Metodi molecolari per il miglioramento genetico (M3G)

Corso di laurea in “Scienze della produzione e protezione delle piante” (codice G59)

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Sito Web del corso:

http://users.unimi.it/geuna/genetica/genetica.html

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Programma

- Richiamo di genetica formale (mendeliana)
- Marcatori molecolari
- Identificazione genetico-molecolare delle varietà: *fingerprinting* genetico
- Mappatura genica con marcatori molecolari
- Metodi di analisi dei genomi (Genomica, Trascrittomica, Proteomica, Metabolomica).
- Gli strumenti della bioinformatica
- Principi di ingegneria genetica e tecnologia del DNA ricombinante. Trasformazione genetica e produzione di organismi geneticamente modificati (OGM)
- Esercitazioni in aula
- Esercitazioni pratiche

http://www.agraria.unimi.it/didattica/programma_pdf.php?codice=G54005&db=MYSQL&anno=0708

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“…produciamo più informazione di quanto siamo in grado di utilizzarne…”

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Autofecondazione di un monoibrido

LU 06-03-2013

Simulare cosa succede (classi e rapporti genotipici e fenotipici) autofecondando un individuo monoibrido Aa

¾ A-

¼ aa

	A	a
A	AA	Aa
a	Aa	aa

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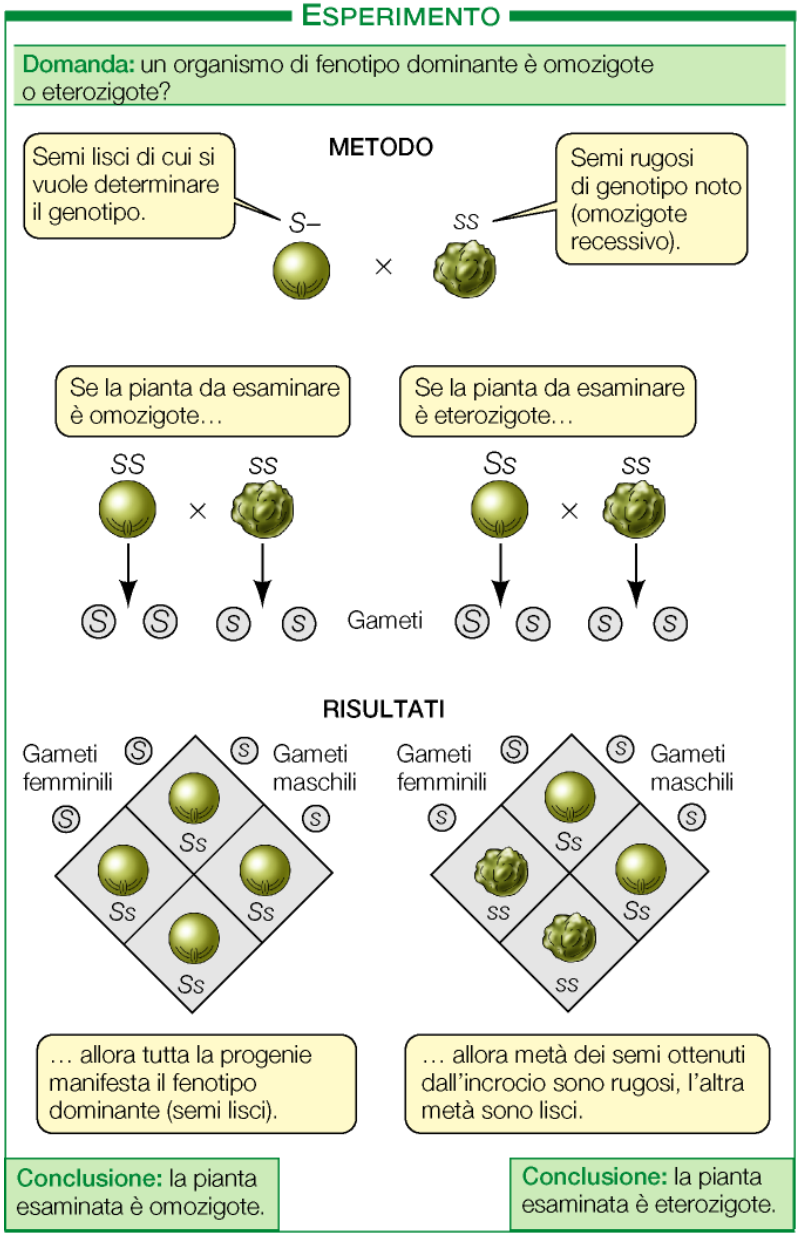
L’incrocio di prova (*test cross*)

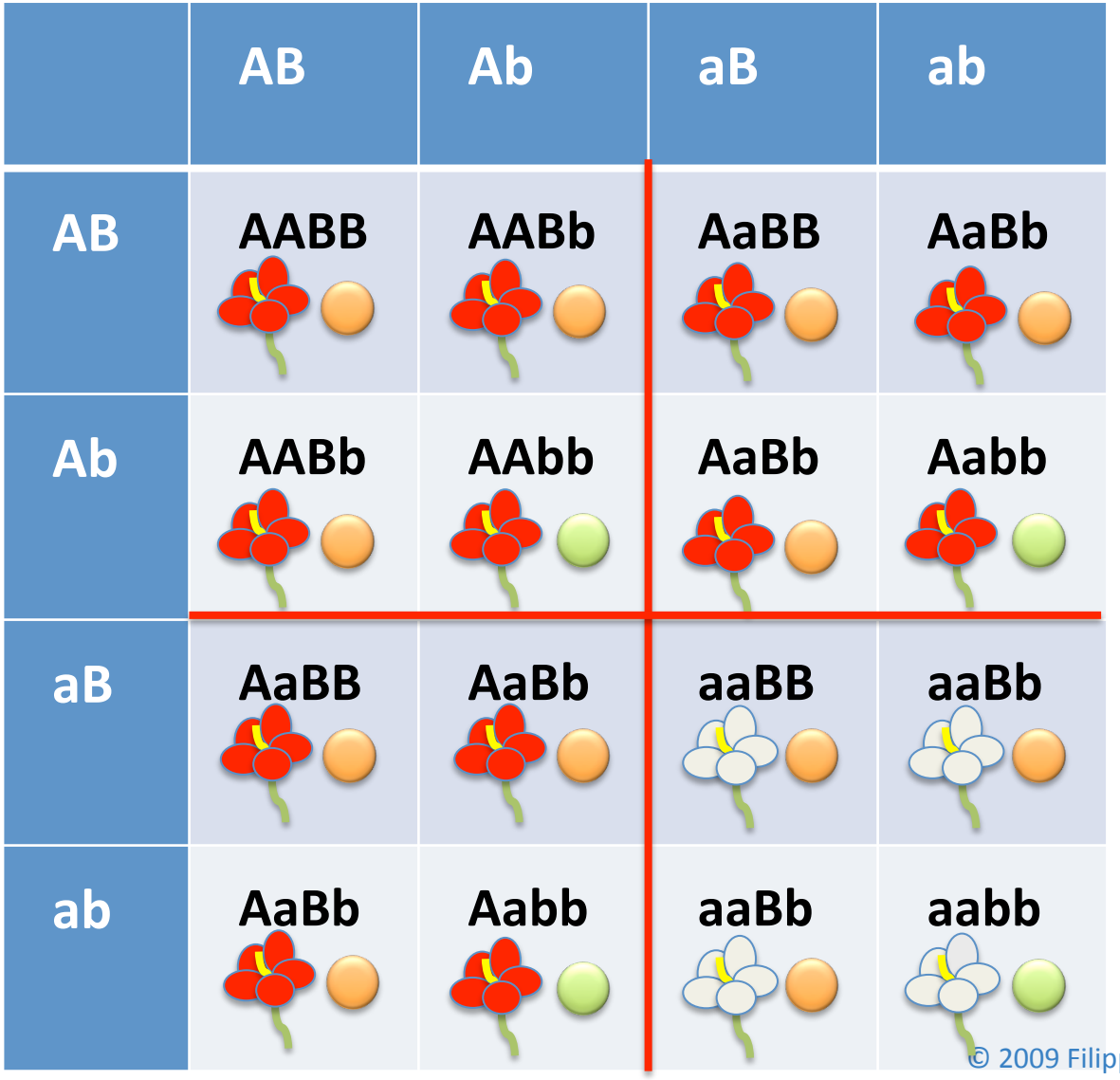
LU 09-03-2009



Il quadrato di Punnett per l'autofecondazione di un diibrido

MA 28-02-2012

	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb



AaBb x AaBb

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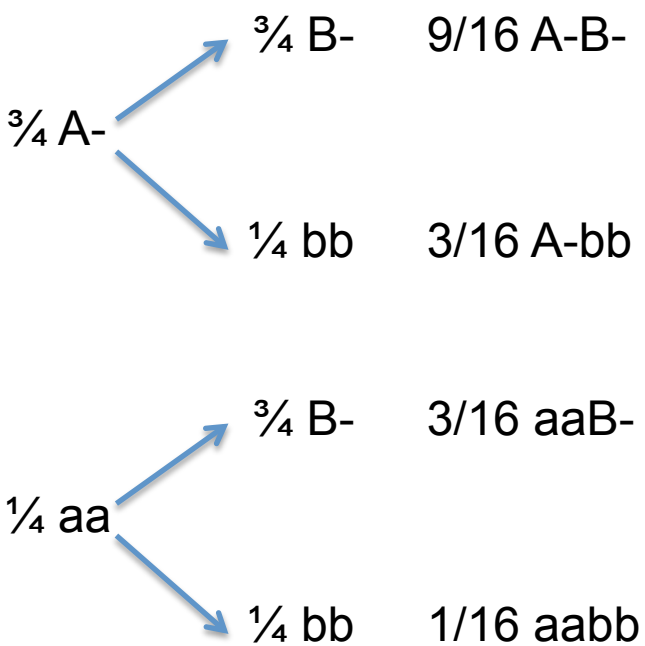
Esercizio sull'autofecondazione di un diibrido

MA 28-02-2012

Simulare cosa succede (classi e rapporti genotipici e fenotipici) autofecondando un individuo diibrido AaBb



	AB	Ab	aB	ab
AB	**AABB**	**AABb**	**AaBB**	**AaBb**
Ab	**AABb**	**AAbb**	**AaBb**	**Aabb**
aB	**AaBB**	**AaBb**	**aaBB**	**aaBb**
ab	**AaBb**	**Aabb**	**aaBb**	**aabb**

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Esercizio sull'autofecondazione di un triibrido

LU 12-03-2013

Simulare cosa succede (classi e rapporti genotipici e fenotipici) autofecondando un individuo triibrido AaBbCc

Suggerimento: utilizzare il metodo della tricotomia genotipica e della dicotomia fenotipica

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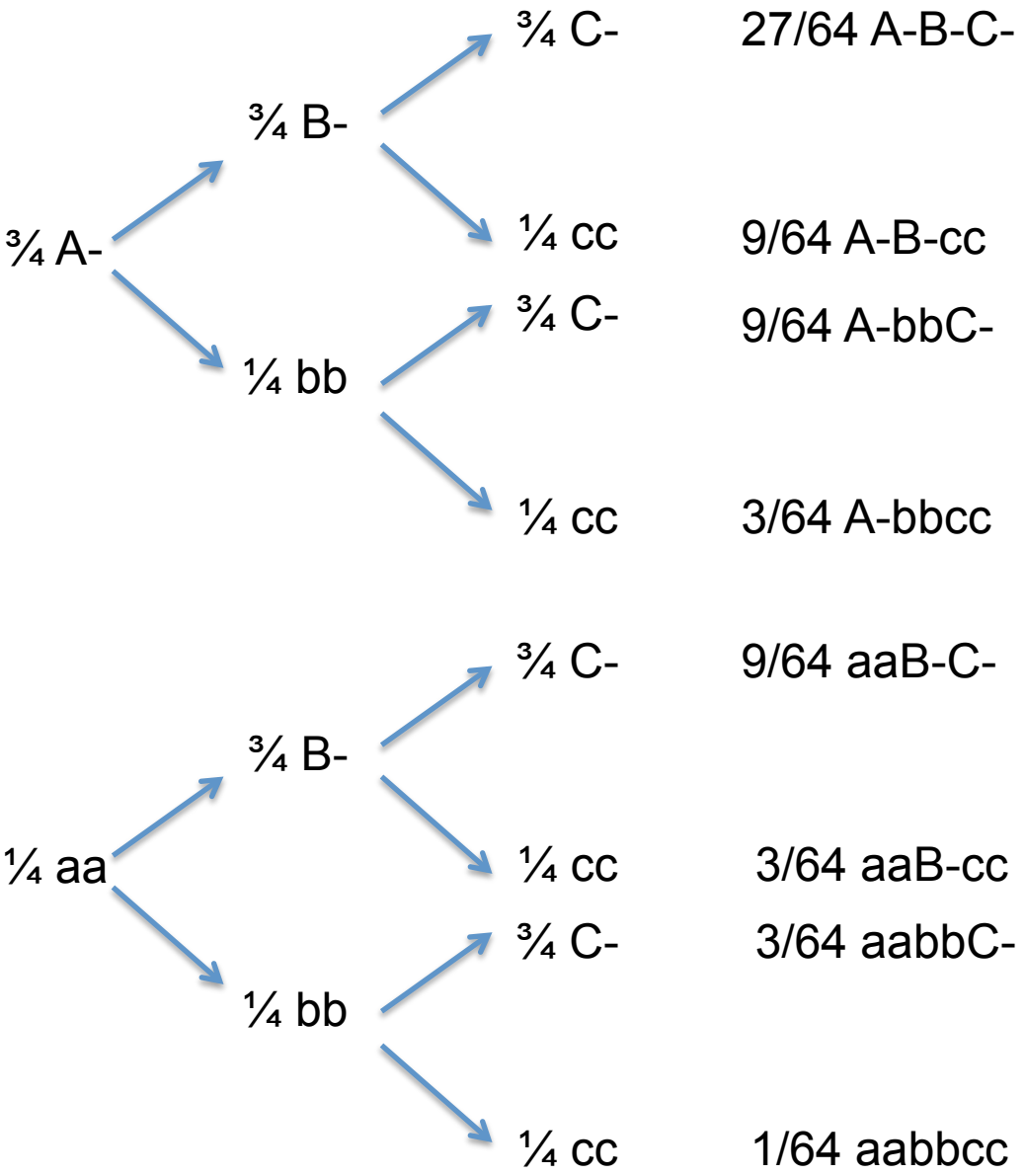
Esercizio sull'autofecondazione di un triibrido

ME 29-02-2012



Quante classi fenotipiche si producono con n geni? 2^n

Ammettendo 2 geni su 2 cromosomi indipendenti 2^2

Ammettendo 3 geni su 3 cromosomi indipendenti 2^3

Ammettendo 23 geni su 23 cromosomi indipendenti 2^{23}

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Esercizio sull'autofecondazione di un triibrido

MA 28-02-2012

	ABC	ABc	AbC	Abc	aBC	aBc	abC	abc
ABC	**AABBCC**	**AABBCc**						
ABc	**AABBCc**							
AbC								
Abc								
aBC								
aBc								
abC								
abc								

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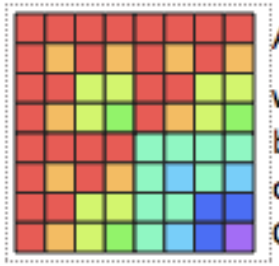
Punnett Square Calculator

MA 28-02-2012

Punnett Square Calculator



A Punnett Square shows the genotypes two individuals can produce when crossed. To draw a square, write all possible allele combinations one parent can contribute to its gametes across the top of a box and all possible allele combinations from the other parent down the left side. The allele combinations along the top and sides become labels for rows and columns within the square. Complete the genotypes in the square by filling it in with the alleles from each parent. Since all allele combinations are equally likely to occur, a Punnett Square predicts the probability of a cross producing each genotype.

Number of traits in cross: 2

Hide Frequencies

Cross:

AaBb × AaBb

	AB	Ab	aB	ab
AB	AABB	AAbB	aABB	aAbB
Ab	AABb	AAbb	aABb	aAbb
aB	AaBB	AabB	aaBB	aabB
ab	AaBb	Aabb	aaBb	aabb

There are 9 distinct genotypes

AABB: 1
AAbB: 2
aABB: 2
aAbB: 4
AAbb: 1
aAbb: 2
aaBB: 1
aabB: 2
aabb: 1

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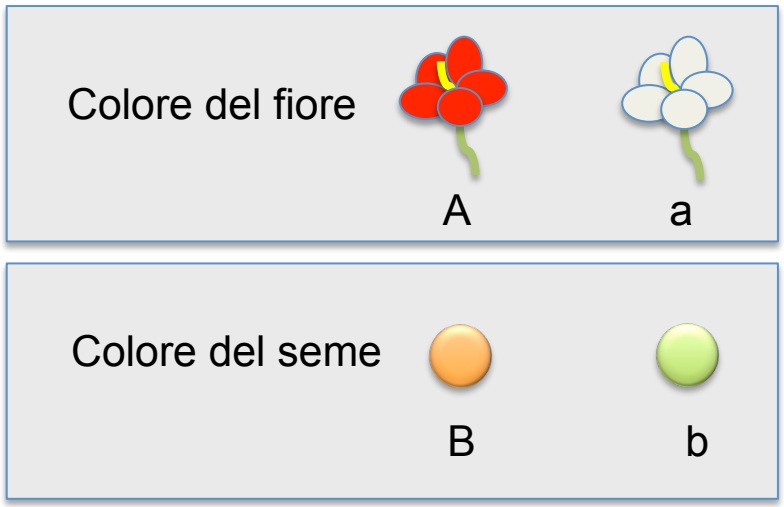
http://scienceprimer.com/punnett-square-calculator

Segregazione di un diibrido in incrocio di prova

MA 10-03-2015

	ab	
AB	AaBb	1/4
Ab	Aabb	1/4
aB	aaBb	1/4
ab	aabb	1/4



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Per ciascuna configurazione possibile compaiono **4** classi fenotipiche in rapporti **uguali** quindi: **segregazione indipendente**

Esercizio sulla segregazione di un diibrido in incrocio di prova

MA 10-03-2015

(Stansfield 3.4)

Le piante di pomodoro alte sono prodotte dall'azione di un allele dominante *D*, e quelle nane dall'allele recessivo *d*. I fusti pelosi sono prodotti da un gene dominante *H* e quelli non pelosi dall'allele recessivo *h*. Una pianta diibrida alta con fusto peloso viene sottoposta ad un incrocio di prova e si osserva che la sua F1 è costituita da: 118 piante alte e con fusto peloso, 121 piante nane con fusto non peloso, 112 piante alte con fusto non peloso, 109 piante nane con fusto peloso. (a) Si tracci lo schema di questo incrocio. (b) Qual è il rapporto tra piante alte e nane; tra piante con fusto peloso e non peloso? (c) Questi due loci sono soggetti ad un assortimento indipendente l'uno dall'altro?

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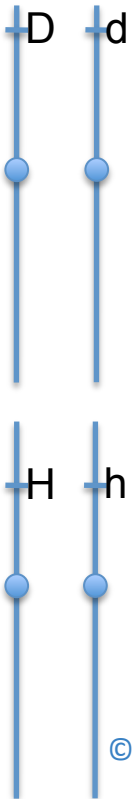
Esercizio sulla segregazione di un diibrido in incrocio di prova

MA 10-03-2015

(Stansfield 3.4) Le piante di pomodoro alte sono prodotte dall'azione di un allele dominante *D*, e quelle nane dall'allele recessivo *d*. I fusti pelosi sono prodotti da un gene dominante *H* e quelli non pelosi dall'allele recessivo *h*. Una pianta diibrida alta con fusto peloso viene sottoposta ad un incrocio di prova e si osserva che la sua F1 è costituita da: 118 piante alte e con fusto peloso, 121 piante nane con fusto non peloso, 112 piante alte con fusto non peloso, 109 piante nane con fusto peloso. (a) Si tracci lo schema di questo incrocio. (b) Qual è il rapporto tra piante alte e nane; tra piante con fusto peloso e non peloso? (c) Questi due loci sono soggetti ad un assortimento indipendente l'uno dall'altro?



Alte Fusto peloso	**118**
Alte Fusto non peloso	**112**
Nane Fusto peloso	**109**
Nane Fusto non peloso	**121**

	DH	Dh	dH	dh
dh	**DdHh 0.25**	**Ddhh 0.25**	**ddHh 0.25**	**ddhh 0.25**

Alte Nane

(118 + 112) : (121 + 109) → 1:1

Peloso Non peloso

(118 + 109) : (121 + 112) → 1:1

Comparsa di 4 classi fenotipiche in rapporti uguali quindi: **segregazione indipendente**

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Esercizio sulla segregazione di un diibrido in incrocio di prova

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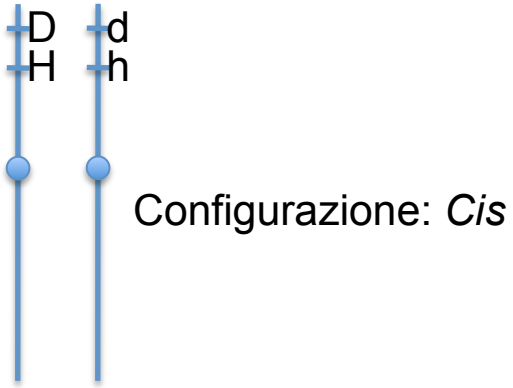
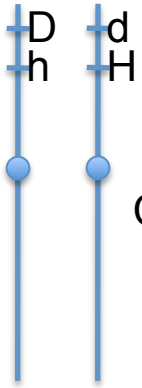
(Stansfield 3.4) Le piante di pomodoro alte sono prodotte dall'azione di un allele dominante *D*, e quelle nane dall'allele recessivo *d*. I fusti pelosi sono prodotti da un gene dominante *H* e quelli non pelosi dall'allele recessivo *h*. Una pianta diibrida alta con fusto peloso viene sottoposta ad un incrocio di prova e si osserva che la sua F1 è costituita da: 118 piante alte e con fusto peloso, 121 piante nane con fusto non peloso, 112 piante alte con fusto non peloso, 109 piante nane con fusto peloso. (a) Si tracci lo schema di questo incrocio. (b) Qual è il rapporto tra piante alte e nane; tra piante con fusto peloso e non peloso? (c) Questi due loci sono soggetti ad un assortimento indipendente l'uno dall'altro?



Configurazione: *Cis*

Vs



Configurazione: *Trans*

	DH	dh
dh	DdHh 0.50	ddhh 0.50

	Dh	dH
dh	Ddhh 0.50	ddHh 0.50

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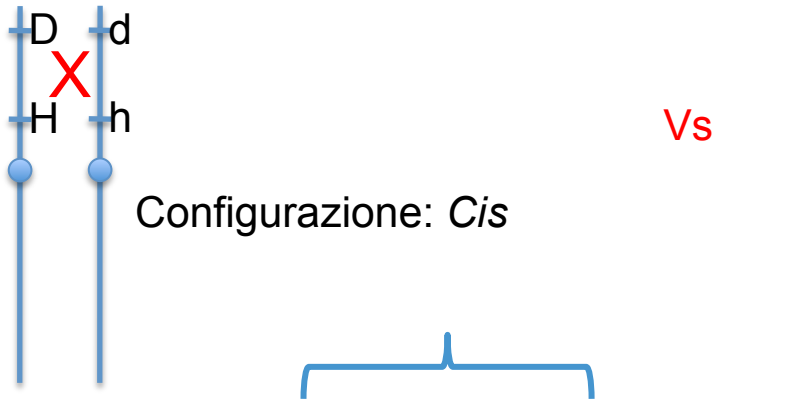
Per ciascuna configurazione possibile compaiono solo **2** classi fenotipiche in rapporti uguali quindi: **segregazione non indipendente (linkage completo)**

Esercizio sulla segregazione di un diibrido in incrocio di prova

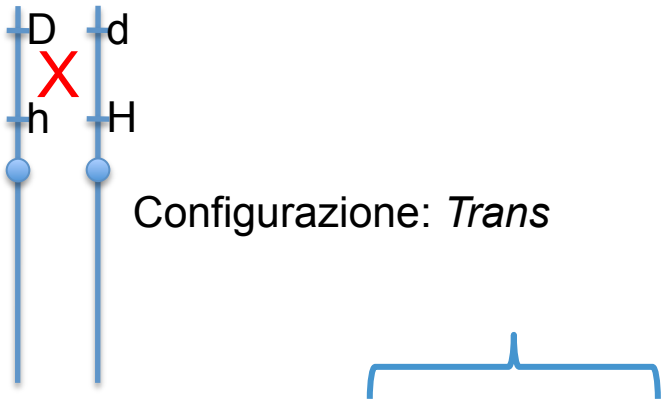
MA 10-03-2015

(Stansfield 3.4) Le piante di pomodoro alte sono prodotte dall'azione di un allele dominante *D*, e quelle nane dall'allele recessivo *d*. I fusti pelosi sono prodotti da un gene dominante *H* e quelli non pelosi dall'allele recessivo *h*. Una pianta diibrida alta con fusto peloso viene sottoposta ad un incrocio di prova e si osserva che la sua F1 è costituita da: 118 piante alte e con fusto peloso, 121 piante nane con fusto non peloso, 112 piante alte con fusto non peloso, 109 piante nane con fusto peloso. (a) Si tracci lo schema di questo incrocio. (b) Qual è il rapporto tra piante alte e nane; tra piante con fusto peloso e non peloso? (c) Questi due loci sono soggetti ad un assortimento indipendente l'uno dall'altro?



Configurazione: *Cis*

Vs



Configurazione: *Trans*

	DH	Dh	dH	dh
dh	DdHh 0.45	Ddhh 0.05	ddHh 0.05	ddhh 0.45

	Dh	DH	dh	dH
dh	Ddhh 0.45	DdHh 0.05	ddhh 0.05	ddHh 0.45

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Per ciascuna configurazione possibile compaiono **4** classi fenotipiche in rapporti **non uguali** quindi: **segregazione non indipendente (linkage incompleto)**

Esercizio sulla segregazione di un triibrido

(Stansfield 3.7)

Si sa che il colore del fusto delle piante di pomodoro è sotto il controllo genetico di almeno un paio di alleli tali per cui *A*- dà come risultato la produzione di pigmento antocianina (fusto color porpora). Il genotipo recessivo *aa* non dà questo pigmento per cui il fusto rimane verde. Il bordo della foglia del pomodoro può essere profondamente intagliato sotto l'azione di un allele dominante *C*. Il genotipo recessivo cc produce foglie dal bordo liscio chiamate "foglie a patata". La produzione di due loculi nel frutto del pomodoro è una caratteristica dell'allele dominante *M*; loculi multipli sono prodotti dal genotipo recessivo *mm*. Viene realizzato un incrocio tra due linee pure: fusto color porpora, foglia di patata, frutto biloculato x fusto verde, foglia incisa, frutto pluriloculato. Quale rapporto fenotipico si attende nella F2?

•VARIANTE: cosa succederebbe se i geni A e C fossero in linkage completo?

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Esercizio sulla segregazione di un diibrido

(Stansfield 3.8)

La posizione del fiore sul fusto del **pisello** è determinata da un paio di alleli. I fiori che crescono alle ascelle (angolo superiore tra il picciolo e il fusto) sono prodotti dall'azione di un allele dominante **T**, quelle che crescono solo all'apice del fusto dall'allele recessivo **t**. I fiori colorati sono prodotti da un gene dominante **C** e i fiori bianchi dall'allele recessivo **c**. Una pianta diibrida con fiori colorati che crescono alle ascelle delle foglie viene incrociata con una pianta di razza pura, avente lo stesso fenotipo. Quali rapporti genotipici e fenotipici si attendono nella progenie F1?

VARIANTE: cosa succederebbe se il padre fosse ttcc?

VARIANTE 2: cosa succederebbe se i geni T e C fossero in linkage? (esempio con linkage a 10 cM e 20 cM)

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Esercizio sulla segregazione di un diibrido

ME 11-03-2015

(Stansfield 3.9)

Nei **meloni estivi** il colore bianco del frutto è determinato da un allele dominante (W) e il colore giallo dall'allele recessivo (w). Un allele dominante in corrispondenza di un altro locus (S) produce frutti piatti e il suo allele recessivo (s) frutti sferici. Se una varietà omozigote con frutto bianco e piatto, dal genotipo WWSS, viene incrociata con una varietà omozigote con frutto giallo sferico, dal genotipo wwss, i componenti della F1 sono tutti diibridi dal genotipo WwSs con frutti bianchi e piatti. Se viene consentito che i componenti della F1 si incrocino tra loro a caso, quale sarà il rapporto fenotipico atteso nella generazione F2? (E i genotipi?)

Variante1: cosa succede se un diibrido WwSs viene incrociato con un doppio recessivo per gli stessi geni?

Variante2: cosa succede se si parte da WWss x wwSS?

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MA 17-03-2015

Esercizio sulla segregazione di un diibrido
(*un gene dominante e uno codominante*)

(Russell 4.12)

Nei **peschi** il gene per la buccia del frutto pelosa (*F*) manifesta dominanza completa su quello che determina la buccia liscia (nettarina) (*f*), e il gene che determina delle formazioni ovali alla base delle foglie (G^o) manifesta dominanza incompleta su quello che ne determina l'assenza (G^N), per cui l'eterozigote presenta formazioni tonde. Una varietà di pesco omozigote con frutti pelosi e assenza di formazioni viene incrociata con una a frutti lisci e formazioni ovali.

a. Quale sarà il fenotipo della F_1?

b. Quale sarà il fenotipo della F_2?

c. Quale sarà il fenotipo dei figli di un incrocio della F_1 con il genitore liscio, con formazioni ovali?

d. Cosa succede se anche il gene F manifesta una dominanza incompleta?

e. Sulla base dei dati del punto (d) si disegni un istogramma delle frequenze dei fenotipi per entrambi i geni contemporaneamente e indipendentemente.

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Esercizio sulla segregazione di un diibrido con codominanza

(*due geni entrambi codominanti*)

MA 17-03-2015

(Russell 4.14)

La forma dei ravanelli può essere allungata (S^L/S^L), ovale (S^L/S^S) o rotonda (S^S/S^S) e il colore dei ravanelli può essere rosso (C^R/C^R), porpora (C^R/C^W) o bianco (C^W/C^W). Se una pianta a ravanelli allungati e rossi viene incrociata con una pianta a ravanelli rotondi e bianchi, quali saranno i fenotipi della F_1 e della F_2?

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Esercizio sulla segregazione di un diibrido con codominanza

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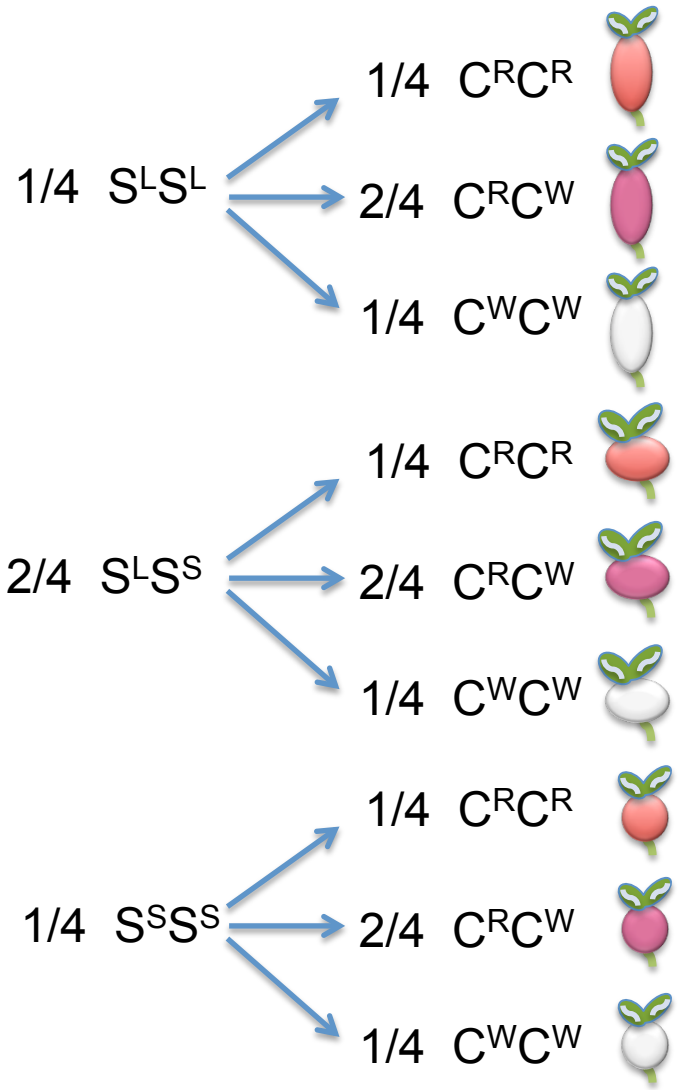
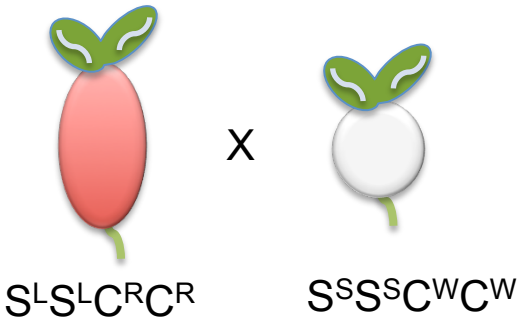
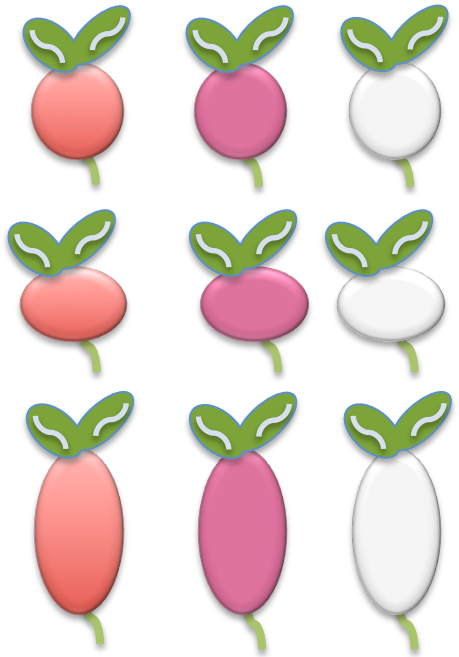
(Russell 4.14)





In F2 con che rapporti segrega il colore? E la forma del ravanello?



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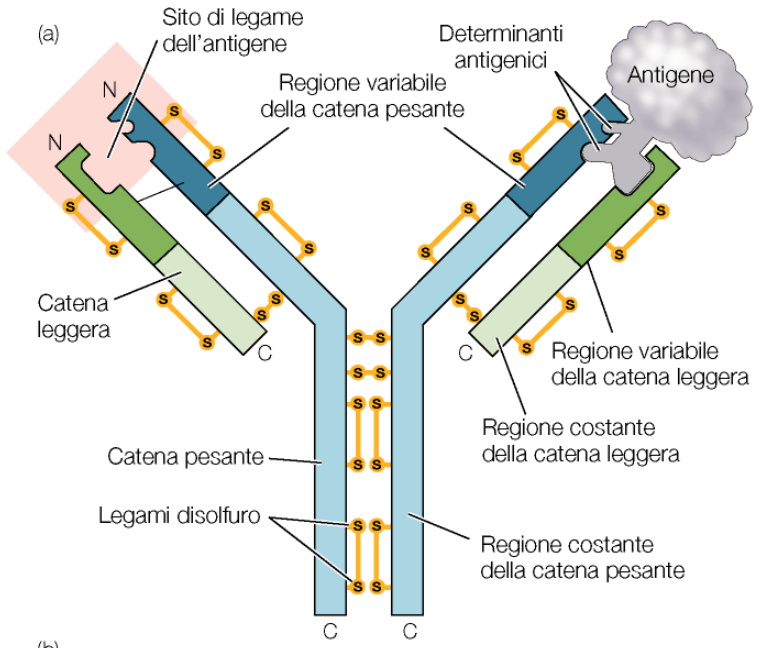
Il sistema AB0 dei gruppi sanguigni

MA 13-03-2012



(b)

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies present	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens present	A antigen	B antigen	A and B antigens	No antigens

Alleli del gruppo AB0:

I^A I^B i

Genotipi possibili:

$I^A I^A \rightarrow A$

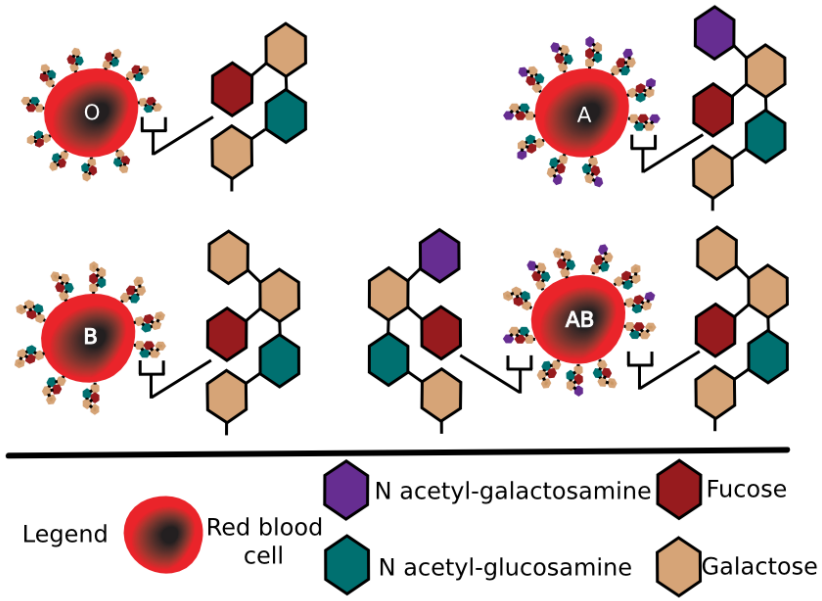
$I^A i \rightarrow A$

$I^B I^B \rightarrow B$

$I^B i \rightarrow B$

$I^A I^B \rightarrow AB$

$ii \rightarrow 0$



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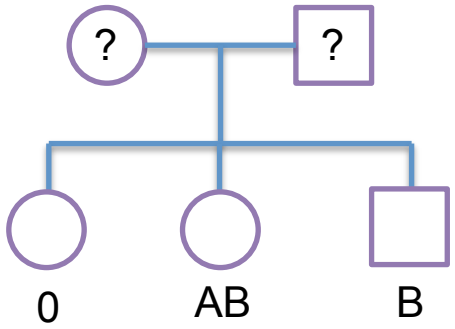
Esercizio sui gruppi sanguigni e la codominanza

MA 13-03-2013

(Stansfield 2.10)

Un uomo chiede il divorzio dalla moglie per infedeltà. Il primo ed il secondo figlio che entrambi riconoscono hanno rispettivamente gruppo sanguigno 0 e AB. Il terzo figlio, che l'uomo disconosce, è del gruppo sanguigno B. Questa affermazione può essere utile alla causa dell'uomo?



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Esercizio sui gruppi sanguigni e la codominanza

MA 13-03-2012

(Russell 4.4)

Stabilite se il bambino, negli esempi che seguono, può realmente nascere dall'incrocio tra due individui. Spiegate la vostra risposta.

a. Un bambino "0" da due individui "A"
b. Un bambino "0" da un individuo "A" e uno "B"
c. Un bambino "AB" da un individuo "A" e uno "0"
d. Un bambino "0" da un individuo "AB" e uno "A"
e. Un bambino "A" da un individuo "AB" e uno "B"

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Esercizio sull'epistasi

ME 25-03-2015

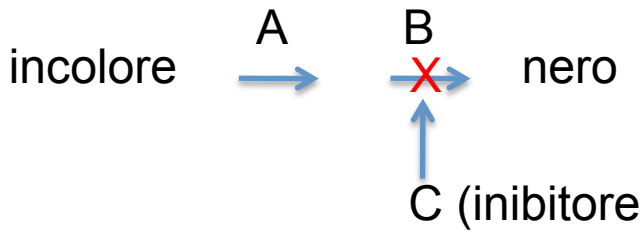
(Russell 4.27)

I geni *A*, *B* e *C* segregano in modo indipendente e controllano la produzione di un pigmento nero.

(a) Supponete che *A*, *B* e *C* agiscano in una via metabolica nel modo seguente:

Gli alleli alternativi che determinano un funzionamento anormale di questi geni sono *a*, *b* e *c* rispettivamente. Un nero *A/A B/B C/C* viene incrociato con un incolore *a/a b/b c/c* e dà una F1 nera. La F1 viene autofecondata. Quale proporzione della F2 è incolore? (Supponete che i prodotti di ogni passaggio, ad eccezione dell'ultimo, siano incolori per cui si osservano solo fenotipi incolori e nero).

(b) Supponete che *C* produca un inibitore che impedisce la formazione del nero distruggendo la capacità di *B* di espletare la sua funzione, nel modo seguente:



Variante: cosa succederebbe se nel caso (a) il gene "C" mostrasse dominanza incompleta con colore intermedio?

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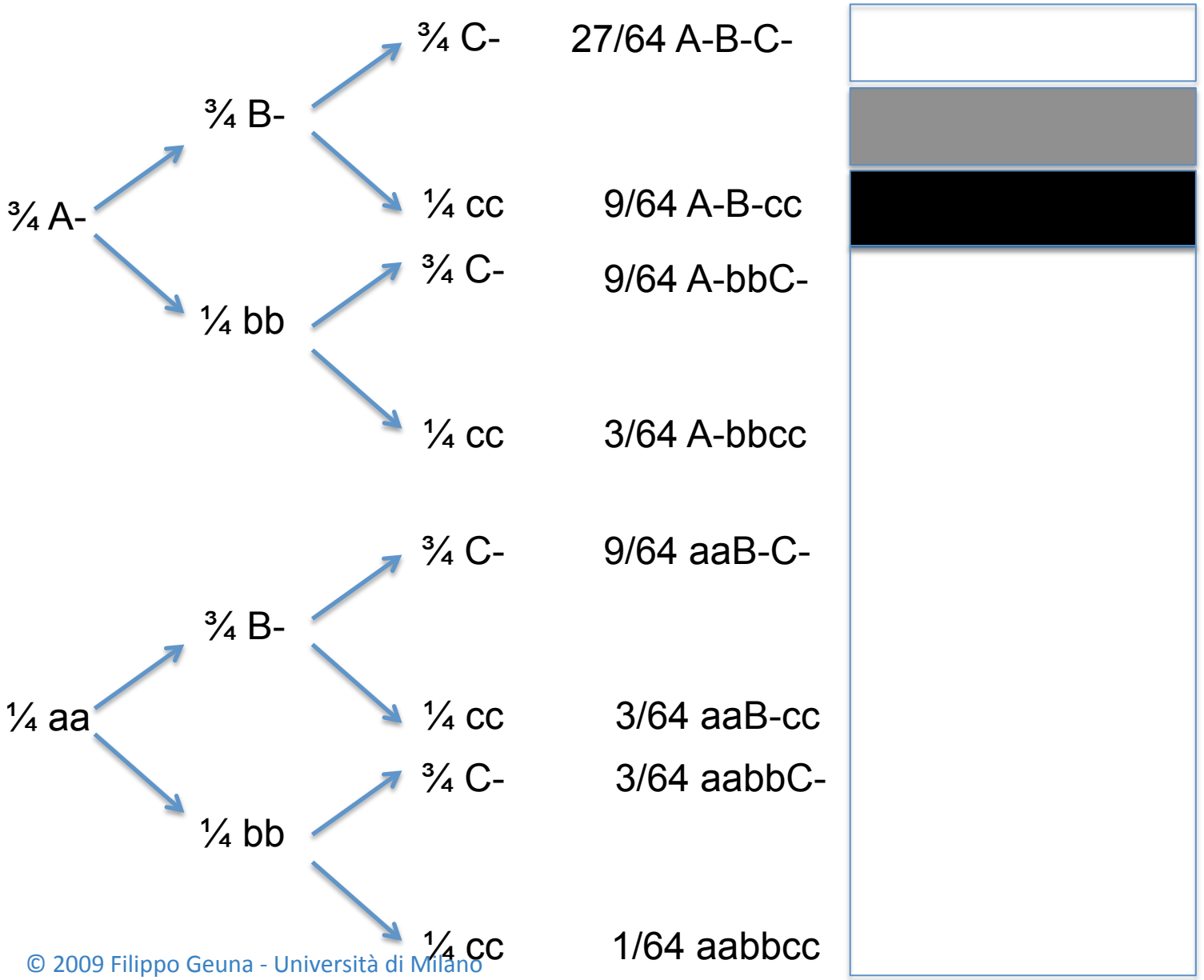
Esercizio sull'epistasi (Russell 4.27)

MA 26-03-2013



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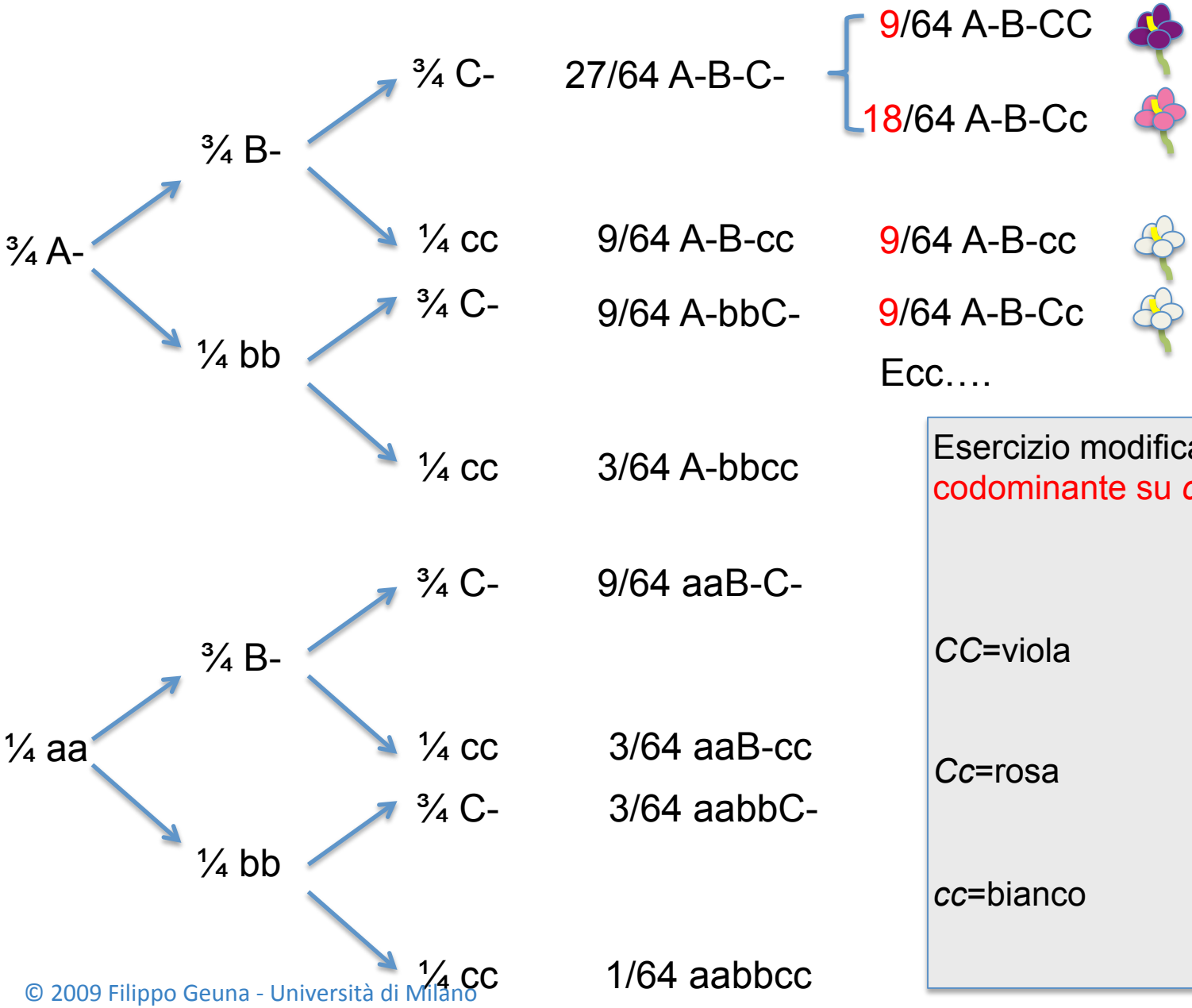
Esercizio sull'epistasi (Russell 4.27 modificato)

MA 20-03-2012



Esercizio modificato in cui *C* è codominante su *c* per cui:

CC=viola CC

Cc=rosa Cc

cc=bianco cc

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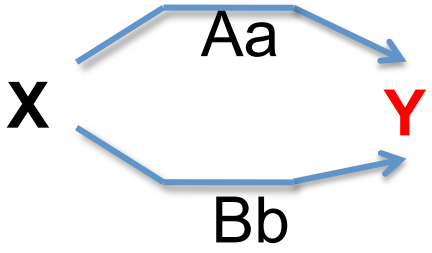
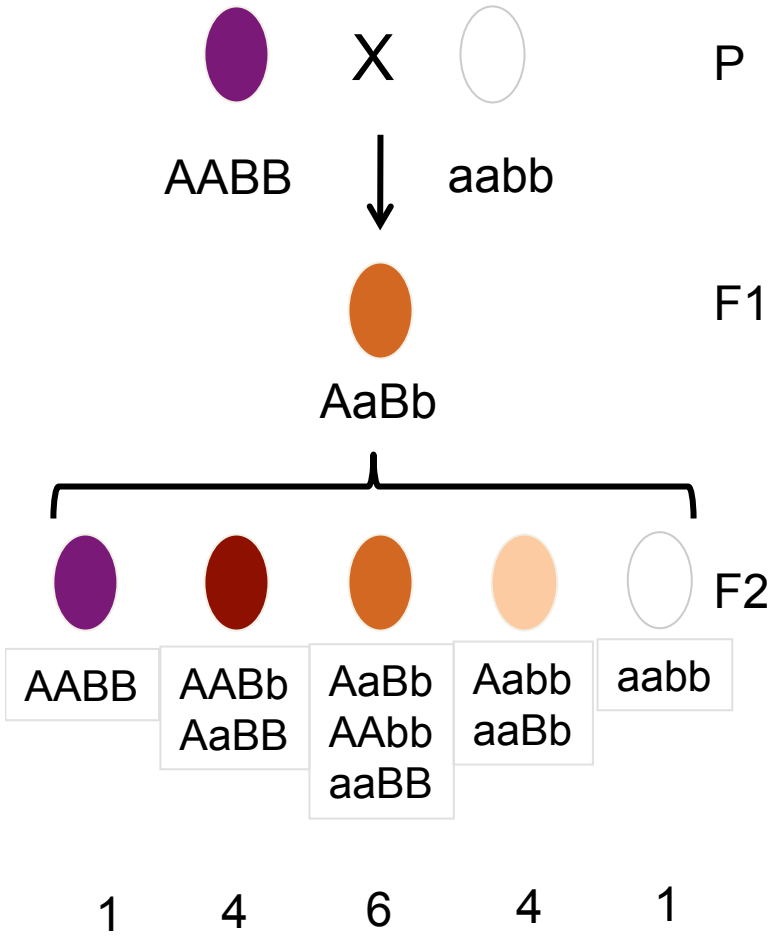
Eredità del colore nella cariosside di frumento (secondo Nilsson-Ehle)

MA 24-03-2015





	AB	**Ab**	**aB**	**ab**
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

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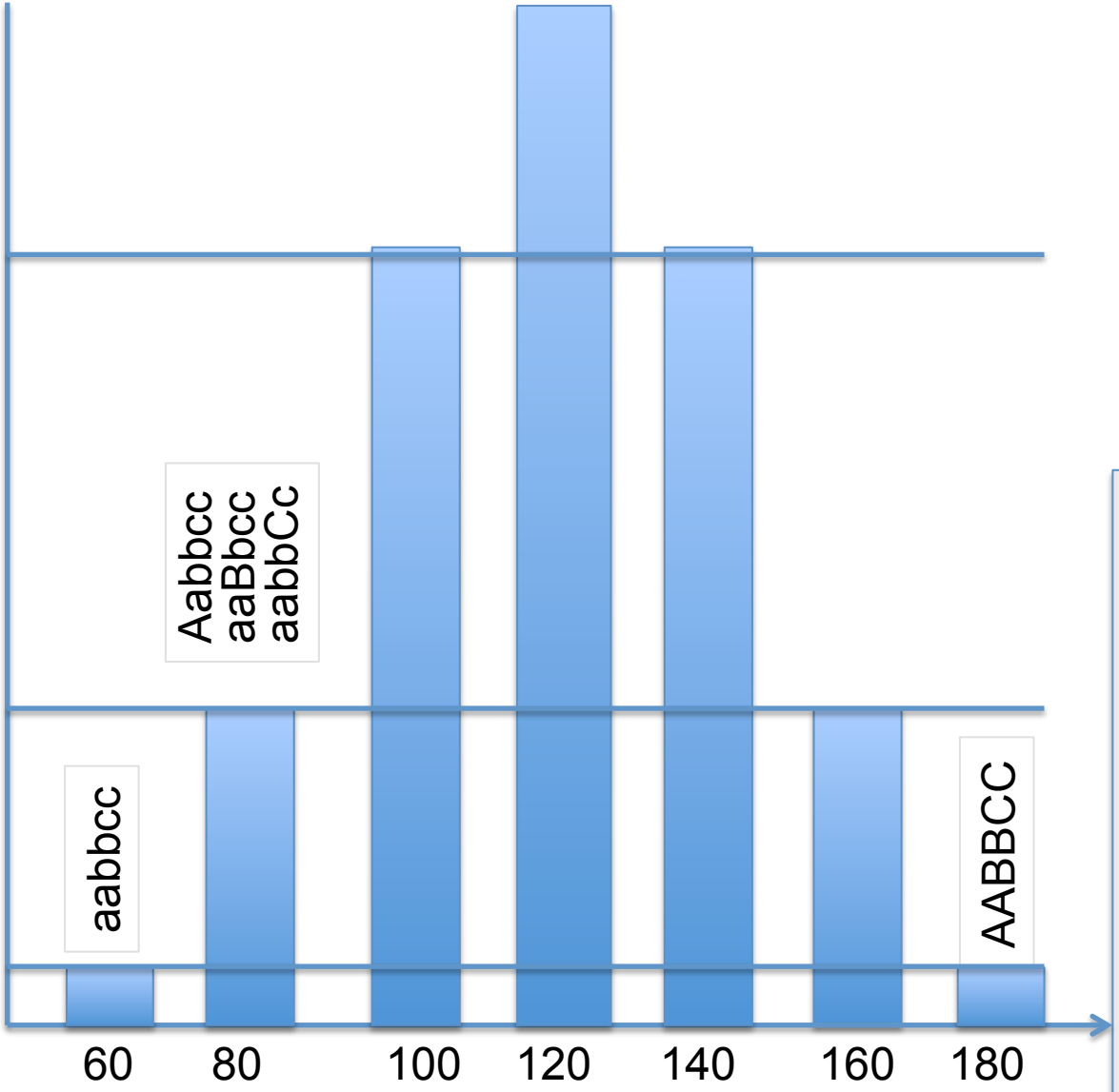
Esercizio sull'epistasi

(ispirato al carattere "colore della cariosside di frumento" di Nilsson-Ehle)

ME 25-03-2015



I geni "A", "B" e "C" sono triplicazioni della stessa funzione biosintetica con due alleli ad effetto quantitativo tali per cui:

A=*B*=*C*=30 unità di pigmento

a=*b*=*c*=10 unità di pigmento

Si calcolino le classi fenotipiche e le relative frequenze attese e si crei un istogramma

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Verifica di ipotesi attraverso il test del X^2 (1/4)

(da Russell, cap.2 pag.36)

SsYy x ssyy

154	lisci, gialli	
124	lisci, verdi	
144	rugosi, gialli	
146	rugosi, verdi	
Totale:	568	

Ipotesi: se i due geni *S* e *Y* sono indipendenti, dal reincrocio dovranno risultare quattro classi fenotipiche con rapporto 1:1:1:1

E' questo il caso?

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Il test del X^2 (calcolo) (2/4)

(da Russell, cap.2 pag.36)

(1)	(2)	(3)	(4)	(5)	(6)
Fenotipo	Numero osservato (*o*)	Numero atteso (*e*)	*d* (= *o* - *e*)	d^2	d^2/e
lm	90	95	-5	25	0,27
ll	100	95	5	25	0,27
Totale			0		**0,54**
	(7) X^2 = ….		**(8)** gradi di libertà (*df*) = ….		

$$X^2 = \sum \frac{d^2}{e}$$

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Il test del X^2 (calcolo) (3/4)

(da Russell, cap.2 pag.36)

(1)	(2)	(3)	(4)	(5)	(6)
Fenotipo	Numero osservato (*o*)	Numero atteso (*e*)	*d* (= *o* - *e*)	d^2	d^2/e
Liscio, giallo	154	142	+12	144	1,01
Liscio, verde	124	142	-18	324	2,28
Rugoso, giallo	144	142	+2	4	0,03
Rugoso, verde	146	142	+4	16	0,11
Totale	568	568	0		**3,43**
	(7) X^2 = 3,43		**(8)** gradi di libertà (*df*) = 3		

$$X^2 = \sum \frac{d^2}{e}$$

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Il test del X^2 (tabella delle probabilità) (4/4)

(da Russell, cap.2 pag.36)

MA 31-03-2015

~ Tabella 2.5

Probabilità di Chi-Quadrato

	Probabilità									
df	0,95	0,90	0,70	0,50	0,30	0,20	0,10	0,05	0,01	0,001
1	0,004	0,016	0,15	0,46	1,07	1,64	2,71	3,84	6,64	10,83
2	0,10	0,21	0,71	1,39	2,41	3,22	4,61	5,99	9,21	13,82
3	0,35	0,58	1,42	2,37	3,67	4,64	6,25	7,82	11,35	16,27
4	0,71	1,06	2,20	3,36	4,88	5,99	7,78	9,49	13,28	18,47
5	1,15	1,61	3,00	4,35	6,06	7,29	9,24	11,07	15,09	20,52
6	1,64	2,20	3,83	5,35	7,23	8,56	10,65	12,59	16,81	22,46
7	2,17	2,83	4,67	6,35	8,38	9,80	12,02	14,07	18,48	24,32
8	2,73	3,49	5,53	7,34	9,52	11,03	13,36	15,51	20,09	26,13
9	3,33	4,17	6,39	8,34	10,66	12,24	14,68	16,92	21,67	27,88
10	3,94	4,87	7,27	9,34	11,78	13,44	15,99	18,31	23,21	29,59
11	4,58	5,58	8,15	10,34	12,90	14,63	17,28	19,68	24,73	31,26
12	5,23	6,30	9,03	11,34	14,01	15,81	18,55	21,03	26,22	32,91
13	5,89	7,04	9,93	12,34	15,12	16,99	19,81	22,36	27,69	34,53
14	6,57	7,79	10,82	13,34	16,22	18,15	21,06	23,69	29,14	36,12
15	7,26	8,55	11,72	14,34	17,32	19,31	22,31	25,00	30,58	37,70
20	10,85	12,44	16,27	19,34	22,78	25,04	28,41	31,41	37,57	45,32
25	14,61	16,47	20,87	24,34	28,17	30,68	34,38	37,65	44,31	52,62
30	18,49	20,60	25,51	29,34	33,53	36,25	40,26	43,77	50,89	59,70
50	34,76	37,69	44,31	49,34	54,72	58,16	63,17	67,51	76,15	86,66

Accettare | Rifiutare
al livello di 0,05

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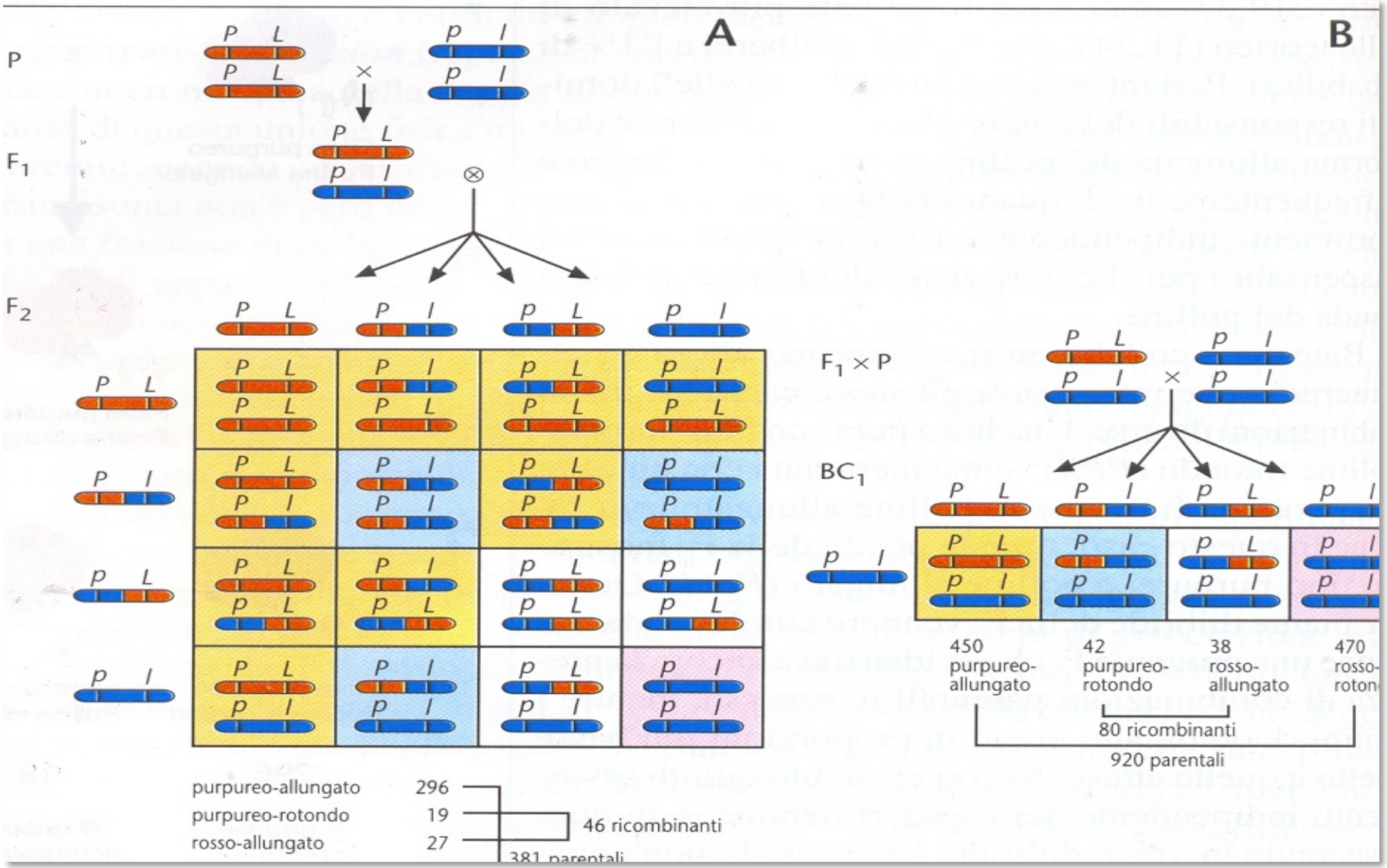
Calcolo della distanza tra geni: metodo della “radice quadrata” vs. reincrocio

MA 31-03-2015



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Calcolo della distanza tra geni: metodo della “radice quadrata” vs. reincrocio (Excel)

MA 31-03-2015

	A	B	C	D	E	F	G	H	I	J	K	L
2			0,25	0,25	0,25	0,25						
3			PL	Pl	pL	pl		Fenotipo	TOTALE	%	x individui -->	150
4	0,25	PL	0,0625	0,0625	0,0625	0,0625		P-L-	0,5625	56,25	84	
5	0,25	Pl	0,0625	0,0625	0,0625	0,0625		P-ll	0,1875	18,75	28	
6	0,25	pL	0,0625	0,0625	0,0625	0,0625		ppL-	0,1875	18,75	28	
7	0,25	pl	0,0625	0,0625	0,0625	0,0625		ppll	0,0625	6,25	9	
8											150	
9												
10												
11	**Segregazione in linkage (?:?:?:?)**											
12			0,44	0,06	0,06	0,44						
13			PL	Pl	pL	pl		Fenotipo	TOTALE	%	x individui -->	150
14	0,44	PL	0,1936	0,0264	0,0264	0,1936		P-L-	0,6936	69,36	104	
15	0,06	Pl	0,0264	0,0036	0,0036	0,0264		P-ll	0,0564	5,64	8	
16	0,06	pL	0,0264	0,0036	0,0036	0,0264		ppL-	0,0564	5,64	8	
17	0,44	pl	0,1936	0,0264	0,0264	0,1936		ppll	0,1936	19,36	29	
18					SQRT(F17) =	0,44					150	
19												

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Dal file Excel: “TGB(Foglio di calcolo).xlsx”

Esercizio di costruzione di mappa con tre geni

ME 01-04-2015

(Russell 5.22)

Per ciascuna delle seguenti tabelle in cui sono riportati i fenotipi e i numeri di progenie di un reincrocio, stabilisci quale locus sta nel mezzo e ricostruisci il genotipo del triplo eterozigote analizzato.

a.

ABC	191
abc	180
Abc	5
aBC	5
ABc	21
abC	31
AbC	104
aBc	109

b.

CDE	9
cde	11
Cde	35
cDE	27
CDe	78
cdE	81
CdE	275
cDe	256

c.

FGH	110
fgh	114
Fgh	37
fGH	33
FGh	202
fgH	185
FgH	4
fGh	0

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Esercizio di costruzione di mappa con tre geni (soluzione)

(Russell 5.22)

ME 01-04-2015

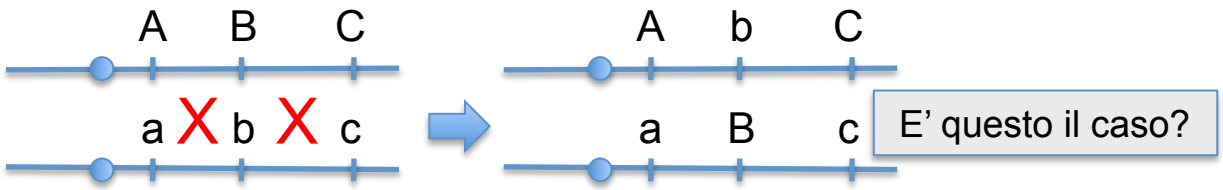
E' necessario determinare innanzitutto l'ordine dei geni utilizzando la classe dei doppi ricombinanti (la meno numerosa)

a.

ABC	191
abc	180
Abc	5
aBC	5
ABc	21
abC	31
AbC	104
aBc	109

Procedimento per stabilire l'ordine dei geni:

- Si individuano le classi dei parentali e dei doppi ricombinanti
- Si determina quale configurazione di marcatori (ordine lineare) consente di trasformare i cromosomi parentali in cromosomi con doppio crossing-over



E' questo il caso?

Una volta stabilito l'ordine bisogna stabilire le distanze a coppie

% ricombinazione 1 regione = (SR 1 regione + DR) / progenie totale = (104 + 109 + 5 + 5) / 646 **= 0,345 (34,5 cM)**

% ricombinazione 2 regione = (SR 2 regione + DR) / progenie totale = (21 + 31 + 5 + 5) / 646 **= 0,096 (9,6 cM)**

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Esercizio di costruzione di mappa con tre geni (variante)

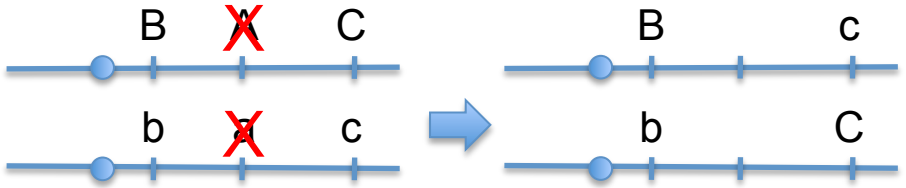
ME 01-04-2015

(Russell 5.22)

a.

ABC	191
abc	180
Abc	5
aBC	5
ABc	21
abC	31
AbC	104
aBc	109

La distanza di mappa tra B e C è **maggiore** che con l'aggiunta del gene A interposto tra i due



(Ricombinanti tra **B** e **C**) / progenie totale = (104 + 109 + 21 + 31) / 646 **= 0,41 (41 cM)**

In questa variante si mostra l'effetto dell'eliminazione del gene centrale sulla percentuale di ricombinazione che risulta minore rispetto alla somma delle due distanze a coppie con un gene intermedio.

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Genetica molecolare e genomica

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Tecniche di analisi presentate nel corso

MA 23-04-2013

Tecniche chimiche

- Cromatografia su carta
- HPLC
- Gas cromatografia (GC)
- Spettrometria di massa (MS)
- Combinazioni delle tecniche precedenti (es. GC-MS, HPLC-MS)

Tecniche molecolari

- Elettroforesi e visualizzazione di acidi nucleici (in agarosio e acrilamide)
- Amplificazione di DNA (PCR)
- Sequenziamento di DNA (metodo Sanger e di nuova generazione)
- Produzione di cDNA da mRNA
- Taglio di DNA con enzimi di restrizione
- Ligazione di frammenti di DNA con DNA ligasi
- Produzione di un costrutto ricombinante (clonazione in un vettore)
- Ibridazione di DNA (*Southern blot*)
- Ibridazione di RNA (*Northern blot*)
- Enzyme Linked Immuno Sorbent Assay (ELISA)
- Elettroforesi di proteine (in acrilamide)
- Rilevamento immunologico di proteine (*Western blot*)

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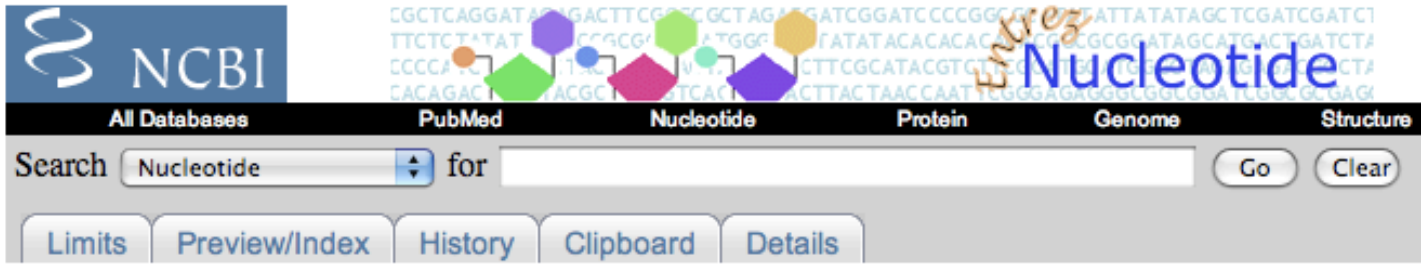
La PCR (selezione di un gene da amplificare)



NCBI Reference Sequence: NM_118207.2

Arabidopsis thaliana TUB9 (tubulin beta-9 chain); structural molecule (TUB9) mRNA, complete cds

Showing 1.33kb region from base 127 to 1461.

Comment Features Sequence

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LOCUS       NM_118207               1335 bp    mRNA    linear   PLN 22-MAY-2008
DEFINITION  Arabidopsis thaliana TUB9 (tubulin beta-9 chain); structural
            molecule (TUB9) mRNA, complete cds.
ACCESSION   NM_118207 REGION: 127..1461
VERSION     NM_118207.2  GI:30685244
KEYWORDS    .
SOURCE      Arabidopsis thaliana (thale cress)
  ORGANISM  Arabidopsis thaliana
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons;
            rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from AT4G20890.1.
            On May 13, 2003 this sequence version replaced gi:18415585.
FEATURES             Location/Qualifiers
     source          1..1335
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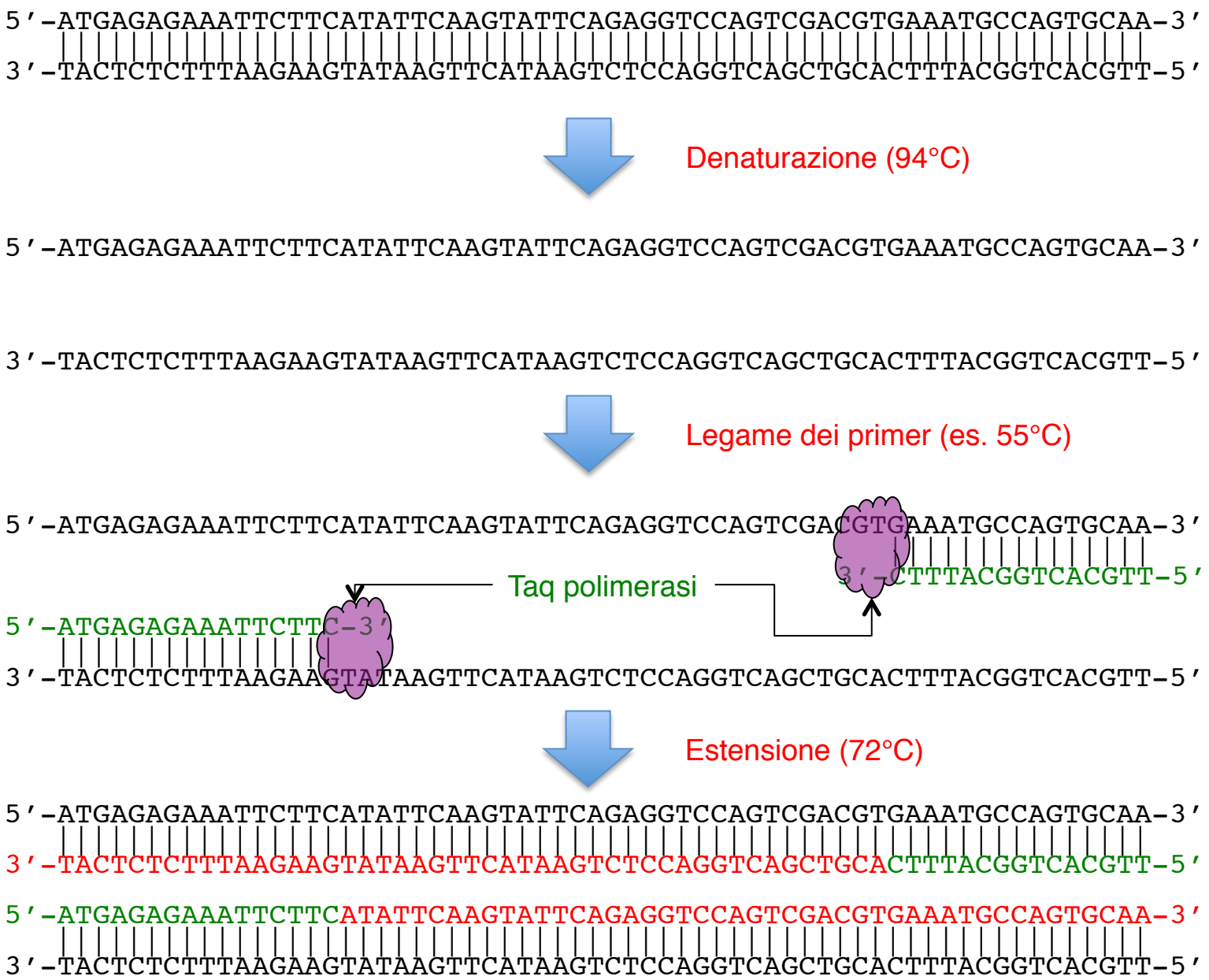
Polymerase Chain Reaction (PCR)

ME 17-04-2013



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Struttura del gene TUB 9 (dal sito NCBI)

NCBI Entrez Gene

All Databases | PubMed | Nucleotide | Protein | Genome | Structure | OMIM | PMC | Journals | Books

Search Gene for [ ] Go Clear

Limits | Preview/Index | History | Clipboard | Details

Display Full Report Show 20 Sort by Relevance Send to

All: 1 | Current Only: 1 | Genes Genomes: 1 | SNP GeneView: 0

1: TUB9 TUB9 (tubulin beta-9 chain); structural molecule [*Arabidopsis thaliana*]

GeneID: 827837 updated 08-Mar-2009

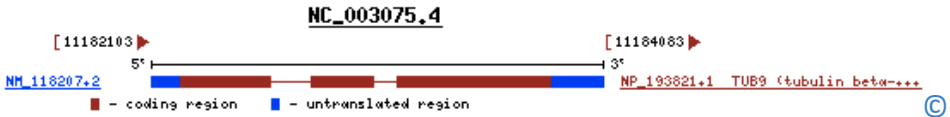
Summary

Gene name	TUB9
Primary source	TAIR:AT4G20890
Locus tag	AT4G20890
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	*Arabidopsis thaliana (ecotype: Columbia)*
Lineage	*Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis*
Also known as	T13K14.50; T13K14_50; tubulin beta-9 chain; TUB9

Genomic regions, transcripts, and products

Go to reference sequence details

Try our new Sequence Viewer



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http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=827837&ordinalpos=1&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

Sequenza del trascritto del gene TUB9 di Arabidopsis

(dal sito NCBI)

MA 23-04-2013

Arabidopsis thaliana TUB9 (tubulin beta-9 chain); structural molecule (TUB9) mRNA, complete cds

>gi|30685244|ref|NM_118207.2| Arabidopsis thaliana TUB9 (tubulin beta-9 chain); structural molecule (TUB9) mRNA, complete cds

TATACCCCTCAATCATAAACAATAAACAGAGGGAGTCGCCAAATTTTCTCATCATTTTCTCGAGAAAATAGACTAAAAGCGATTCCCGAGAGA
AAGAAAGAACAAAAAAAAAACATCCGAGTGAAAATGAGAGAAATTCTTCATATTCAAGGAGGTCAGTGCGGAAACCAGATCGGAGCTAAGTTC
TGGGAAGTTATTTGCGGCGAGCACGGTATTGATCAAACCGGTCAGTCATGTGGCGATACAGATCTACAGCTTGAAAGGATCAATGTCTATTTT
AACGAAGCTAGCGGAGGCAAATACGTTCCTCGTGCTGTTCTTATGGATCTGGAGCCTGGAACTATGGATTCTCTCAGATCTGGGCCGTTTGGT
CAGATTTTCCGGCCTGATAACTTCGTTTTTGGTCAGTCTGGTGCTGGGAATAACTGGGCGAAAGGTCATTACACGGAGGGAGCTGAGTTGATT
GATTCTGTGCTTGATGTTGTGAGGAAGGAAGCTGAGAACTGTGATTGTCTTCAAGGTTTTCAAGTTTGTCATTCTTTGGGAGGAGGAACTGGT
TCTGGAATGGGAACACTTCTTATTTCTAAGATTAGAGAGGAATATCCAGATCGTATGATGATGACCTTTTCGGTCTTCCCATCTCCTAAGGTC
TCTGATACAGTTGTTGAGCCATACAATGCTACTCTTTCCGTTCACCAGCTCGTTGAGAATGCTGATGAATGTATGGTTTTGGATAACGAAGCT
CTTTACGATATCTGTTTCCGTACCTTGAAGCTTGCTAATCCTACATTTGGTGACCTTAACCATCTCATCTCAGCTACAATGAGTGGTGTTACA
TGCTGTCTTCGGTTCCCTGGTCAACTAAACTCTGACCTTAGAAAACTTGCTGTGAACCTTATTCCTTTCCCACGACTCCACTTCTTCATGGTT
GGTTTTGCACCTTTGACATCAAGAGGATCTCAACAATACAGTGCCTTGAGTGTCCCTGAGCTAACTCAACAGATGTGGGATGCCAAAAACATG
ATGTGTGCAGCTGATCCTCGTCATGGTCGTTACTTGACAGCTTCTGCTGTCTTCCGTGGAAAGATGAGTACCAAAGAGGTTGATGAACAGATG
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AAAATGGCATCAACTTTCATTGGAAACTCAACTTCAATCCAGGAAATGTTCAGGCGTGTGAGTGAACAGTTCACTGCCATGTTCAGGAGAAAG
GCTTTCCTTCATTGGTACACAGGAGAAGGAATGGACGAGATGGAGTTCACTGAAGCAGAGAGTAACATGAATGATCTTGTTGCAGAGTATCAG
CAATATCAAGATGCTACAGTCGGTGAAGAGGAGTACGAGGAGGACGAAGAAGAAGAAGAAGCCTAAAGAAAAGATGATTGATTATTACTTTTG
TAAGGCTGTAAGTTCGTCGCACTCATTTCCTTTTACCTCTTTTTCTGAATTGGGATTCTCTCTTTTTGTGCTTTGTGTGTTTTGTTATTGAAT
TTCTTTCCCAAACCTAAGATTTGTTCCAGAGTGTAATCACATTTGCCTATGCTTCTTTGTTTTGTCTATGAAAATATTTCAATGTCAAAAGCC
TATACAATTTCTATGATCC

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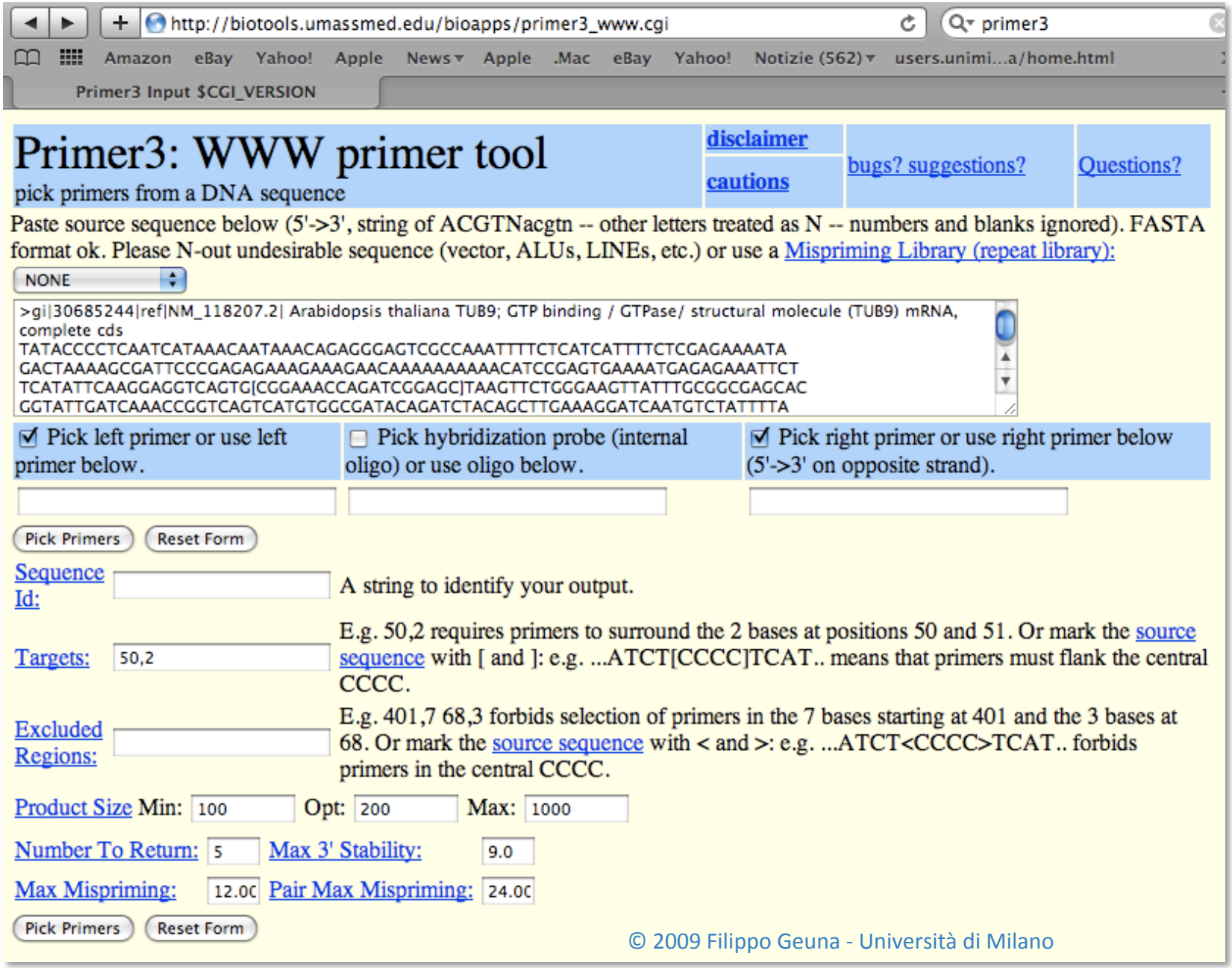
Ricerca di primer per PCR (software Primer3)

MA 24-03-2009



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Ricerca di primer per PCR (risultati del software Primer3)

Primer3 Output

```
PRIMER PICKING RESULTS FOR gi|30685244|ref|NM_118207.2| Arabidopsis thaliana TUB9;

No mispriming library specified
Using 1-based sequence positions
OLIGO            start  len       tm      gc%    any    3' seq
LEFT PRIMER        442   20    59.87    55.00   4.00   3.00 TACACGGAGGGAGCTGAGTT
RIGHT PRIMER       641   20    60.31    50.00   3.00   1.00 GATGGGAAGACCGAAAAGGT
SEQUENCE SIZE: 1693
INCLUDED REGION SIZE: 1693

PRODUCT SIZE: 200, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 0.00

    1 TATACCCCTCAATCATAAACAATAAACAGAGGGAGTCGCCAAATTTTCTCATCATTTTCT

   61 CGAGAAAATAGACTAAAAGCGATTCCCGAGAGAAAGAAAGAACAAAAAAAAAAACATCCGA

  121 GTGAAAATGAGAGAAATTCTTCATATTCAAGGAGGTCAGTGCGGAAACCAGATCGGAGCT

  181 AAGTTCTGGGAAGTTATTTGCGGCGAGCACGGTATTGATCAAACCGGTCAGTCATGTGGC

  241 GATACAGATCTACAGCTTGAAAGGATCAATGTCTATTTTAACGAAGCTAGCGGAGGCAAA

  301 TACGTTCCTCGTGCTGTTCTTATGGATCTGGAGCCTGGAACTATGGATTCTCTCAGATCT

  361 GGGCCGTTTGGTCAGATTTTCCGGCCTGATAACTTCGTTTTTGGTCAGTCTGGTGCTGGG

  421 AATAACTGGGCGAAAGGTCATTACACGGAGGGAGCTGAGTTGATTGATTCTGTGCTTGAT
                          >>>>>>>>>>>>>>>>>>>>

  481 GTTGTGAGGAAGGAAGCTGAGAACTGTGATTGTCTTCAAGGTTTTCAAGTTTGTCATTCT

  541 TTGGGAGGAGGAACTGGTTCTGGAATGGGAACACTTCTTATTTCTAAGATTAGAGAGGAA

  601 TATCCAGATCGTATGATGATGACCTTTTCGGTCTTCCCATCTCCTAAGGTCTCTGATACA
                          <<<<<<<<<<<<<<<<<<<<

  661 GTTGTTGAGCCATACAATGCTACTCTTTCCGTTCACCAGCTCGTTGAGAATGCTGATGAA
```

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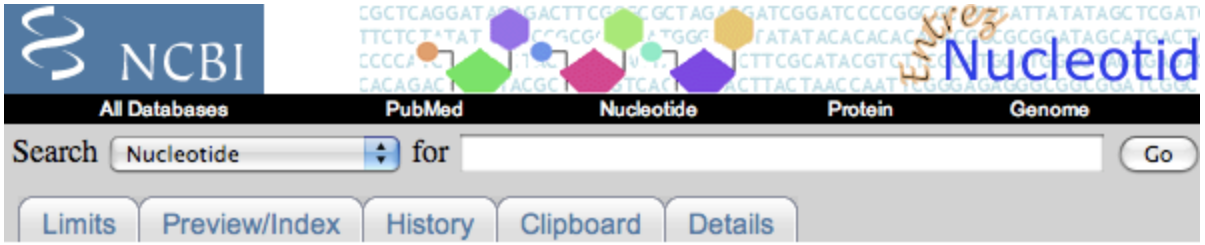
La PCR (scelta di un gene da amplificare)



NCBI Reference Sequence: NM_118207.2

Arabidopsis thaliana TUB9 (tubulin beta-9 chain); structural molecule (TUB9) mRNA, complete cds

Showing 1.33kb region from base 127 to 1461.

```
>gi|30685244:127-1461 Arabidopsis thaliana TUB9 (tubulin beta-9 chain);
structural molecule (TUB9) mRNA, complete cds
ATGAGAGAAATTCTTCATATTCAAGGAGGTCAGTGCGGAAACCAGATCGGAGCTAAGTTCTGGGAAGTTA
TTTGCGGCGAGCACGGTATTGATCAAACCGGTCAGTCATGTGGCGATACAGATCTACAGCTTGAAAGGAT
CAATGTCTATTTTAACGAAGCTAGCGGAGGCAAATACGTTCCTCGTGCTGTTCTTATGGATCTGGAGCCT
GGAACTATGGATTCTCTCAGATCTGGGCCGTTTGGTCAGATTTTCCGGCCTGATAACTTCGTTTTTGGTC
AGTCTGGTGCTGGGAATAACTGGGCGAAAGGTCATTACACGGAGGGAGCTGAGTTGATTGATTCTGTGCT
TGATGTTGTGAGGAAGGAAGCTGAGAACTGTGATTGTCTTCAAGGTTTTCAAGTTTGTCATTCTTTGGGA
GGAGGAACTGGTTCTGGAATGGGAACACTTCTTATTTCTAAGATTAGAGAGGAATATCCAGATCGTATGA
TGATGACCTTTTCGGTCTTCCCATCTCCTAAGGTCTCTGATACAGTTGTTGAGCCATACAATGCTACTCT
TTCCGTTCACCAGCTCGTTGAGAATGCTGATGAATGTATGGTTTTGGATAACGAAGCTCTTTACGATATC
TGTTTCCGTACCTTGAAGCTTGCTAATCCTACATTTGGTGACCTTAACCATCTCATCTCAGCTACAATGA
GTGGTGTTACATGCTGTCTTCGGTTCCCTGGTCAACTAAACTCTGACCTTAGAAAACTTGCTGTGAACCT
TATTCCTTTCCCACGACTCCACTTCTTCATGGTTGGTTTTGCACCTTTGACATCAAGAGGATCTCAACAA
TACAGTGCCTTGAGTGTCCCTGAGCTAACTCAACAGATGTGGGATGCCAAAAACATGATGTGTGCAGCTG
ATCCTCGTCATGGTCGTTACTTGACAGCTTCTGCTGTCTTCCGTGGAAAGATGAGTACCAAAGAGGTTGA
TGAACAGATGATGAACGTCCAGAACAAAAACTCCTCATACTTTGTTGAATGGATTCCAAATAACGTGAAA
TCAAGCGTCTGCGATATAGCTCCCACTGGTTTGAAAATGGCATCAACTTTCATTGGAAACTCAACTTCAA
TCCAGGAAATGTTCAGGCGTGTGAGTGAACAGTTCACTGCCATGTTCAGGAGAAAGGCTTTCCTTCATTG
GTACACAGGAGAAGGAATGGACGAGATGGAGTTCACTGAAGCAGAGAGTAACATGAATGATCTTGTTGCA
GAGTATCAGCAATATCAAGATGCTACAGTCGGTGAAGAGGAGTACGAGGAGGACGAAGAAGAAGAAGAAG
CCTAA
```

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La reazione di PCR: foglio di calcolo in Excel

ME 02-05-2012

	A	B	C	D	E	F	G
1	**Componente**	**Concentrazione stock**	**Unità misura**	**Concentrazione finale**	**Unità misura**	**Fattore diluizione**	**Volume (uL) x 20 uL**
2							
3	DNA templato	1000	ng/uL	10	ng/uL	100	0,2
4	10x bf	10	-	1	-	10	2,0
5	MgCl2	50	mM	1,5	mM	33,3	0,6
6	dNTP	2	mM	0,2	mM	10	2,0
7	Primer Fw	1000	ng/uL	20	ng/uL	50	0,4
8	Primer Rv	1000	ng/uL	20	ng/uL	50	0,4
9	Taq polimerasi	10	U/uL	1	U/uL	10	2,0
10	H2O	-	-	-	-	-	12,4
11	**Volume TOTALE**						**20 uL**

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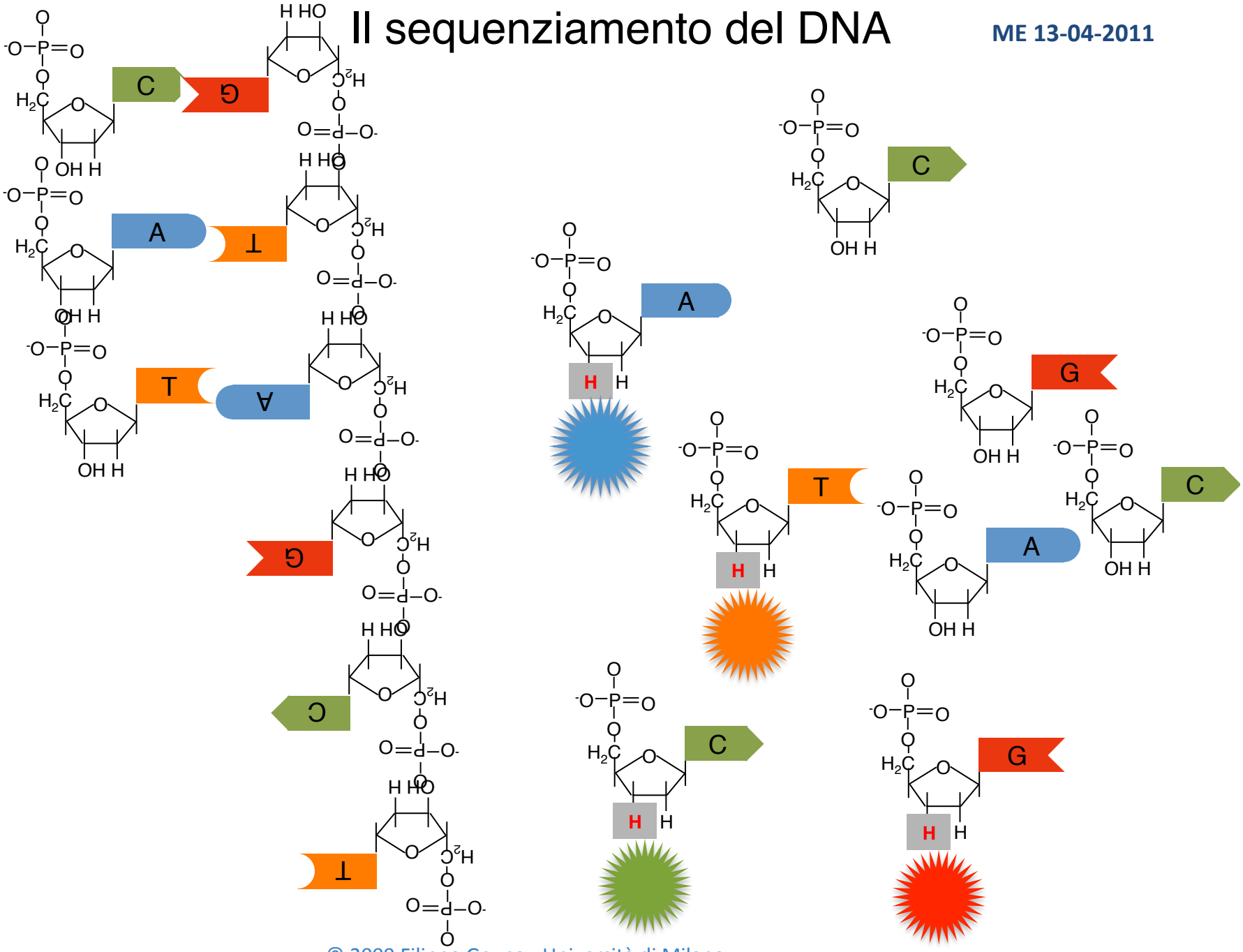
Il sequenziamento del DNA

ME 13-04-2011



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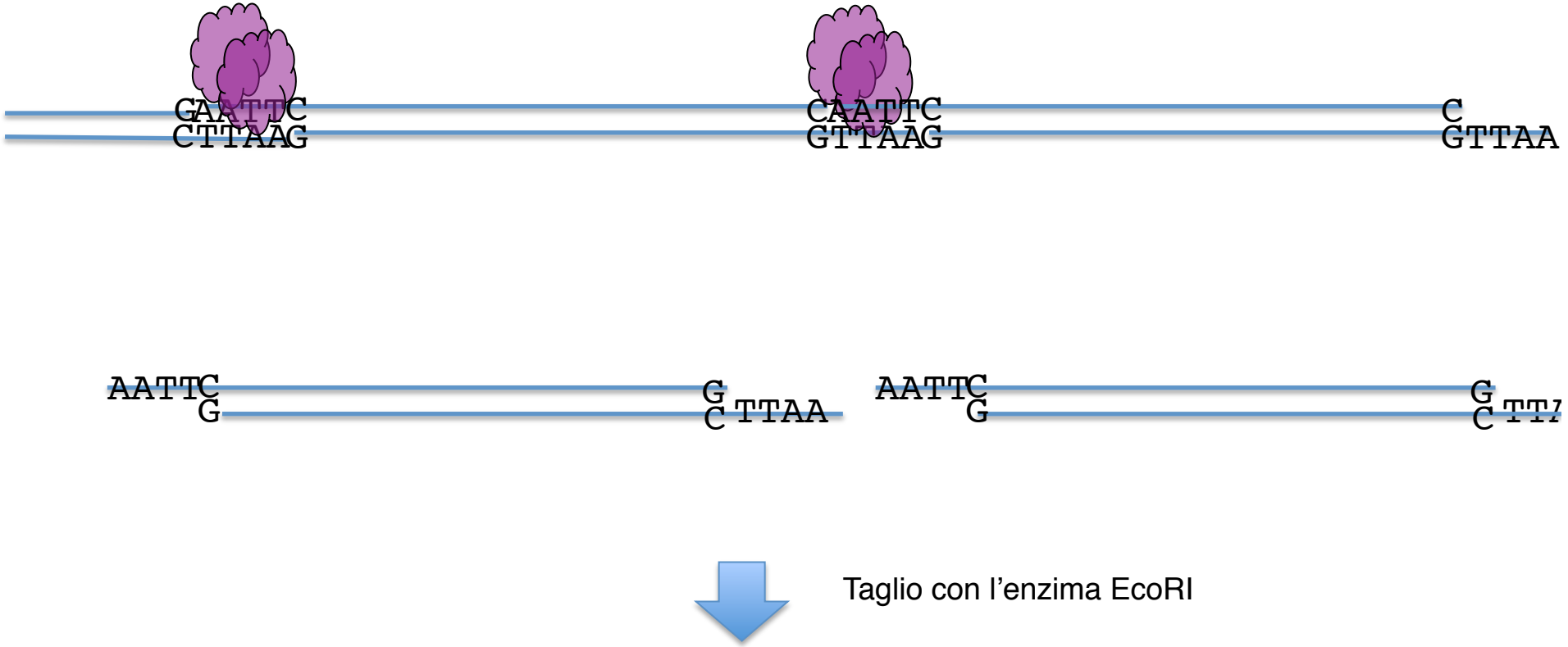
Taglio del DNA con enzimi di restrizione

MA 08-05-2012



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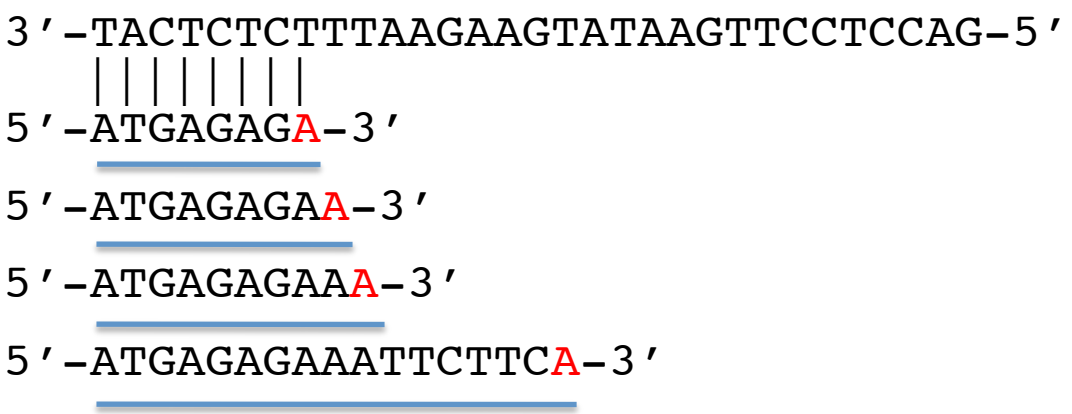
Sequenziamento del DNA

MA 22-05-2012



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Il genoma umano stampato su carta

ME 06-03-2013

Encyclopedia Genomica: UK Scientists Print the Book of Life in 130 Volumes

University of Leicester staff with the human genome (hard copy). Cas Kramer and Ed Hollox, back row (right).

December 28, 2012 | Nowadays it is cheaper to sequence the human genome than to print out a full hard copy. We know this because earlier this year, Cas Kramer and a group of scientists at the University of Leicester decided to print out a complete version of the human genome.
The genome print out spans **130 volumes**, with each page printed on **both sides in 4-point font**, with precisely **43,000 characters per page**. The X chromosome is made up of seven volumes, while the Y chromosome occupies one. The exercise costs a little less than 4,000 pounds (about $6,000).

http://www.bio-itworld.com/2012/12/28/encyclopedia-genomica-UK-scientists-print-book-of-life-in-130-volumes.html

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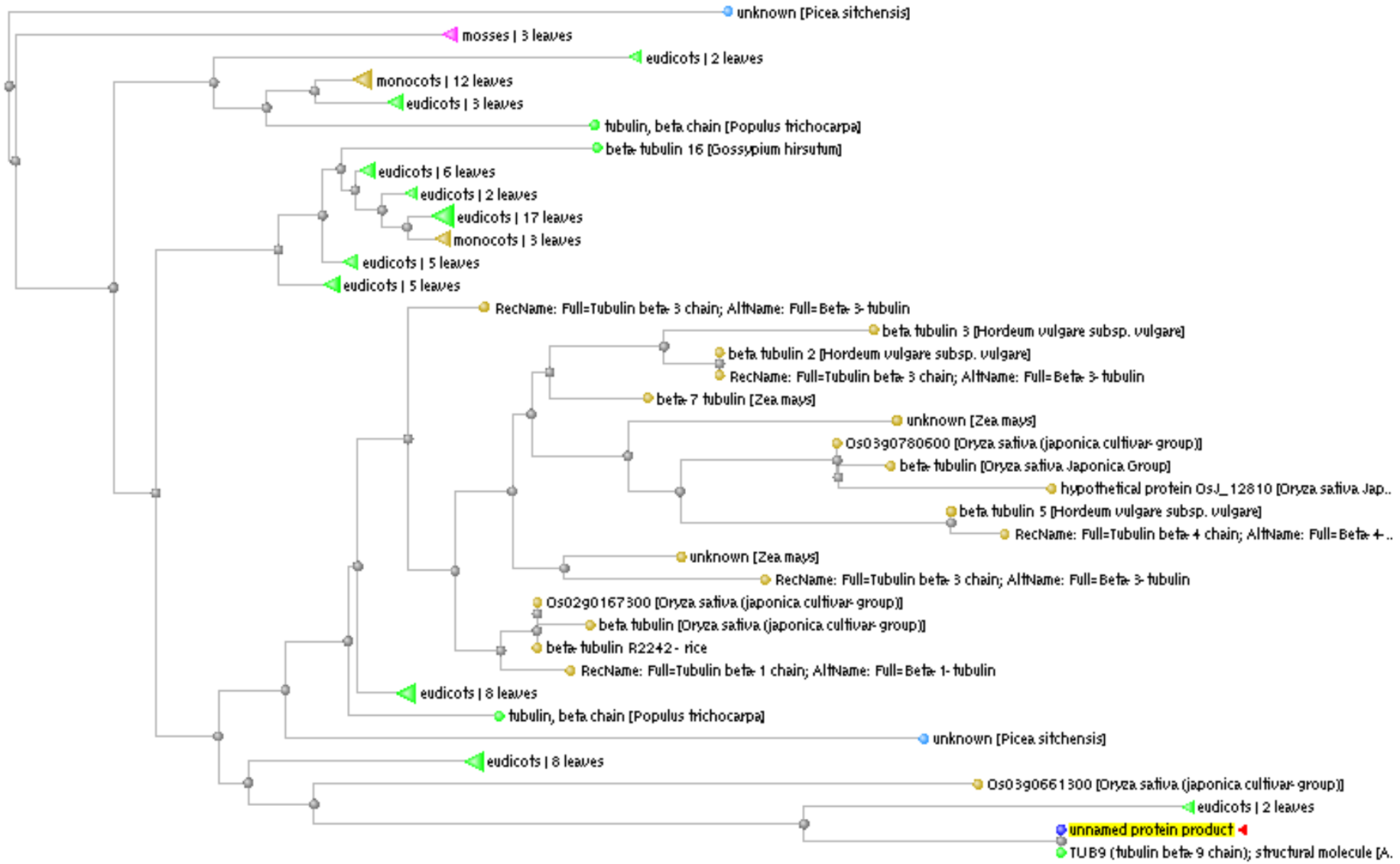
Dendrogramma da allineamento multiplo del gene TUB9

(dal sito NCBI)

.....-05-2010



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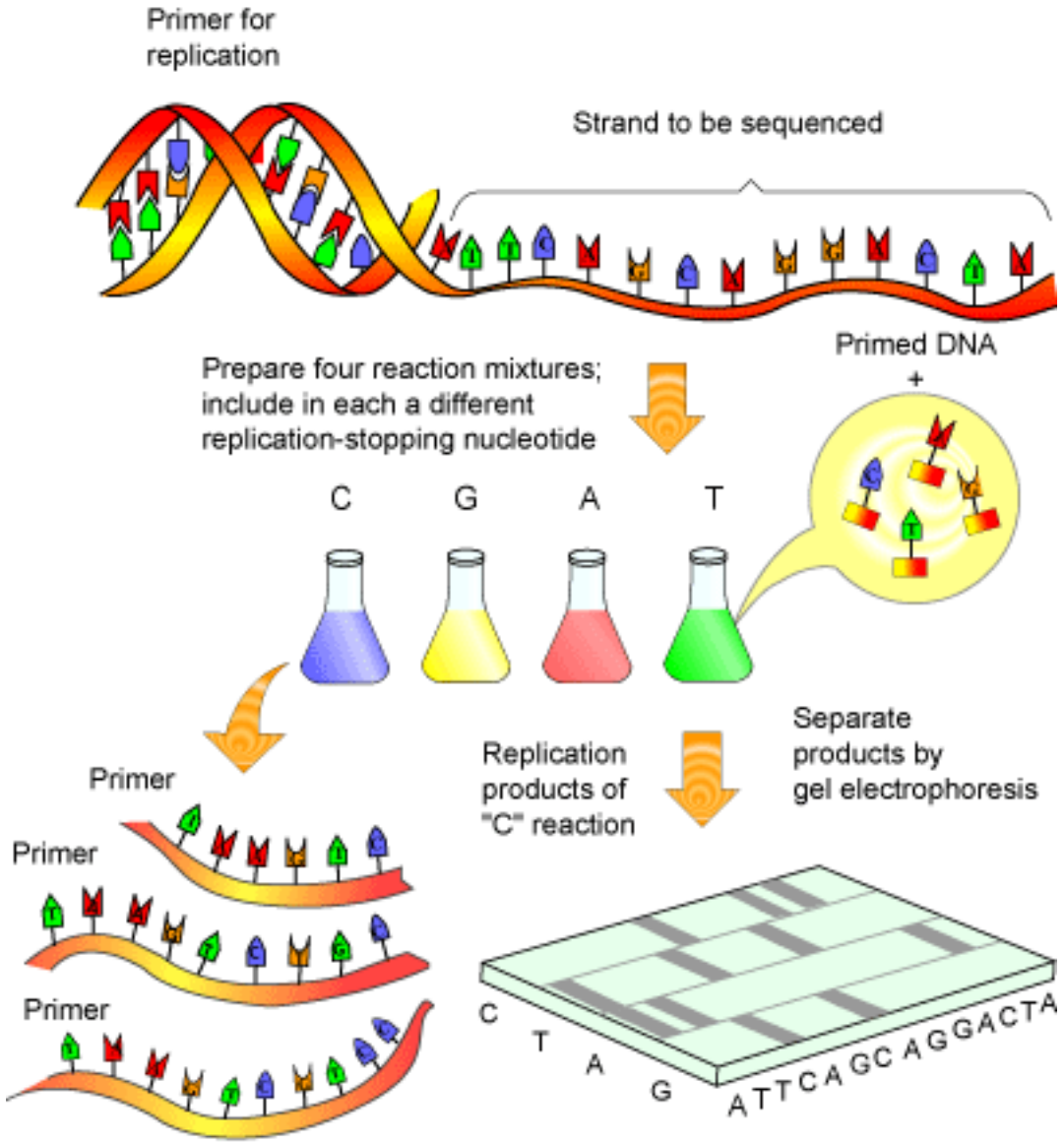
Sequenziamento del DNA

MA 14-05-2013



http://universe-review.ca/I11-50-DNAsequencing1.jpg

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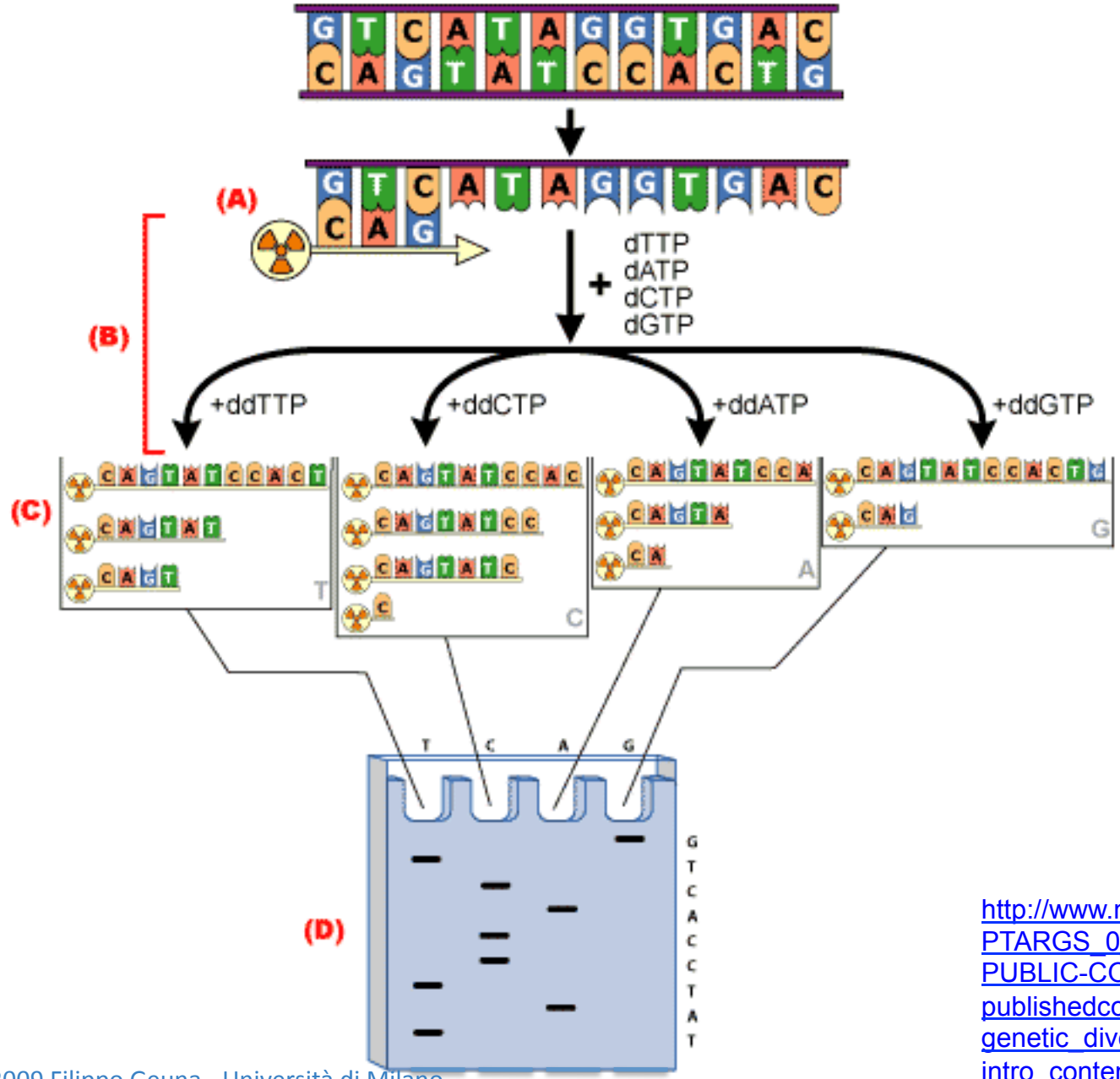
Sequenziamento del DNA

MA 14-05-2013



http://www.nbii.gov/portal/server.pt/gateway/PTARGS_0_2_4015_402_571_43/http%3B/PUBLIC-CONTENT%3B7087/publishedcontent/publish/ecological_topics/genetic_diversity/dna_sequencing_intro/intro_content_3.gif

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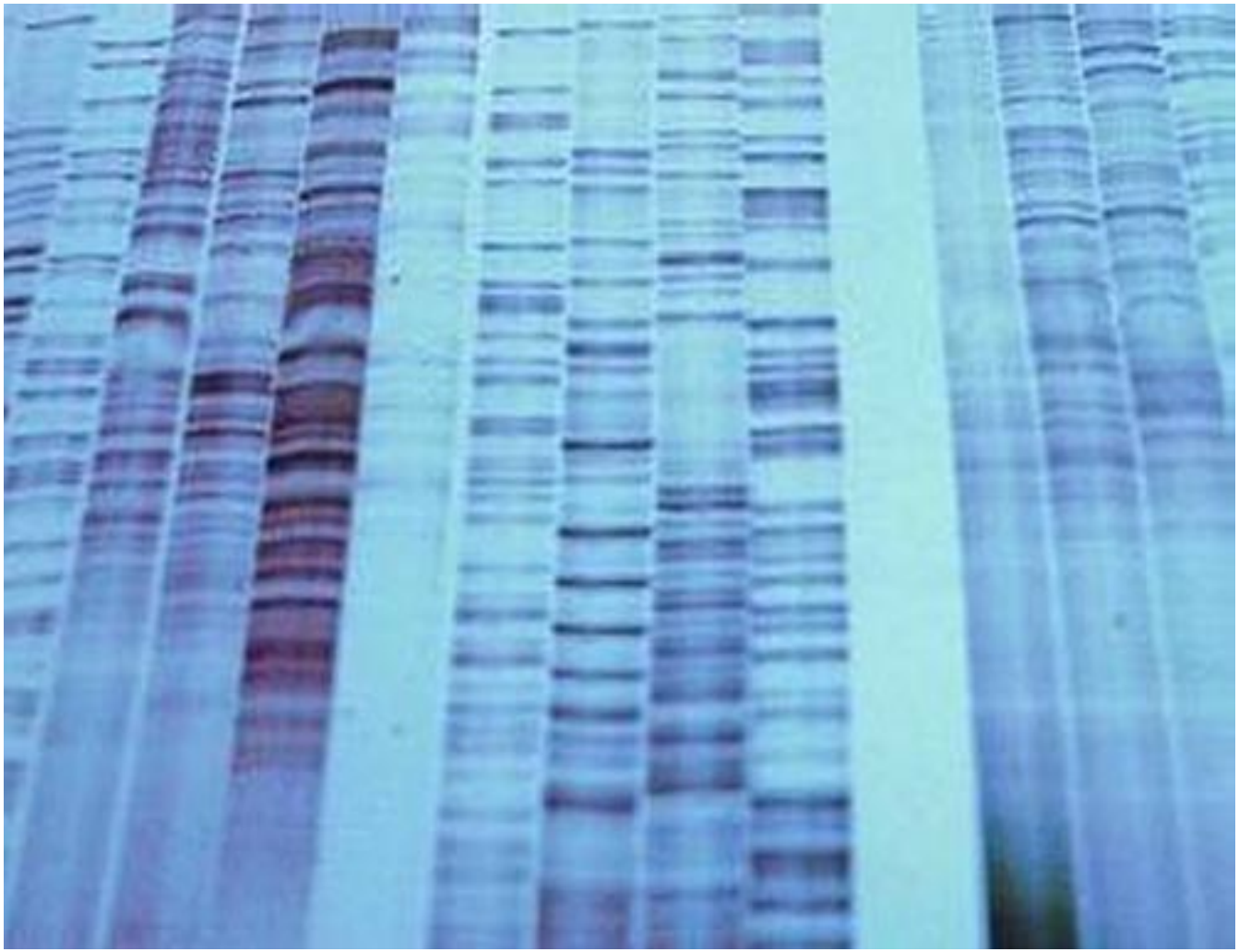
Sequenziamento del DNA



http://www.ocf.berkeley.edu/%7Eedy/genome/sanger.jpg

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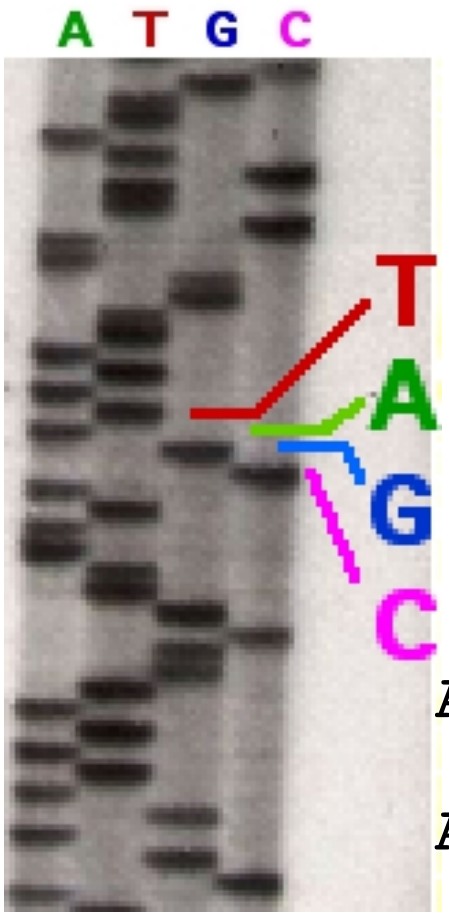
Esercizio sul sequenziamento del DNA

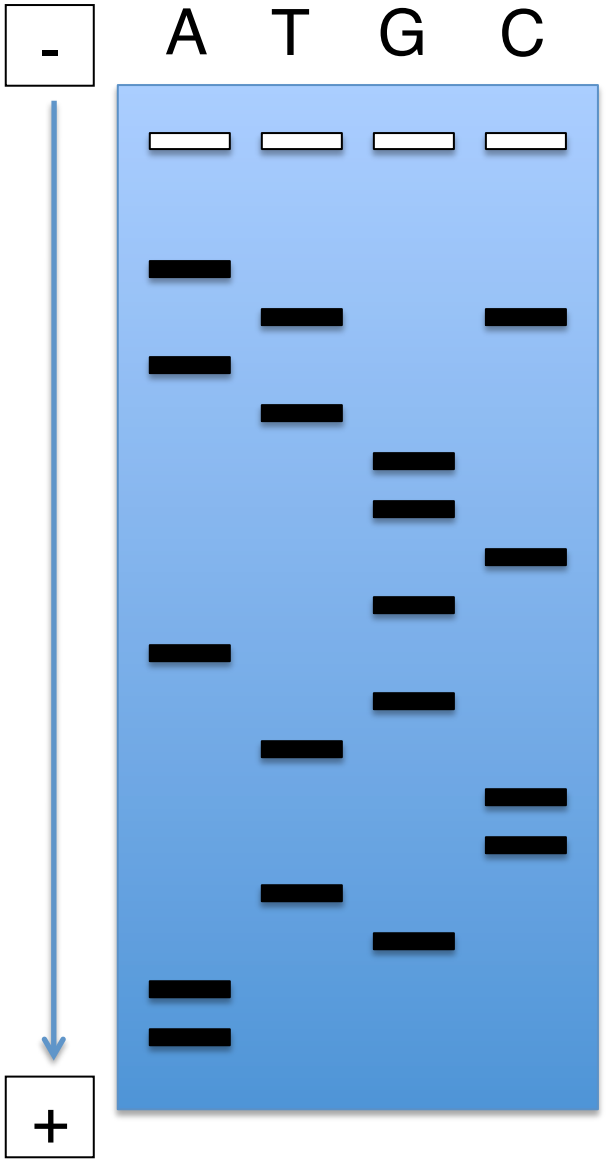
MA 14-05-2013

Ricostruire la sequenza di DNA sulla base del seguente profilo elettroforetico



AAGTCCTGAGCGGTATA

AAGTCCTGAGCGGTACA



http://dnasequencing.wordpress.com/2007/10/26/chain-termination-methods/

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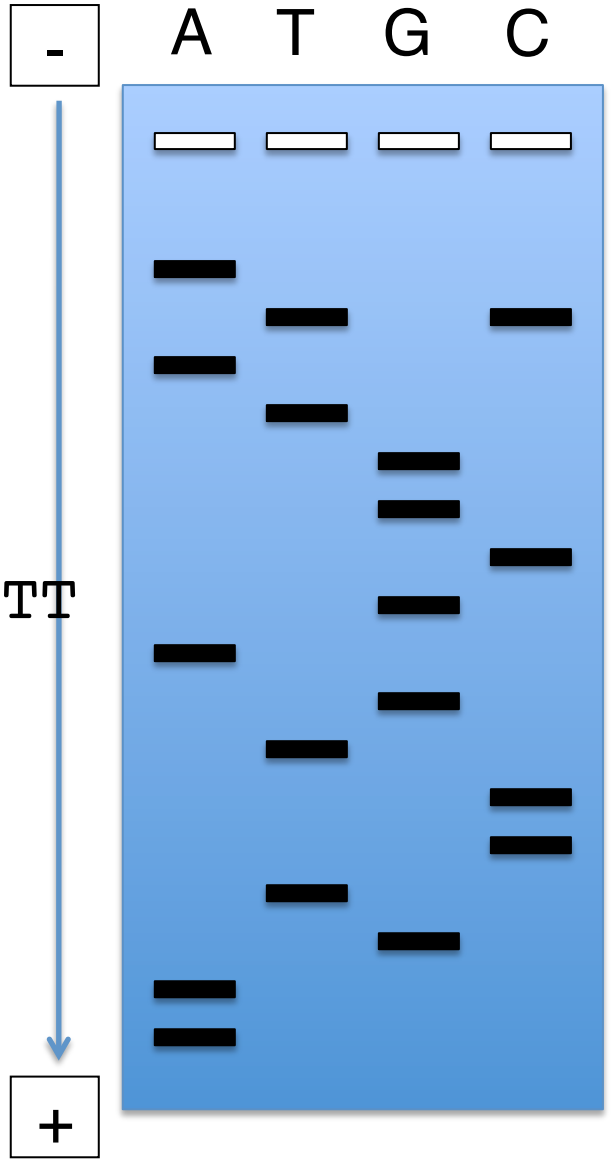
Esercizio sul sequenziamento del DNA

MA 14-05-2013

Ricostruire la sequenza di DNA sulla base del seguente profilo elettroforetico



5’AAGTCCTGAGCGGTAC

3’TTCAGGACTCGCCATATACGTAGCCAGCTT

AAGTCCTGAGCGGTATA

AAGTCCTGAGCGGTACA

AAGTCCTGAGCGGTA[T/C]A

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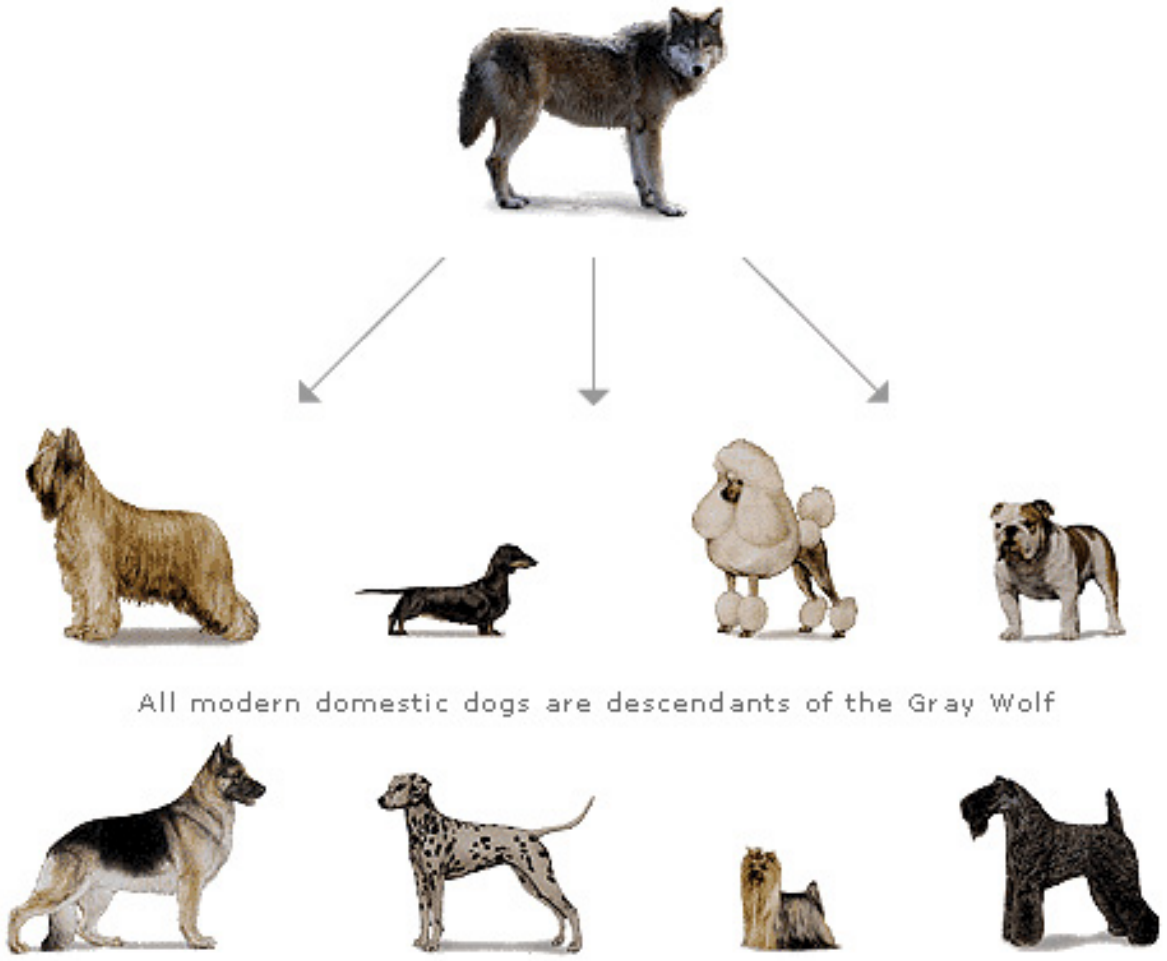
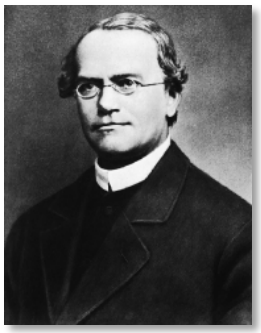
Variabilità genetica → Polimorfismo



Variabilità genetica → Polimorfismo

ME 22-05-2013



- La genetica mendeliana studia caratteri discreti
- Ogni tratto è caratterizzato da due o più alleli → POLIMORFISMO!!

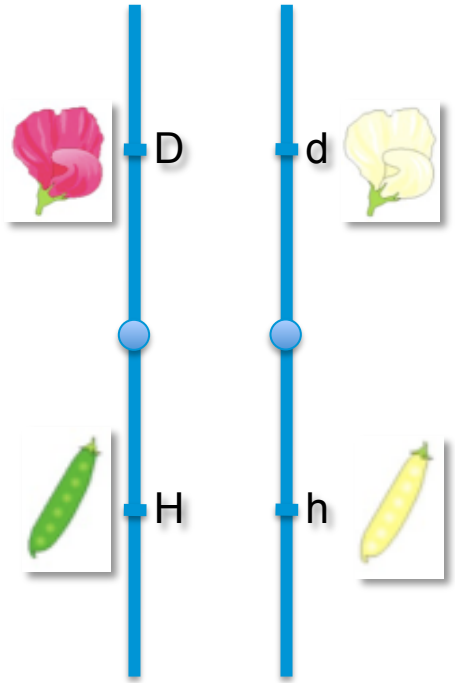
	Seed shape	Seed color	Flower color	Flower position	Pod shape	Pod color	Plant height
Dominante	round	yellow	violet-red	axial flowers	inflated	green	tall
Recessivo	wrinkled	green	white	terminal flowers	pinched	yellow	short

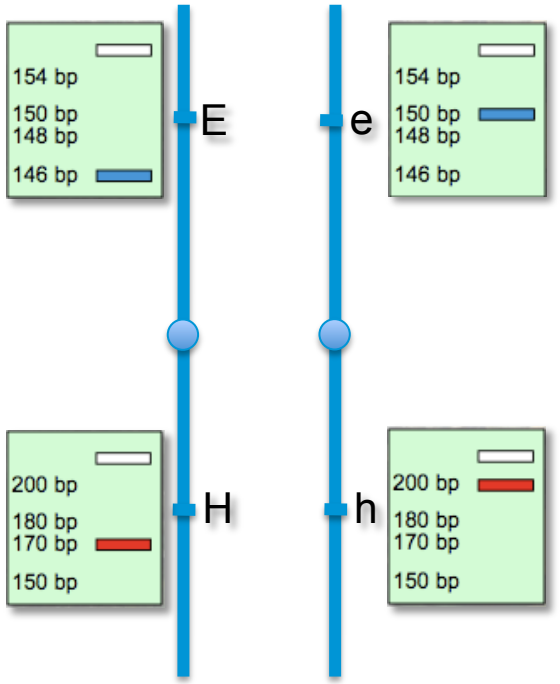
I marcatori molecolari si comportano come geni

Ma…

•Sono neutrali

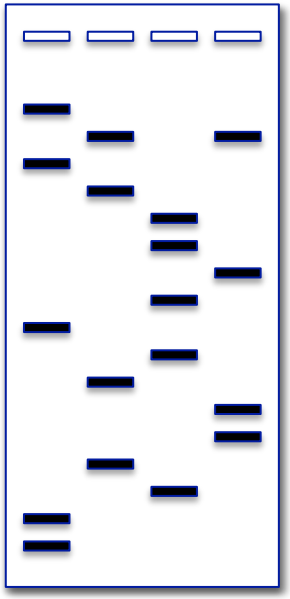
•Possono essere studiati in laboratorio partendo da pochissimo DNA





Il *fingerprinting* molecolare: un tipo di "codice a barre"

ME 12-05-2010



http://it.wikipedia.org/wiki/Codice_a_barre

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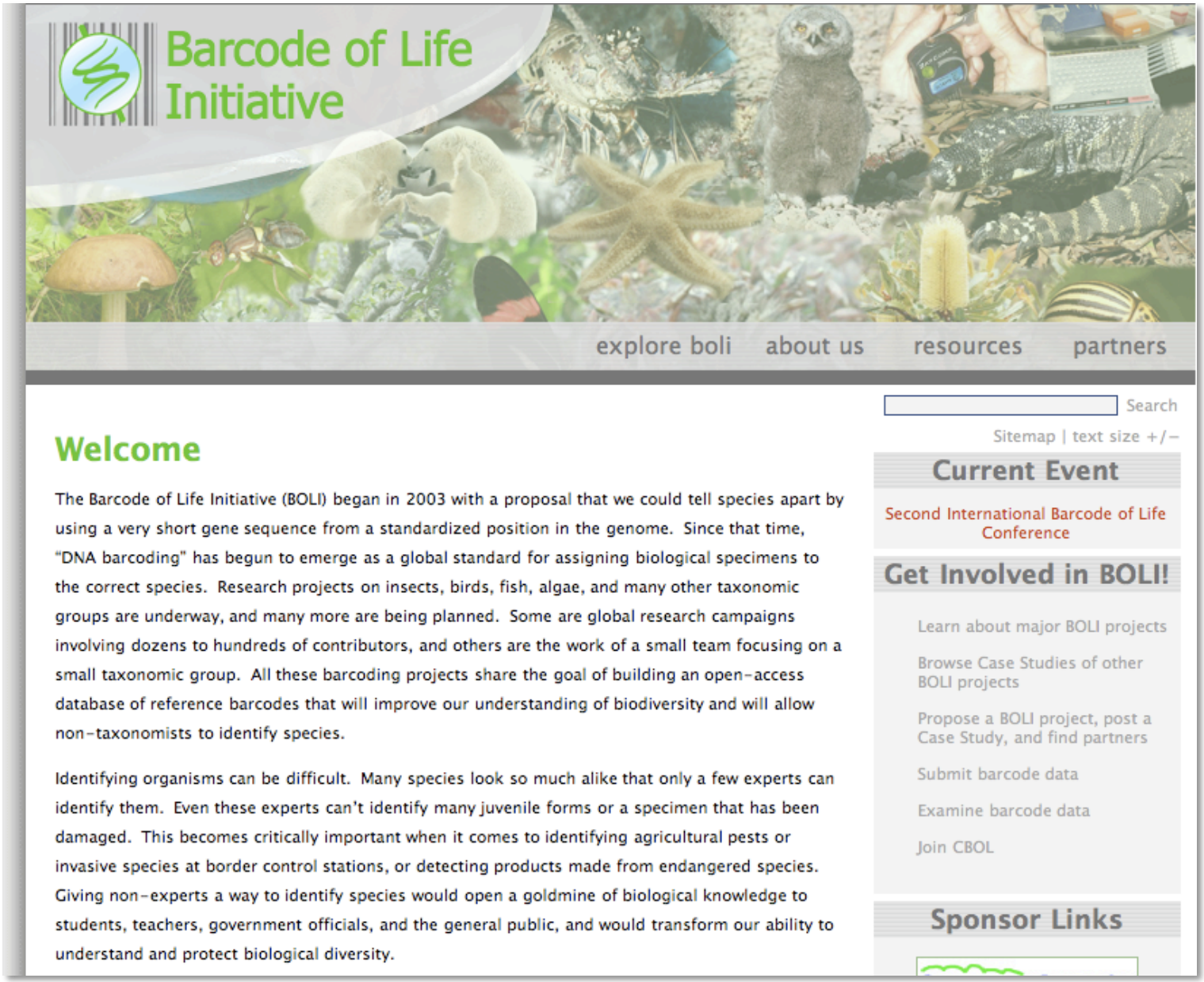
Il progetto *Barcode Of Life* (BOL)

ME 12-05-2010



http://www.dnabarcodes.org/

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Il progetto *Barcode Of Life* (BOL)

ME 12-05-2010

What is DNA Barcoding?

In 2003, researchers at the University of Guelph in Ontario, Canada, proposed "DNA barcoding" as a way to identify species. Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code. Two items may look very similar to the untrained eye, but in both cases the barcodes are distinct.

Until now, biological specimens were identified using morphological features. In some cases a trained technician could make routine identifications using morphological "keys", but in most cases an experienced professional taxonomist is needed. If a specimen is damaged or is in an immature stage of development, even specialists may be unable to make identifications. Barcoding solves these problems, because non-specialists can obtain barcodes from tiny amounts of tissue. This is not to say that traditional taxonomy has become less important, but rather that DNA barcoding can serve a dual purpose as a new tool in the taxonomists toolbox supplementing his/ her knowledge as well as being an innovative device for non-experts who need to make a quick identification.

The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but botanists are now close to identifying a combination of gene regions that will serve as a barcode region for plants.

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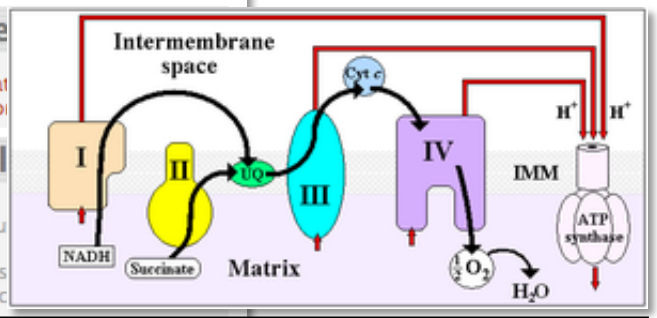
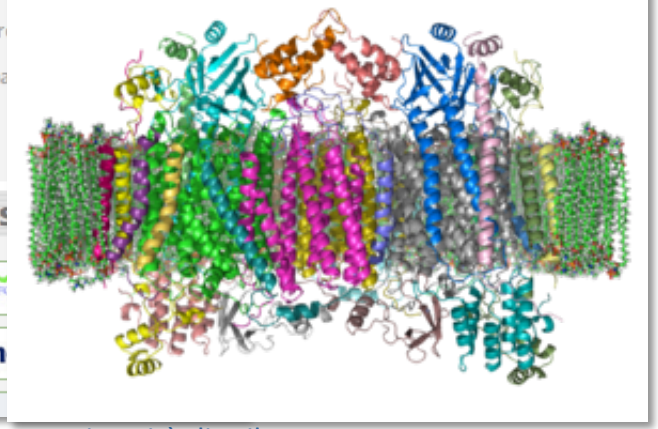


http://en.wikipedia.org/wiki/Cytochrome_c_oxidase



http://www.dnabarcodes.org/pa/ge/what_is_dna_barcoding

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Barcode Of Life (BOL): sequenze e codici a barre

Gene :	COI	GenBank Accession :	AY666424
Last Updated :	2004-07-13	Translation Matrix :	Vertebrate Mitochondria

Sequencing Runs

Run Date	Run Site	Direction	Trace File	PCR primers	Seq Primer
2004-07-06 10:57:41	University of Guelph	Forward	TZBNA061-03_BF1.pdf	BF1 /BR1	BF1

Nucleotide Sequence

Length :	624
Comp. A :	151
Comp. G :	103
Comp. C :	225
Comp. T :	145
Updated :	2005-08-26

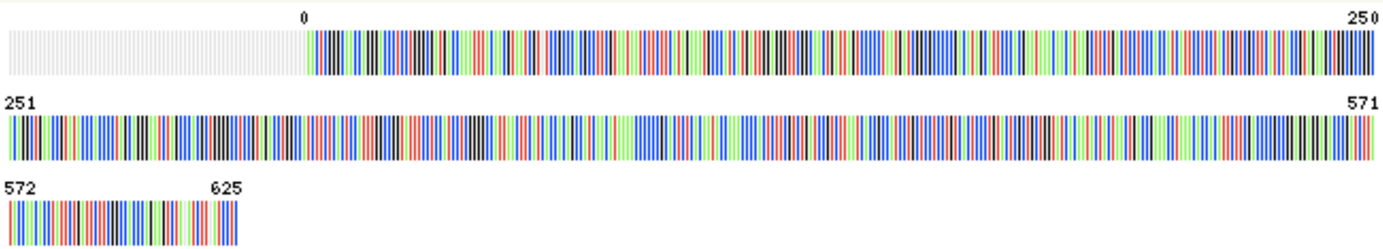
```
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAACTC
GGGCAACCAGGGACCCTCCTGGGCGATGACCAAATTTACAACGTAATCGTNTCCGCCCACGCCTTCGTAATAATC
TTCTTCATAGAAATGCCCATCATGATTGGAGGGTTCGGCAACTGATTAGTCCCCCTAATGATCGGCGCCCCCGAC
ATAGCATTCCCACGAATAAACAACATAAGCTTCTGACTCCTCCCACCATCATTCCTCCTACTGCTCGCTTCATCT
ACCGTAGAAGCTGGCGCCGGCACAGGCTGAACCGTATACCCACCCCTAGCAGGGAATCTAGCCCACGCTGGGGCC
TCCGTAGACCTGGCCATCTTCTCACTCCATTTGGCCGGTATTTCCTCCATCCTCGGGGCCATTAACTTCATCACC
ACAGCCATCAACATAAAACCCCCCGCACTCTCACAATACCAAACCCCACTCTTCGTCTGATCCGTCTTAATCACC
GCCATCCTGCTCCTCCTGTCACTCCCTGTACTCGCTGCTGGTATCACAATACTACTAACTGACCGAAACCTAAAC
ACCACATTCTTCGACCCCGCCGGAGGAGGAGACCCGATCTTATACCAACACCTATTCTGATTCTTCGGCCACCCA
GAAGTCTAcATCTTNATCCTC
```

Amino Acid Sequence

Length :	232 .

```
-----------------------XLGQPGTLLGDDQIYNVIXSAHAFVMIFFMEMPIMIGGFGNWLVPLMIGAPD
MAFPRMNNMSFWLLPPSFLLLLASSTVEAGAGTGWTVYPPLAGNLAHAGASVDLAIFSLHLAGISSILGAINFIT
TAINMKPPALSQYQTPLFVWSVLITAILLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGHP
EVYIXIL
```

Illustrative Barcode



http://www.boldsystems.org/docs/boldtutorial.html

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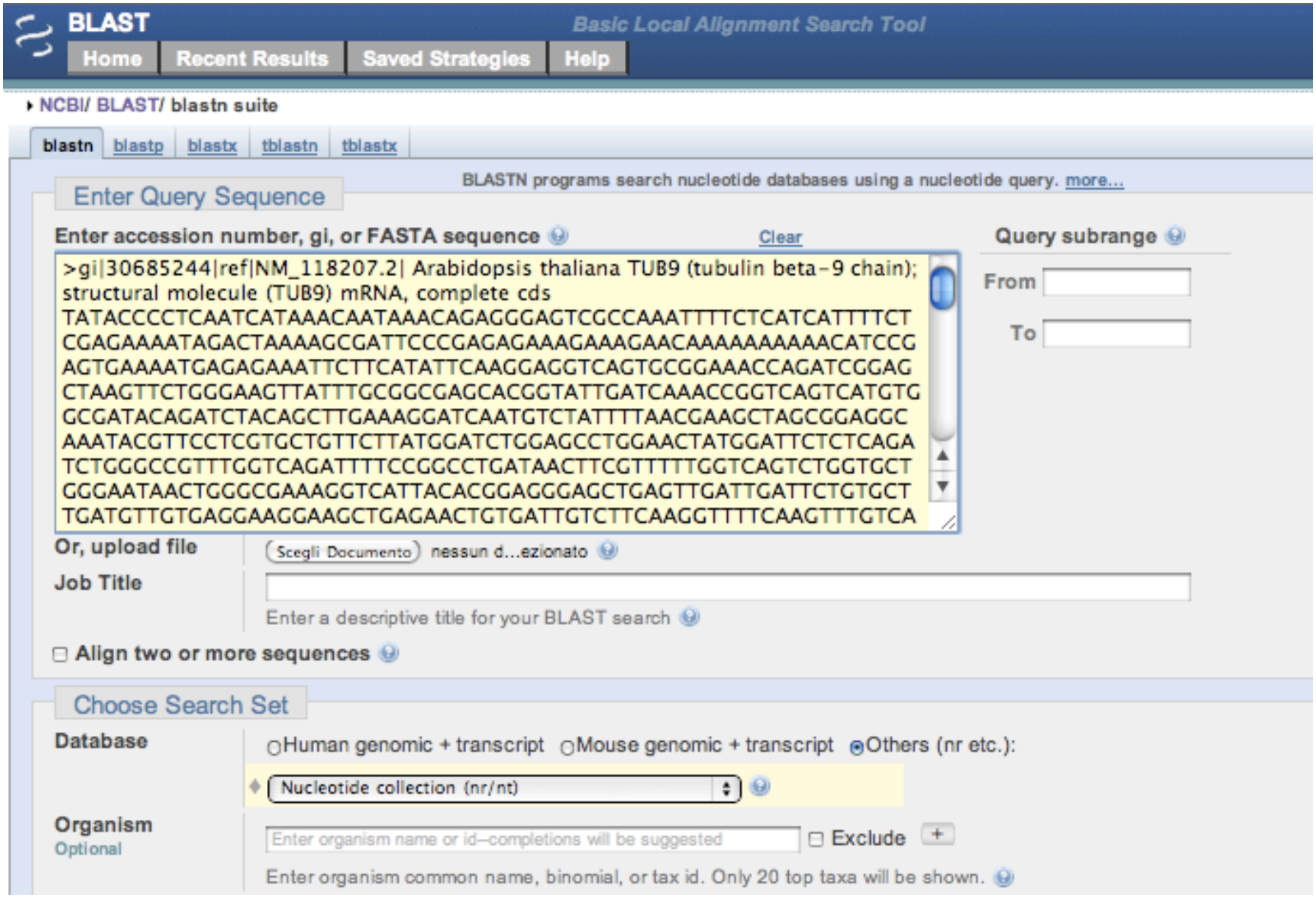
Analisi BLAST di sequenza TUB9 (presso il sito NCBI)

.....-04-2010



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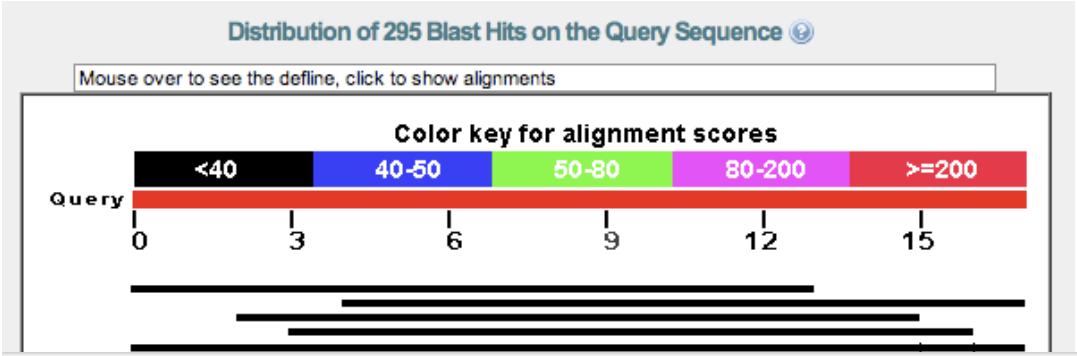
Ricerca di sequenza di DNA in banca dati
(server BLAST al sito NCBI)

.....04-2010

5’ -AAGTCCTGAGCGGTATA-3’

http://blast.ncbi.nlm.nih.gov



Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
Transcripts							
XR_016400.2	PREDICTED: Homo sapiens misc_RNA (LOC646208), miscR	26.3	26.3	76%	144	100%	GM
NM_000498.3	Homo sapiens cytochrome P450, family 11, subfamily B, p	26.3	26.3	76%	144	100%	UEGM
NM_005281.2	Homo sapiens G protein-coupled receptor 3 (GPR3), mRNA	26.3	26.3	76%	144	100%	UEGM
NM_015931.1	Homo sapiens chromosome 3 open reading frame 32 (C3c	26.3	26.3	76%	144	100%	UEGM
Genomic sequences [show first]							
NT_022517.17	Homo sapiens chromosome 3 genomic contig, reference a	30.2	353	100%	9.3	100%	
NT_005612.15	Homo sapiens chromosome 3 genomic contig, reference a	28.2	275	100%	37	100%	
NT_009755.18	Homo sapiens chromosome 12 genomic contig, reference	28.2	103	82%	37	100%	
NT_022135.15	Homo sapiens chromosome 2 genomic contig, reference a	28.2	76.8	100%	37	100%	
NT_034772.5	Homo sapiens chromosome 5 genomic contig, reference a	28.2	52.5	94%	37	100%	
NT_007819.16	Homo sapiens chromosome 7 genomic contig, reference a	28.2	78.8	100%	37	100%	
NT_010966.13	Homo sapiens chromosome 18 genomic contig, reference	28.2	52.5	100%	37	100%	
NT_022184.14	Homo sapiens chromosome 2 genomic contig, reference a	28.2	175	100%	37	100%	
NT_032977.8	Homo sapiens chromosome 1 genomic contig, reference a	28.2	149	88%	37	100%	
NT_008046.15	Homo sapiens chromosome 8 genomic contig, reference a	28.2	200	100%	37	100%	
NT_011109.15	Homo sapiens chromosome 19 genomic contig, reference	28.2	204	100%	37	100%	
NW_001838952.2	Homo sapiens chromosome 5 genomic contig, alternate as	28.2	52.5	94%	37	100%	

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Ricerca di sequenza di DNA del gene TUB9 in banca dati

(server BLAST al sito NCBI)

ME 21-04-2010

http://blast.ncbi.nlm.nih.gov/Blast.cgi

Select All Get selected sequences Distance tree of results

>ref|NM_118207.2| Arabidopsis thaliana TUB9; GTP binding / GTPase/ structural molecule (TUB9) mRNA, complete cds
Length=1693

GENE ID: 827837 TUB9 | TUB9; GTP binding / GTPase/ structural molecule [Arabidopsis thaliana] (10 or fewer PubMed links)

Score = 3127 bits (1693), Expect = 0.0
Identities = 1693/1693 (100%), Gaps = 0/1693 (0%)
Strand=Plus/Plus

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Query  1    TATACCCCTCAATCATAAACAATAAACAGAGGGAGTCGCCAAATTTTCTCATCATTTTCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TATACCCCTCAATCATAAACAATAAACAGAGGGAGTCGCCAAATTTTCTCATCATTTTCT  60

Query  61   CGAGAAAATAGACTAAAAGCGATTCCCGAGAGAAAGAAAGAACaaaaaaaaaaCATCCGA  120
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Sbjct  61   CGAGAAAATAGACTAAAAGCGATTCCCGAGAGAAAGAAAGAACAAAAAAAAAAACATCCGA  120

Query  121  GTGAAAATGAGAGAAATTCTTCATATTCAAGGAGGTCAGTGCGGAAACCAGATCGGAGCT  180
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Sbjct  121  GTGAAAATGAGAGAAATTCTTCATATTCAAGGAGGTCAGTGCGGAAACCAGATCGGAGCT  180

Query  181  AAGTTCTGGGAAGTTATTTGCGGCGAGCACGGTATTGATCAAACCGGTCAGTCATGTGGC  240
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Query  241  GATACAGATCTACAGCTTGAAAGGATCAATGTCTATTTTAACGAAGCTAGCGGAGGCAAA  300
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Query  301  TACGTTCCTCGTGCTGTTCTTATGGATCTGGAGCCTGGAACTATGGATTCTCTCAGATCT  360
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Query  361  GGGCCGTTTGGTCAGATTTTCCGGCCTGATAACTTCGTTTTTGGTCAGTCTGGTGCTGGG  420
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Query  421  AATAACTGGGCGAAAGGTCATTACACGGAGGGAGCTGAGTTGATTGATTCTGTGCTTGAT  480
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  TTGGGAGGAGGAACTGGTTCTGGAATGGGAACACTTCTTATTTCTAAGATTAGAGAGGAA  600

Query  601  TATCCAGATCGTATGATGATGACCTTTTCGGTCTTCCCATCTCCTAAGGTCTCTGATACA  660
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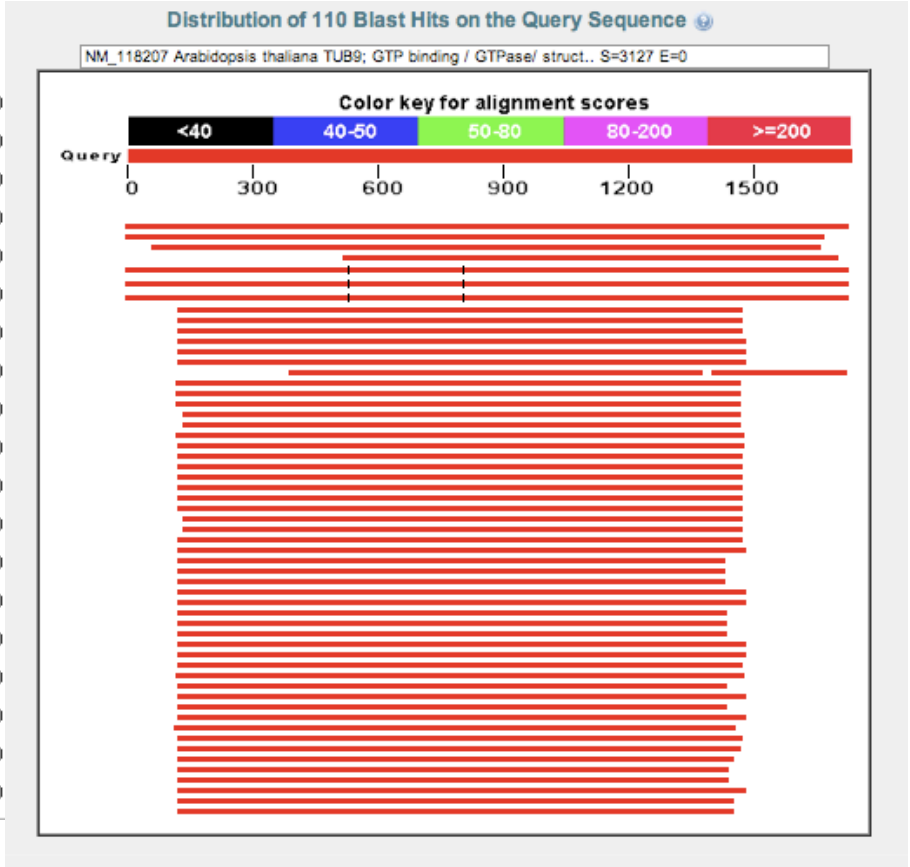

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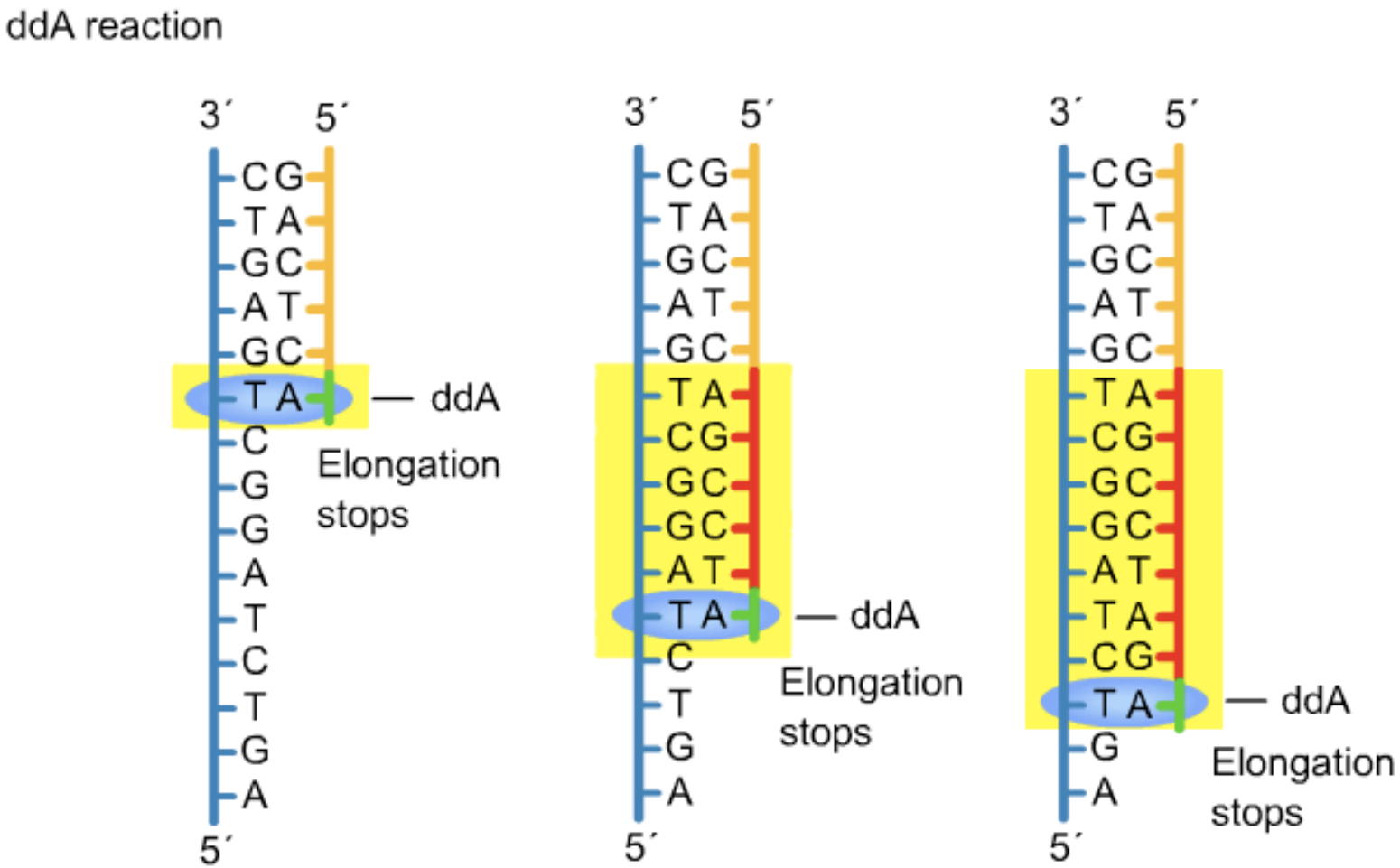
Il sequenziamento del DNA secondo Sanger

LU 02-05-2011

(video) parte 1



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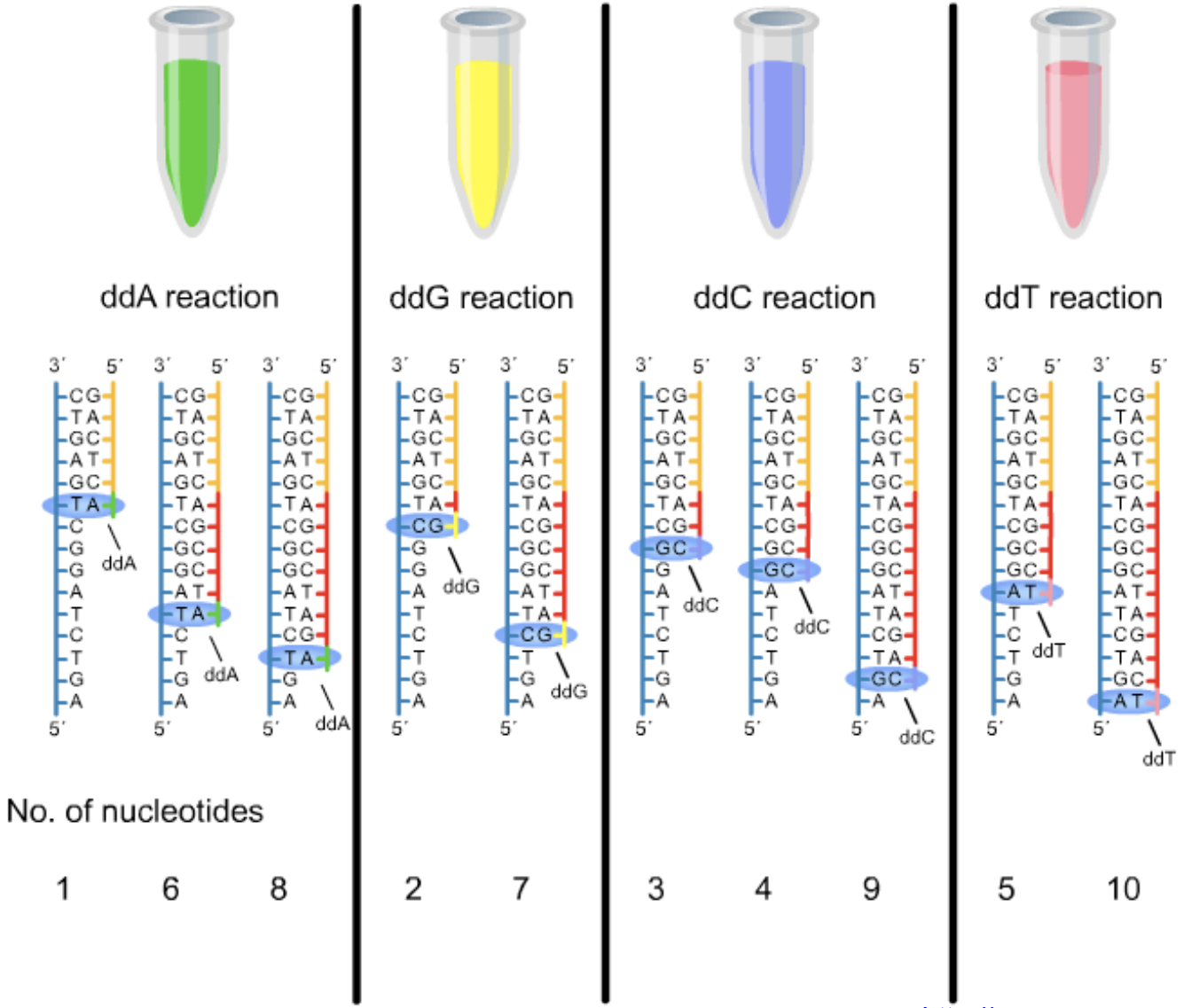
http://smcg.ccg.unam.mx/enp-unam/03-EstructuraDelGenoma/animaciones/secuencia.swf

Il sequenziamento del DNA secondo Sanger

LU 02-05-2011

(video) parte 2



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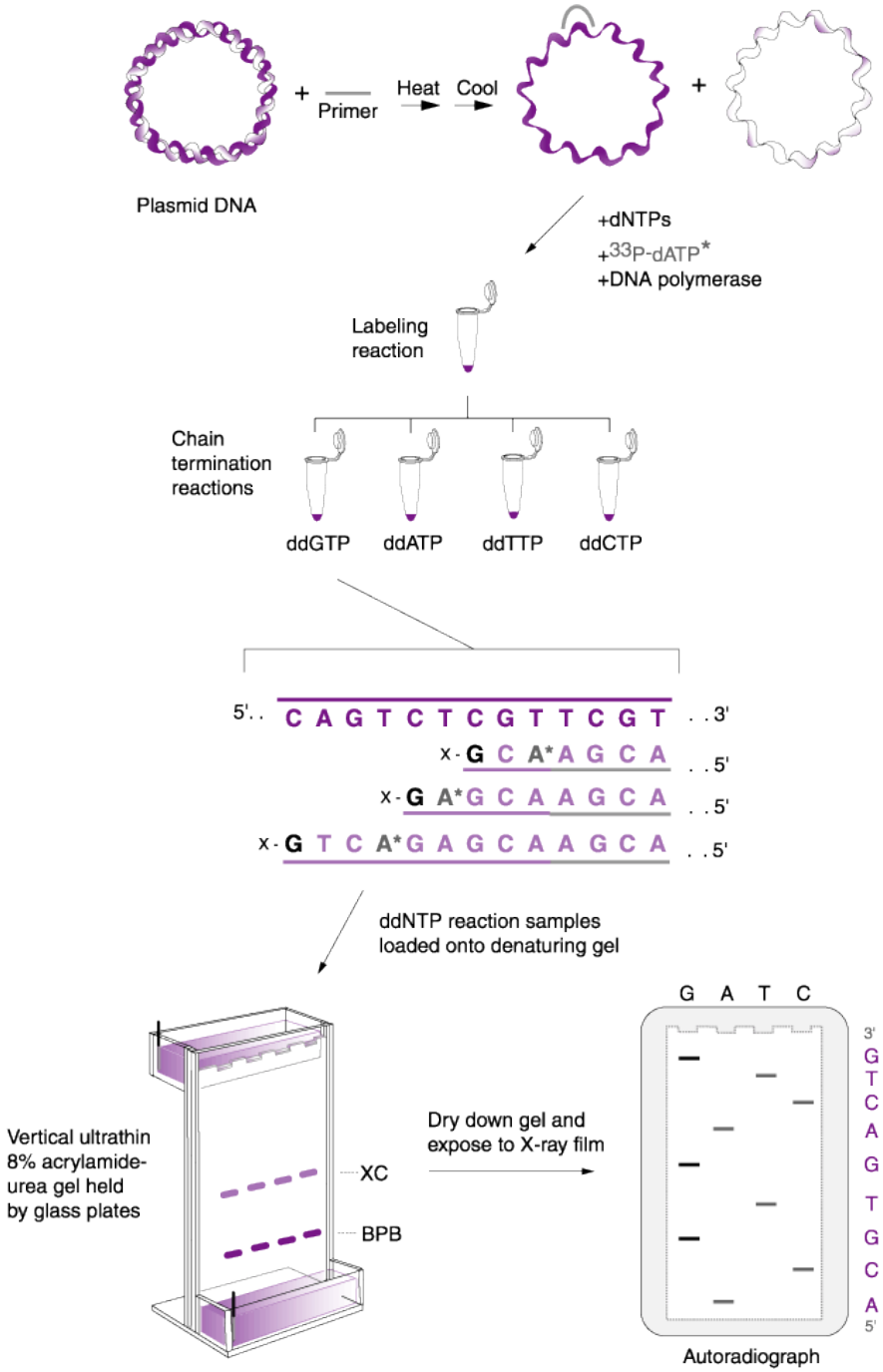
http://smcg.ccg.unam.mx/enp-unam/03-EstructuraDelGenoma/animaciones/secuencia.swf

MA 14-05-2013

Sequenziamento secondo il metodo Sanger



http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture2/Lecture2.html

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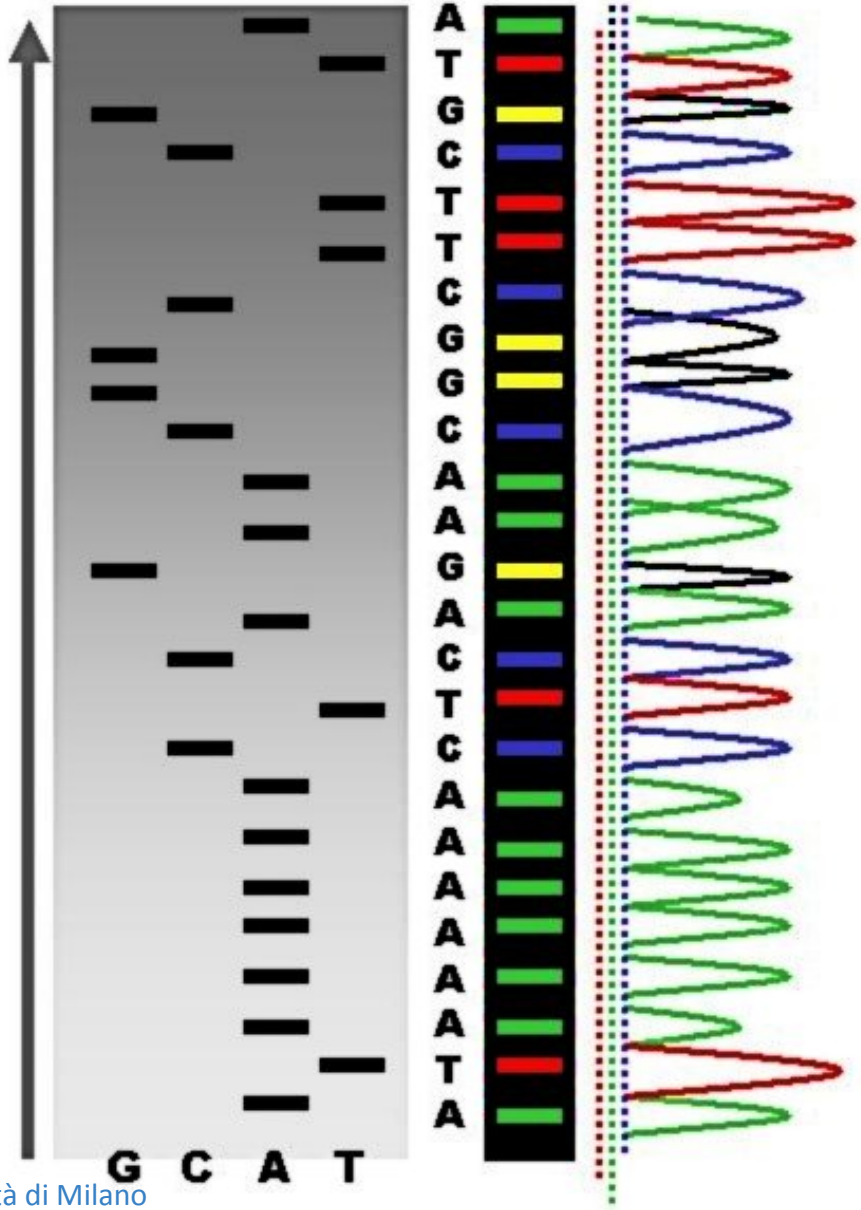
Il sequenziamento del DNA

MA 14-05-2013

(esempio di lettura di un elettroferogramma fluorescente)



http://dnasequencing.wordpress.com/2007/10/26/chain-termination-methods/

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Il sequenziamento del DNA secondo Sanger: video dal sito *DNAinteractive*

LU 06-04-2009

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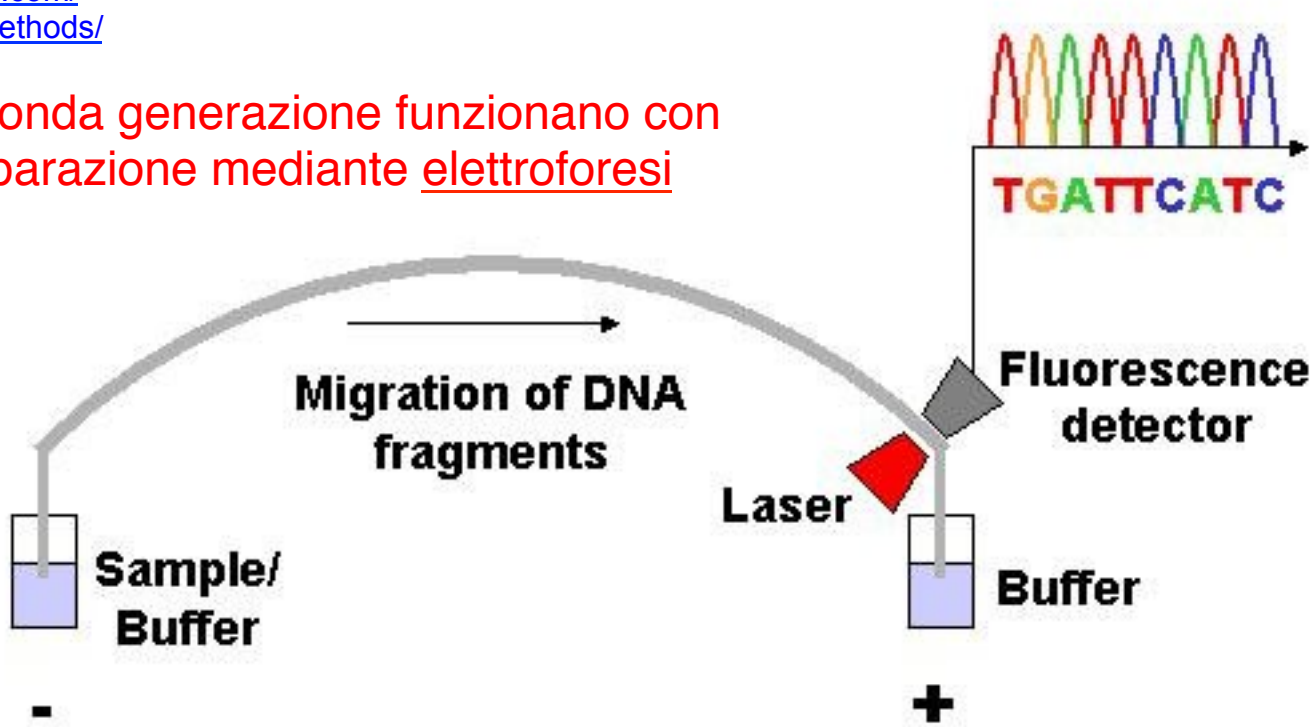
http://www.dnai.org/text/mediashowcase/index2.html?id=552

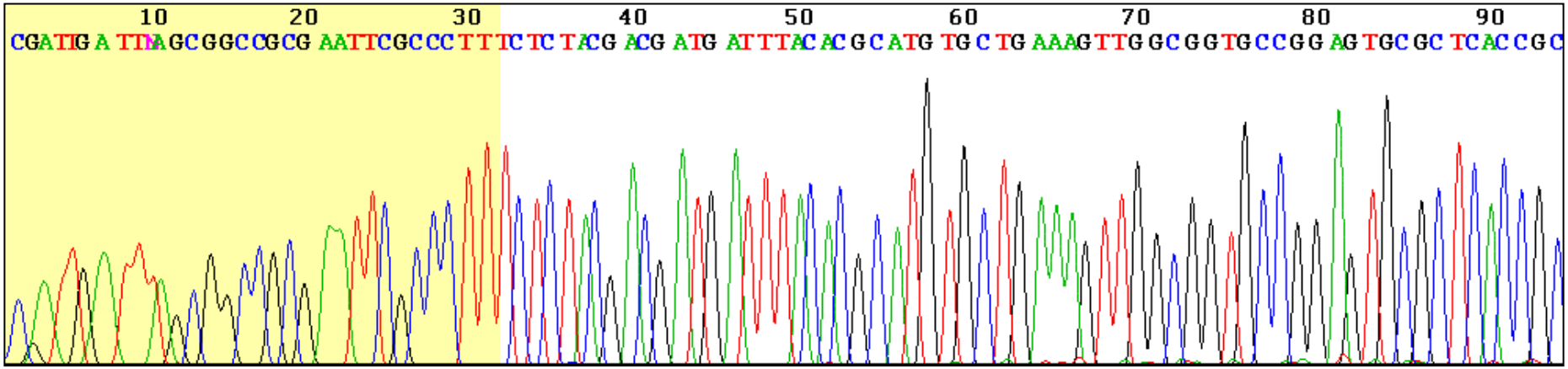
Il sequenziamento del DNA (metodo ad elettroforesi capillare)

MA 14-05-2013

http://dnasequencing.wordpress.com/2007/10/26/chain-termination-methods/

I sequenziatori di seconda generazione funzionano con tecnologia laser e separazione mediante elettroforesi capillare





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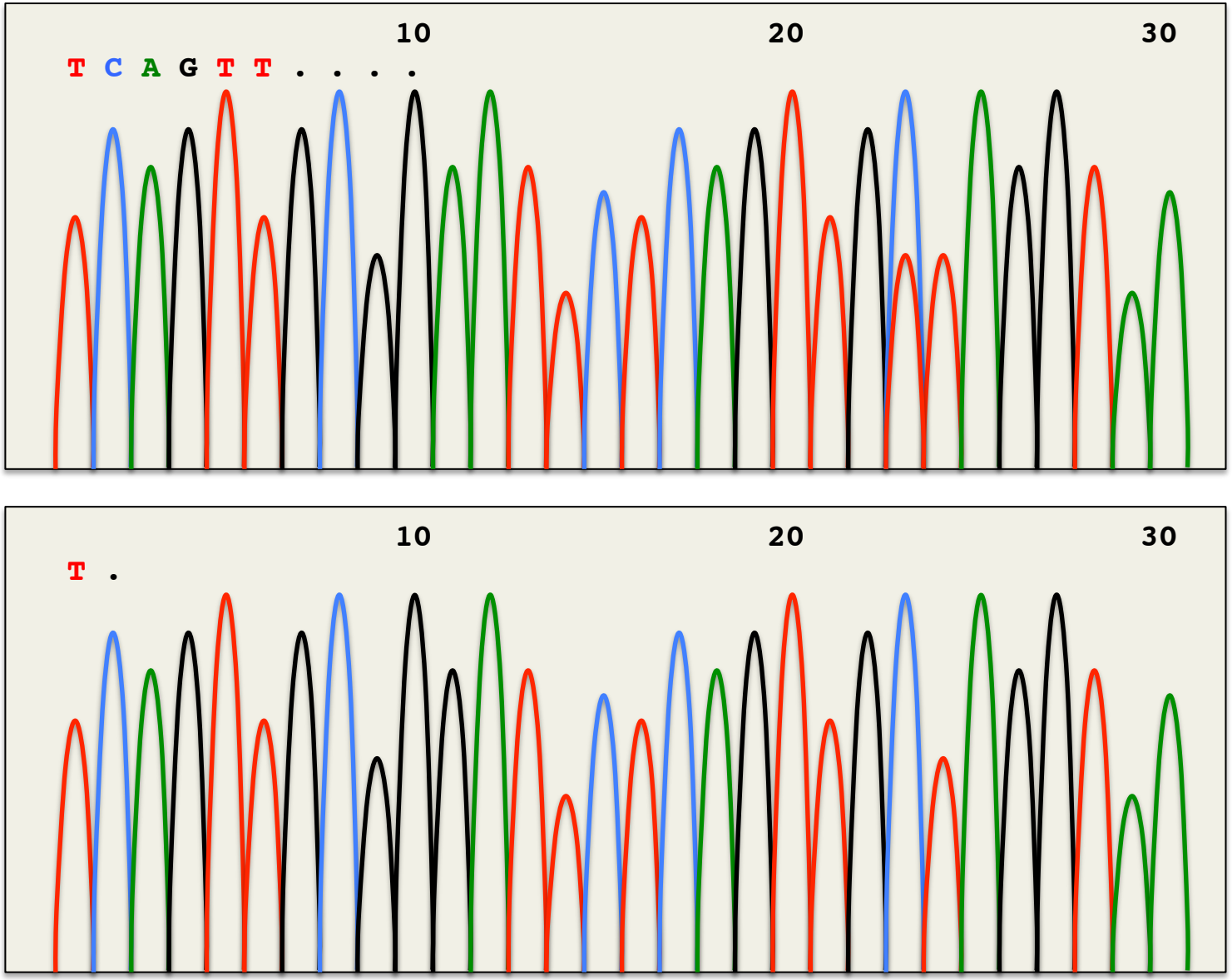
Esercizio di interpretazione di un elettroferogramma di DNA

(sequenziamento capillare)

ME 15-05-2013



Completare le sequenze e trovare eventuali differenze

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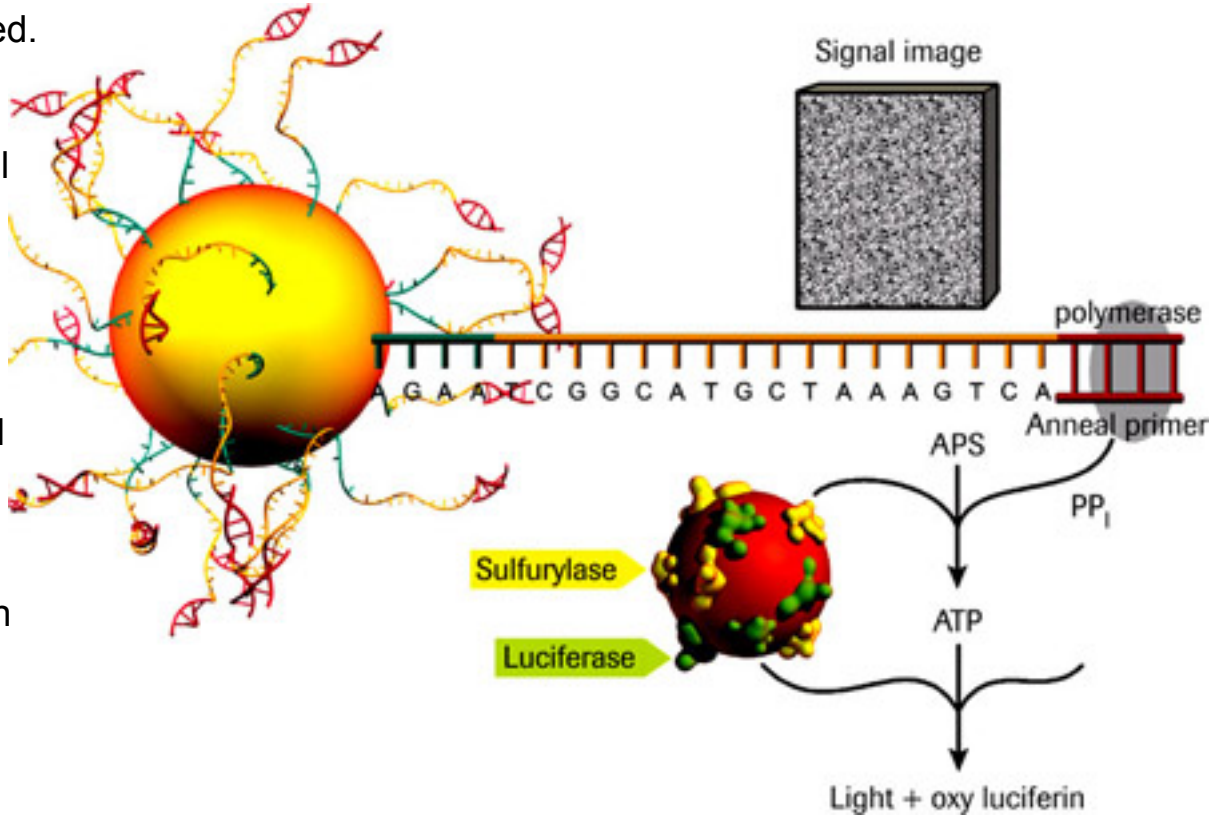
Il sequenziamento del DNA (metodo “Pyrosequencing”)

ME 21-04-2010

The principle is pyrosequencing. The sequencing is carried out in cycles. For each cycle only one nucleotide is offered. The nucleotide flow is CTAG. If one (or more) nucleotide matches with the complementary strand, a nucleotide will be coupled by the polymerase.
The resulting pyrophosphate (PPi) plus APS (activated sulphate) form ATP.

ATP plus **luciferin** in the presence of **luciferase** result into **oxy luciferin** and the energy is released as light. The resulting light is captured with a **CCD camera**. The light signal is linear and reflects the number of homopolymers in a row.



http://www.eurofinsdna.com/service-corner/faqs/faqs-genome-sequencing/general-technical-questions/what-is-the-principal-of-the-new-sequencing-technique.html?gclid=CImVkJuB3pUCFRsbZwodXR1JWg

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Il sequenziamento del DNA (metodo “Ion Torrent”)

ME 21-04-2010

http://www.iontorrent.com/technology-ion-technology-video/ http://www.iontorrent.com/the-simplest-sequencing-chemistry/

Il nuovissimo metodo di sequenziamento della ditta “Ion Torrent” sfrutta la produzione di idrogenioni H+ e non richiede cascate di reazioni enzimatiche

ion torrent Semiconductor Sequencing for Life™

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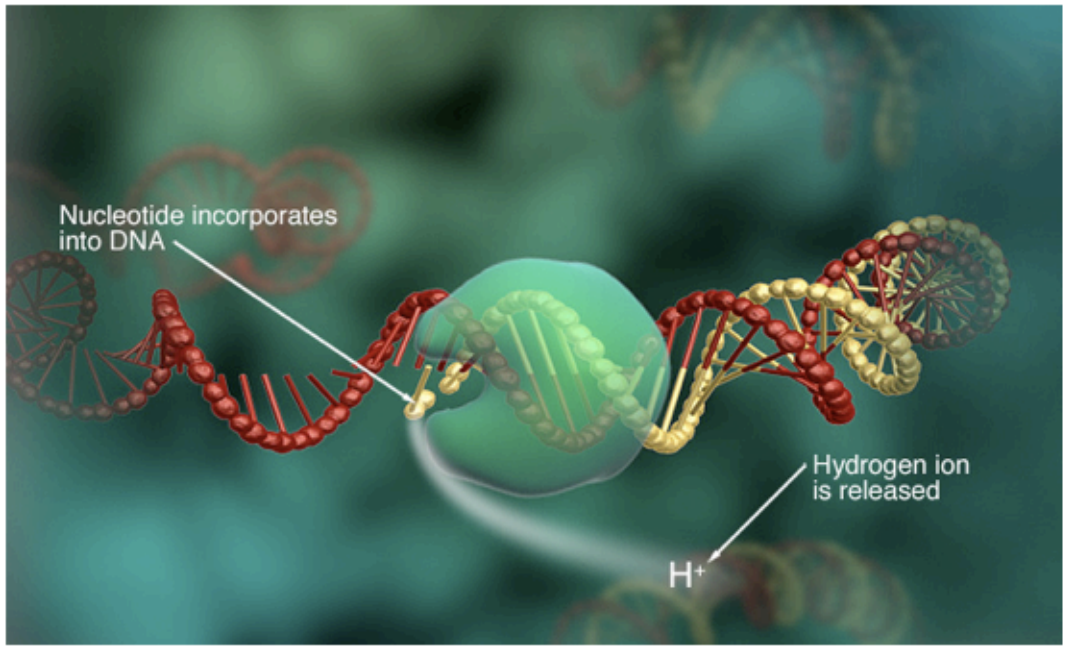
- The Chip is The Machine
- Simplest Sequencing Chemistry
- Ion Technology Complete Video
- - Semiconductor Section
- - Chemistry Section

TECHNOLOGY: THE SIMPLEST SEQUENCING CHEMISTRY

We pair proven semiconductor technology with a simple sequencing chemistry that is based on a well-characterized biochemical process.

show video >



In nature, when a nucleotide is incorporated into a strand of DNA by a polymerase, a hydrogen ion is released as a byproduct.

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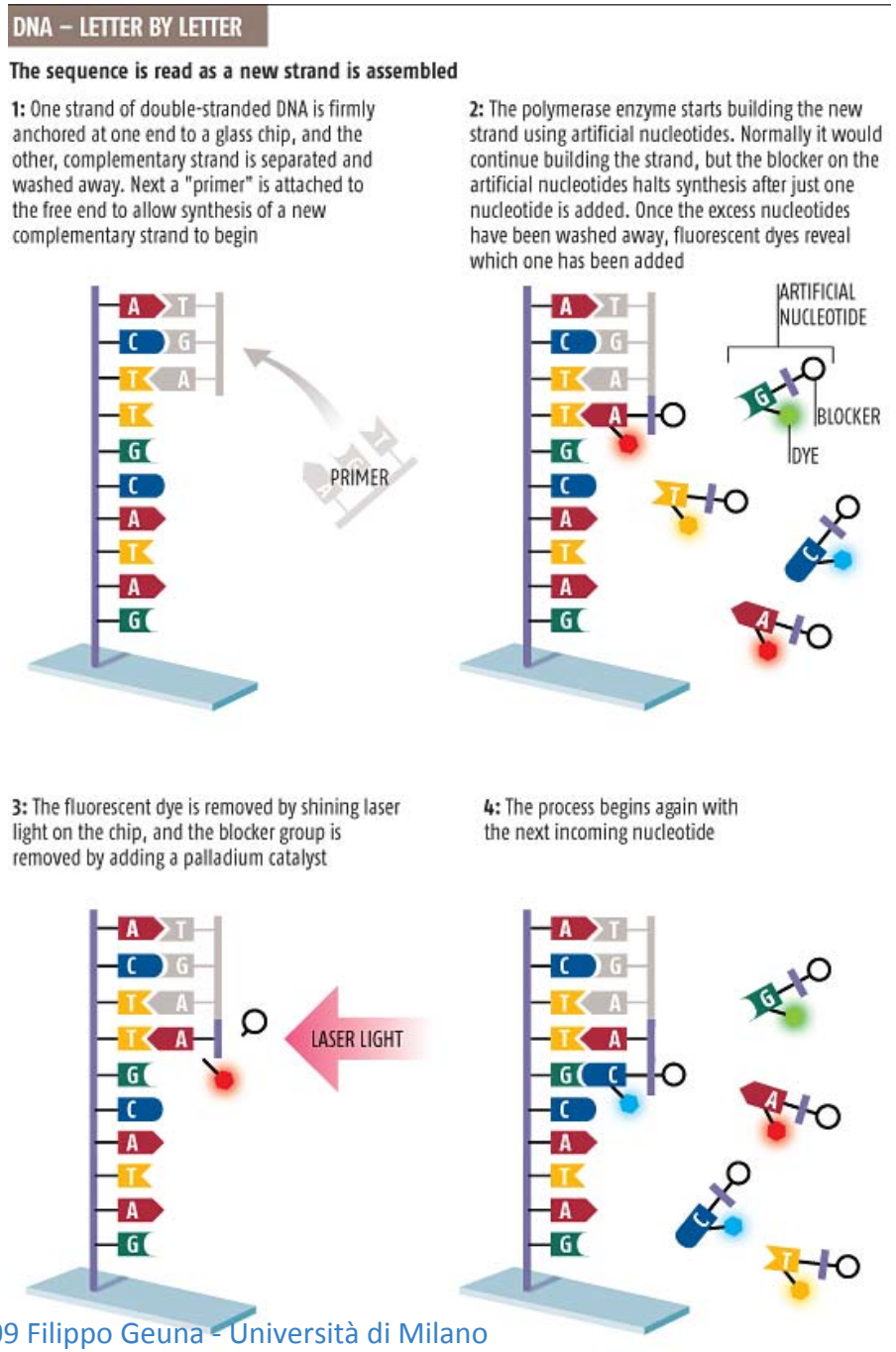
Sequenziamento su microarray



http://cmg.health.ufl.edu/index_files/HOWDNASEQ.jpg

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Taglio del DNA con enzimi di restrizione

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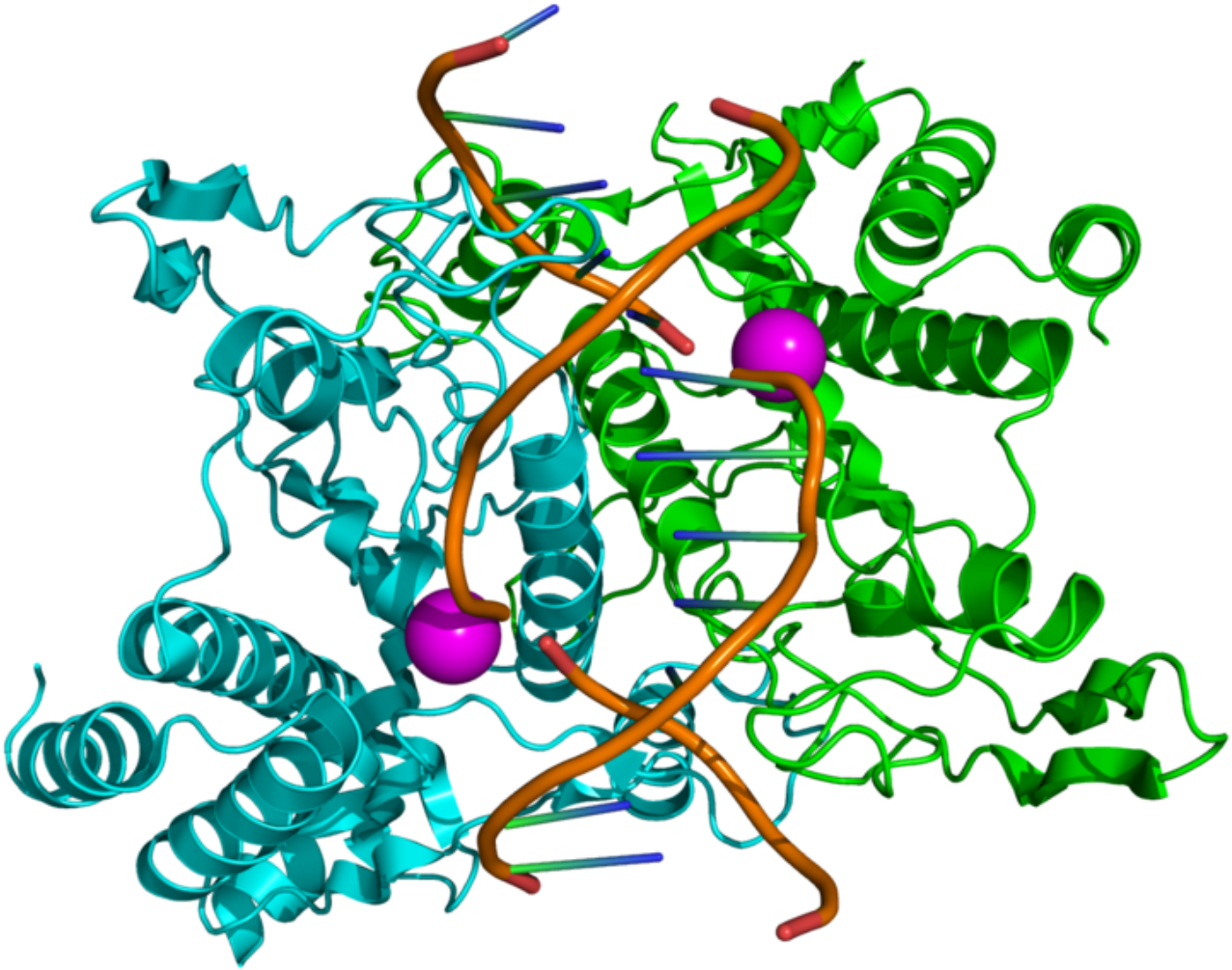
Enzimi di restrizione (struttura)



```
5'-GTATAC-3'
   ||||||
3'-CATATG-5'
```

```
G|AATTC
CTTAA|G
```

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http://en.wikipedia.org/wiki/Restriction_enzyme

Structure of the homodimeric restriction enzyme EcoRI (cyan and green cartoon diagram) bound to double stranded DNA (brown tubes)

Enzimi di restrizione (sequenze di taglio)

Examples

Examples of restriction enzymes include:[32]

Enzyme	Source	Recognition Sequence	Cut
*Eco*RI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
*Eco*RII	*Escherichia coli*	5'CCWGG 3'GGWCC	5'--- CCWGG---3' 3'---GGWCC ---5'
*Bam*HI	*Bacillus amyloliquefaciens*	5'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
*Hind*III	*Haemophilus influenzae*	5'AAGCTT 3'TTCGAA	5'---A AGCTT---3' 3'---TTCGA A---5'
*Taq*I	*Thermus aquaticus*	5'TCGA 3'AGCT	5'---T CGA---3' 3'---AGC T---5'
*Not*I	*Nocardia otitidis*	5'GCGGCCGC 3'CGCCGGCG	5'---GC GGCCGC---3' 3'---CGCCGG CG---5'

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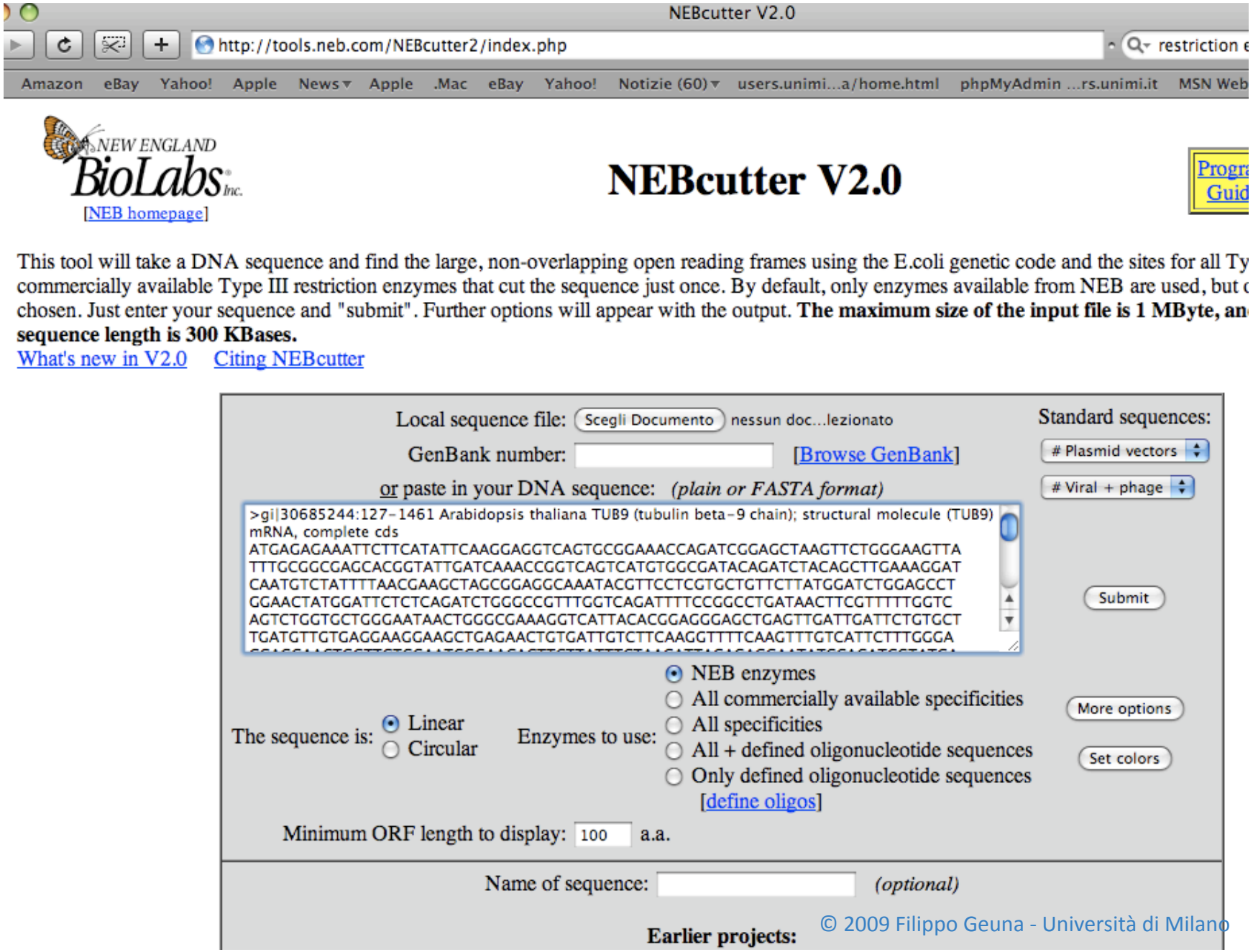
http://en.wikipedia.org/wiki/Restriction_enzyme

Taglio del DNA con enzimi di restrizione (NEBcutter 2.0) ME 15-05-2013



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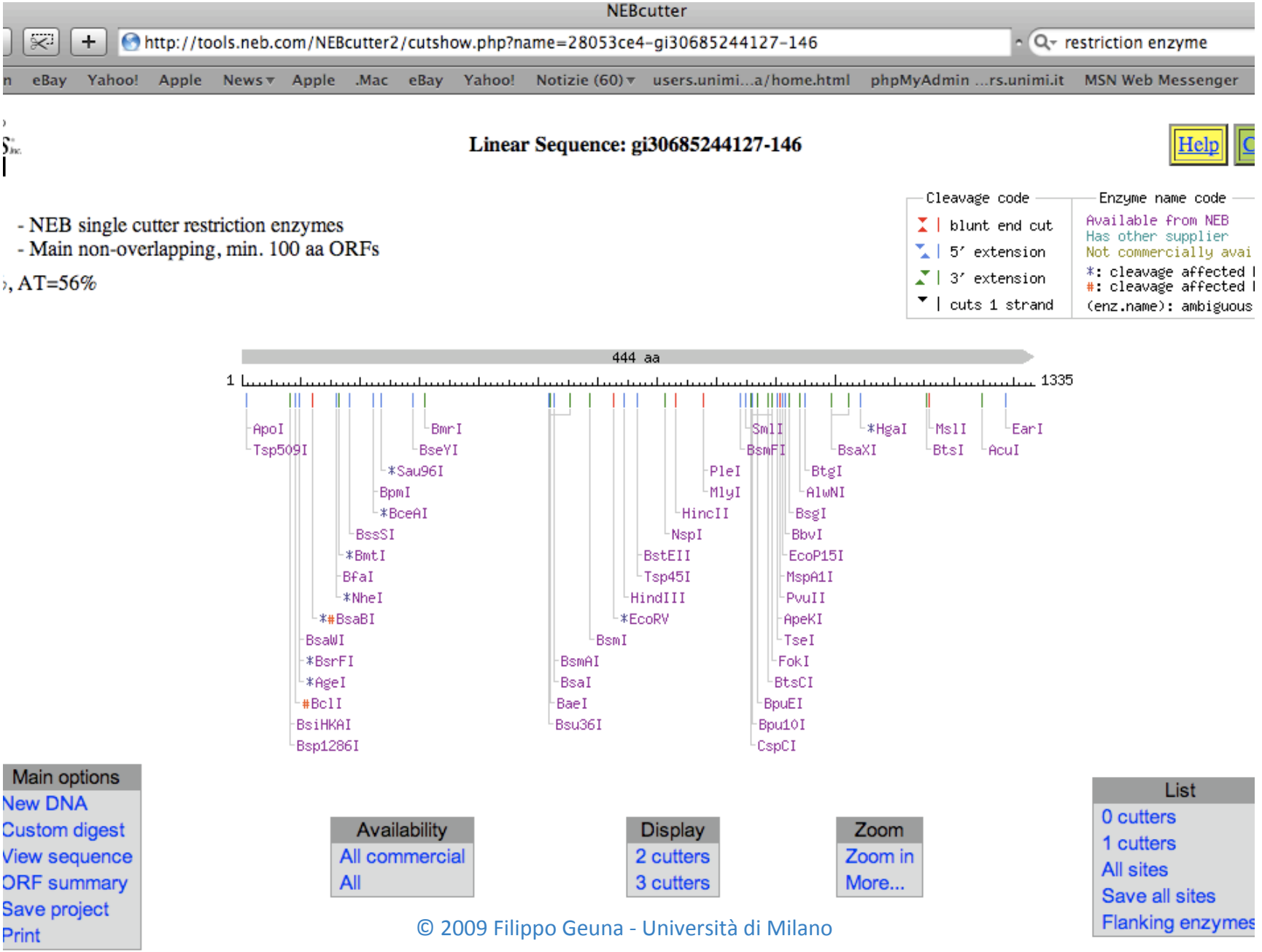
Taglio del DNA con enzimi di restrizione (NEBcutter)

ME 15-05-2013



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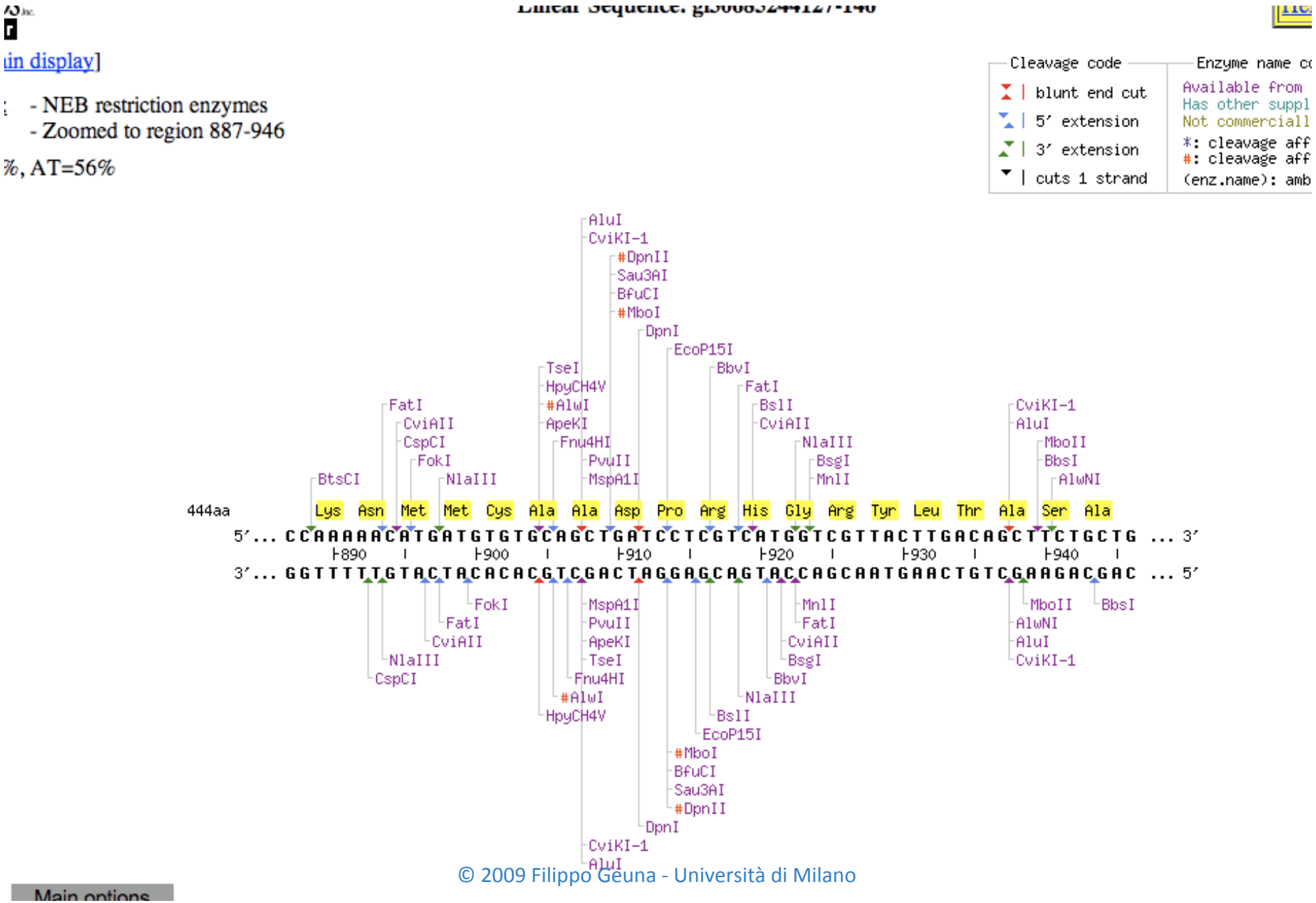
Taglio del DNA con enzimi di restrizione (NEBcutter)

ME 15-05-2013



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Analisi di una sequenza proteica (sito NEBcutter)

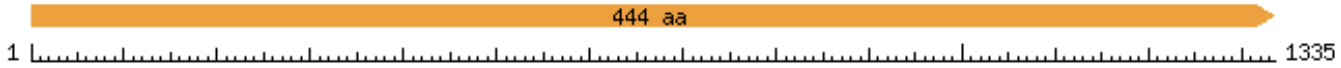
NEW ENGLAND BioLabs Inc. NEBcutter

[Back to main display]

ORF Sequence

gi30685244127-146

Coding region: 1..1335



[Edit] - [Delete] - [Add new ORF] - [Locate multiple cutters that excise this ORF] - [Silent Mutagenesis]

Protein sequence:

```
> 444 aa
MREILHIQGG QCGNQIGAKF WEVICGEHGI DQTGQSCGDT DLQLERINVY
FNEASGGKYV PRAVLMDLEP GTMDSLRSGP FGQIFRPDNF VFGQSGAGNN
WAKGHYTEGA ELIDSVLDVV RKEAENCDCL QGFQVCHSLG GGTGSGMGTL
LISKIREEYP DRMMMTFSVF PSPKVSDTVV EPYNATLSVH QLVENADECM
VLDNEALYDI CFRTLKLANP TFGDLNHLIS ATMSGVTCCL RFPGQLNSDL
RKLAVNLIPF PRLHFFMVGF APLTSRGSQQ YSALSVPELT QQMWDAKNMM
CAADPRHGRY LTASAVFRGK MSTKEVDEQM MNVQNKNSSY FVEWIPNNVK
SSVCDIAPTG LKMASTFIGN STSIQEMFRR VSEQFTAMFR RKAFLHWYTG
EGMDEMEFTE AESNMNDLVA EYQQYQDATV GEEEYEEDEE EEEA
```

Blast this sequence at NCBI

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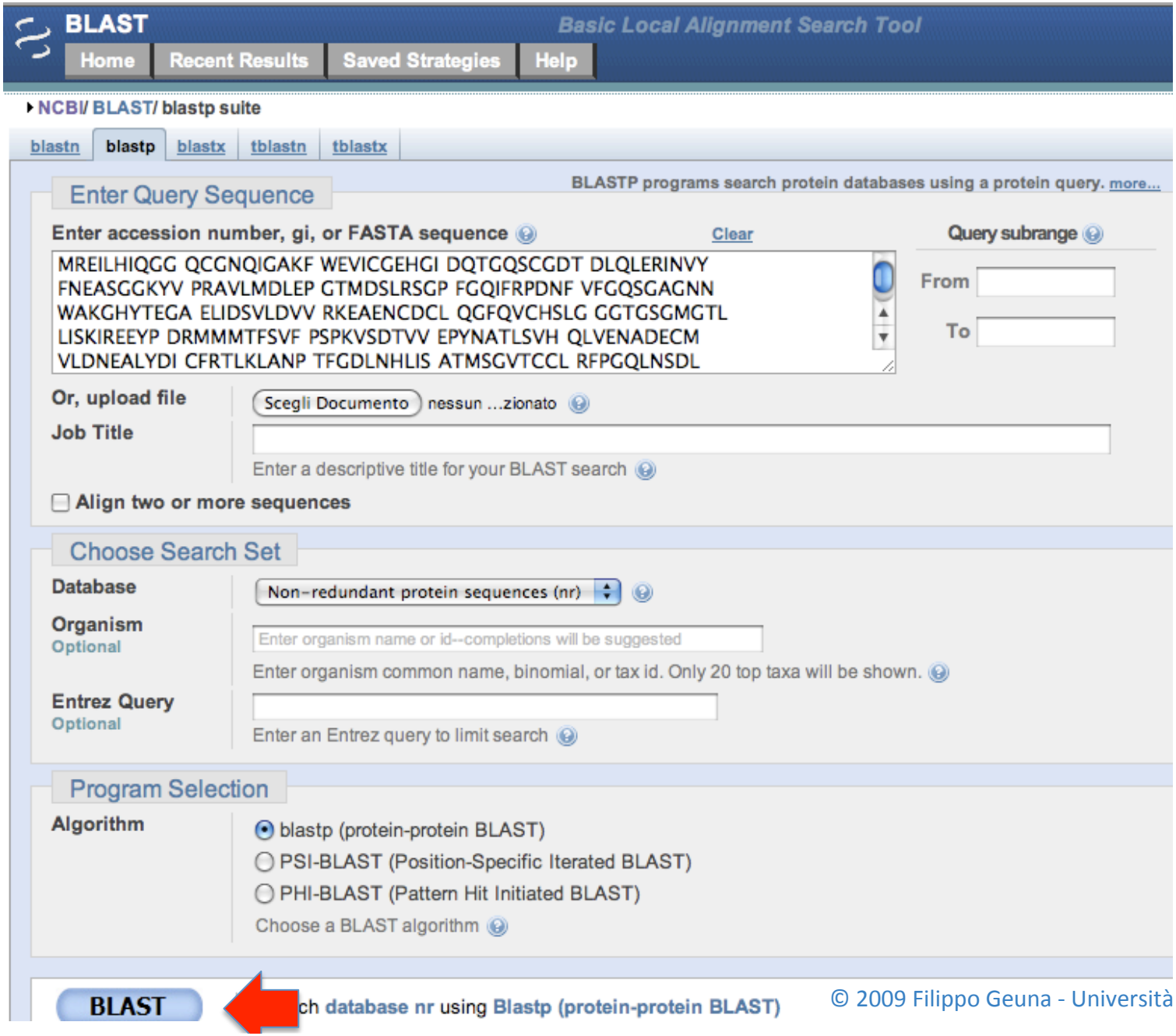
Analisi BLAST di sequenza proteica (presso il sito NCBI)

…..-04-2010



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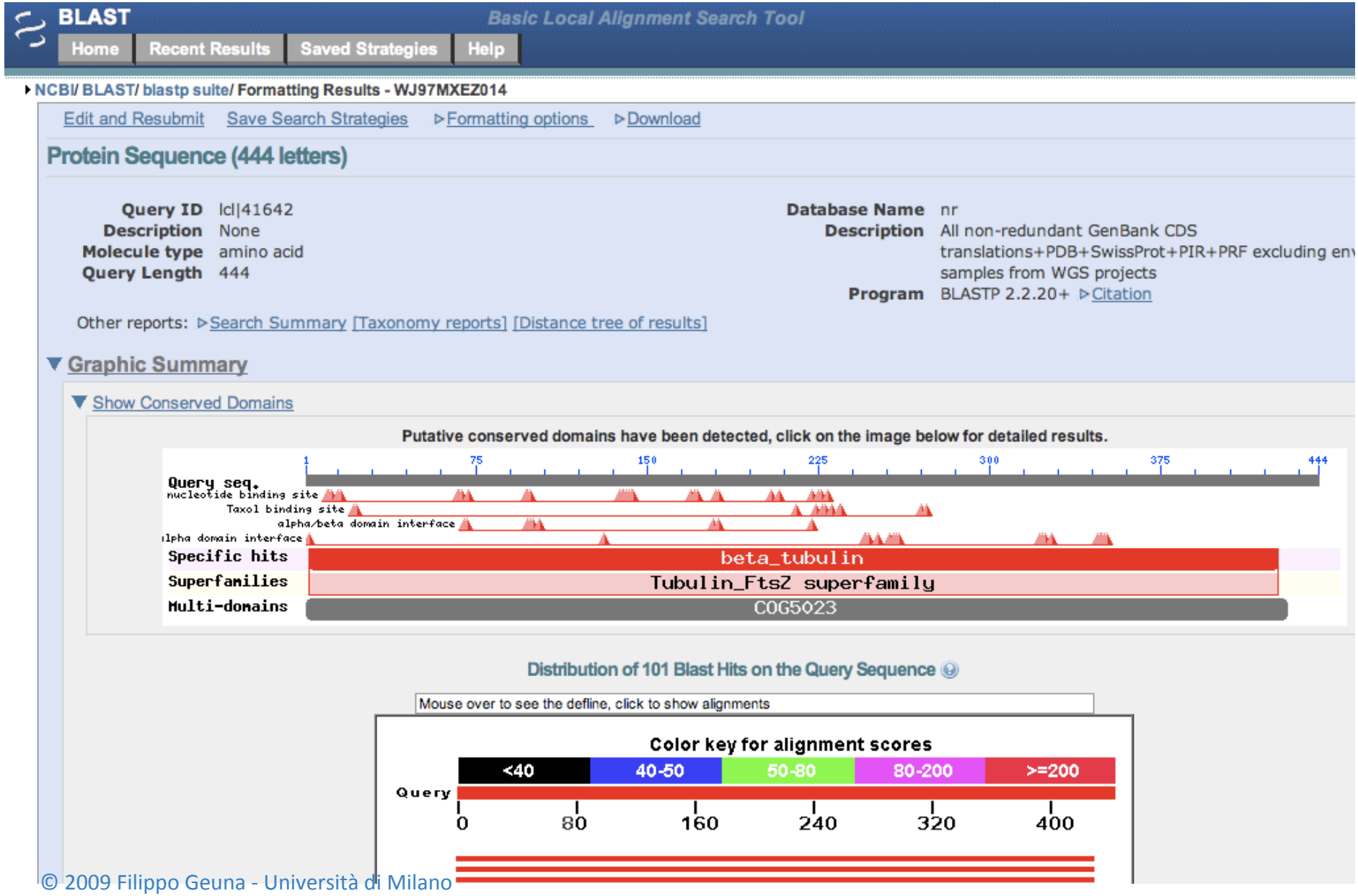
Taglio del DNA con enzimi di restrizione (NEBcutter)

.....04-2010



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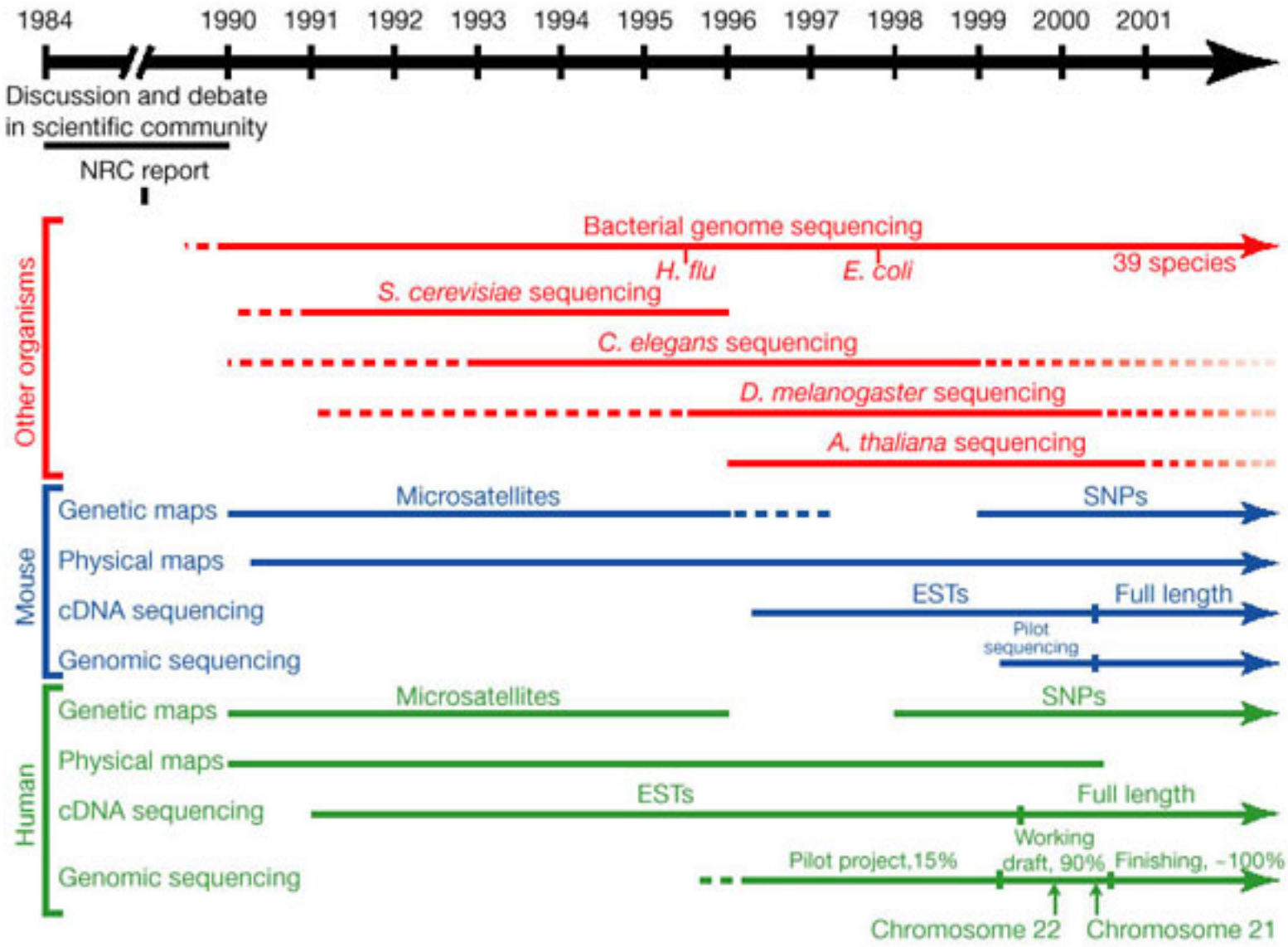
Evoluzione dei progetti di analisi fisica dei genomi

…..-05-2010



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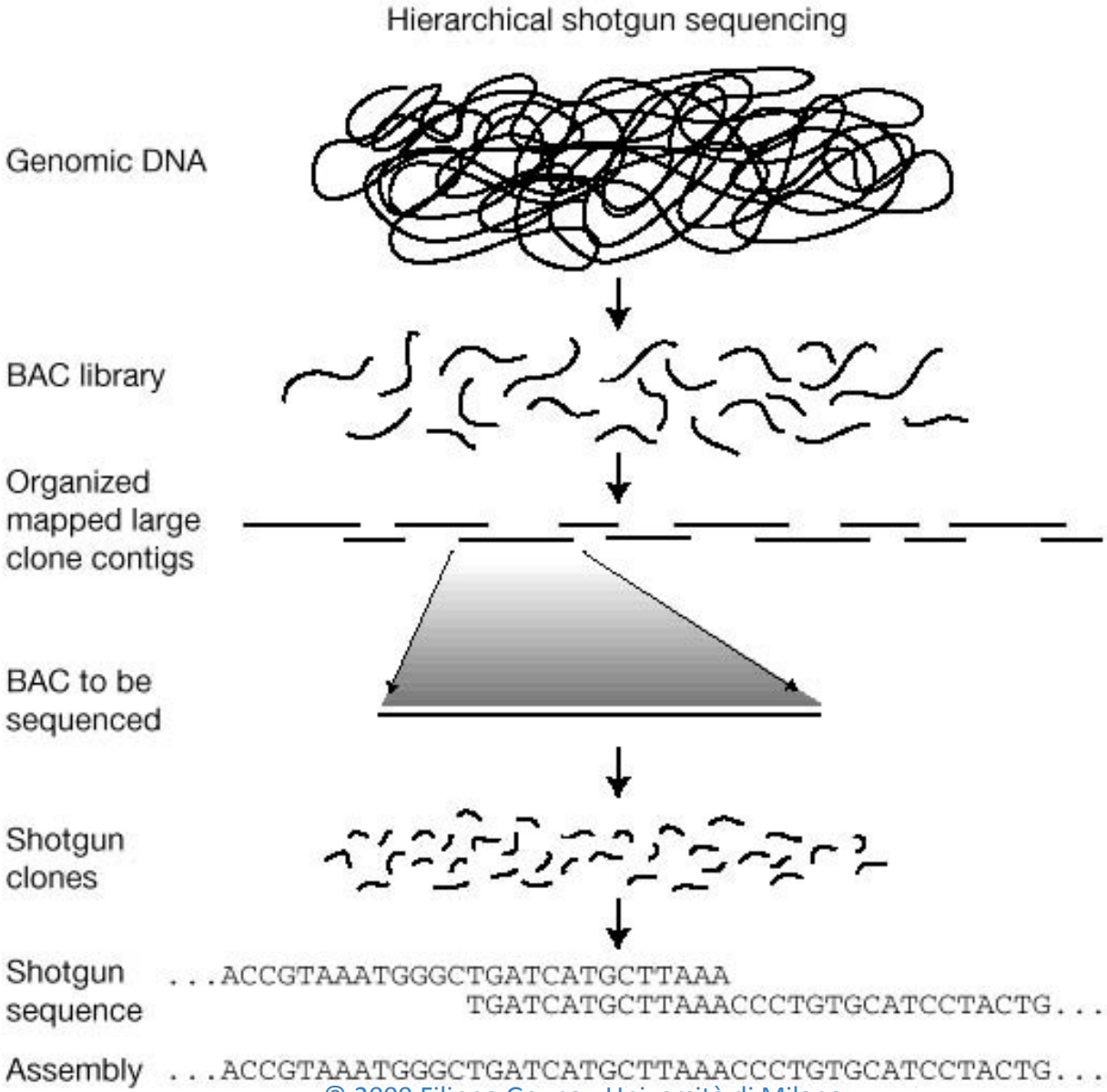
Sequenziamento “shotgun”



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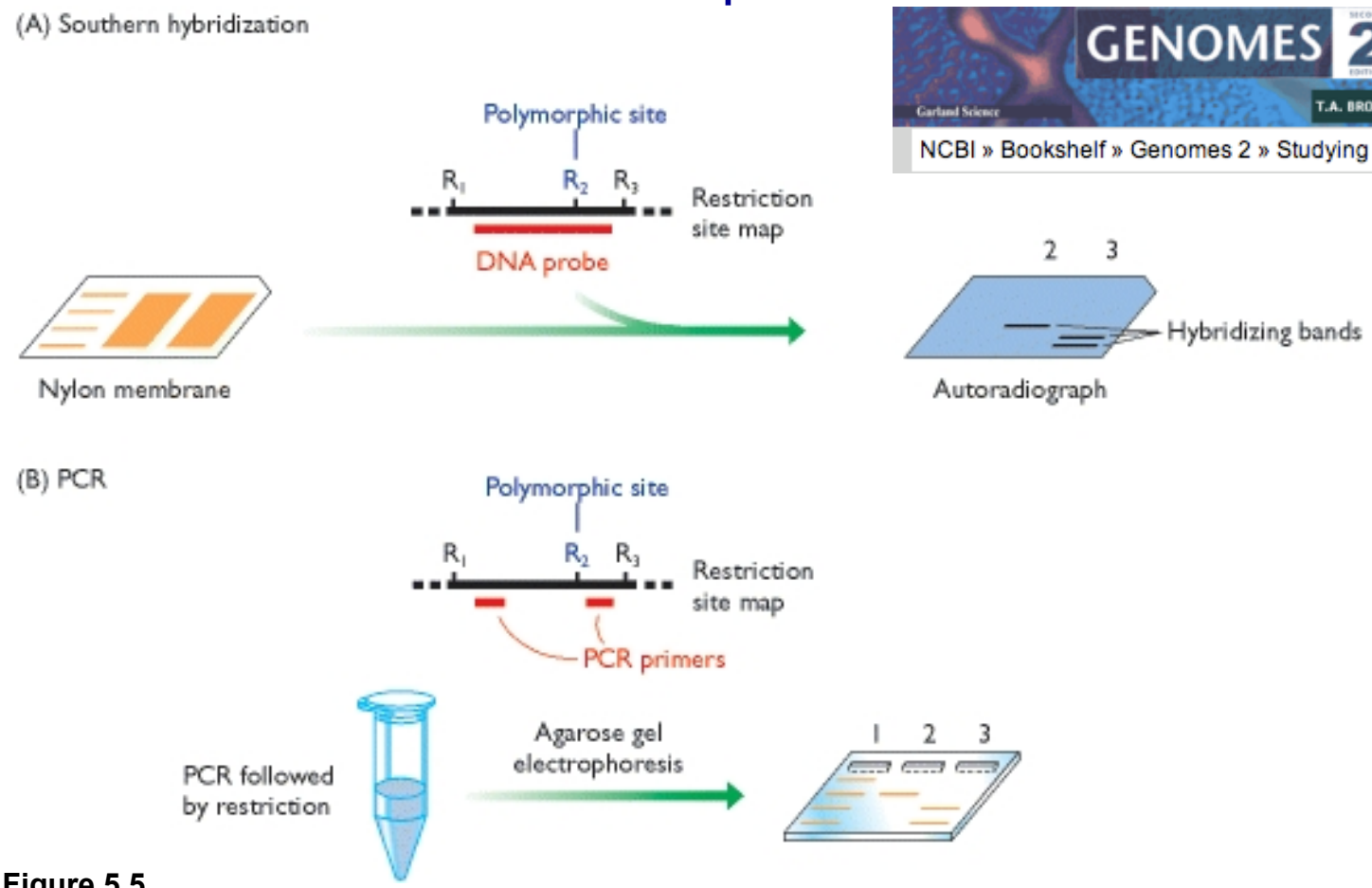
http://www.nature.com/nature/journal/v409/n6822/fig_tab/409860a0_F2.html

Due metodi per rilevare RFLP



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5 Table of Contents
In this page
Genetic and Physical Maps
Genetic Mapping
Physical Mapping
Study Aids For Chapter 5
References
Further Reading

Figure 5.5.
Two methods for scoring an RFLP
(A) RFLPs can be scored by Southern hybridization. The DNA is digested with the appropriate restriction enzyme and separated in an agarose gel. The smear of restriction fragments is transferred to a nylon membrane and probed with a piece of DNA that spans the polymorphic restriction site. If the site is absent then a single restriction fragment is detected (lane 2); if the site is present then two fragments are detected (lane 3). (B) The RFLP can also be typed by PCR, using primers that anneal either side of the polymorphic restriction site. After the PCR, the products are treated with the appropriate restriction enzyme and then analyzed by agarose gel electrophoresis. If the site is absent then one band is seen on the agarose gel; if the site is present then two bands are seen.

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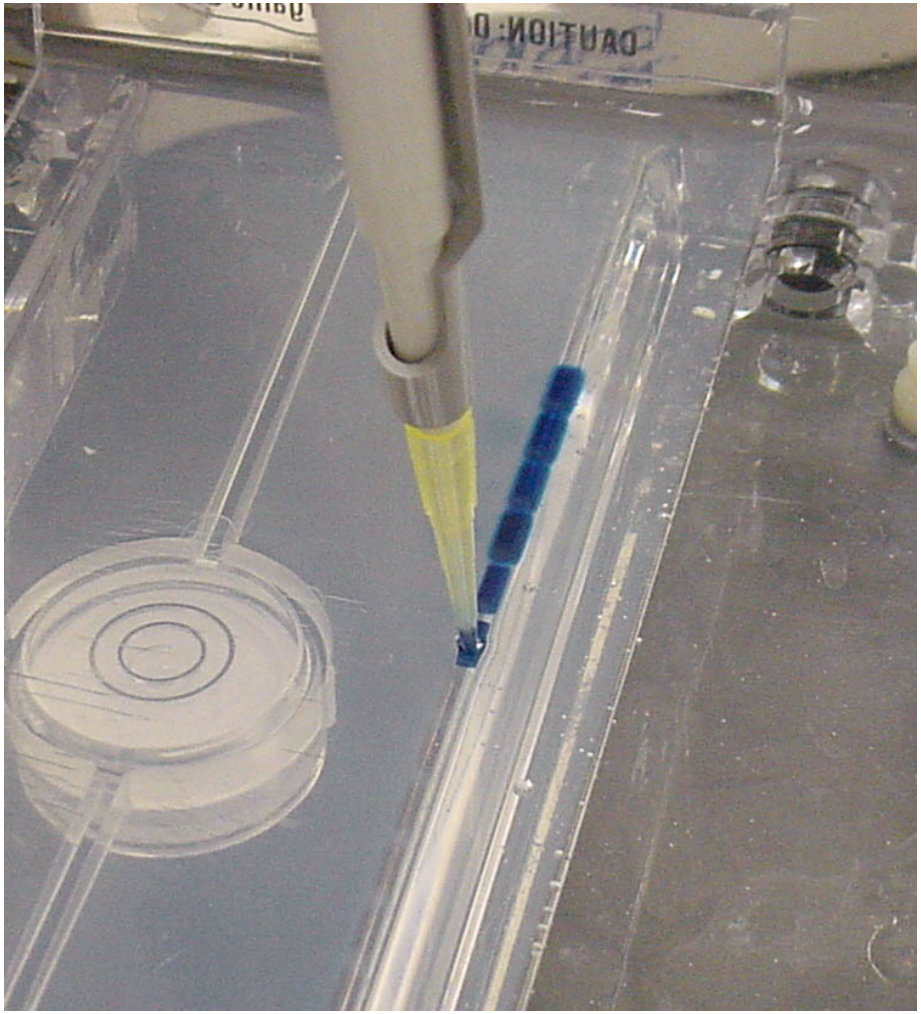
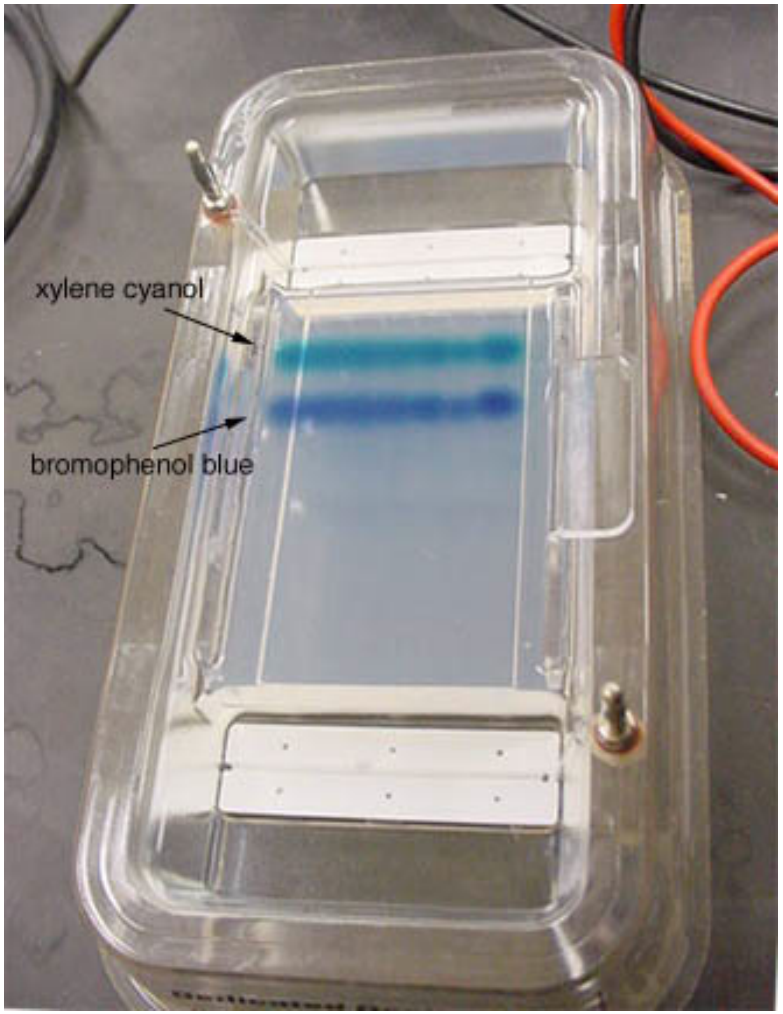
http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=genomes&part=A6196

Gel di agarosio



http://delliss.people.cofc.edu/virtuallabbook/LoadingGel/GelLoadB.jpg



http://delliss.people.cofc.edu/virtuallabbook/LoadingGel/Minigel2a.jpg

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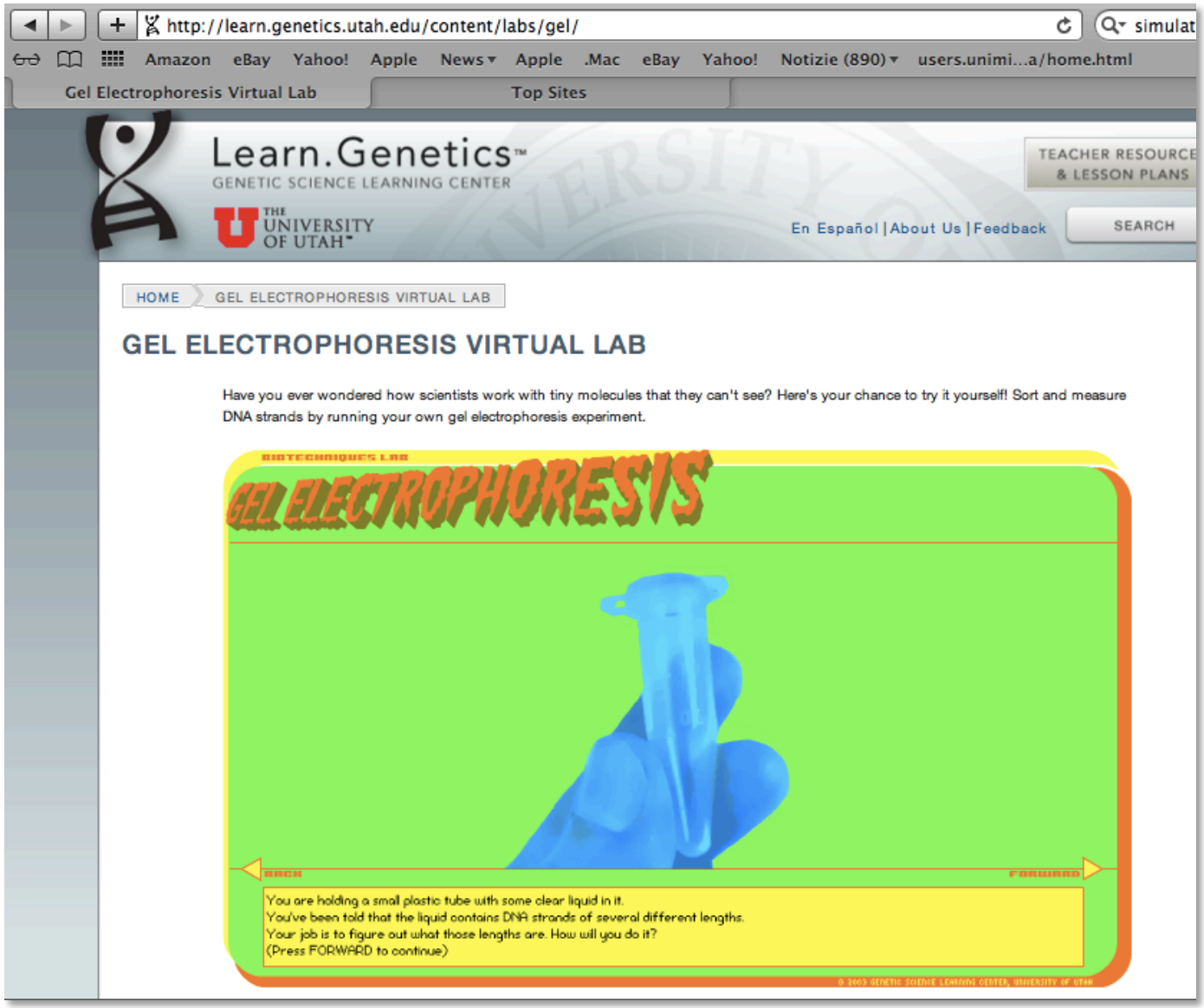
(Purves et al. Fig. 16.3)

Separazione di frammenti di DNA: simulazione interattiva

MA 23-04-2013



http://learn.genetics.utah.edu/content/labs/gel/

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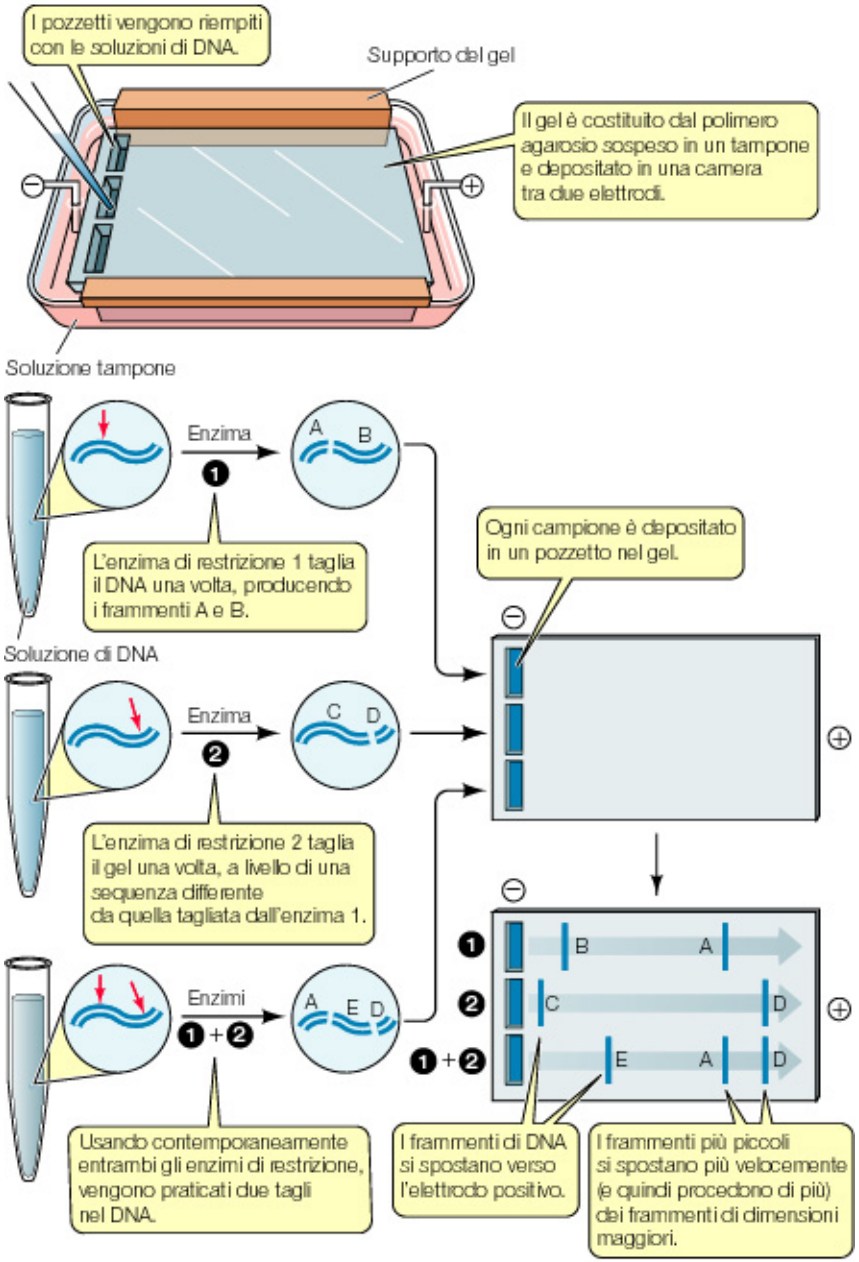
Separazione di frammenti di DNA

ME 17-04-2013



(Purves et al. Fig. 16.2)

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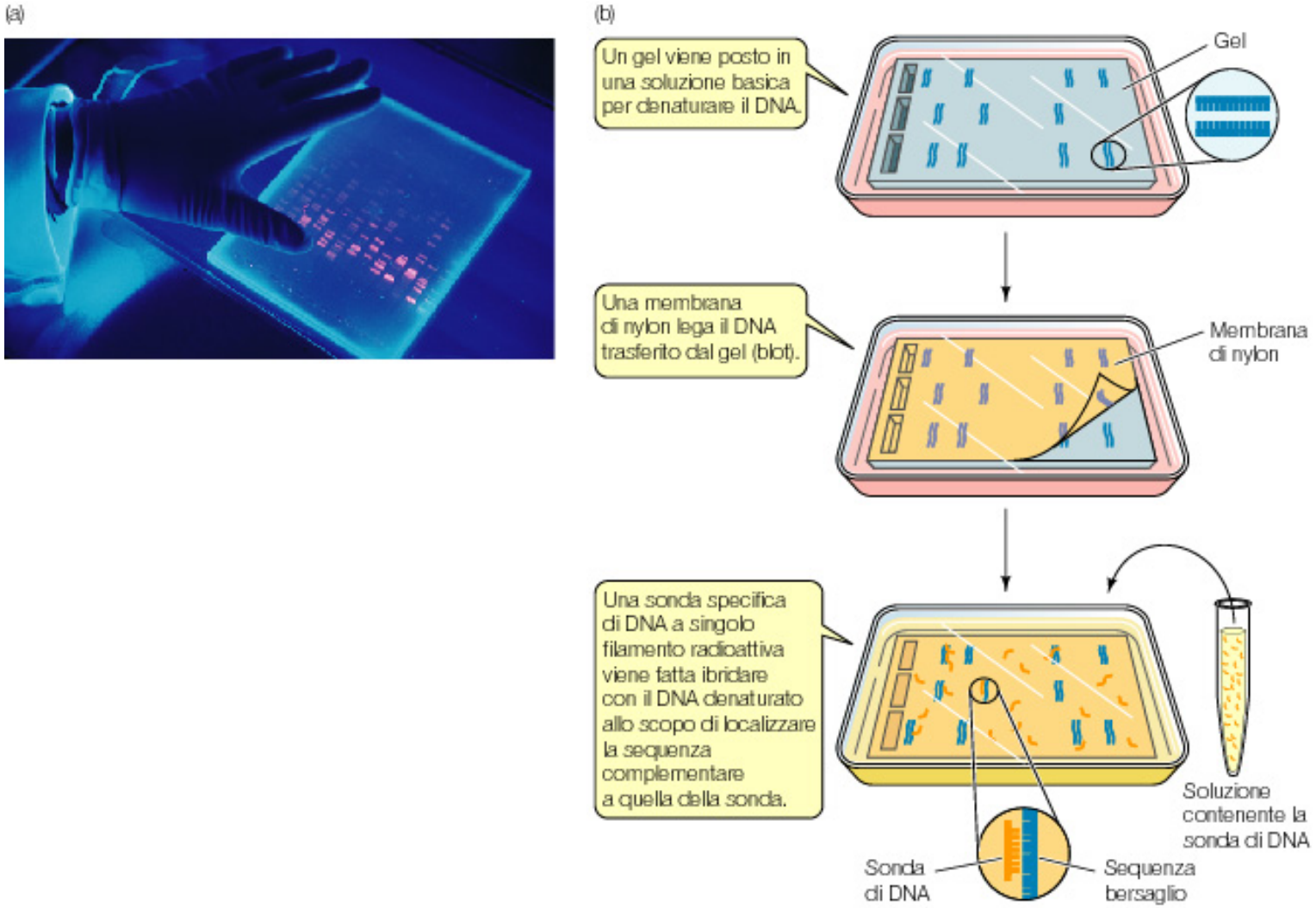
Ibridazione molecolare su frammenti di restrizione



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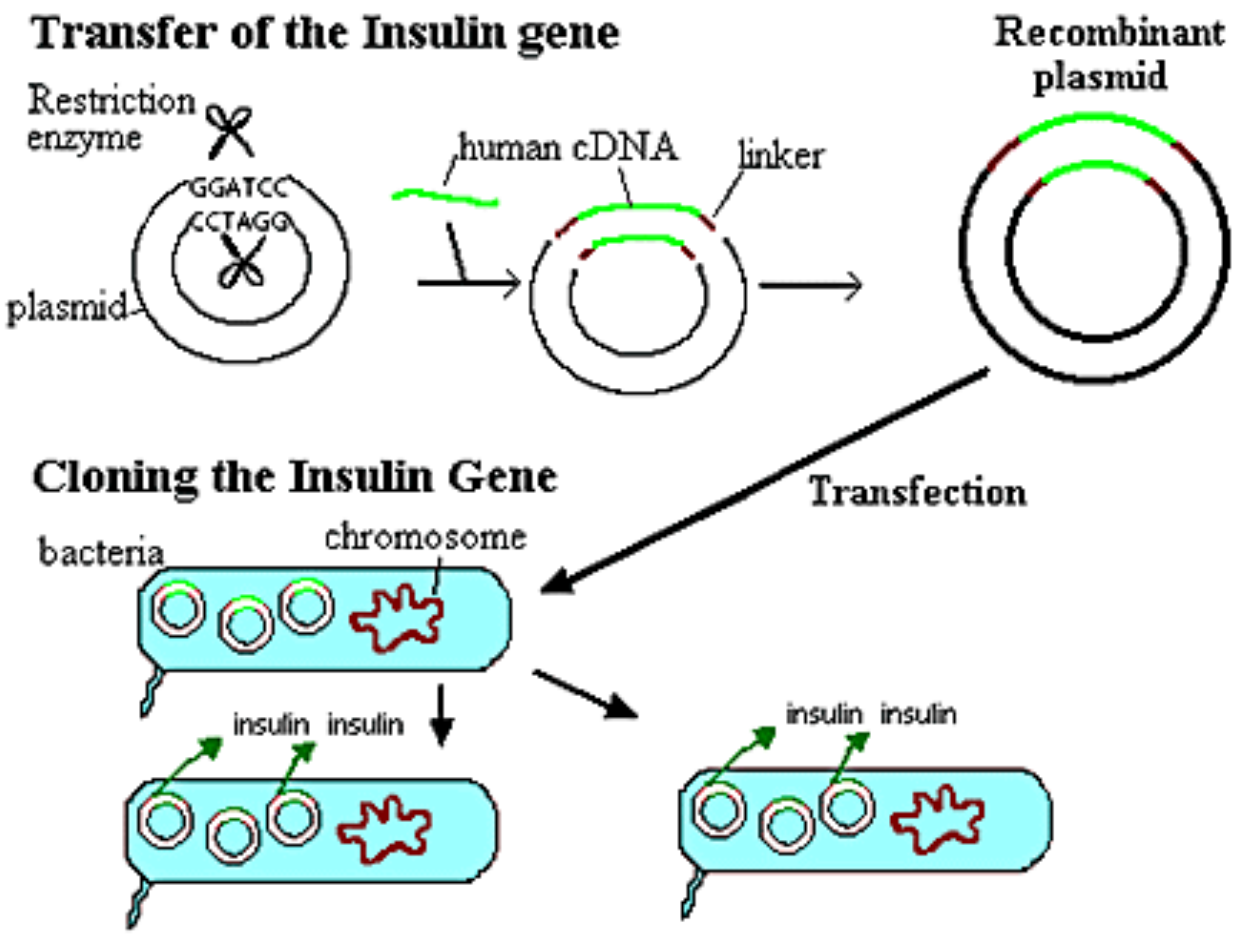
(Purves et al. Fig. 16.3)

Clonazione del DNA



Transfer and cloning of the Insulin gene

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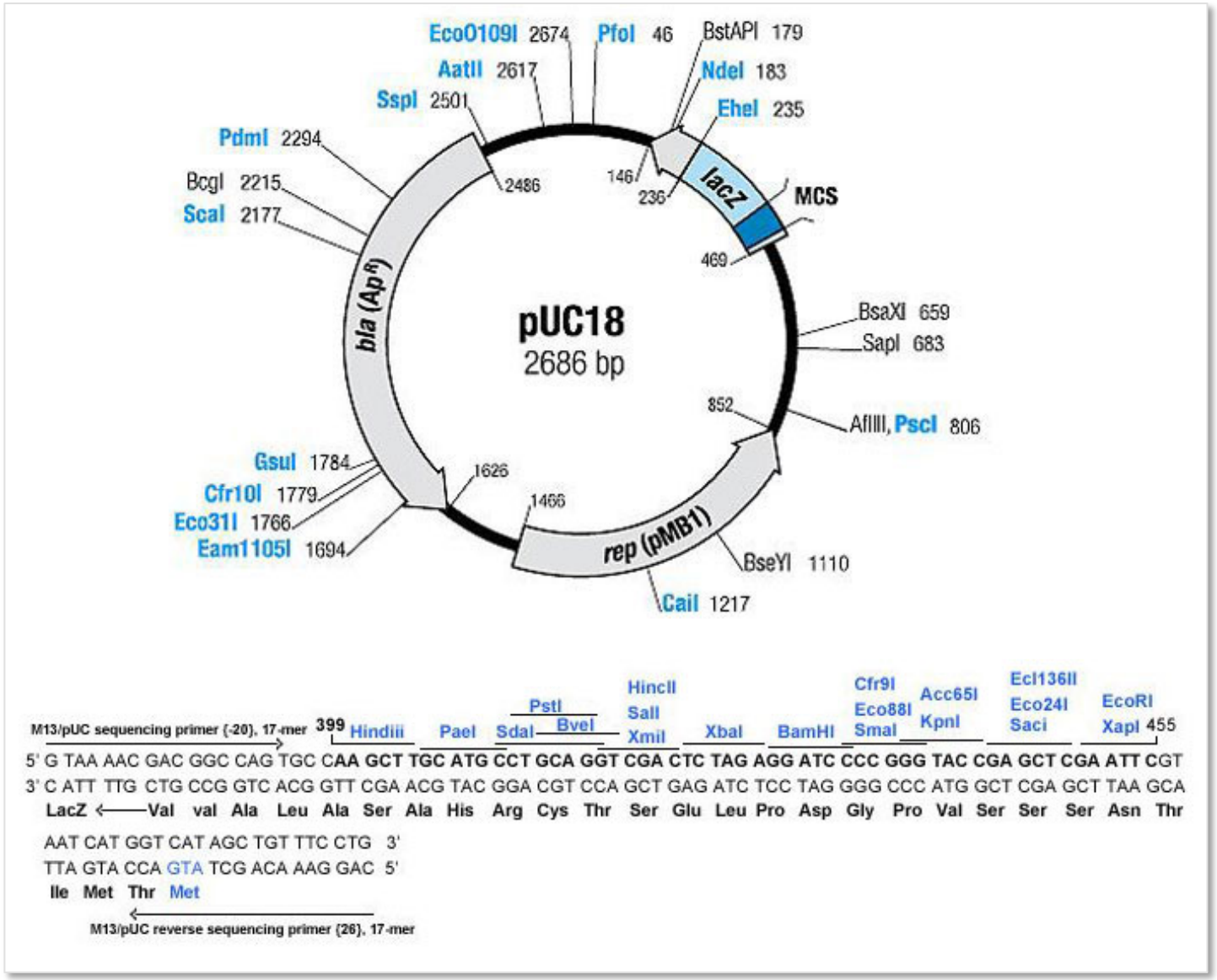
http://www.accessexcellence.org/RC/VL/GG/transfer_and.php

Vettore pUC18 con sito multiplo di clonaggio (MCS)

ME 23-05-2012



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http://www.genscript.com/site2/document/5322_20080813063105.JPG

Clonazione del DNA: esempio di plasmide di espressione

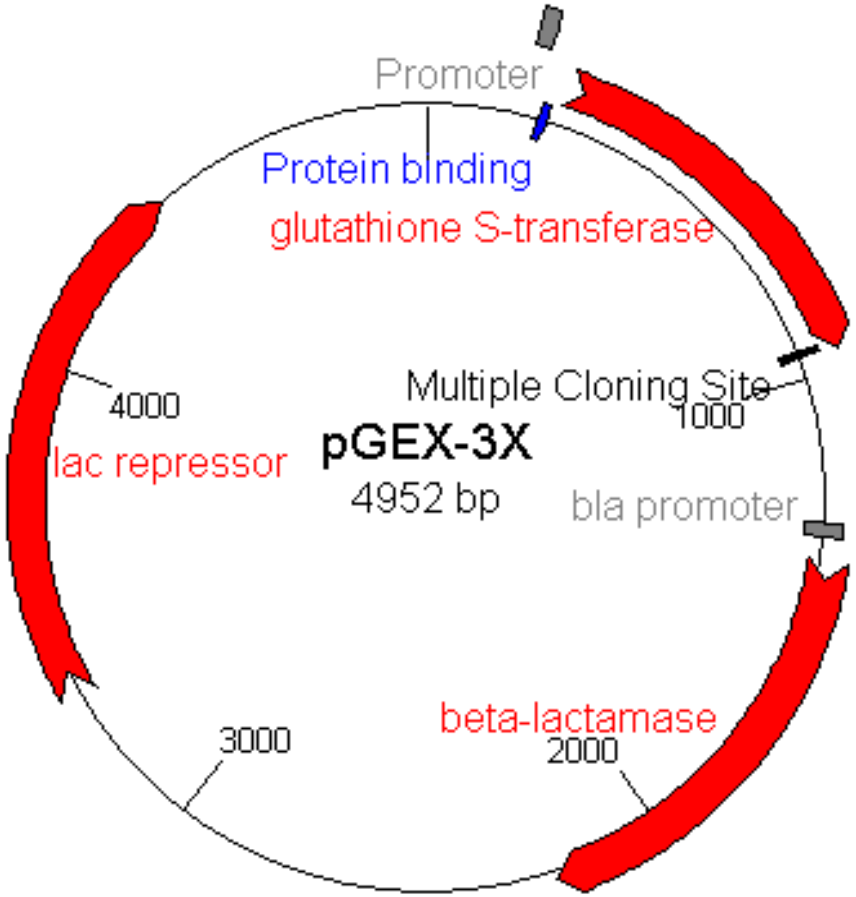
…..-05-2010

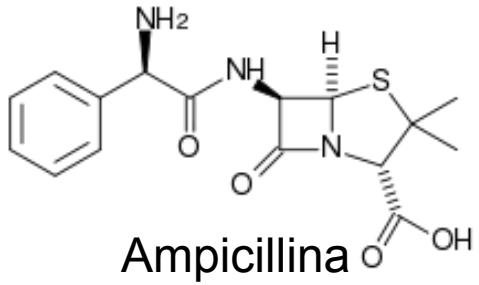
pBR322

pGEM3

Amp^R

Kan^R





Ampicillina

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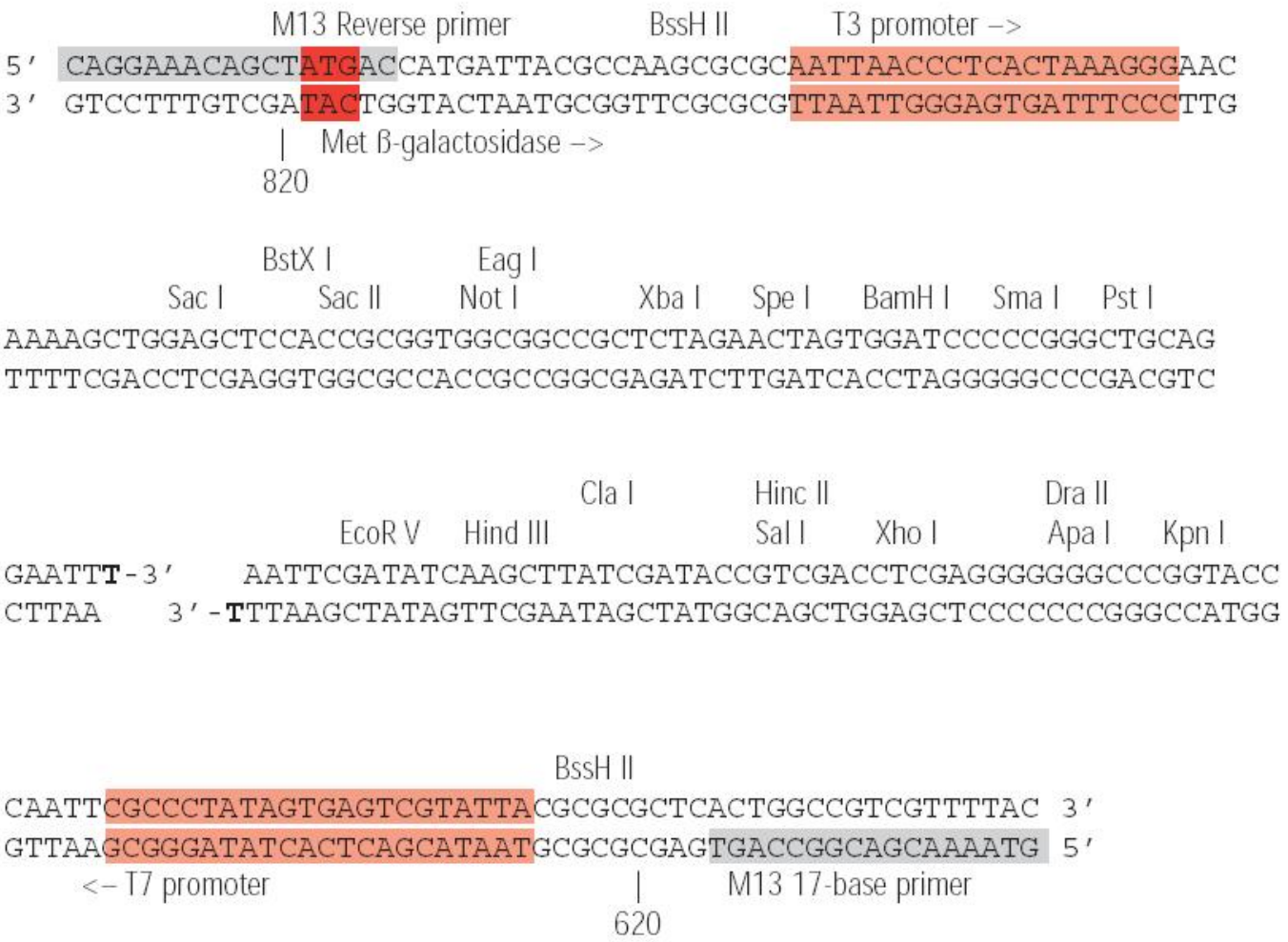
http://en.wikipedia.org/wiki/File:PGEX-3X_cloning_vector.png

Clonazione del DNA: sito multiplo di clonaggio (MCS) di un T-vector

(sequence shown 828 - 600)



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http://www.genecraft.de/photos/207_2627.JPG

Trasformazione vegetale

This image was first published in a November 1986 issue of the journal *Science in a paper titled "Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants" by David W. Ow, Keith V. Wood, Marlene DeLuca, Jeffrey R. de Wet, Donald R. Helinski and Stephen H. Howell. The research was funded by grants from the US Dept. of Agriculture and the National Science Foundation.*

http://compuhighwv.com/classes/bio/lessons/lesson15.html

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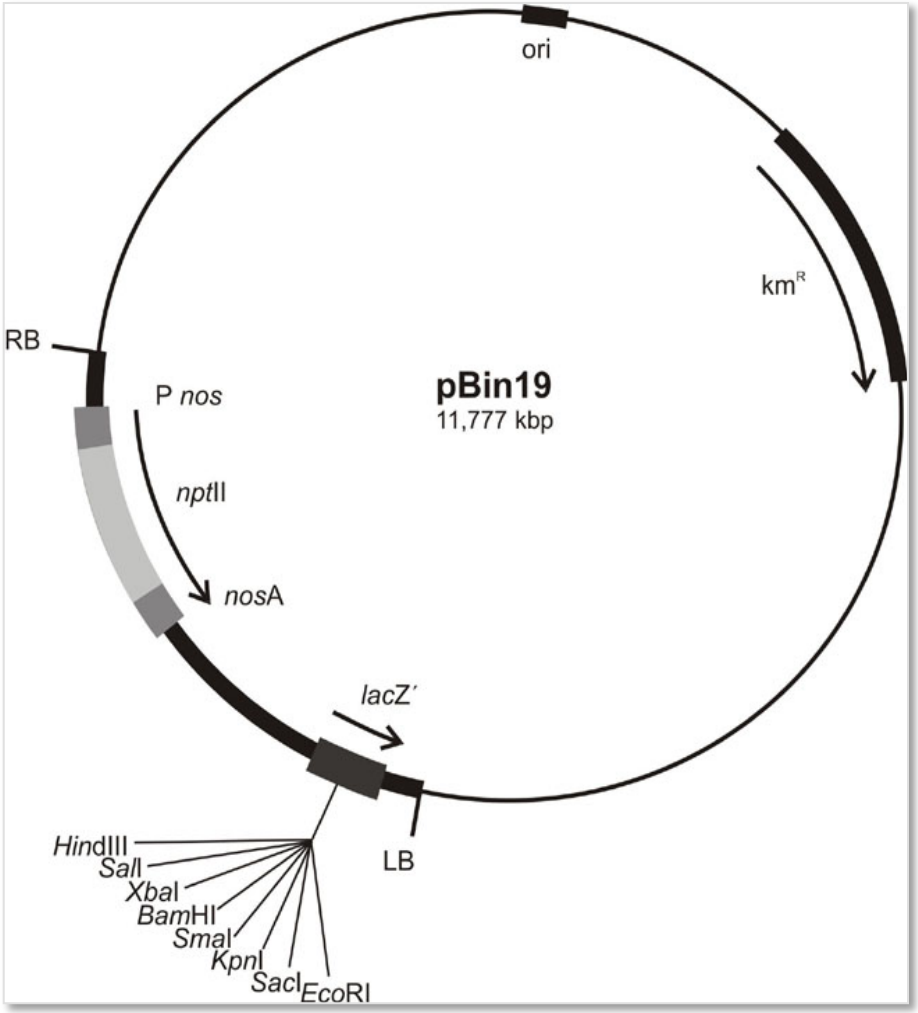
Clonazione: esempio di vettore vegetale (pBIN19)



…..-04-2010

http://edoc.hu-berlin.de/dissertationen/jacob-daniela-2003-07-15/HTML/chapter6.html

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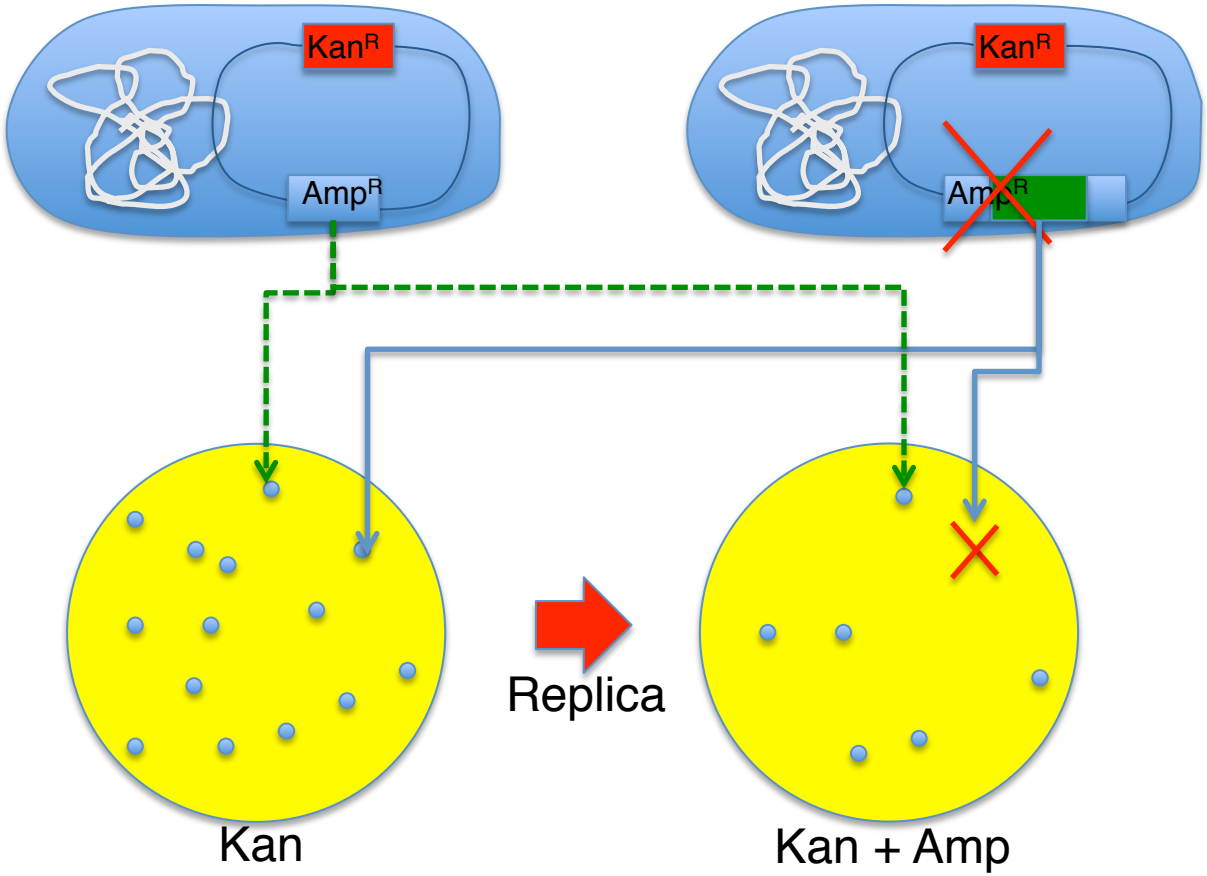
Identificazione dei costrutti ricombinanti tramite “replica plate”

MA 22-05-2012



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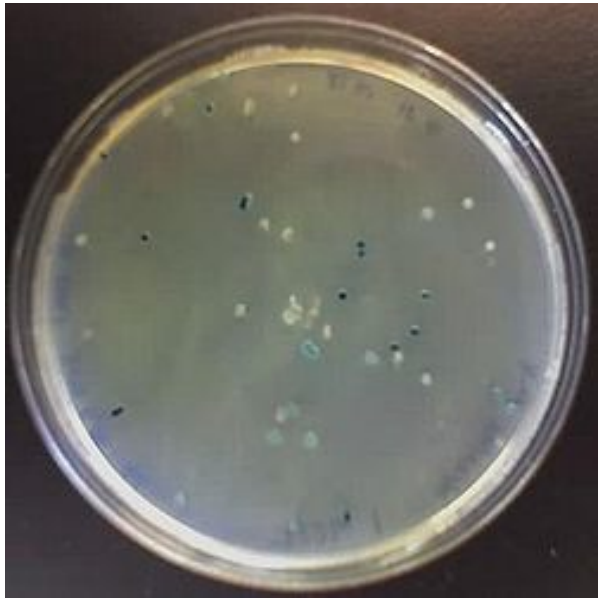
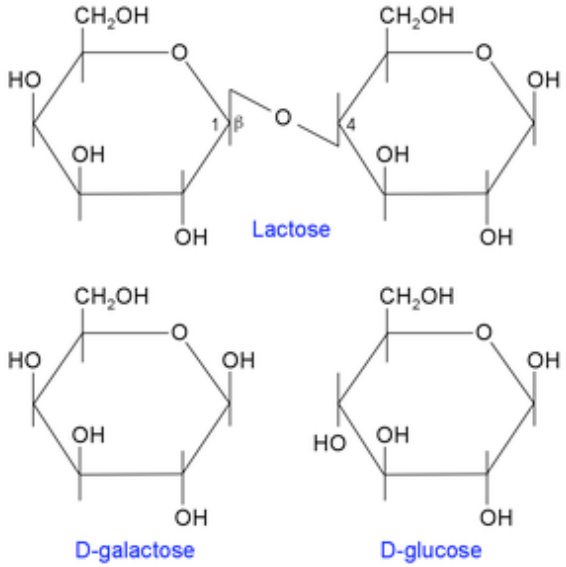
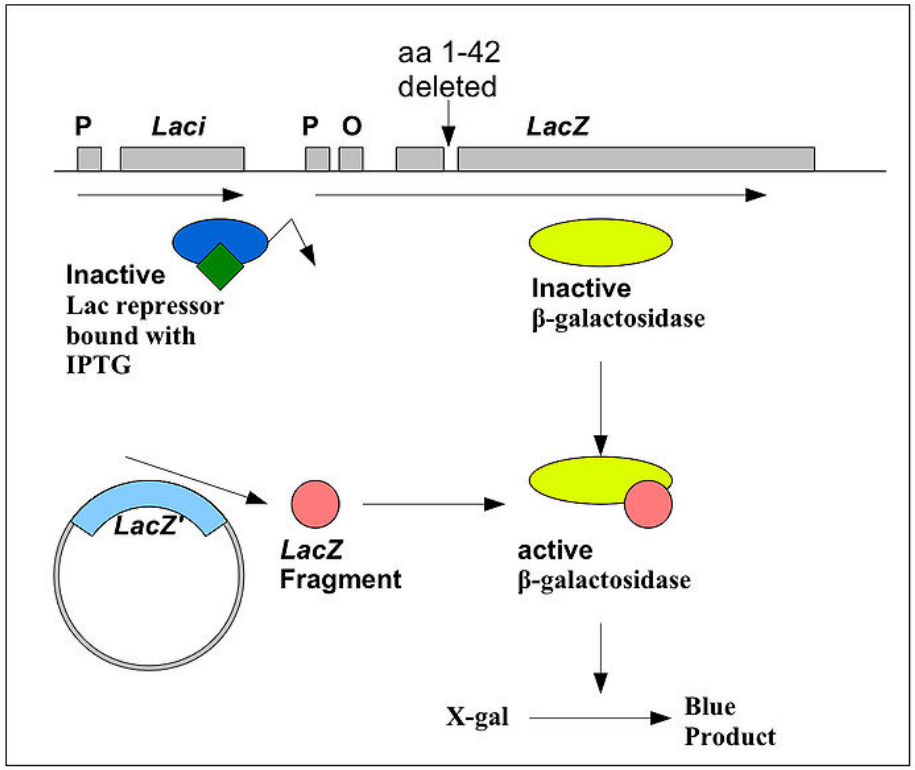
Clonazione del DNA: Blue/white screen

MA 22-05-2012







http://en.wikipedia.org/wiki/File:Beta-galactosidase.png

http://en.wikipedia.org/wiki/Blue_white_screen

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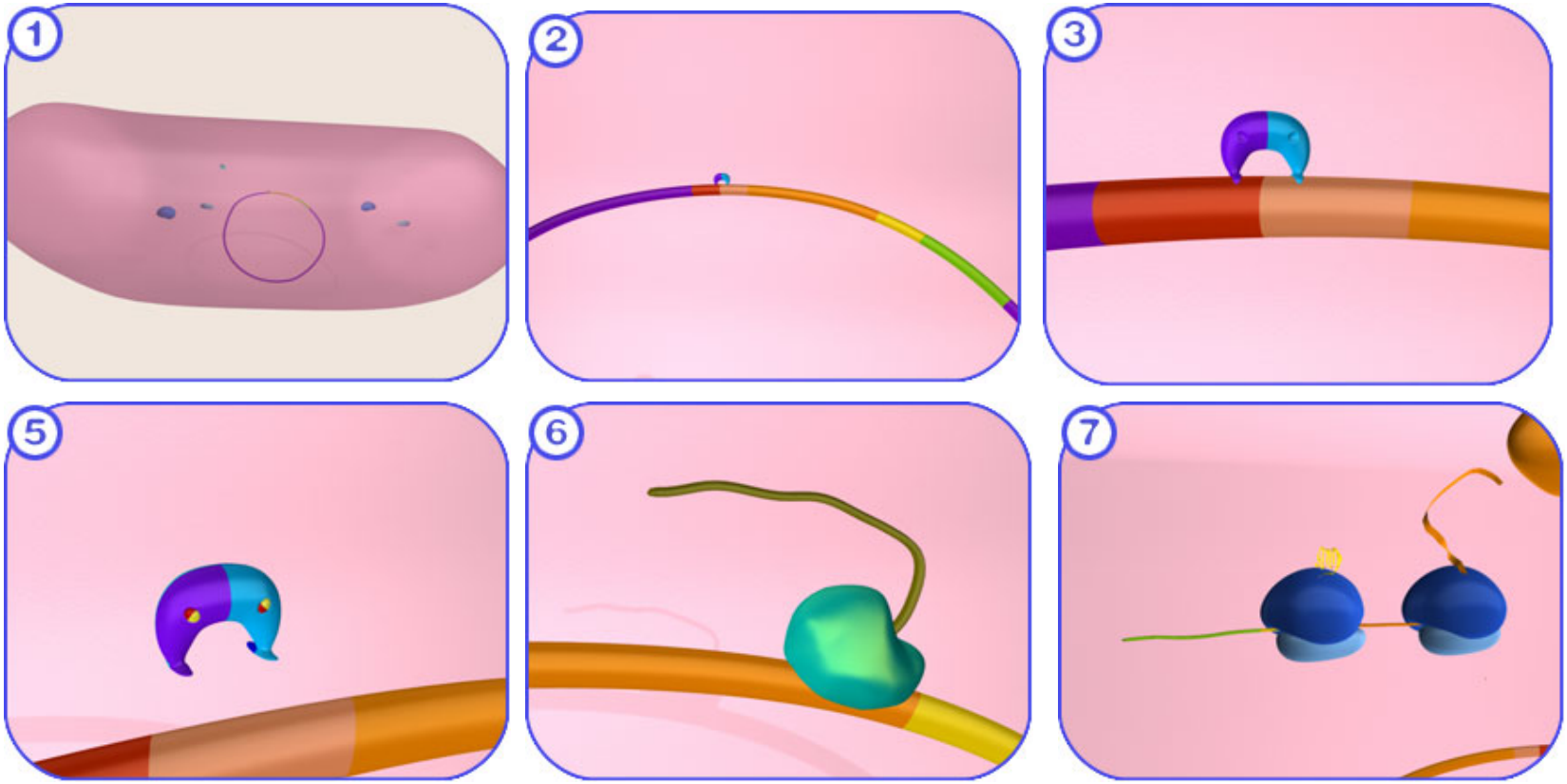
L'operone *lac* visto attraverso un video



Virtual Cell Animation Collection
Molecular and Cellular Biology Learning Center

http://vcell.ndsu.nodak.edu/animations/lacOperon/index.htm

http://en.wikipedia.org/wiki/Lac_operon

http://www.youtube.com/watch?v=oBwtxdI1zvk

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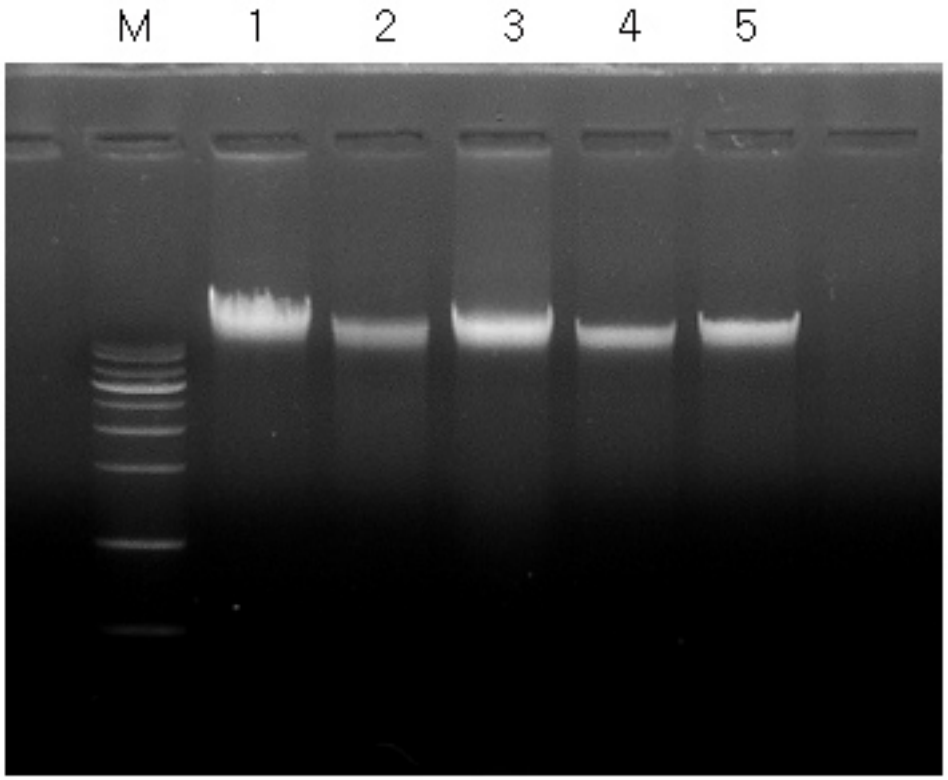
Elettroforesi su gel di agarosio di DNA genomico



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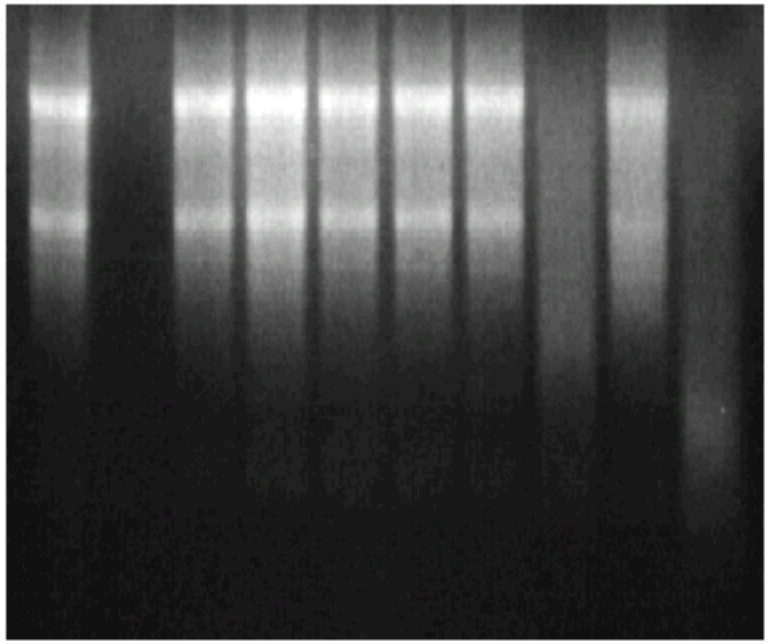
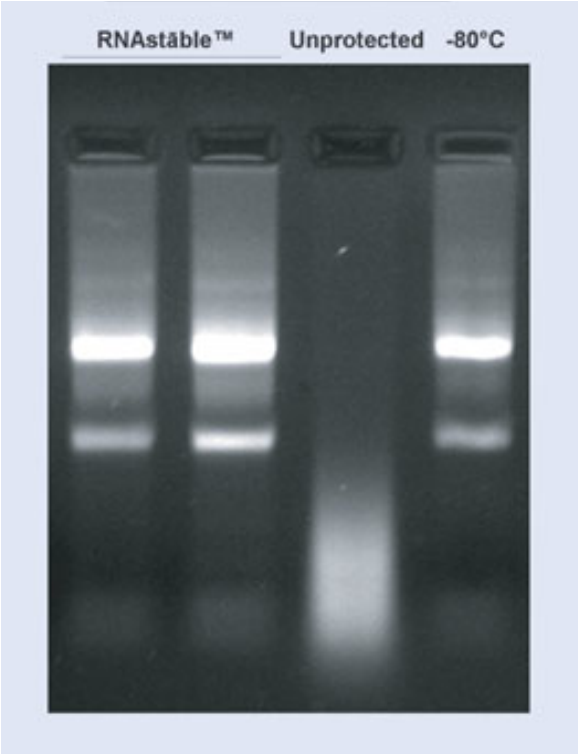
http://m-biotech.co.kr/bbs/images/genomic_DNA01.jpg

Elettroforesi su gel di agarosio di RNA totale



http://www.purebiotechllc.com/images/mbpd/rna/rnaseoff_fig1.GIF



http://www.genengnews.com/articles/chtitem_print.aspx?tid=2241&chid=0

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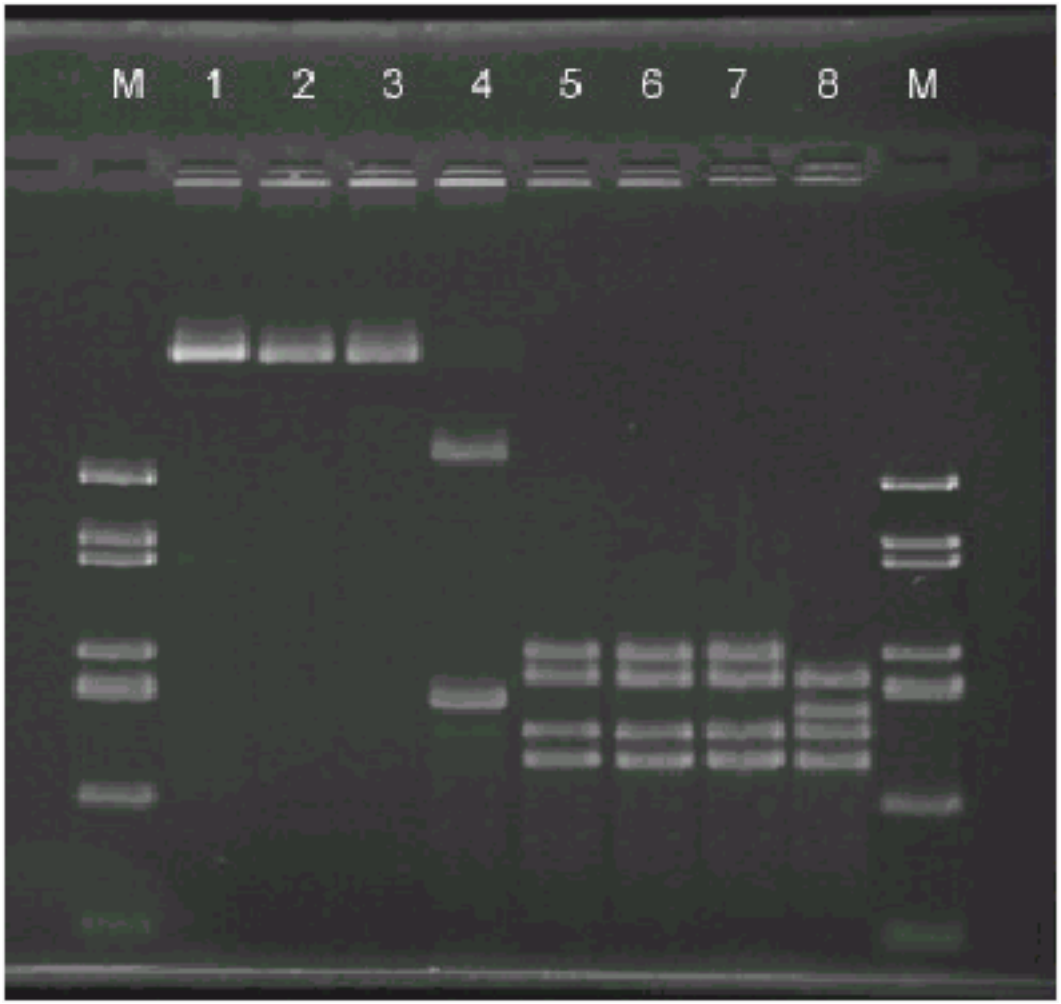
Gel di agarosio con frammenti di DNA



http://www.antibiotic.ru/en/mbio/pub/images/p233r2.gif

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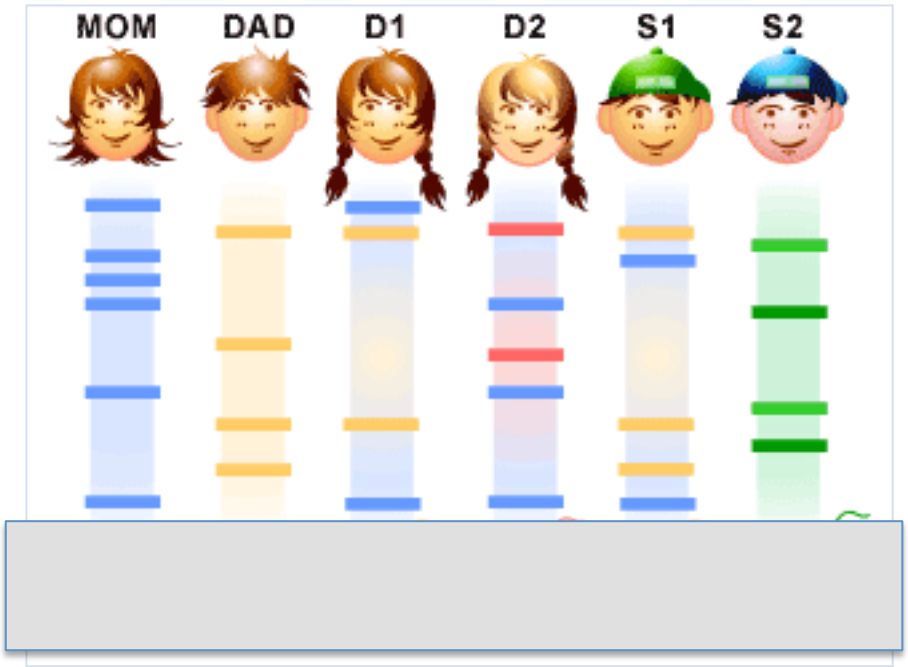
The Science Creative Quarterly

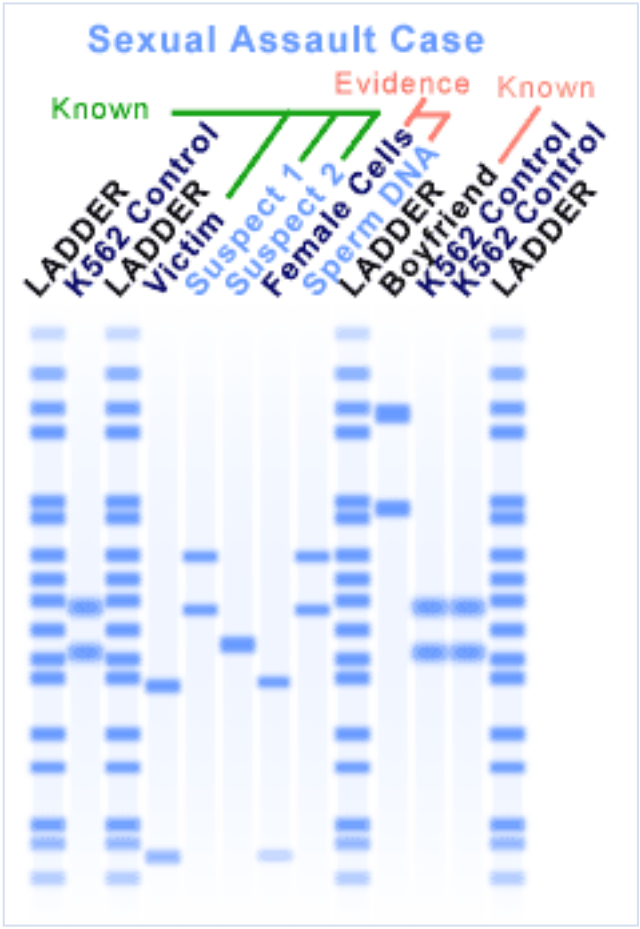
in which, with respect to our name, we are as confused as you are.

.....-05-2010

A BRIEF TOUR OF DNA FINGERPRINTING

By Christine Antler





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http://www.scq.ubc.ca/a-brief-tour-of-dna-fingerprinting/

Metodi di analisi per ibridazione di acidi nucleici

ME 11-05-2011

- Southern blot
- Northern blot
- Dot blot
- Colony hybridization
- Microarray

Metodi di analisi per proteine

- Western blot (utilizza anticorpi)
- Elettroforesi bidimensionale
- ELISA (anche per metaboliti non proteici)
- Spettrometria di massa (MS)

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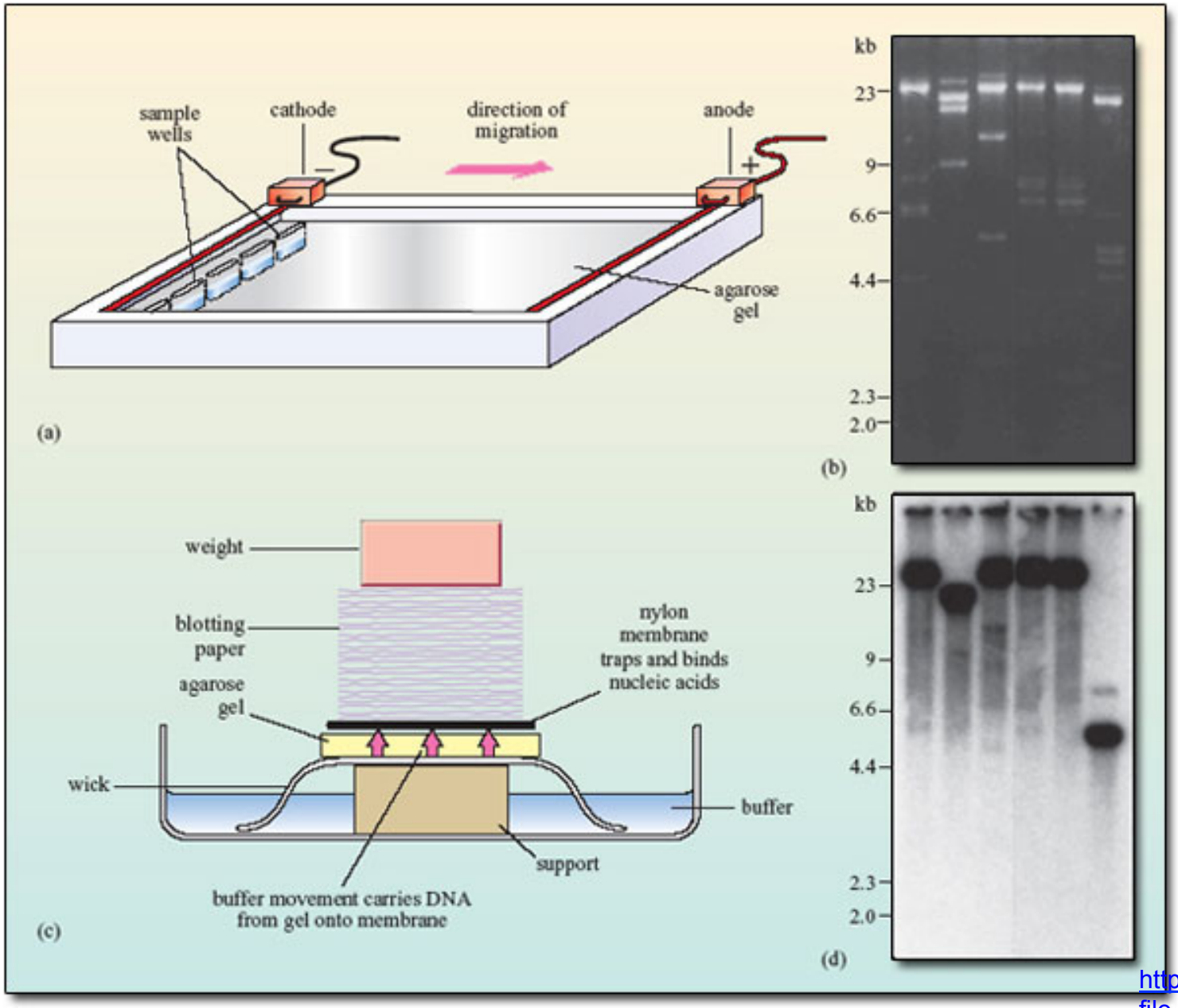
Ibridazione molecolare di acidi nucleici

LU 09-05-2012



http://openlearn.open.ac.uk/file.php/2645/S377_1_007i.jpg

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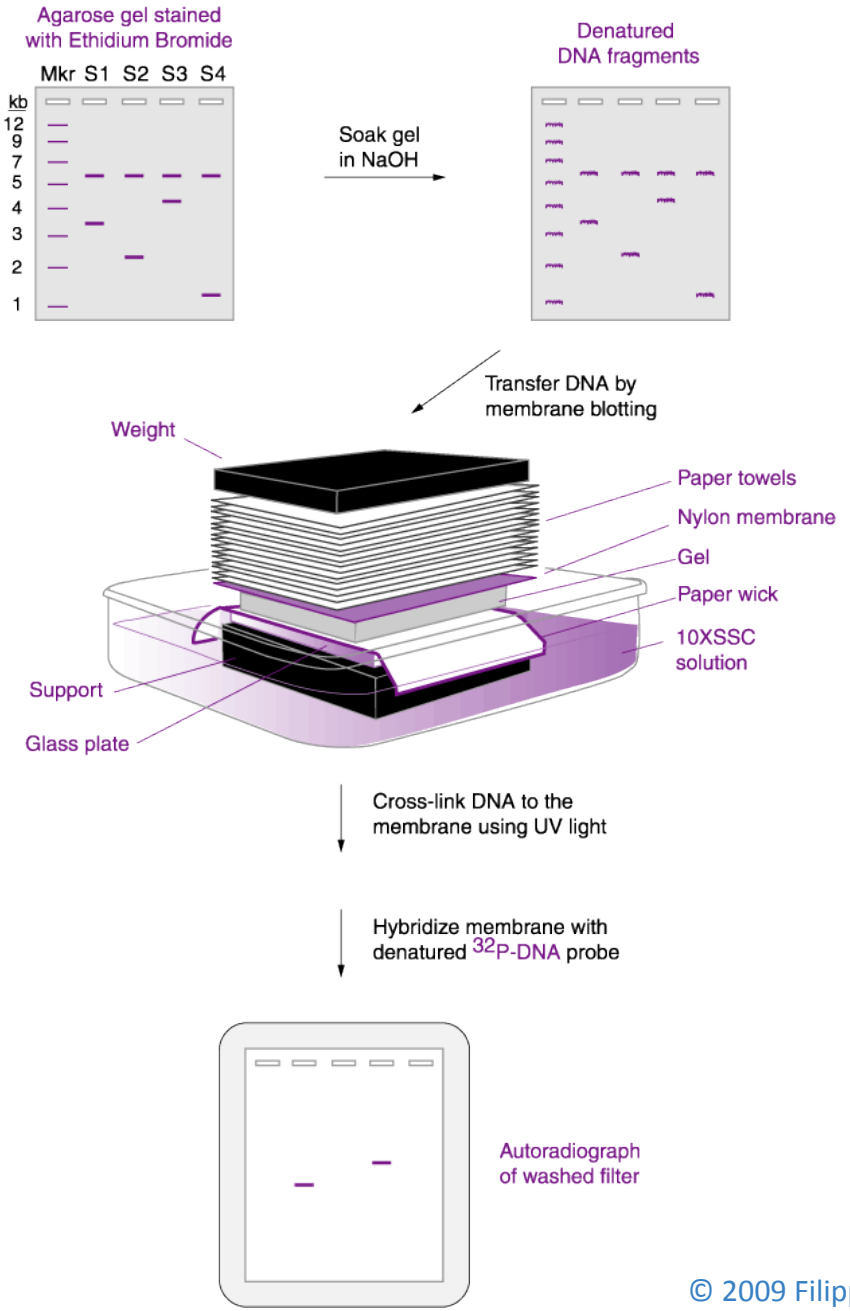
Ibridazione molecolare di acidi nucleici (Southern Blot)

LU 20-04-2010



http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture2/Lecture2.html

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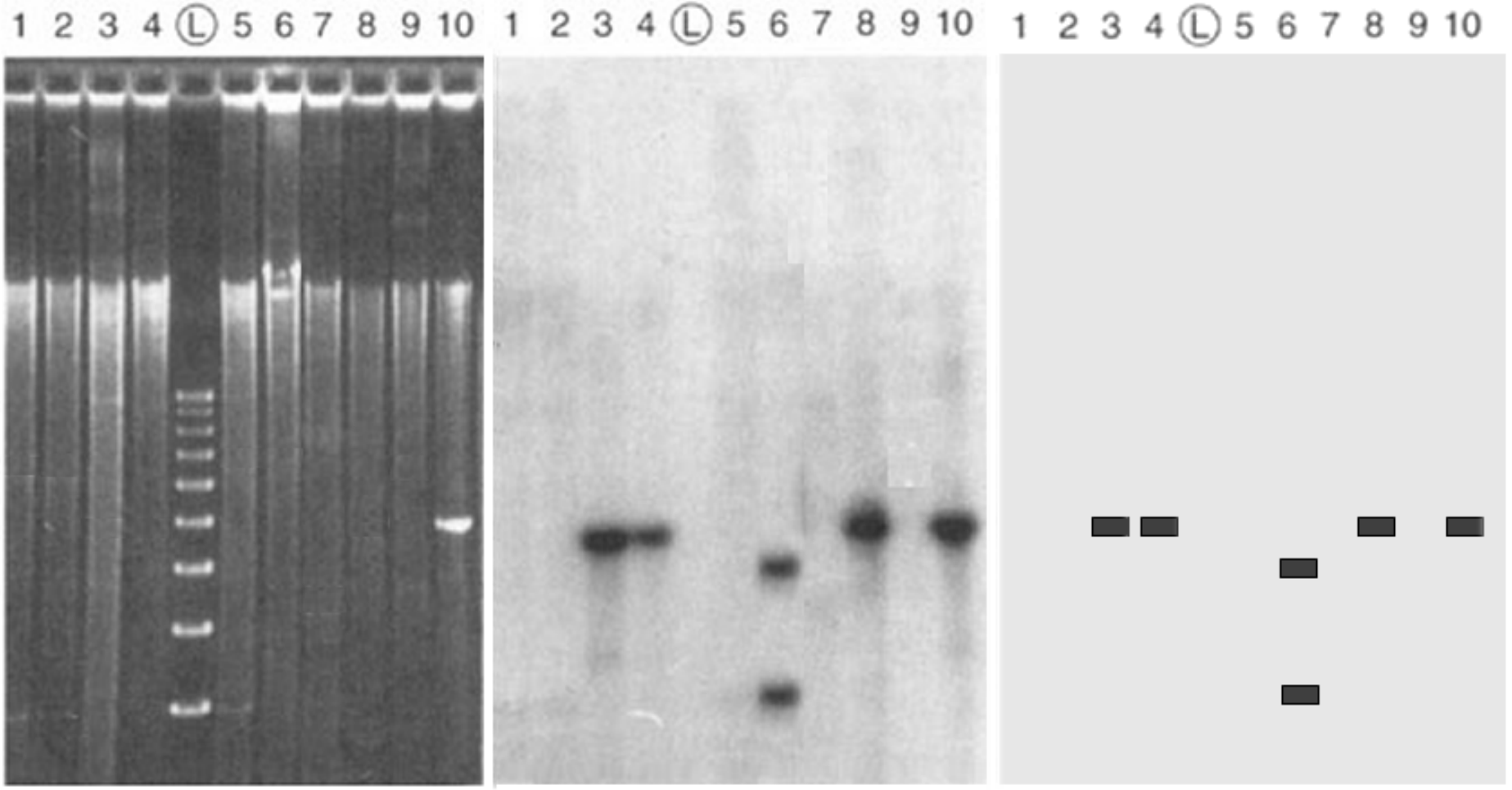
Ibridazione molecolare di acidi nucleici (Southern Blot)

LU 28-05-2012



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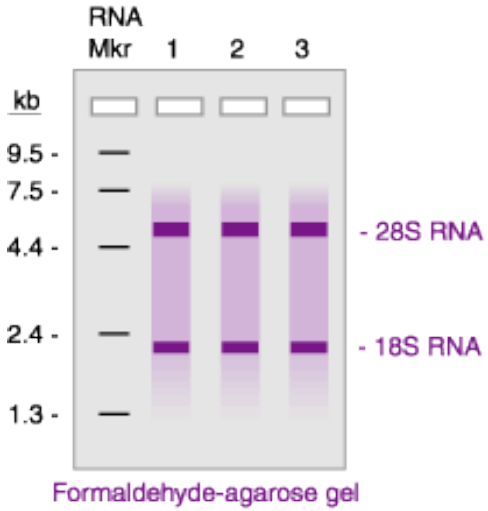
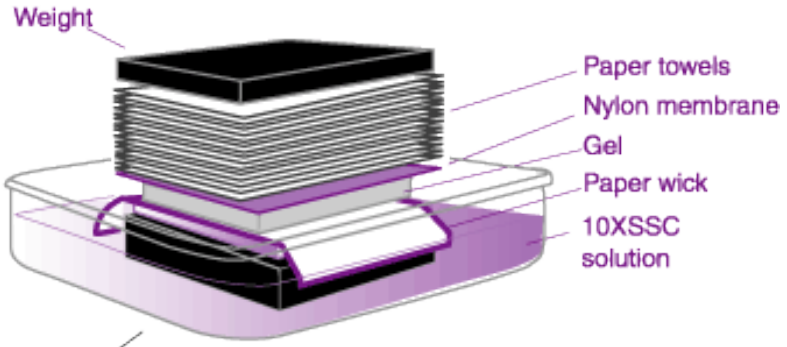
http://www.mun.ca/biology/scarr/Southern_Blot_analysis.html

La tecnica *Northern blot*

MA 06-05-2014

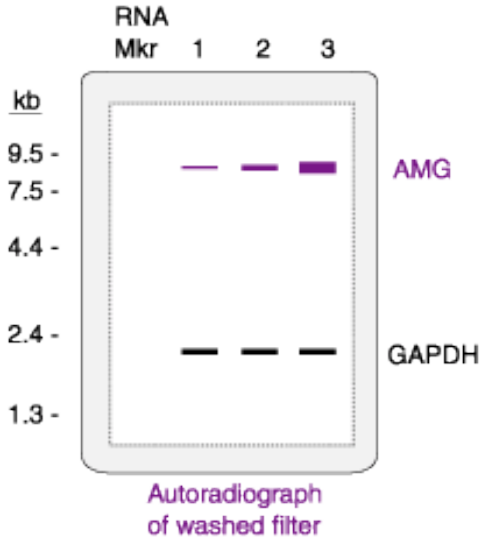


Transfer RNA by membrane blotting



Cross-link RNA to the membrane using UV light

Hybridize membrane with denatured ^{32}P-cDNA probe



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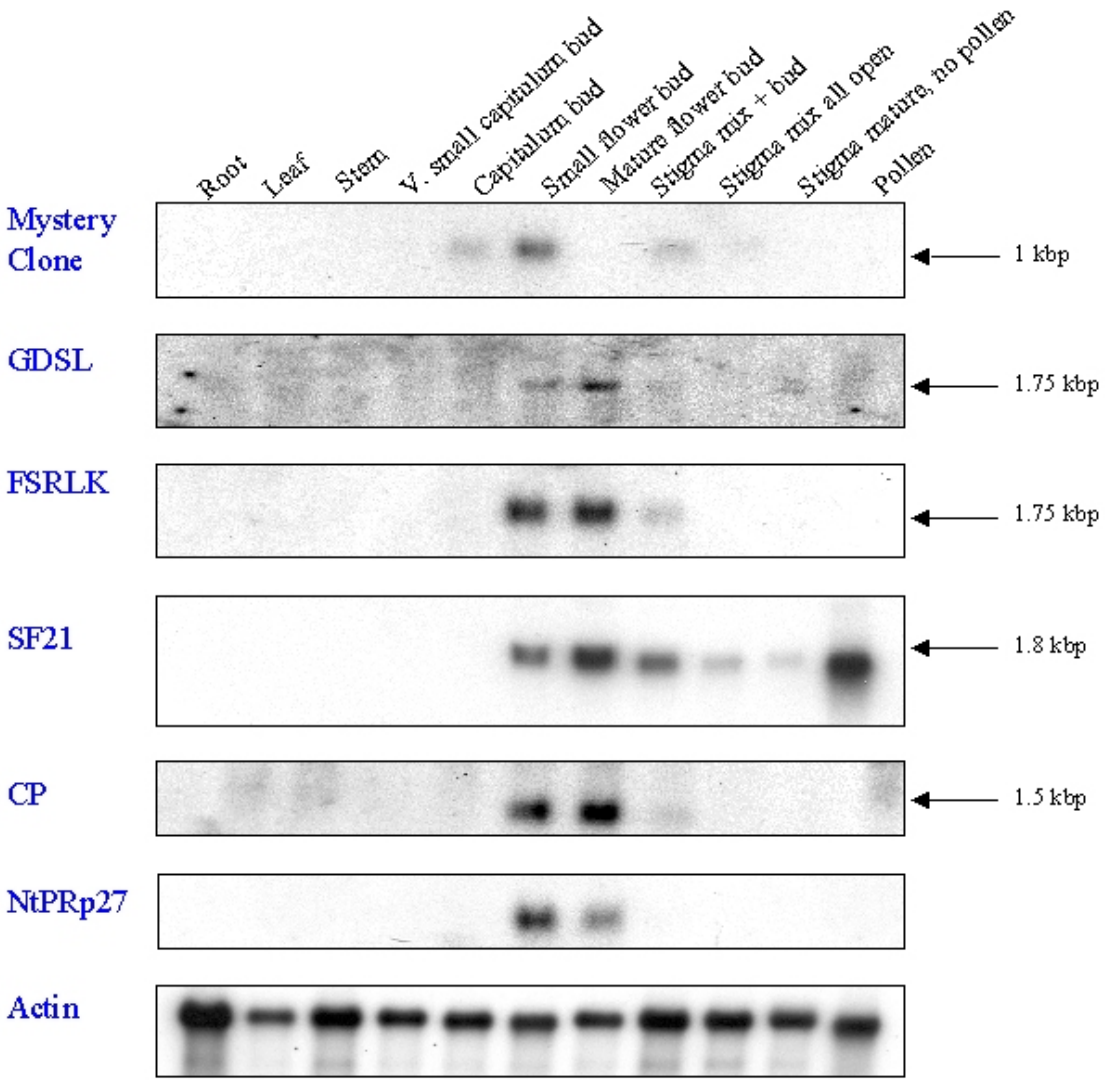
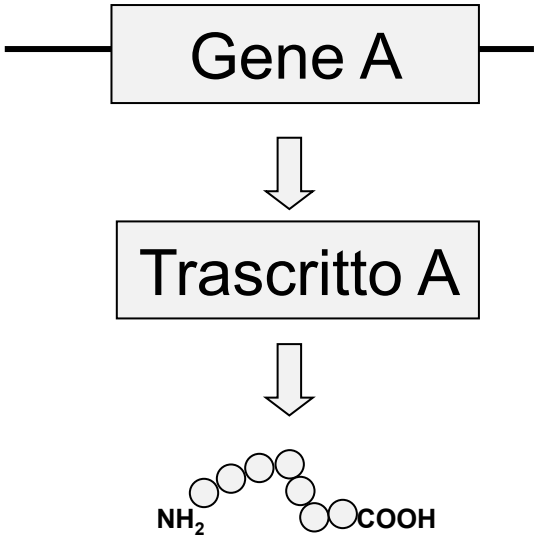
http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture2/Lecture2.html

La tecnica *Northern blot*





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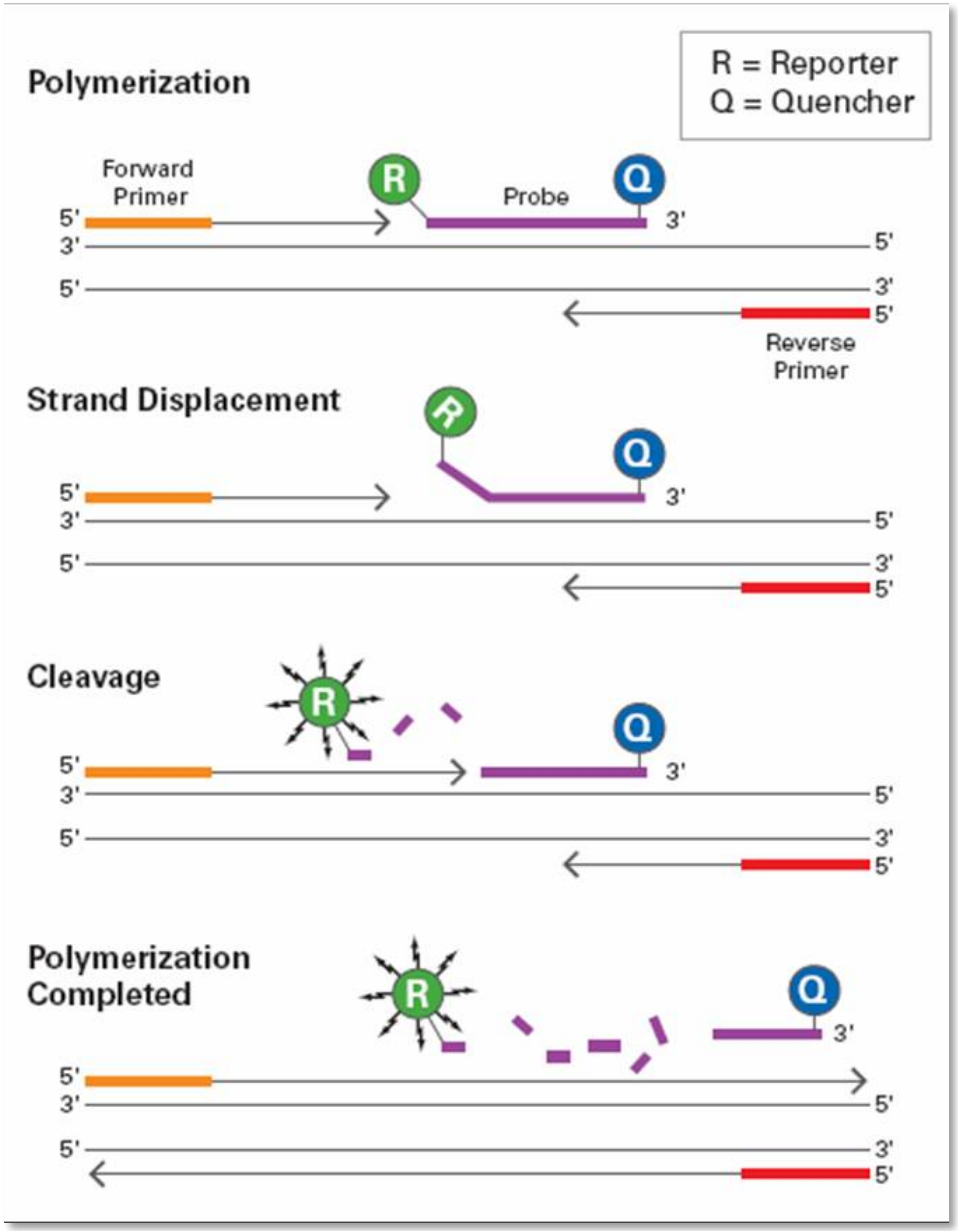
http://www.bio.bris.ac.uk/research/plantrepro/images/northerns.jpg

Real-time PCR (metodica *TaqMan*®)



http://www.asuragen.com/images/TaqMan%20GX%20cartoon.jpg

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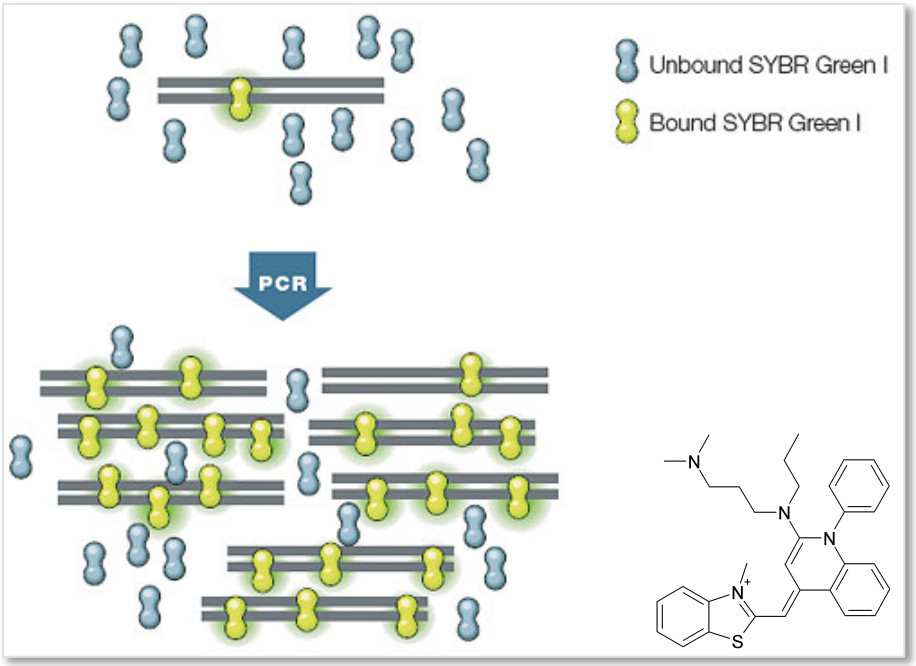
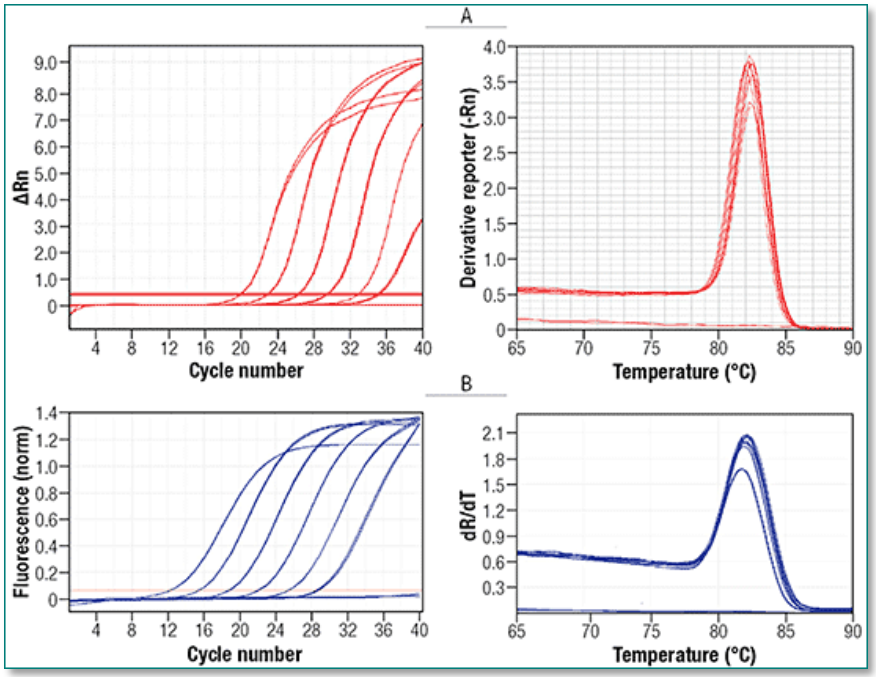
Real-time PCR (metodica SYBR Green)

MA 29-05-2012



http://www3.bio-rad.com/gexp/images/support/amp_central/ac307_1.jpg



http://www.fermentas.com/templates/files/tiny_mce/subsection_images/k025_1.gif

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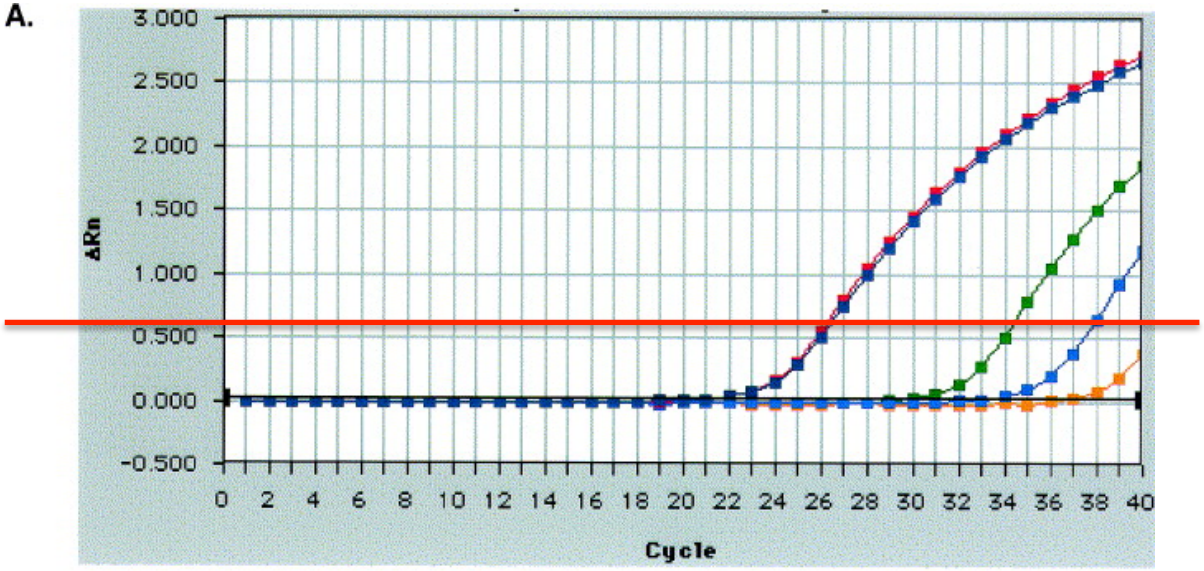
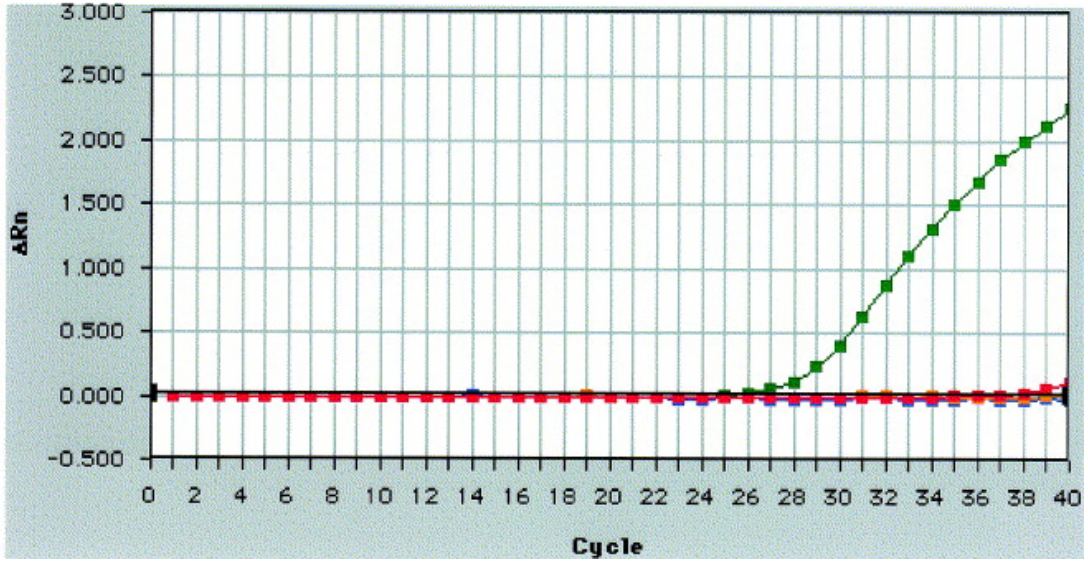
Real-time PCR (profili)

A.



B.



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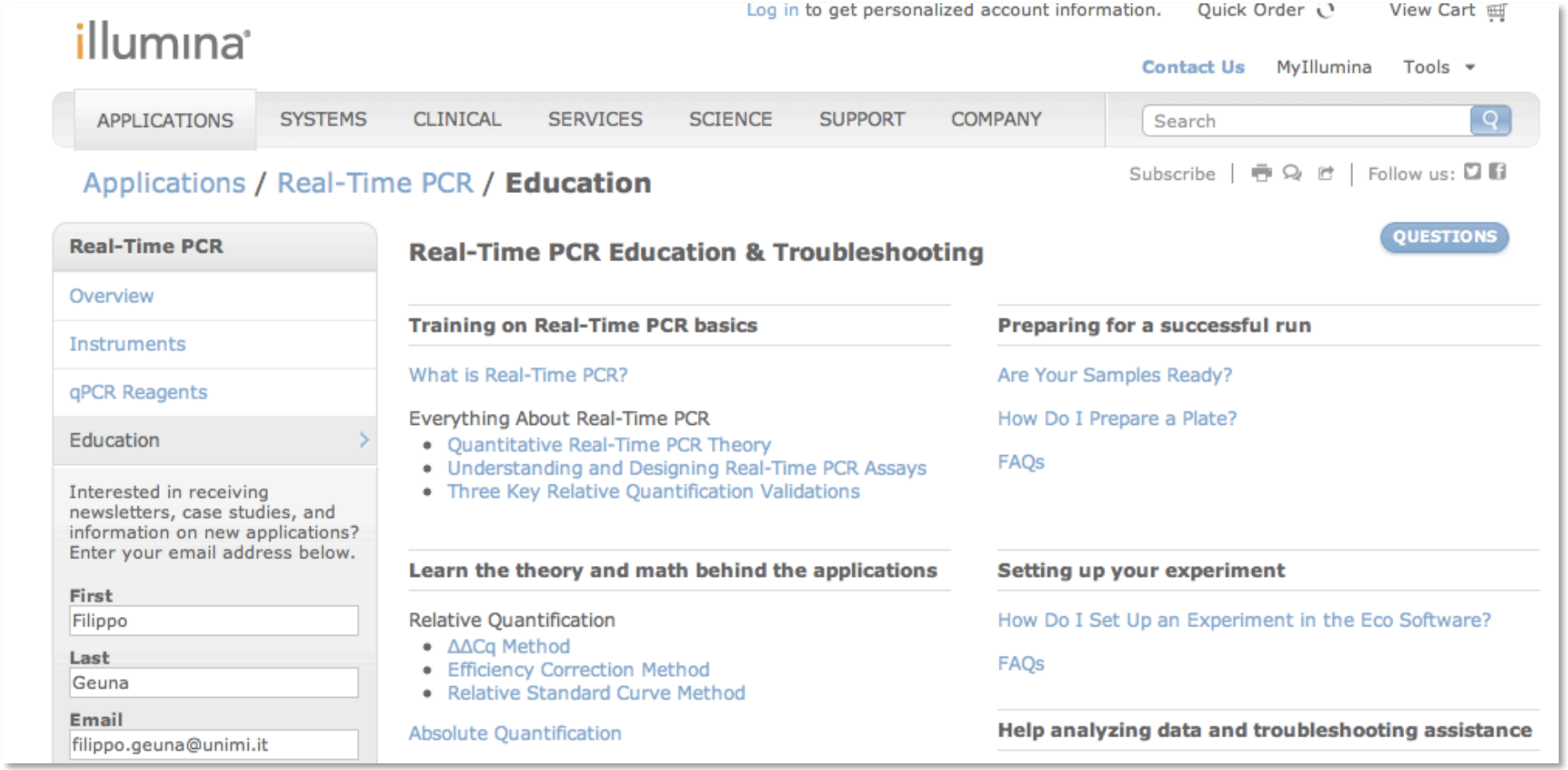
http://ars.els-cdn.com/content/image/1-s2.0-S0027510799001475-gr3ab.jpg

Real-time PCR - Illumina® Education

LU .....-05-2013



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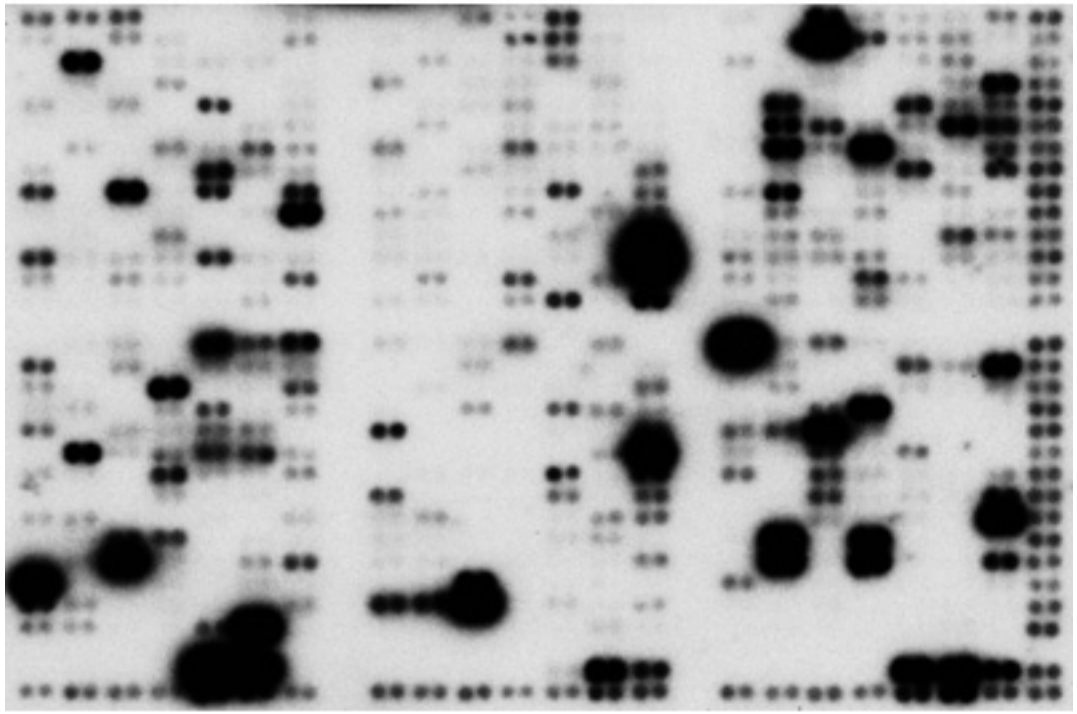
http://www.illumina.com/applications/real-time-pcr/qpcr-education.ilmn

La tecnica *Dot blot*



http://www.clontech.com/products/detail.asp?tabno=2&product_id=10472

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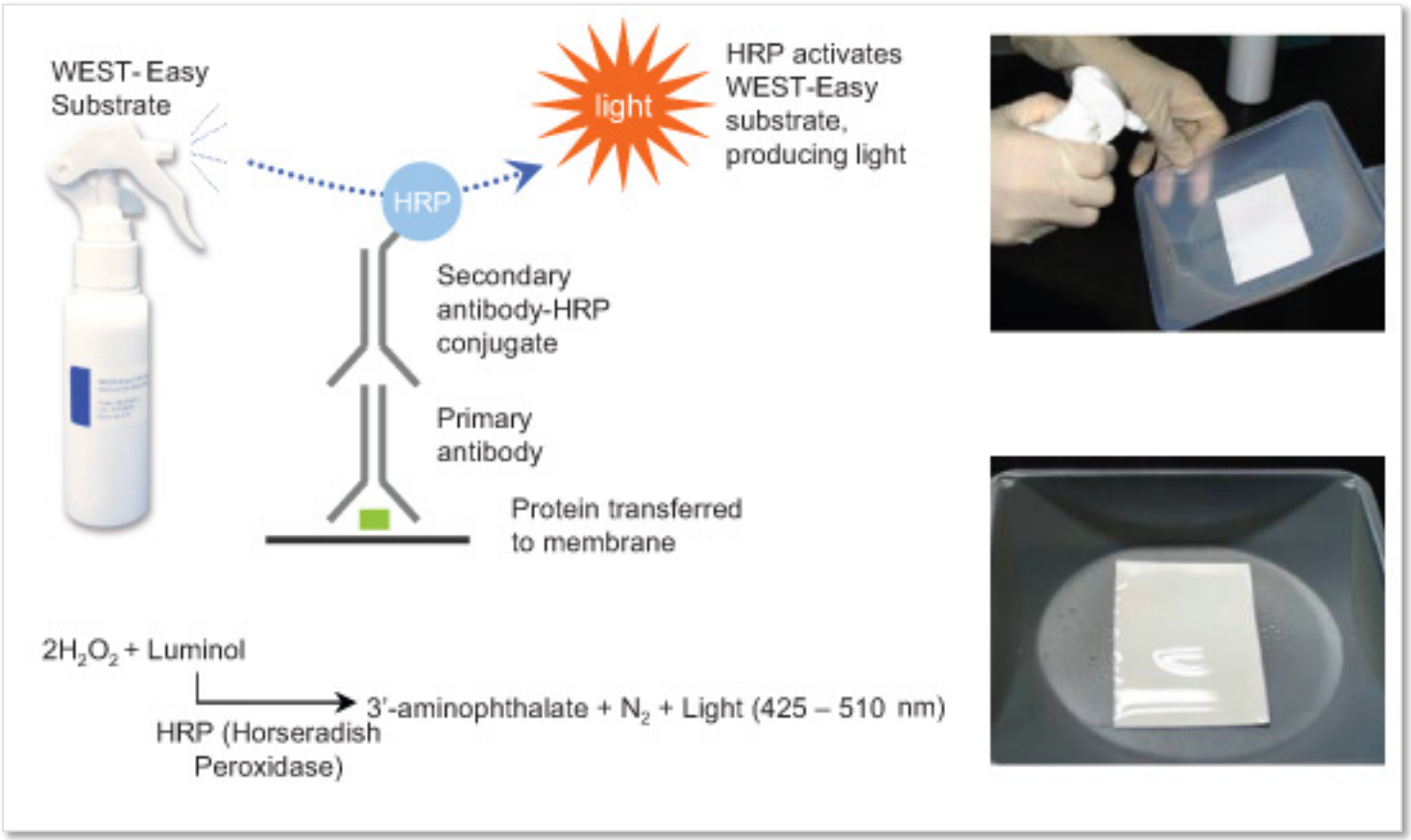
La tecnica *Western blot*

http://en.wikipedia.org/wiki/Horseradish_peroxidase



http://www.nacalaiusa.com/images/products/west_easy001.jpg

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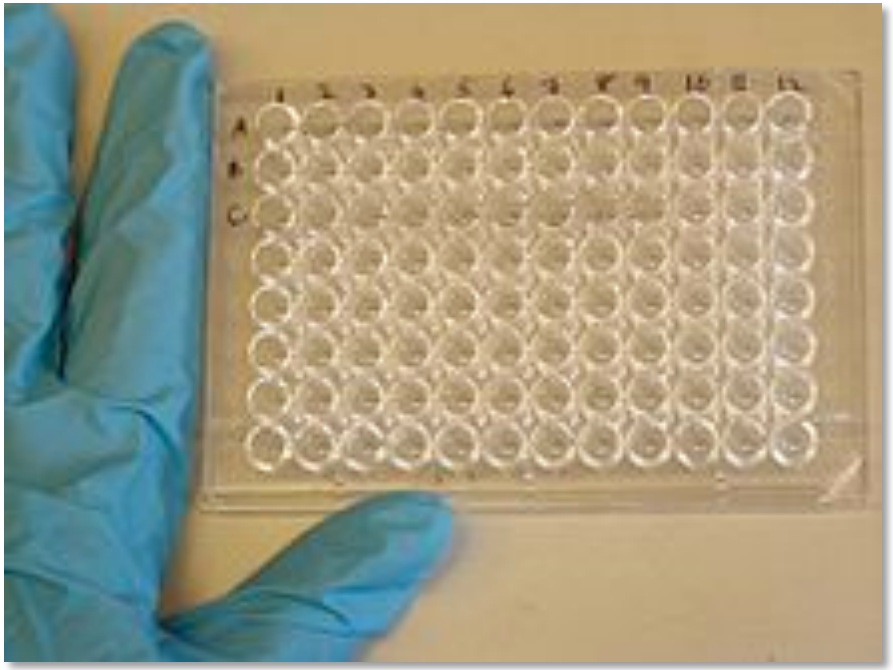
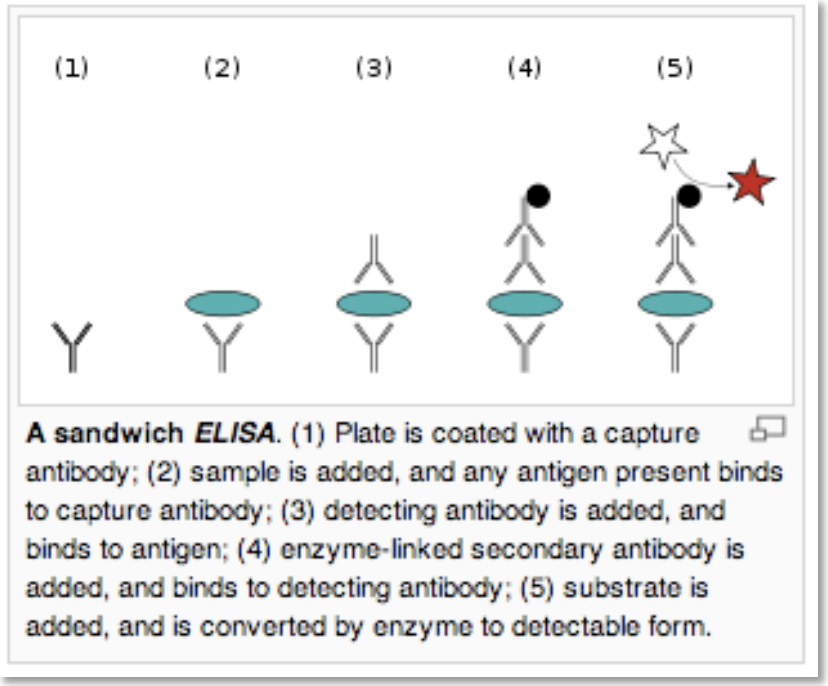
La tecnica *ELISA*

MA 06-05-2014





A sandwich *ELISA*. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

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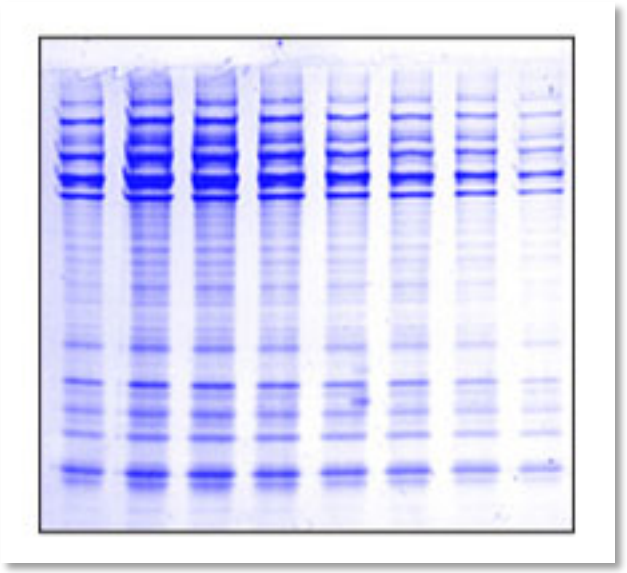
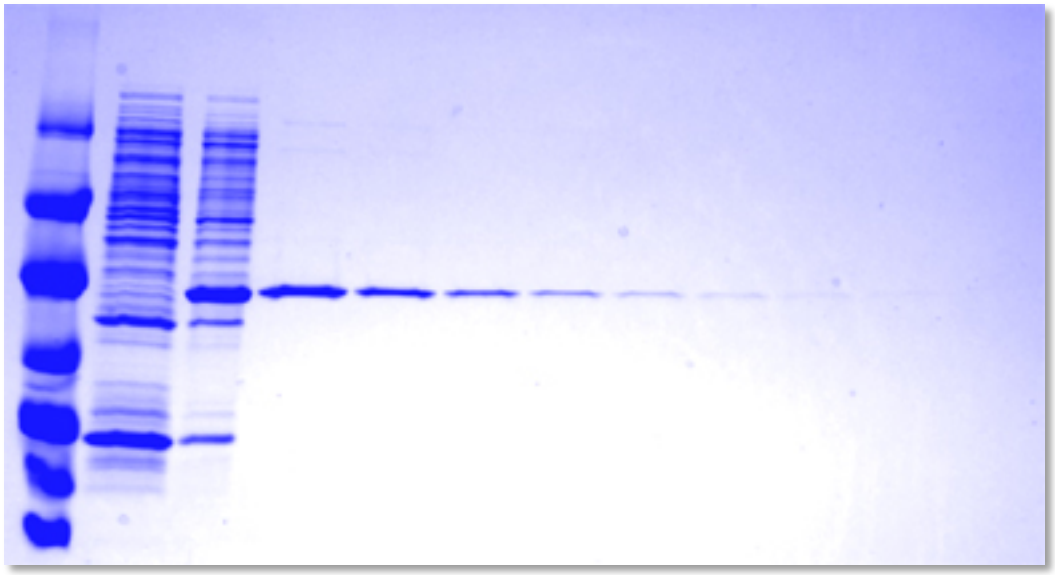
http://en.wikipedia.org/wiki/Enzyme_immunoassay

Gel di proteine colorato con *Coomassie blue*



http://www.uvp.com/images/CoomassieBlue.jpg



http://www.nibsc.ac.uk/images/coomassie14-3-3.jpg

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Western blot di proteine

http://www.kpl.com/catalog/productdetail.cfm?Catalog_ID=17&Category_ID=419&Product_ID=514

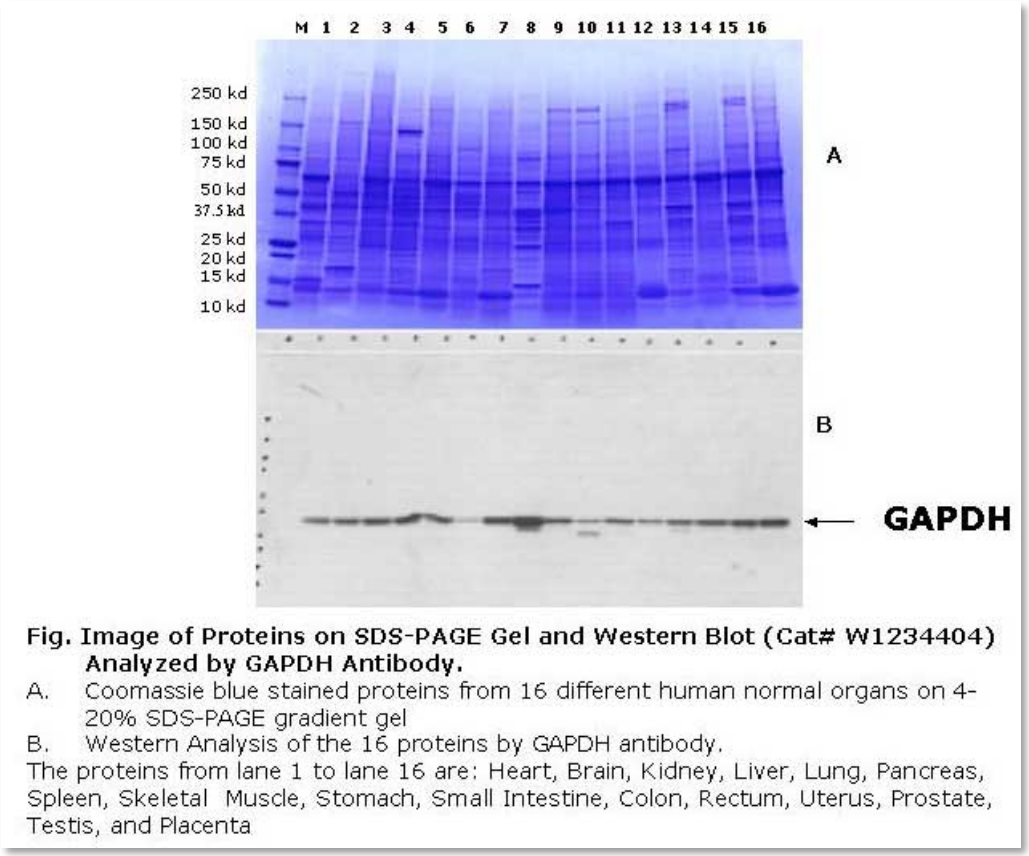

Fig. Image of Proteins on SDS-PAGE Gel and Western Blot (Cat# W1234404) Analyzed by GAPDH Antibody.

A. Coomassie blue stained proteins from 16 different human normal organs on 4-20% SDS-PAGE gradient gel

B. Western Analysis of the 16 proteins by GAPDH antibody.

The proteins from lane 1 to lane 16 are: Heart, Brain, Kidney, Liver, Lung, Pancreas, Spleen, Skeletal Muscle, Stomach, Small Intestine, Colon, Rectum, Uterus, Prostate, Testis, and Placenta

http://bricker.tcnj.edu/tech/WesternBlotImage.jpg

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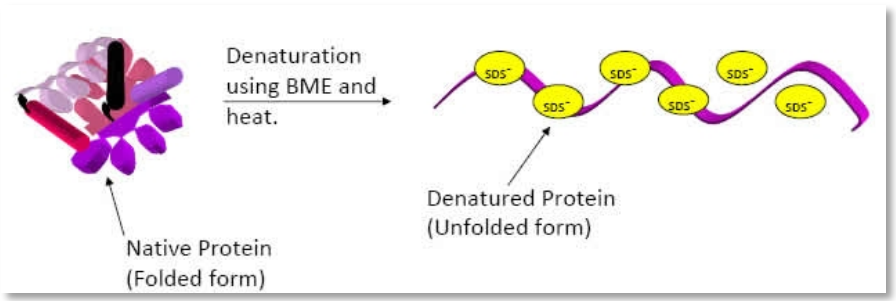
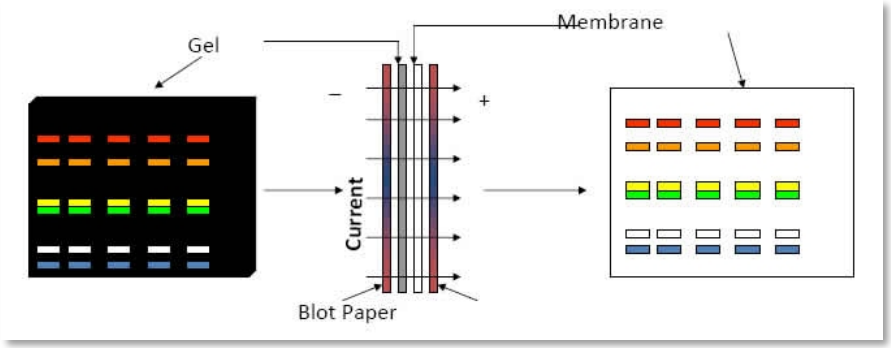
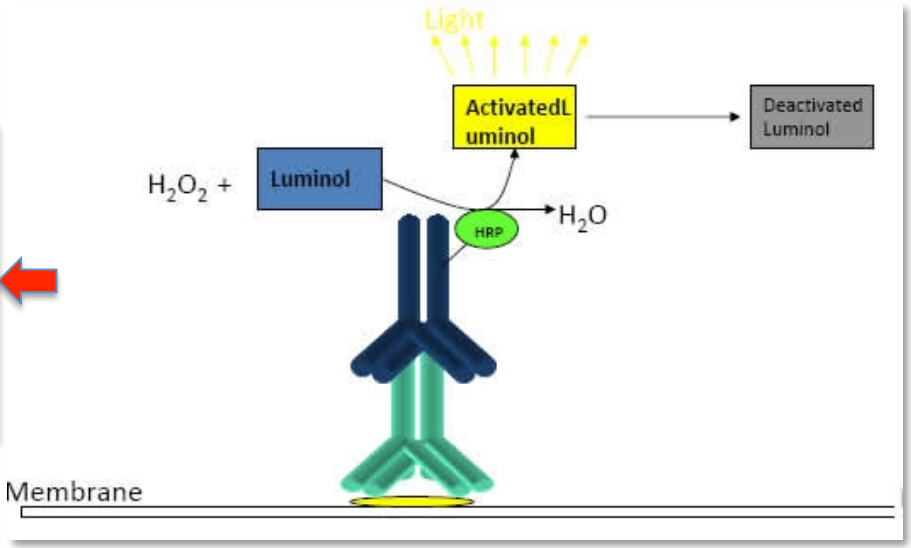
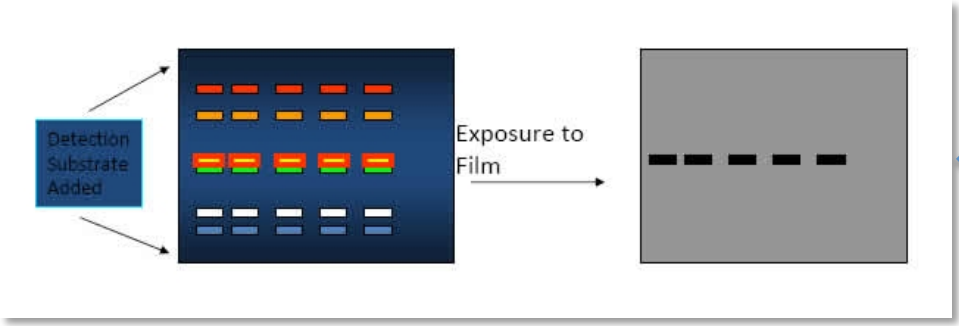
Western blot di proteine (con video)

MA 06-05-2014









Video!!!

http://www.novusbio.com/support/support-by-application/western-blot/video.html

http://www.novusbio.com/support/support-by-application/western-blot/illustrated-assay.html

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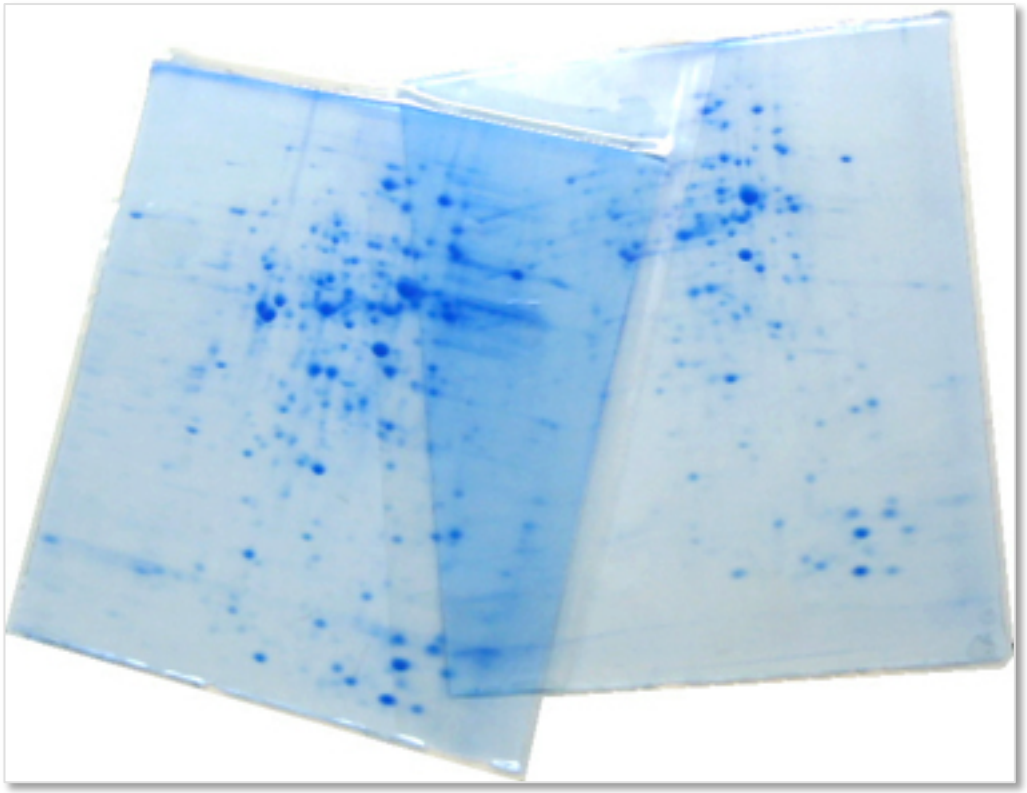
Gel 2D di proteine colorato con *Coomassie blue*

ME 08-05-2013



http://www.uvp.com/images/CoomassieBlue.jpg

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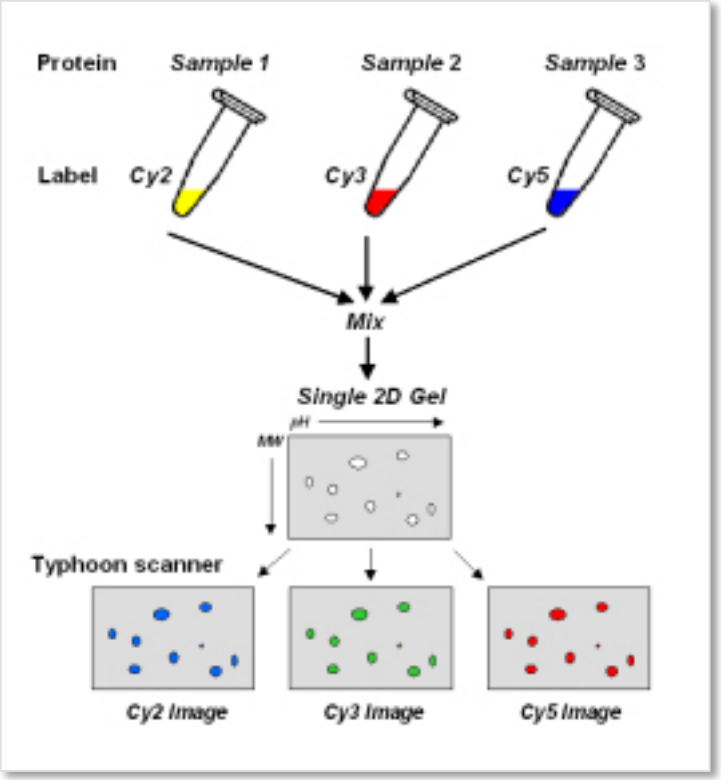
Gel 2D di proteine marcate con *cianine fluorescenti (Cy)*

ME 08-05-2013



http://www.bioscience.ucla.edu/institution/party-image-view?image_id=209240

Difference Gel Electrophoresis (DiGE) is a proteomics technology, which is used to reliably compare protein expression levels between two or more samples using a two-dimensional (2D) gel electrophoresis and mass spectrometry.

DiGE is based on labeling protein samples with fluorescent dyes with different spectral characteristics prior to electrophoresis. Samples obtained from different sources (cell types, time points, etc) are reacted with dyes (Cy2, Cy3, and Cy5), then combined together and analyzed using 2D gel electrophoresis. The protein from different samples running in the same gel will co-register when their fluorescence is visualized at different wavelengths. Such multiplexing reduces the number of gels for analysis, minimizes experimental errors, and allows for quantitative analysis. Differences in protein expression of as low as 20% can be reliably detected using software DeCyder (GE Healthcare) specially designed for DiGE analysis. Proteins of interest (i.e. differentially expressed) are then excised from the gel, processed and identified using mass spec.

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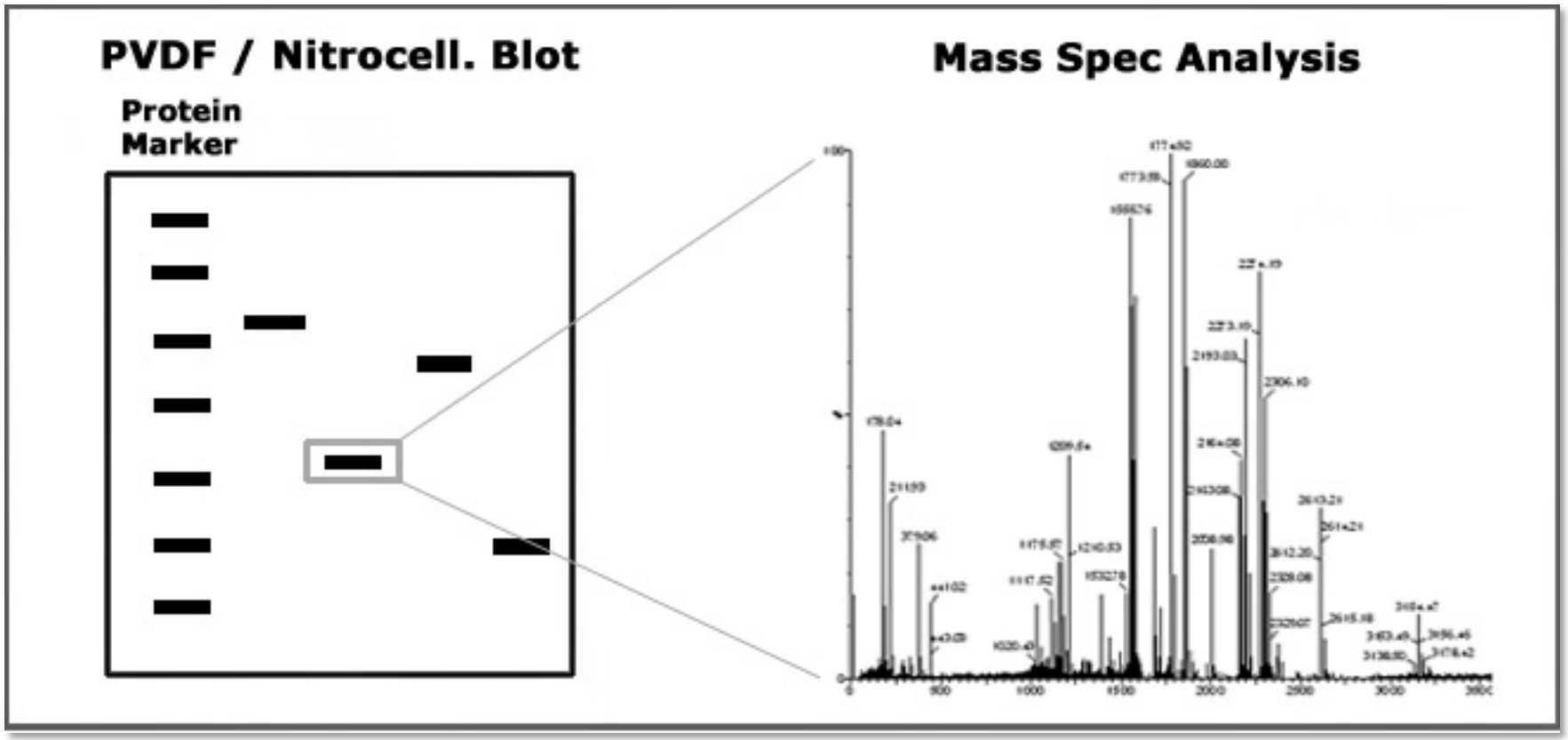
Spettrometria di massa di proteine

ME 08-05-2013



http://signagen.com/index.php?main_page=product_info&products_id=521

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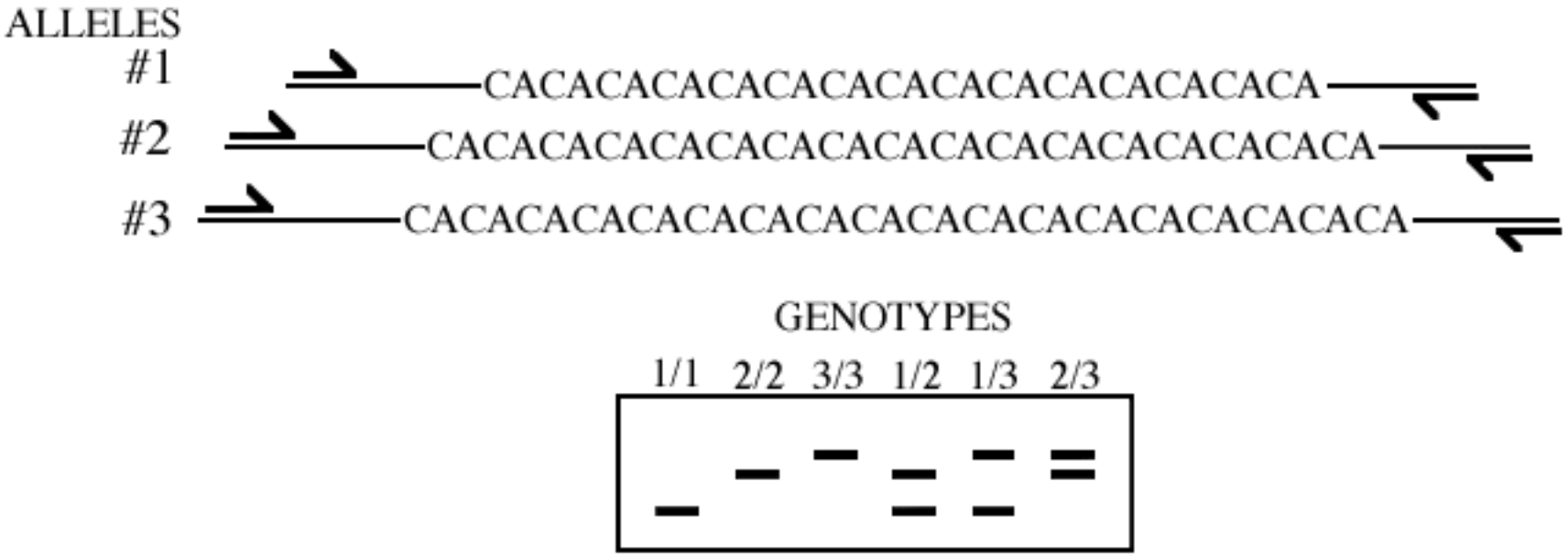
Marcatori microsatelliti (SSR):

© 2009 Filippo Geuna - Università di Milano http://en.wikipedia.org/wiki/Microsatellite

Polimorfismo dei microsatelliti (SSR)

MA 28-05-2013



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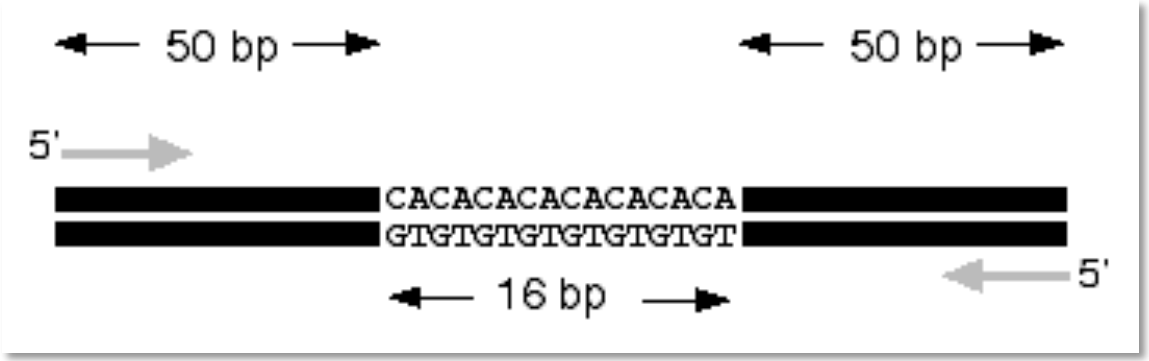
http://www.informatics.jax.org/silverbook/images/figure8-10.gif

Amplificazione PCR di loci microsatelliti

MA 28-05-2013



Detecting microsatellites from genomic DNA. Two PCR primers (forward and reverse gray arrows) are designed to flank the microsatellite region. If there were zero repeats, the PCR product would be 100 bp in length. Therefore, by determining the size of each PCR product (in this case 116 bp), you can calculate how many CA repeats are present in each microsatellite (8 CA repeats in this example).

http://www.bio.davidson.edu/COURSES/genomics/method/microsatellite.html

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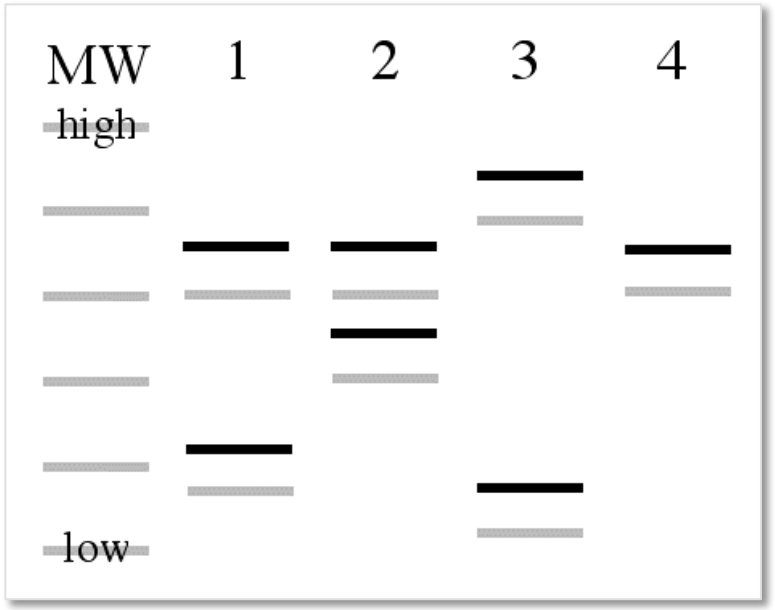
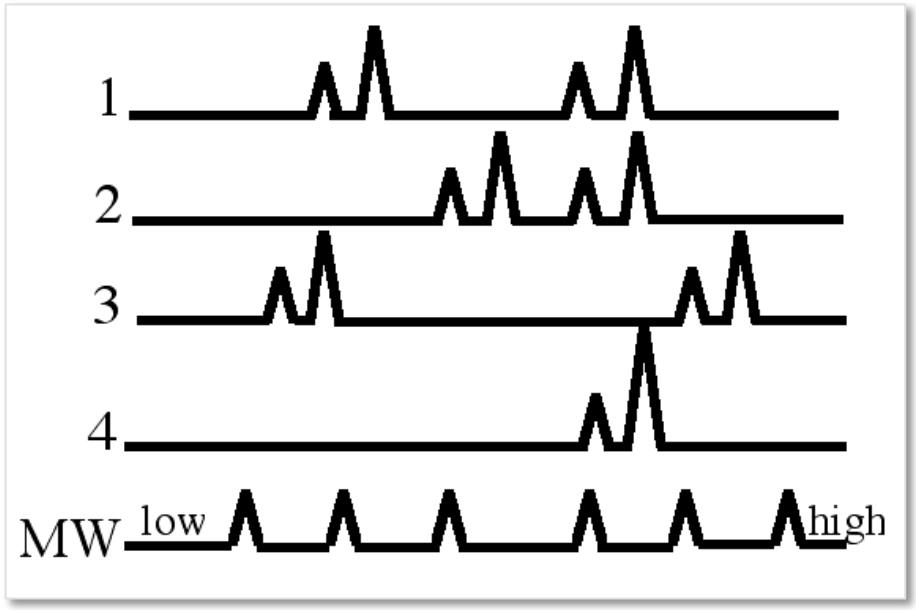
Esempi schematizzati di profili microsatelliti

MA 28-05-2013

Elettroforesi su gel



Elettroforesi capillare



Left half: four sets of data were produced by gel electrophoresis and so you can see the major (black) and stutter (gray) bands. MW; molecular weight standards. **Right half:** These data were produced by analysis on an automated capillary electrophoresis-based DNA sequencer. The data are line graphs with the location of each peak on the X-axis representing a different sized PCR product and the height of each peak indicates the amount of PCR product. The major bands produce higher peaks than the stutter peaks.

http://www.bio.davidson.edu/COURSES/genomics/method/microsatellite.html

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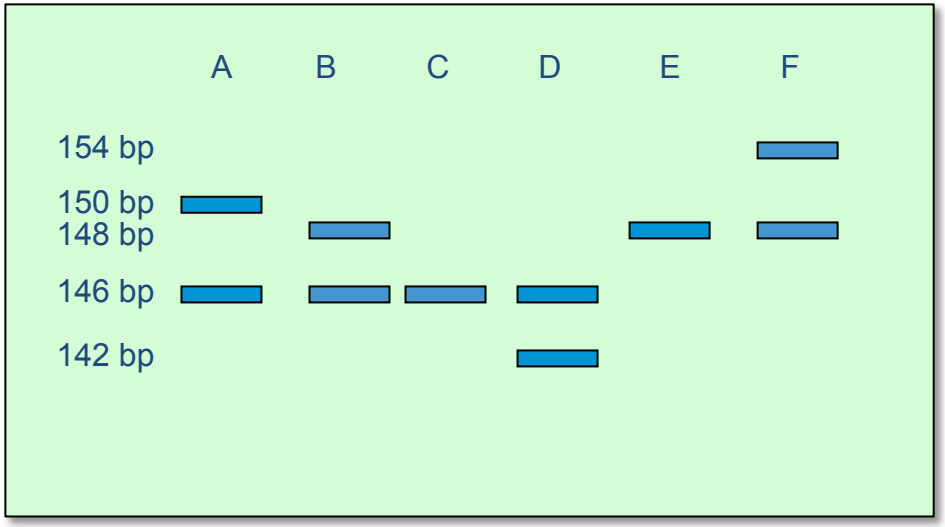
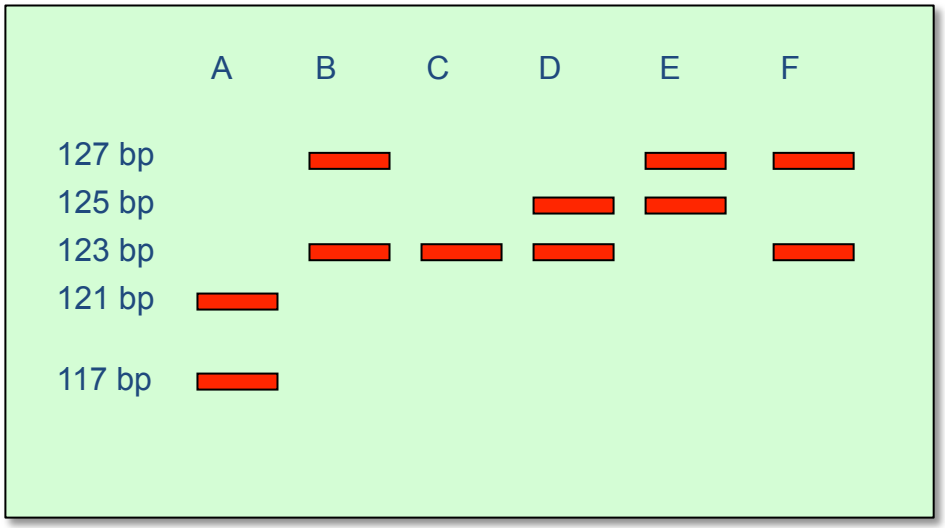
Calcolo della distanza genetica attraverso loci microsatelliti

MA 12-05-2012



	Allele 1	Allele 2
A	150	146
B	148	146
C	146	146
D	146	142
E	148	148
F	154	148



	Allele 1	Allele 2
A	121	117
B	127	123
C	123	123
D	125	123
E	127	125
F	127	123

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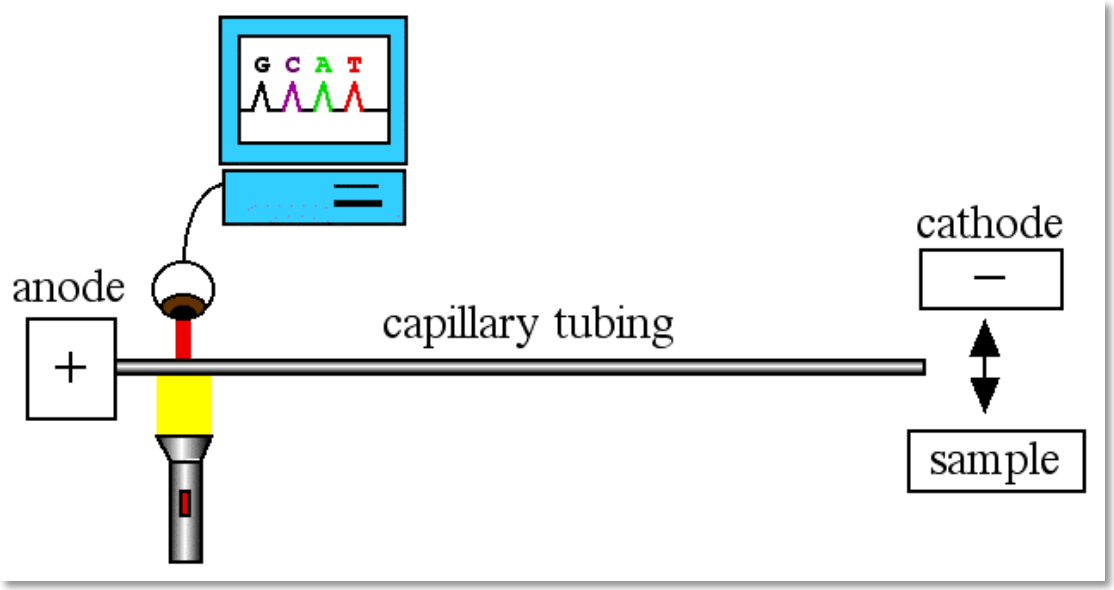
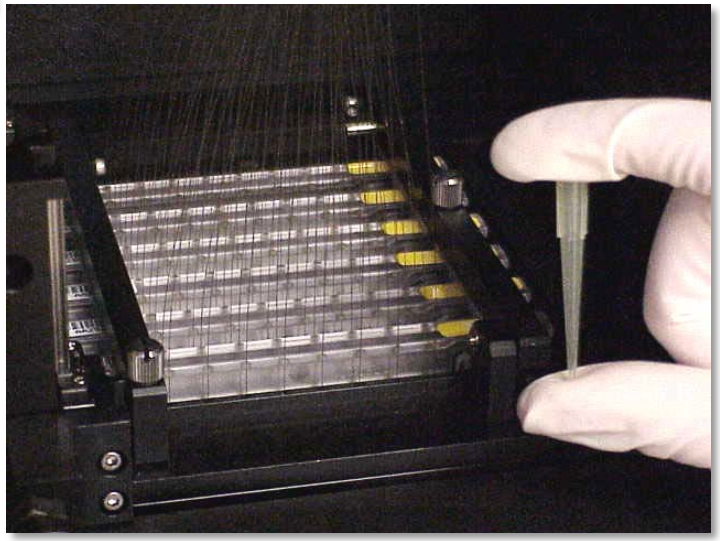
Il principio dell'elettroforesi capillare (CE)

ME 12-05-2010





http://www.bio.davidson.edu/COURSES/genomics/method/Capillary.html

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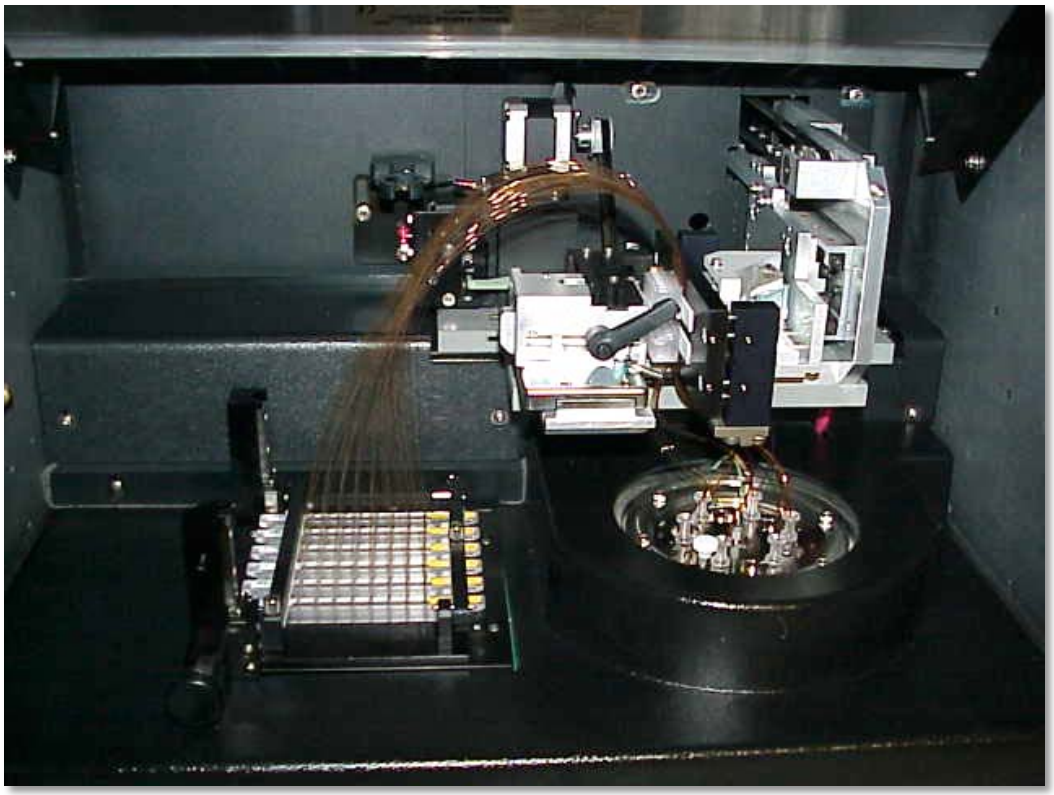
Analisi di marcatori attraverso elettroforesi capillare

ME 12-05-2010

MegaBace™ 96-capillaries genetic analyzer



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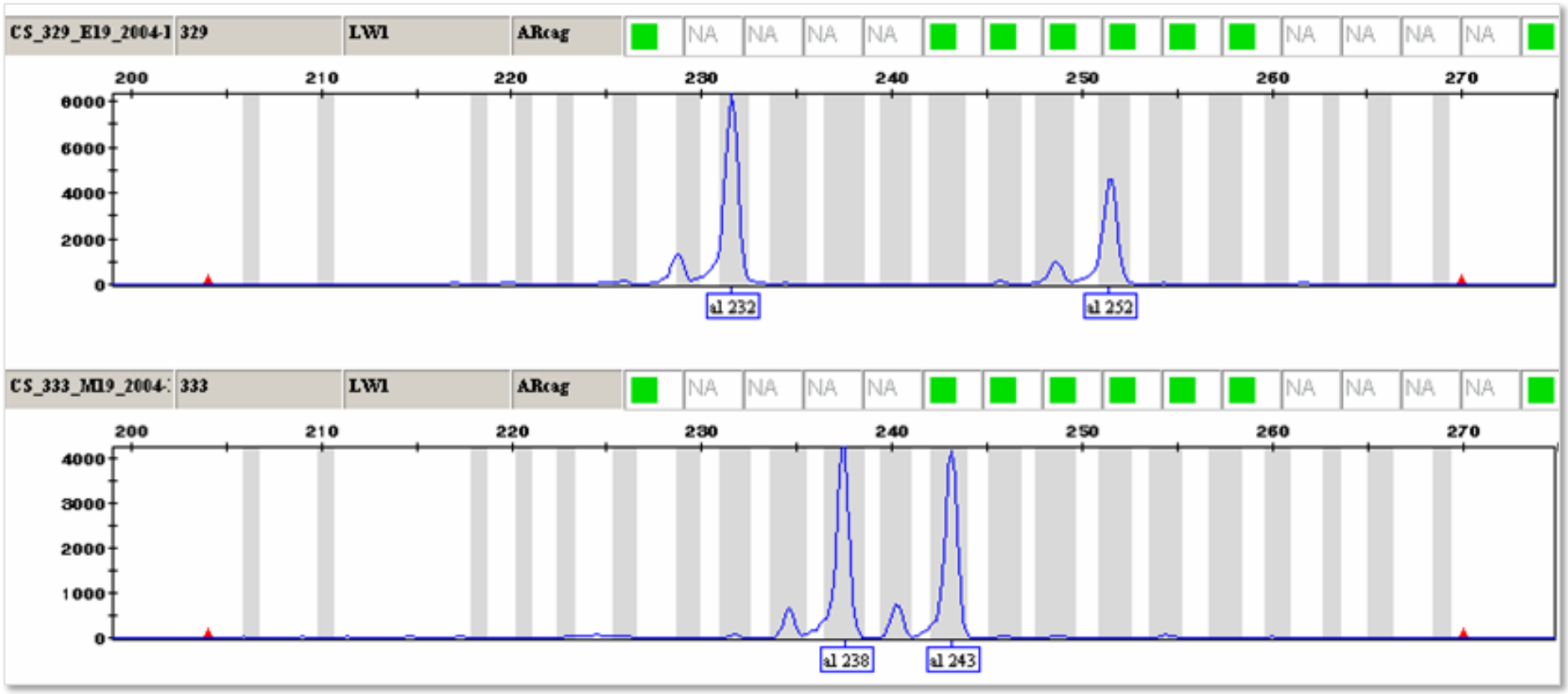
Profilo elettroforetico (capillare) di microsatelliti

LU 28-05-2012



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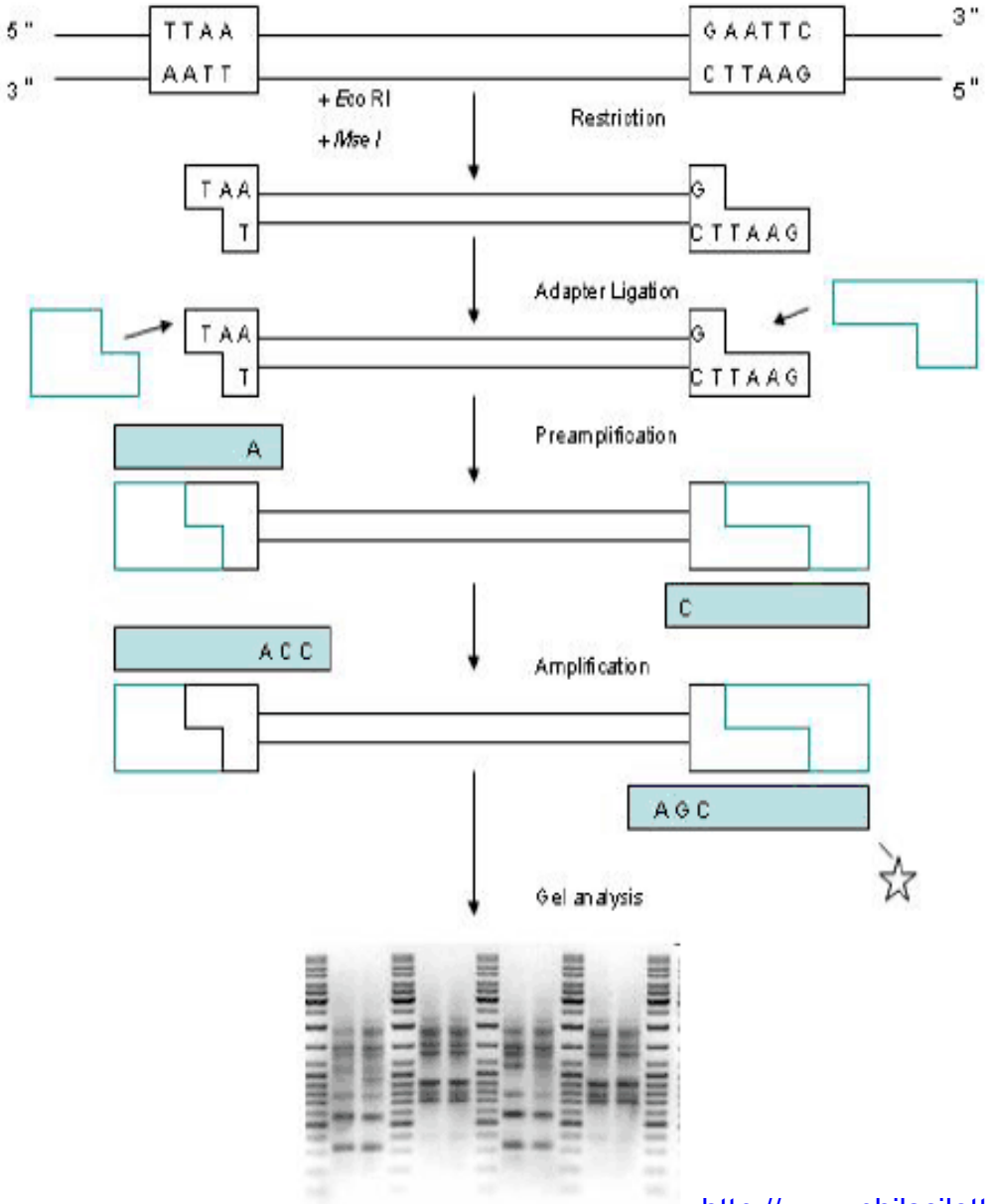
http://www.cf.gu.se/digitalAssets/791/791499_Microsatellite_result.png

Amplified Fragment Length Polymorphism (AFLP)

ME 22-05-2013



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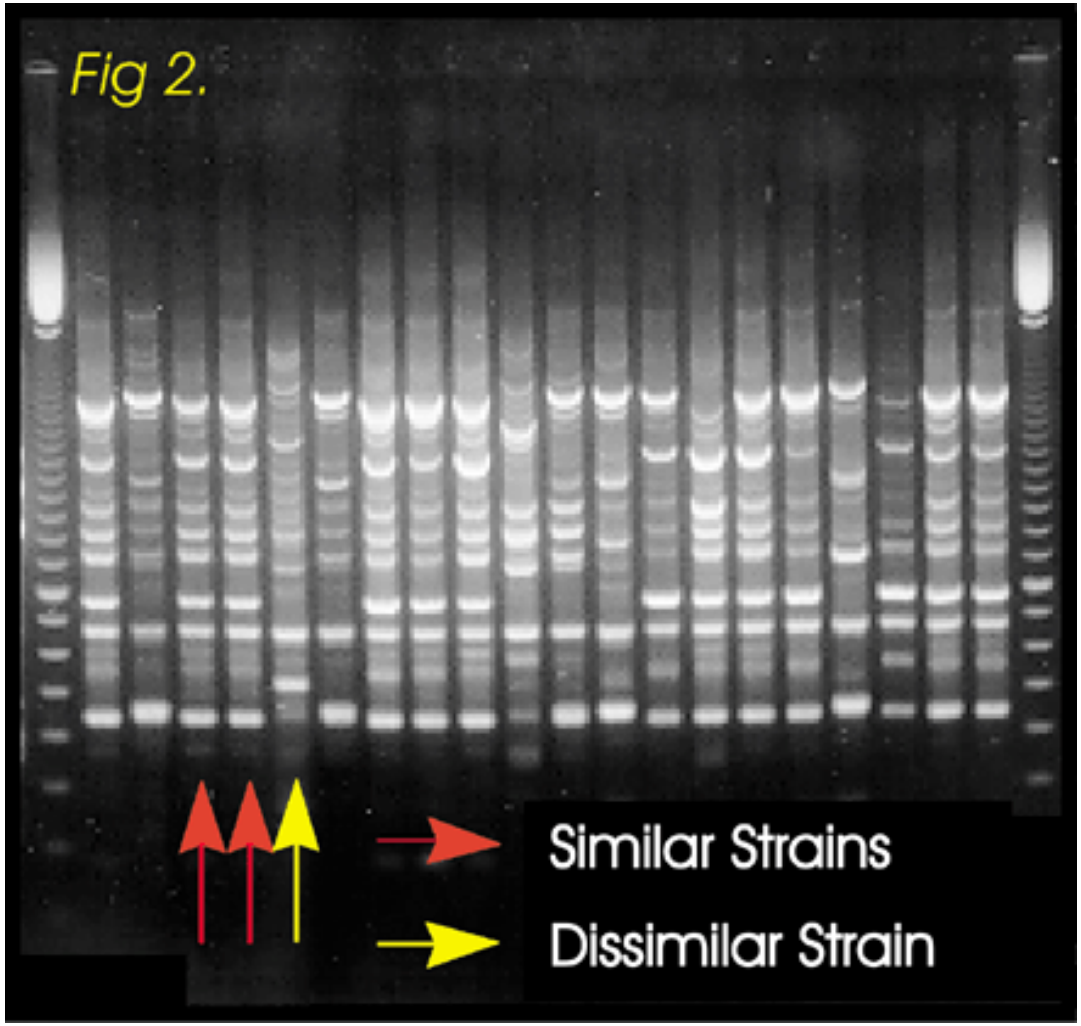
http://www.philsciletters.org/May%202,%202009/Genetic%20fingerprinting.htm

Rapid Amplification of Polymorphic DNA (RAPD)



ME 22-05-2013

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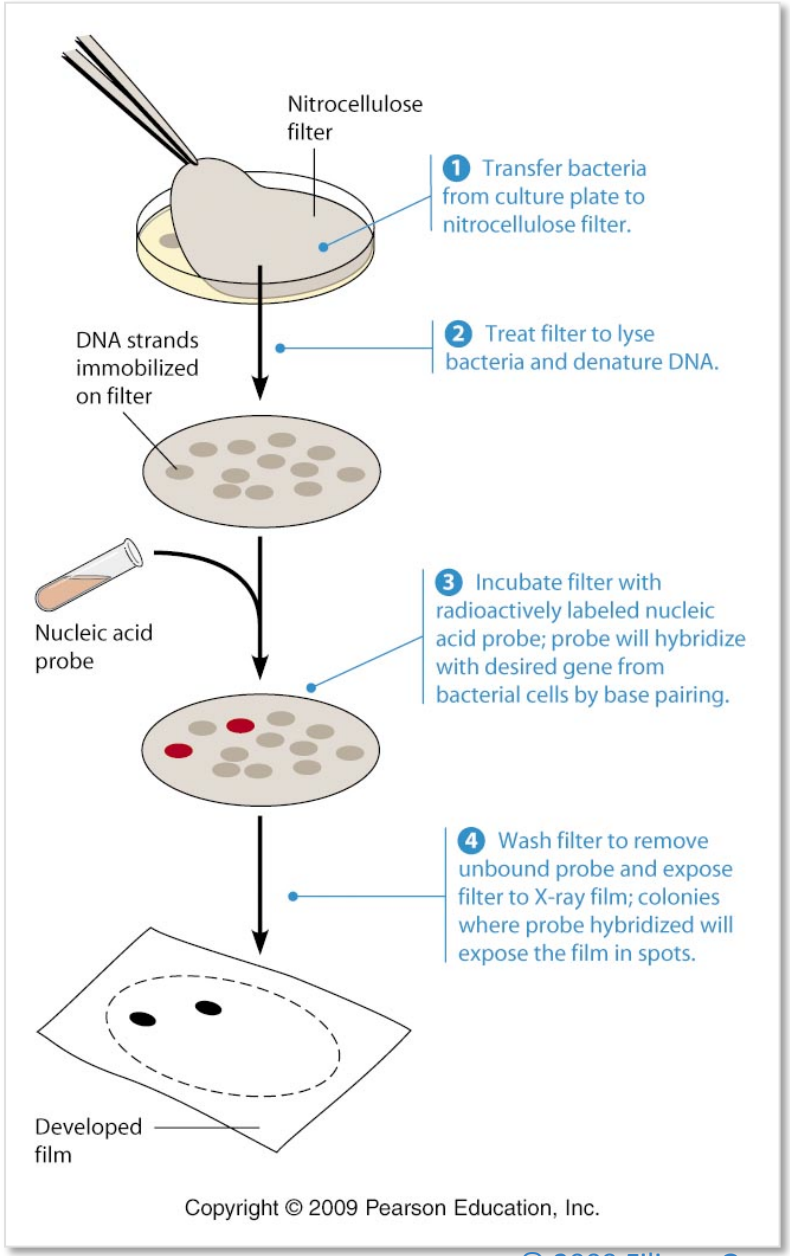
http://www.teagasc.ie/research/reports/dairyproduction/4209/4209fig02.gif

Ibridazione su colonia (*Colony hybridization*)

LU 16-05-2011



http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-20/2029.jpg

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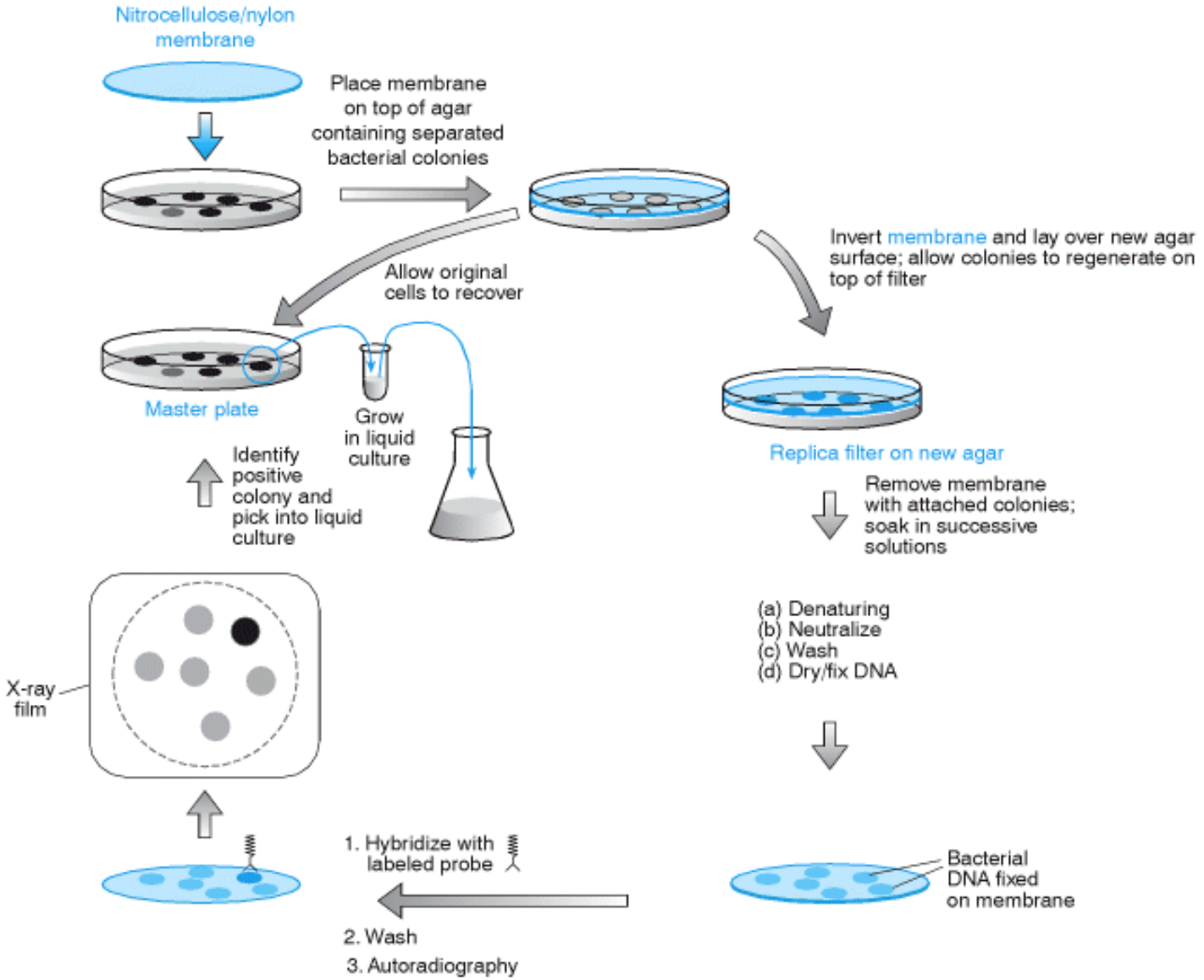
Ibridazione su colonia (*Colony hybridization*)

-05-2010



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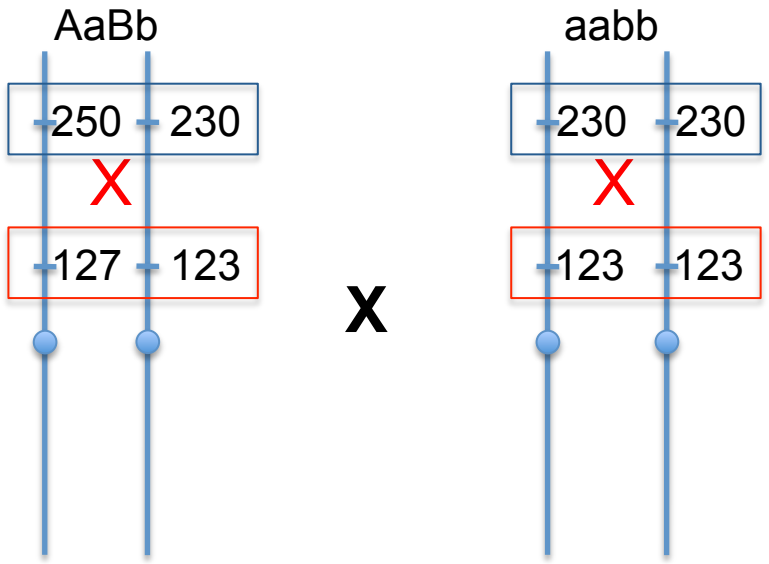
http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/hmg/ch5f18.gif

Calcolo della percentuale di ricombinazione tra marcatori SSR

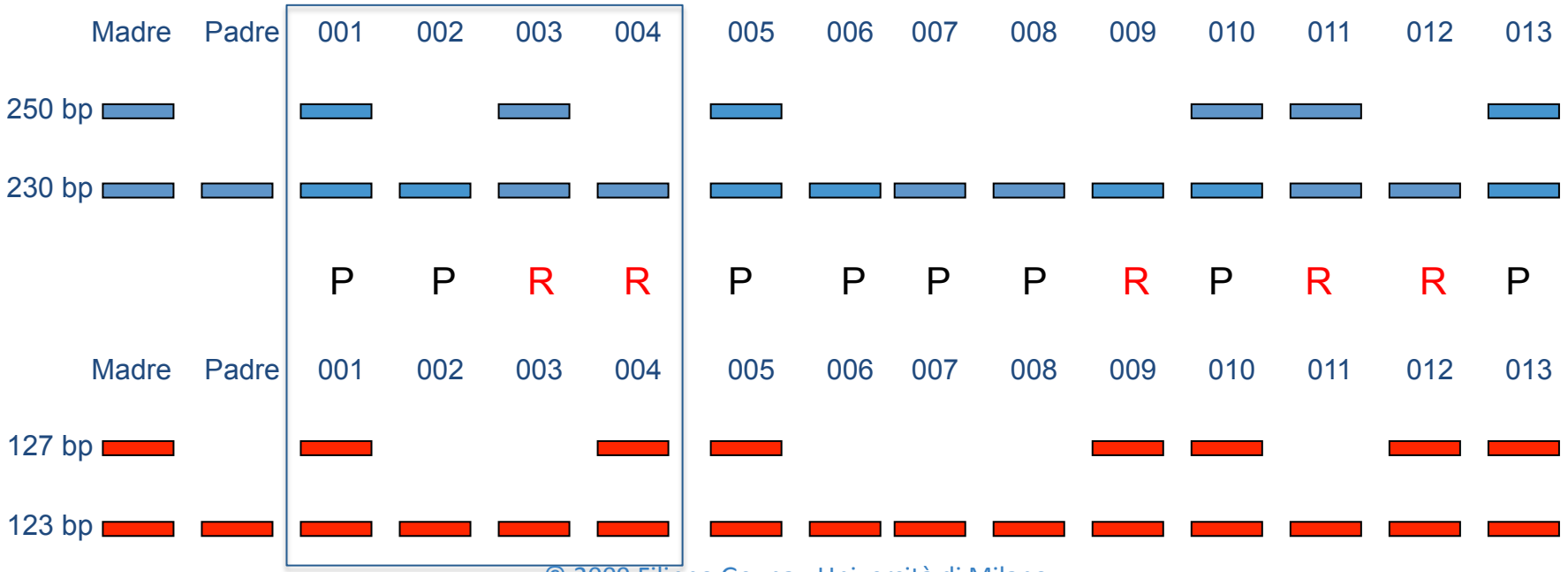


MA 13-05-2014

% ricombinanti = 5/13

Frazione ricombinanti = 0,38

Distanza di mappa tra i due marcatori = 38 cM



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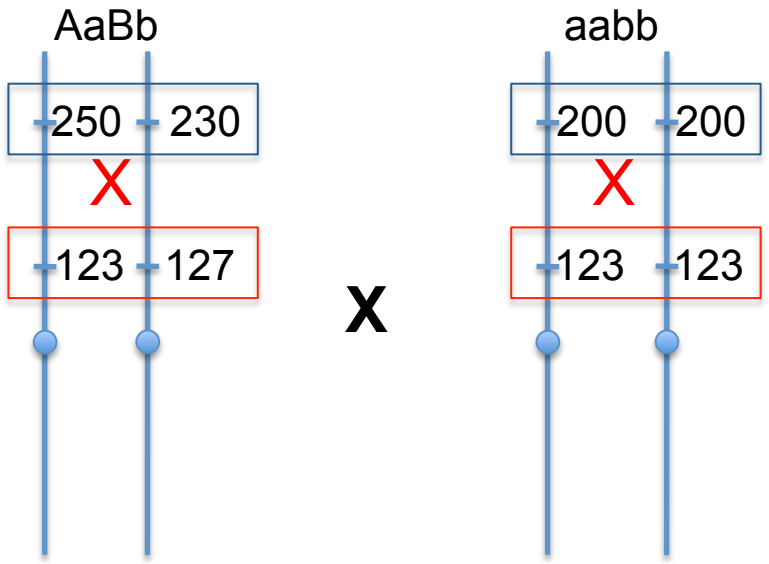
Calcolo della percentuale di ricombinazione tra marcatori SSR

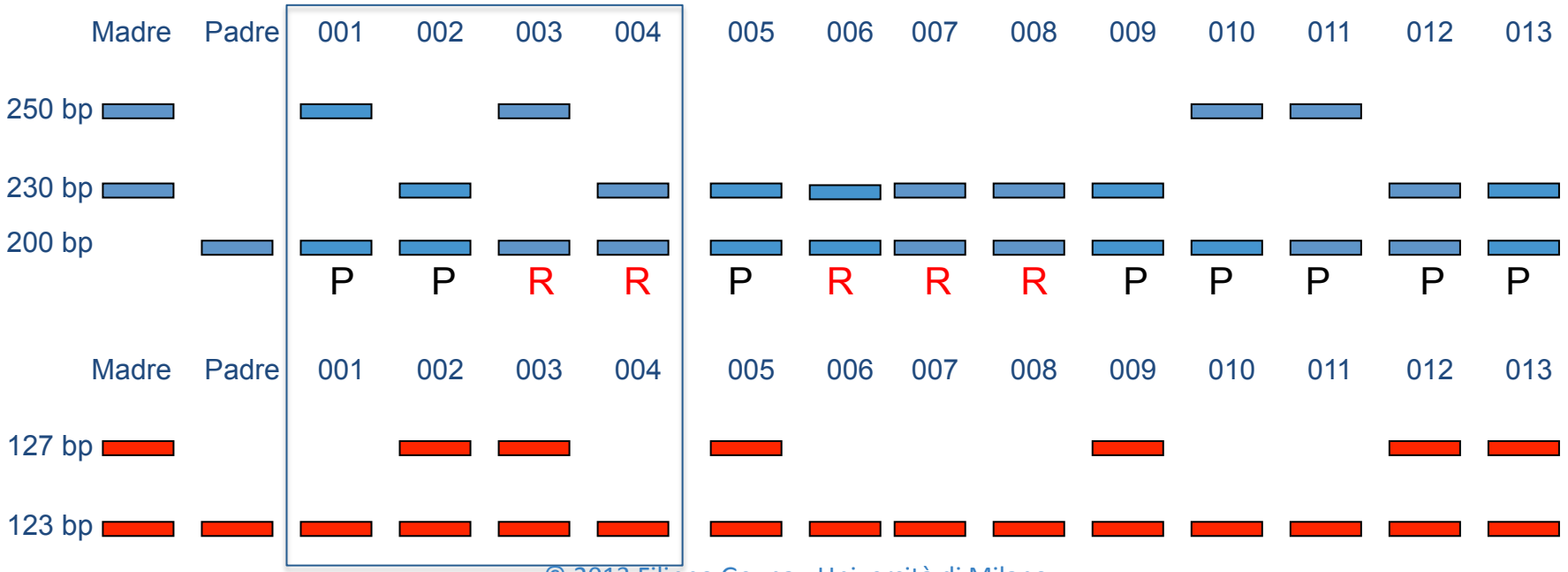


MA 13-05-2014

% ricombinanti = 5/13

Frazione ricombinanti = 0,38

Distanza di mappa tra i due marcatori = 38 cM



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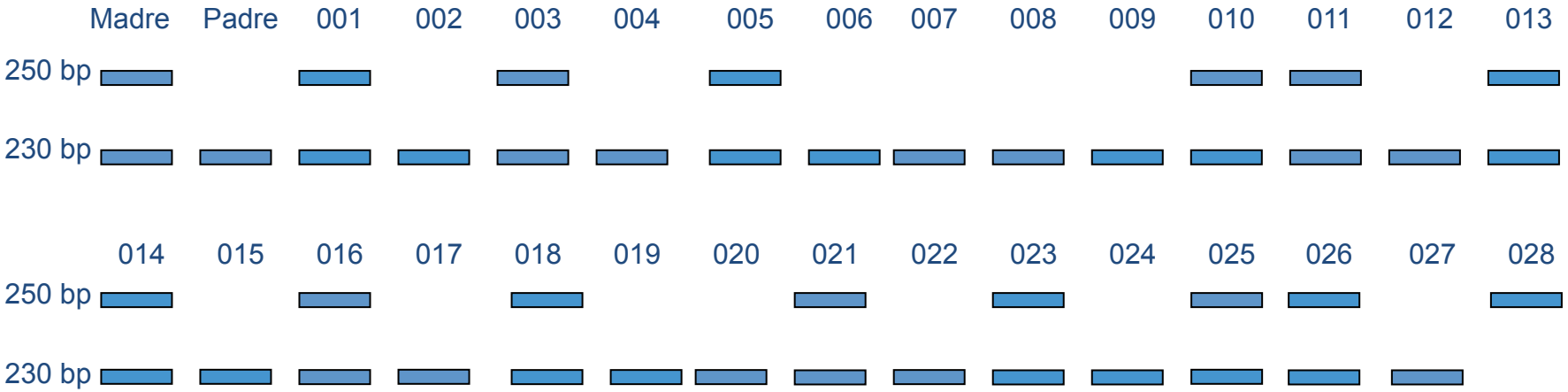
Appello di MGV2 – Esercizio 1

MA 12-07-2011

Si calcoli la distanza di mappa in cM tra il marcatore SSR e il carattere “fiore rosso” ammettendo che la madre sia eterozigote per il gene che determina il “fiore rosso”. Di seguito sono riportati i due genitori di incrocio e 28 figli. Si disegni la disposizione del marcatore e del gene per il colore del fiore sui cromosomi e si discuta ciò che si è fatto.



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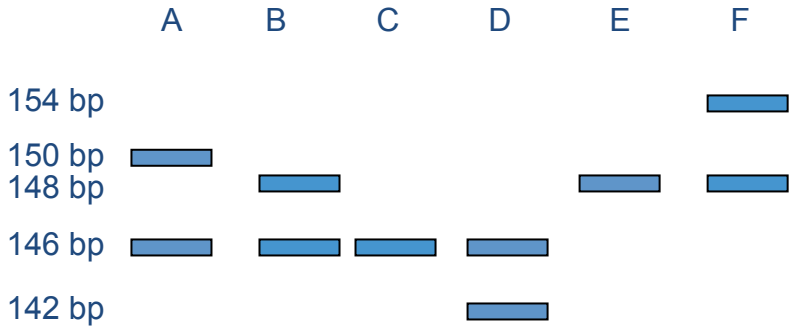
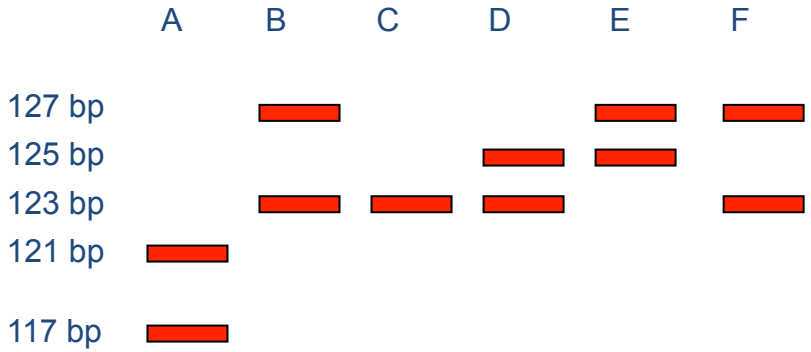
Calcolo della distanza genetica tra individui con marcatori SSR

ME 14-05-2014



	Allele 1	Allele 2
A	150	146
B	148	146
C	146	146
D	146	142
E	148	148
F	154	148



	Allele 1	Allele 2
A	121	117
B	127	123
C	123	123
D	125	123
E	127	125
F	127	123

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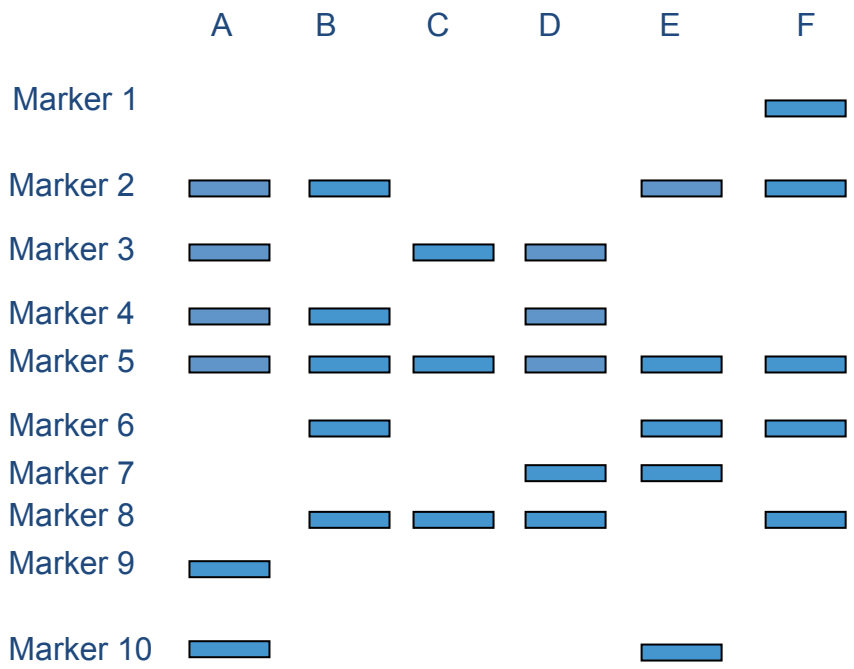
Calcolo della distanza genetica tra individui con marcatori AFLP

MA 05-06-2012



	A	B	C	D	E	F
M1	0	0	0	0	0	1
M2	1	1	0	0	1	1
M3	1	0	1	1	0	0
M4	1	1	0	1	0	0
M5	1	1	1	1	1	1
M6	0	1	0	0	1	1
M7	0	0	0	1	1	0
M8	0	1	1	1	0	1
M9	1	0	0	0	0	0
M10	1	0	0	0	1	0

Calcolo della distanza:

1 vs 1 = 0
0 vs 0 = 0
1 vs 0 = 1

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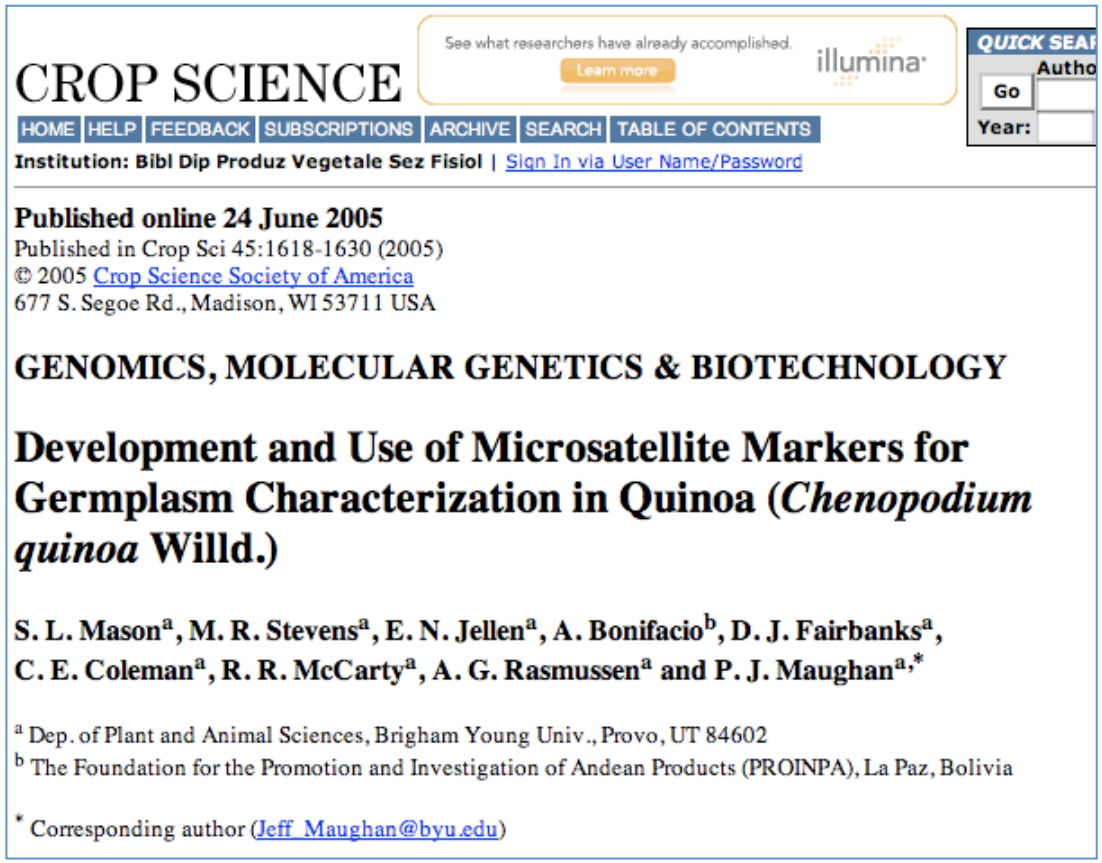
Calcolo della distanza genetica tra individui con marcatori molecolari

MA 16-06-2009



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Published in Crop Sci 45:1618-1630 (2005)
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677 S. Segoe Rd., Madison, WI 53711 USA

GENOMICS, MOLECULAR GENETICS & BIOTECHNOLOGY

Development and Use of Microsatellite Markers for Germplasm Characterization in Quinoa (*Chenopodium quinoa* Willd.)

S. L. Mason[a], M. R. Stevens[a], E. N. Jellen[a], A. Bonifacio[b], D. J. Fairbanks[a], C. E. Coleman[a], R. R. McCarty[a], A. G. Rasmussen[a] and P. J. Maughan[a,*]

[a] Dep. of Plant and Animal Sciences, Brigham Young Univ., Provo, UT 84602
[b] The Foundation for the Promotion and Investigation of Andean Products (PROINPA), La Paz, Bolivia

[*] Corresponding author (Jeff_Maughan@byu.edu)

Heterozygosity, a measure of allelic diversity, was estimated by:

$$H = 1 - \sum_{i=1}^{k} P_i^2$$

where P_i is the frequency of the ith allele and k is the number of alleles (Nei 1978)

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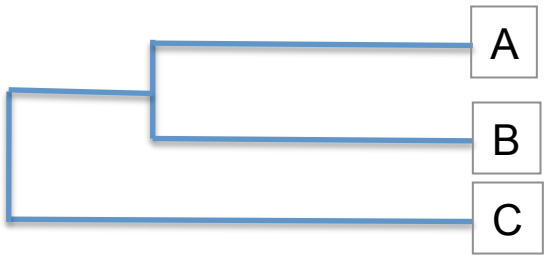
http://crop.scijournals.org/cgi/content/full/45/4/1618

Calcolo della distanza genetica tra individui attraverso allineamento di sequenze

ME 06-06-2012

```
A  5'-ACGAGTACGTACGTCATTAAC-3'
     ||||||||||||||||||||
B  5'-ACGCGTACGTACGTCATTAAC-3'
     ||||||||||||||||||||
C  5'-ACGCGTACGTACGTCGTTAAC-3'
```

A	vs	B	=	1/21	=	0.0476
B	vs	C	=	1/21	=	0.0476
A	vs	C	=	2/21	=	0.0952



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Mappatura mediante “Bulk Segregant Analysis”

Fitopatol. bras. vol.27 no.2 Brasília Mar./Apr. 2002

ME 13-05-2015

IDENTIFICATION OF RAPD MARKER LINKED TO BLAST RESISTANCE GENE IN A SOMACLONE OF RICE CULTIVAR ARAGUAIA

LEILA G. ARAÚJO, ANNE S. PRABHU & MARTA C. FILIPPI

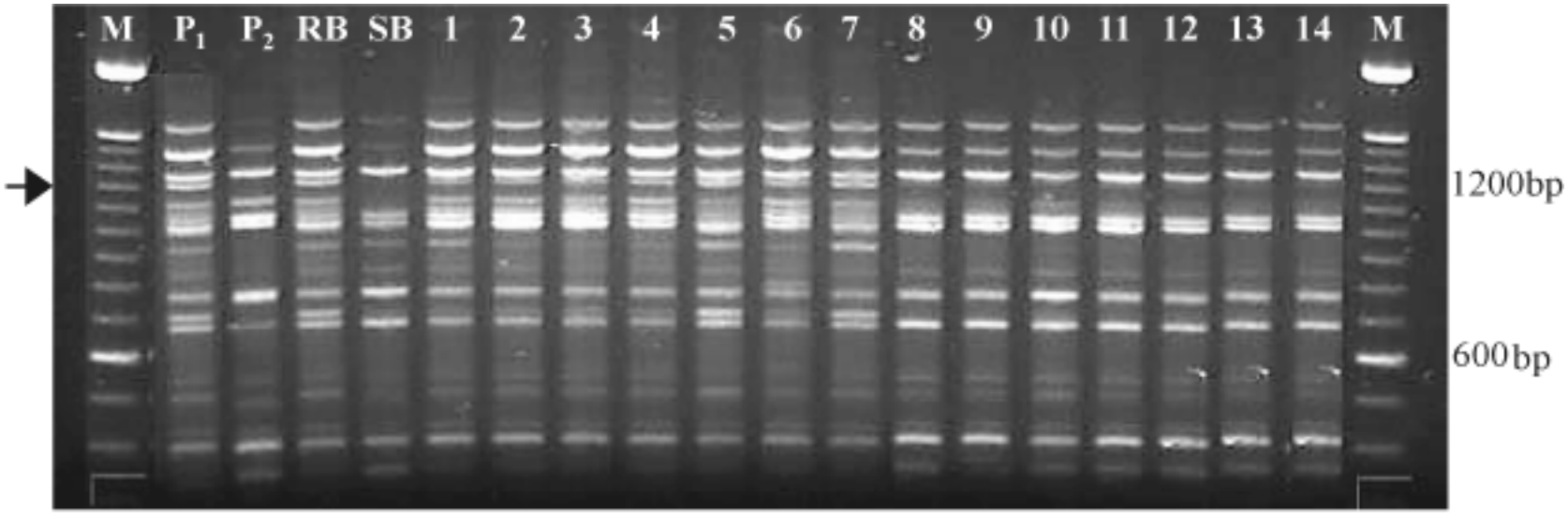

FIG. 2 - RAPD tagging of *Pi-ar* gene by bulked segregant analysis using primer $OPC02_{(1200)}$. Lanes: Resistant somaclone SC09 (P_1), susceptible parental cultivar Araguaia (P_2), resistant bulk (RB), susceptible bulk (SB) followed by 14 F_2 plants of the cross composed of resistant plants (lanes 1 to 7) and susceptible plants (lanes 8 to 14). The arrow indicates the marker linked to the resistance gene *Pi-ar* of somaclone SC09 derived from Araguaia to race IB-45 of *Pyricularia grisea*. M = Marker (100 bp)

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http://www.scielo.br/img/fbpe/fb/v27n2/9142f2.gif

Creazione e ibridazione di microarray

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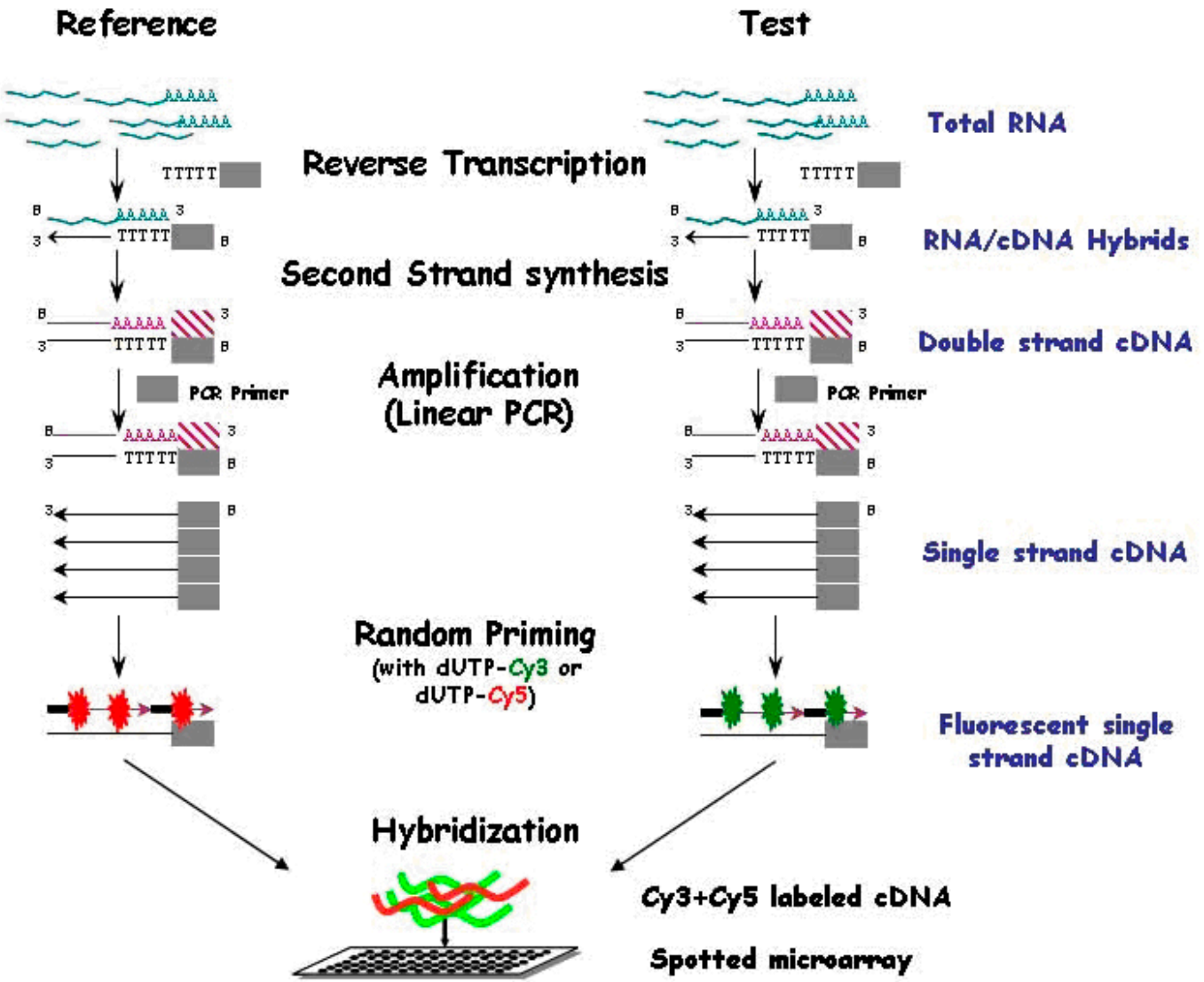

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Lettura e analisi di dati microarray

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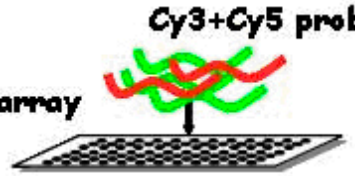
Hybridization



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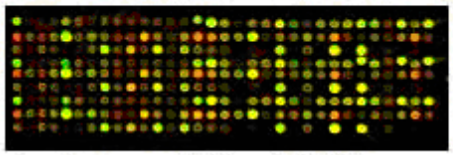
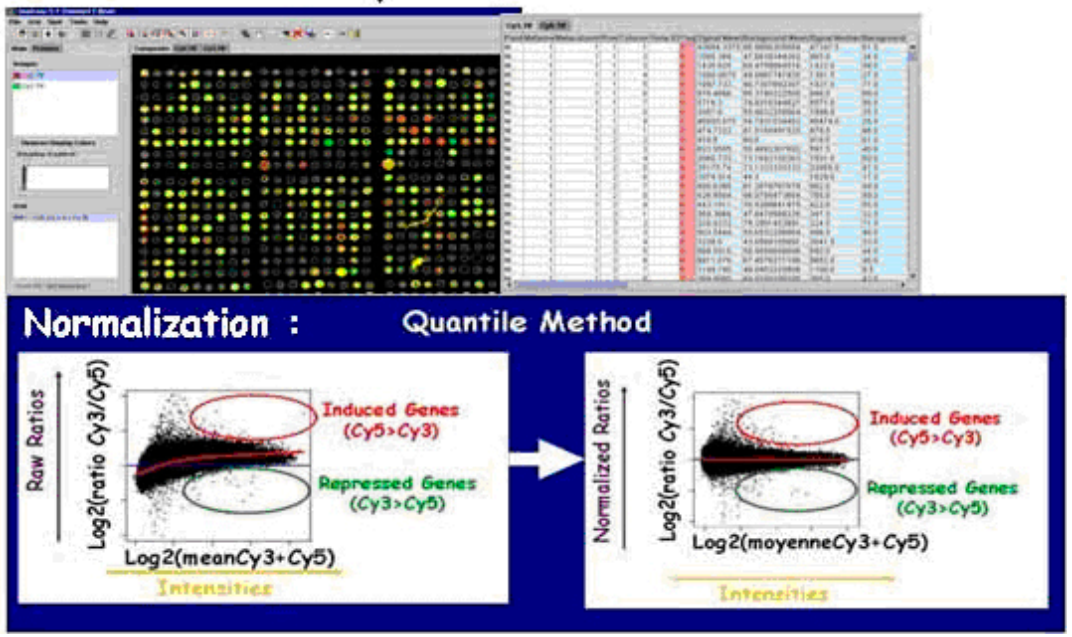


False color images : Cy3 and Cy5 images overlayed

Quantification & Normalization



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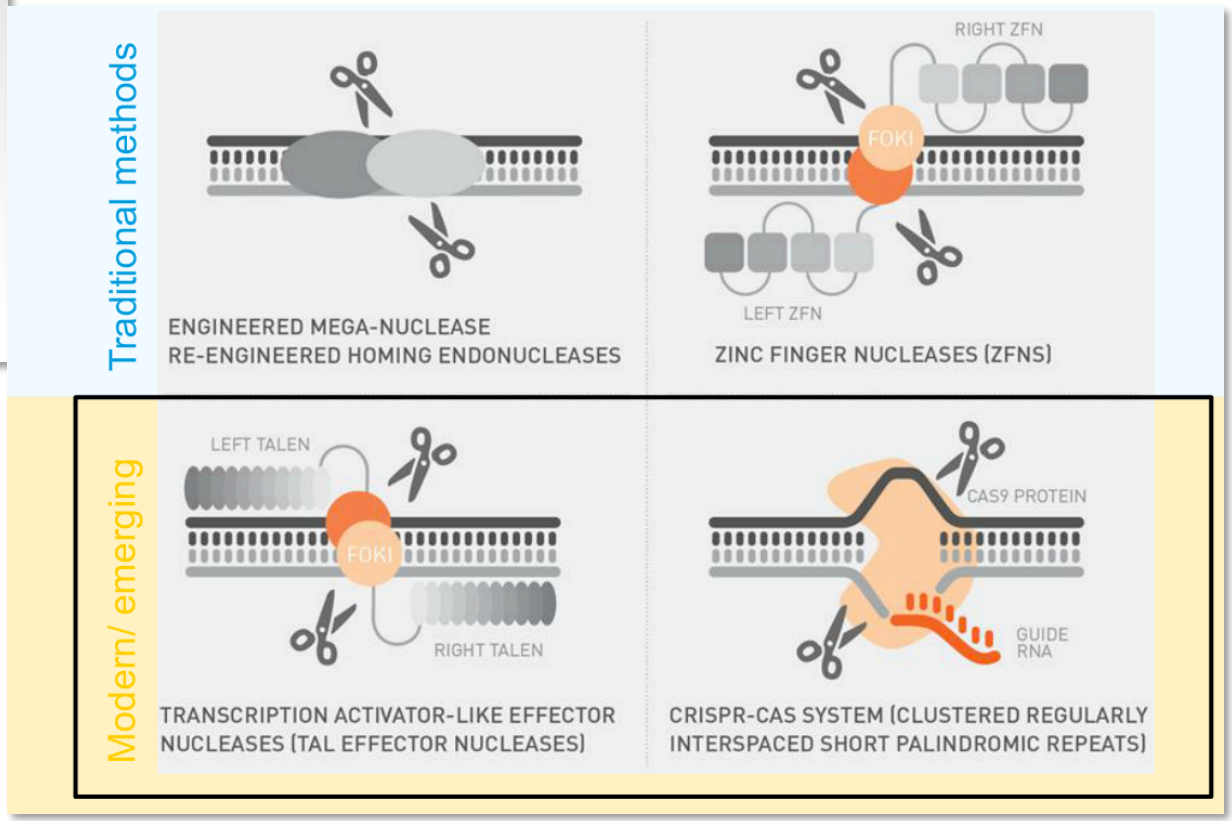

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Programmi internazionali di ricostruzione delle genealogie

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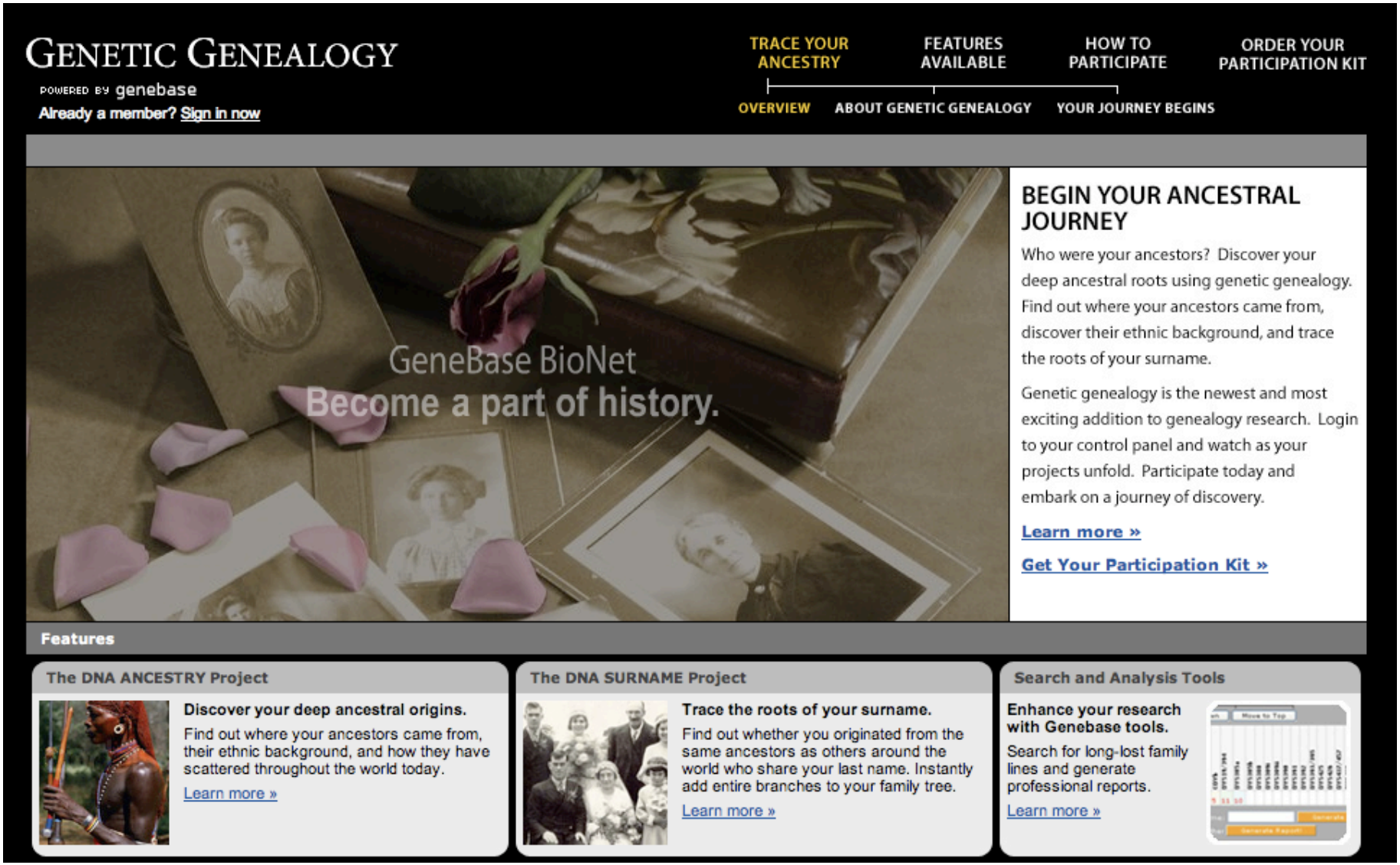

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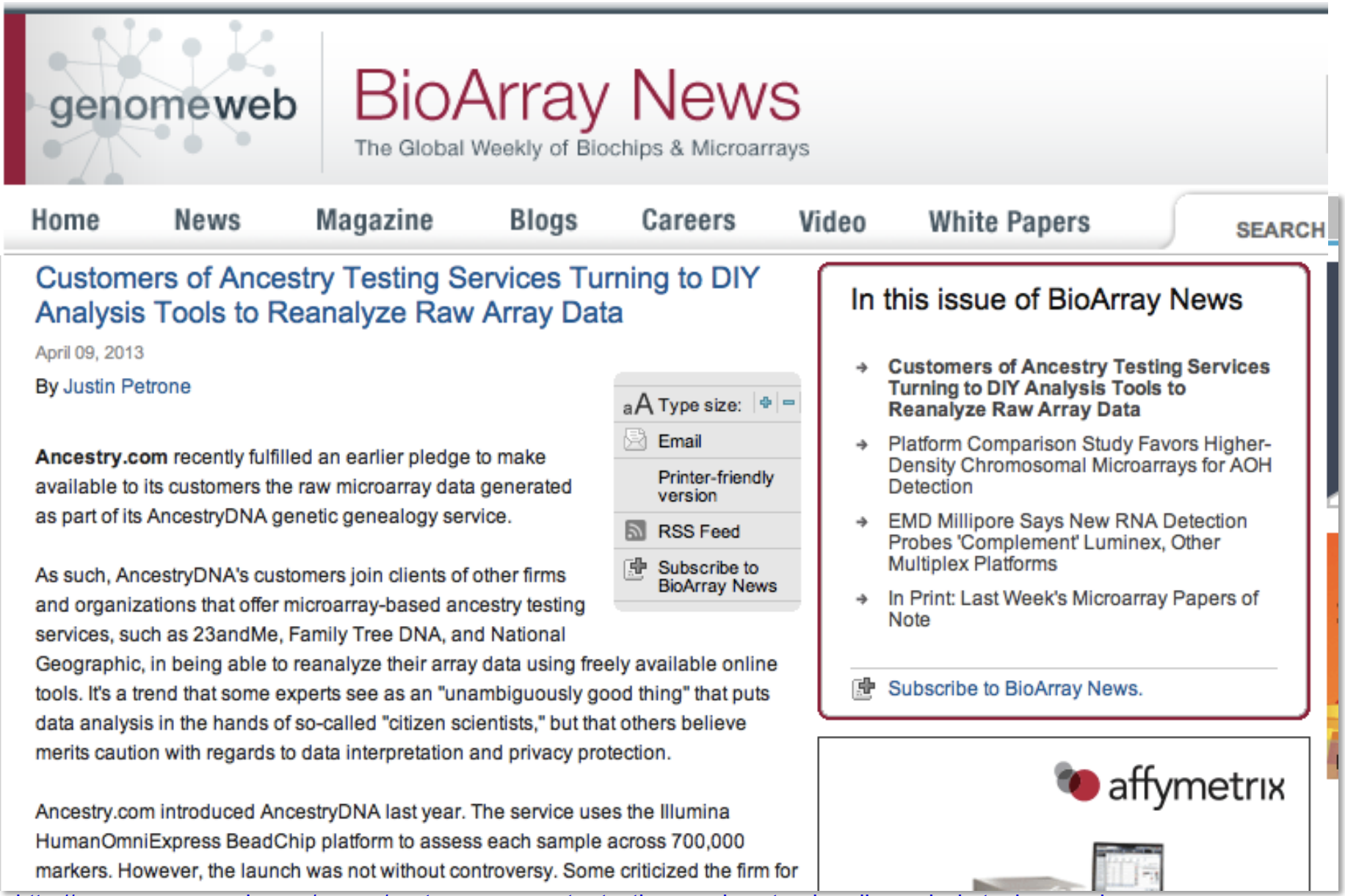
Software di ricostruzione di genealogia



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BioArray News

The Global Weekly of Biochips & Microarrays

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Customers of Ancestry Testing Services Turning to DIY Analysis Tools to Reanalyze Raw Array Data

April 09, 2013

By Justin Petrone

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Ancestry.com recently fulfilled an earlier pledge to make available to its customers the raw microarray data generated as part of its AncestryDNA genetic genealogy service.

As such, AncestryDNA's customers join clients of other firms and organizations that offer microarray-based ancestry testing services, such as 23andMe, Family Tree DNA, and National Geographic, in being able to reanalyze their array data using freely available online tools. It's a trend that some experts see as an "unambiguously good thing" that puts data analysis in the hands of so-called "citizen scientists," but that others believe merits caution with regards to data interpretation and privacy protection.

Ancestry.com introduced AncestryDNA last year. The service uses the Illumina HumanOmniExpress BeadChip platform to assess each sample across 700,000 markers. However, the launch was not without controversy. Some criticized the firm for

In this issue of BioArray News

- **Customers of Ancestry Testing Services Turning to DIY Analysis Tools to Reanalyze Raw Array Data**
- Platform Comparison Study Favors Higher-Density Chromosomal Microarrays for AOH Detection
- EMD Millipore Says New RNA Detection Probes 'Complement' Luminex, Other Multiplex Platforms
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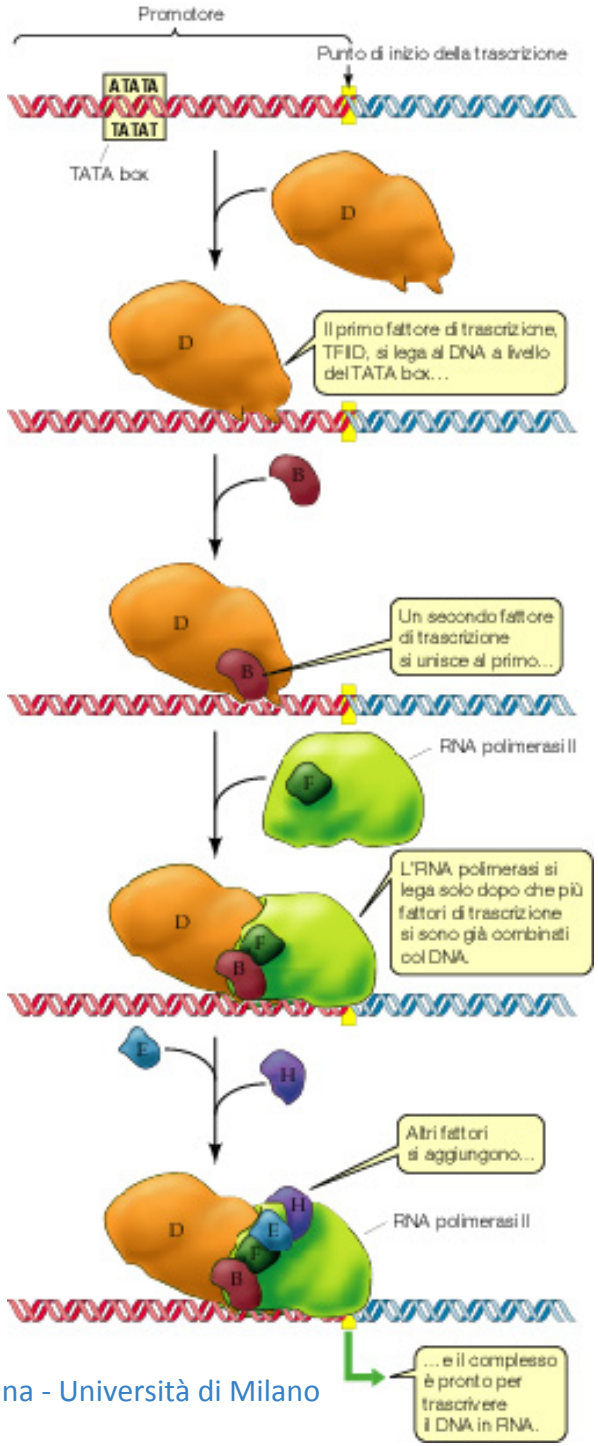
Il controllo della trascrizione



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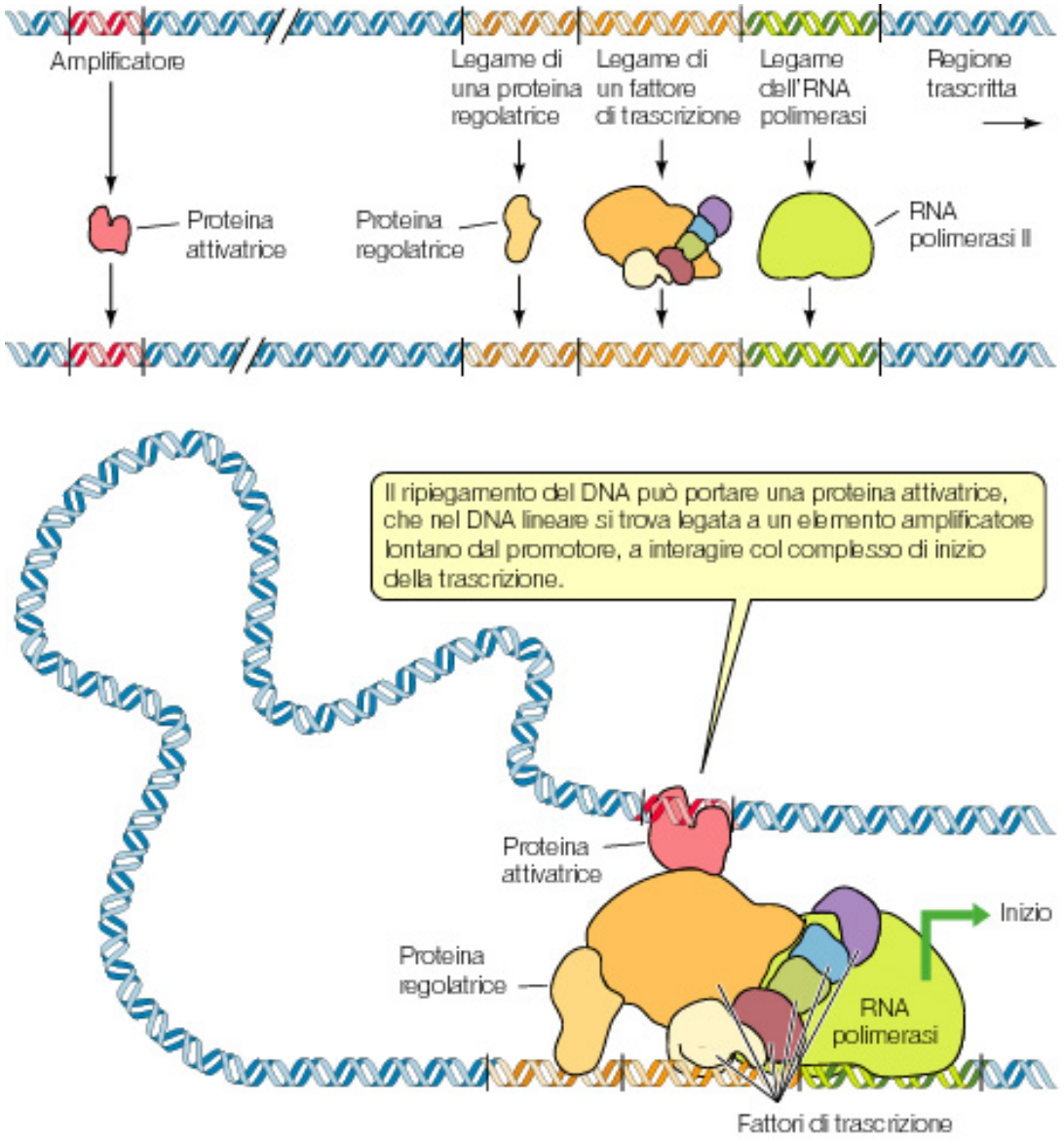
(Purves et al. Fig. 14.12)

Il controllo della trascrizione



(Purves et al. Fig. 14.13)

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Il controllo della trascrizione: classi di motivi strutturali

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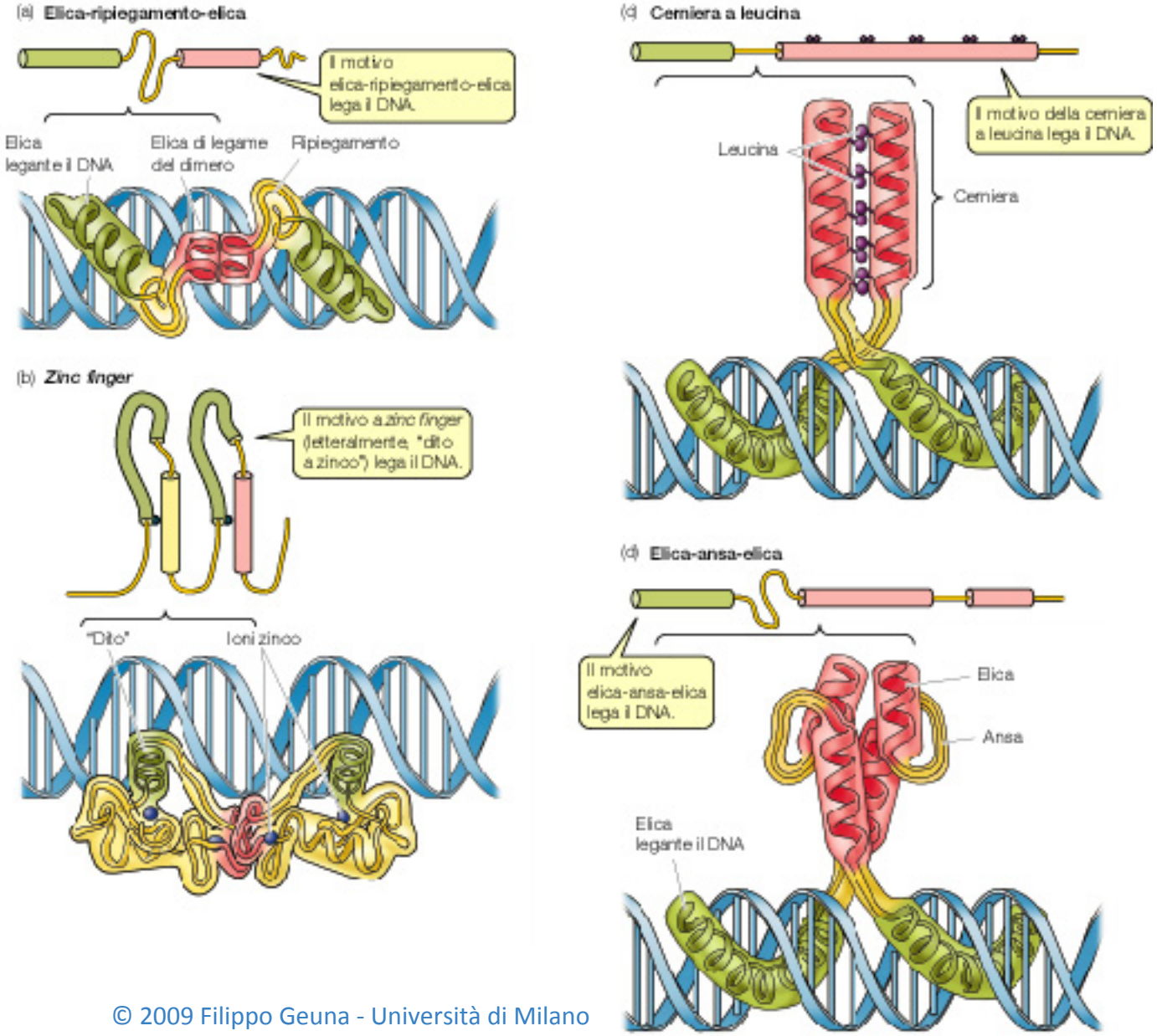

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Repression-lignin.pdf

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RESEARCH

Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees

Wen-Jing Hu[1,4], Scott A. Harding[1], Jrhau Lung[1], Jacqueline L. Popko[1], John Ralph[2], Douglas D. Stokke[3], Chung-Jui Tsai[1], and Vincent L. Chiang[1]*

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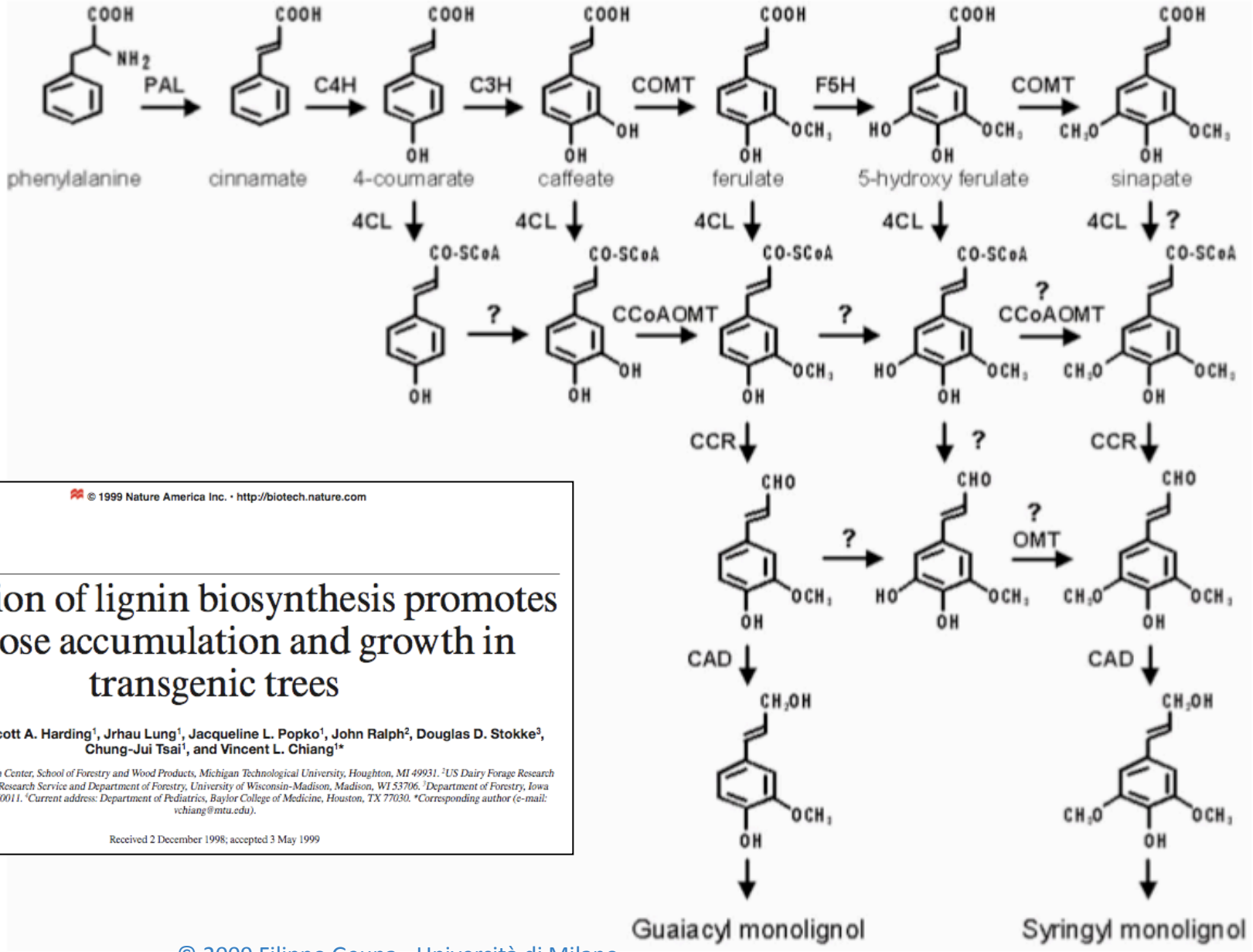
Repression-lignin.pdf



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RESEARCH

Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees

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[1]Plant Biotechnology Research Center, School of Forestry and Wood Products, Michigan Technological University, Houghton, MI 49931. [2]US Dairy Forage Research Center, USDA-Agricultural Research Service and Department of Forestry, University of Wisconsin-Madison, Madison, WI 53706. [3]Department of Forestry, Iowa State University, Ames, IA 50011. [4]Current address: Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030. *Corresponding author (e-mail: vchiang@mtu.edu).

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nature biotechnology

Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors

Eugenio Butelli[1], Lucilla Titta[2], Marco Giorgio[2], Hans-Peter Mock[3], Andrea Matros[3], Silke Peterek[3], Elio G W M Schijlen[4], Robert D Hall[5], Arnaud G Bovy[4], Jie Luo[1] & Cathie Martin[1]

Nature Publishing Group http://www.nature.com/naturebiotechnology

Dietary consumption of anthocyanins, a class of pigments produced by higher plants, has been associated with protection against a broad range of human diseases. However, anthocyanin levels in the most commonly eaten fruits and vegetables may be inadequate to confer optimal benefits. When we expressed two transcription factors from snapdragon in tomato, the fruit of the plants accumulated anthocyanins at levels substantially higher than previously reported for efforts to engineer anthocyanin accumulation in tomato and at concentrations comparable to the anthocyanin levels found in blackberries and blueberries. Expression of the two transgenes enhanced the hydrophilic antioxidant capacity of tomato fruit threefold and resulted in fruit with intense purple coloration in both peel and flesh. In a pilot test, cancer-susceptible *Trp53*$^{-/-}$ mice fed a diet supplemented with the high-anthocyanin tomatoes showed a significant extension of life span.

Anthocyanins are naturally occurring polyphenols present in many foods that are commonly consumed as part of the human diet. They offer protection against certain cancers, cardiovascular disease and level of consumption, and the numbers of people consuming the recommended five daily portions of fruit and vegetables have declined over the last 10 years. These figures argue strongly for the development of strategies to increase the levels of health-promoting bioactive compounds such as anthocyanins in the fruits and vegetables that people consume in substantial amounts.

Tomato is an excellent candidate for transgenic enhancement of flavonoid content. It is an important food crop worldwide and its levels of flavonoids (which include anthocyanins) are considered sub-optimal, with only small amounts of naringenin chalcone and rutin accumulating in tomato peel[11]. Flavonoids represent an important source of hydrophilic dietary antioxidants, whereas the most abundant antioxidant in tomato fruit is lycopene, a lipophilic antioxidant. Generally, foods rich in both soluble and membrane-associated anti-oxidants are considered to offer the best protection against disease[12].

Although most tomato cultivars do not produce anthocyanins in fruit, low, light-dependent accumulation of anthocyanins in sectors of the skin has been achieved by introgression of a trait from a wild relative of tomato[13]. Genetic engineering strategies have also provided modest success; silencing of the tomato *DET1* gene, which represses light-mediated development, increases flavonoid levels up to

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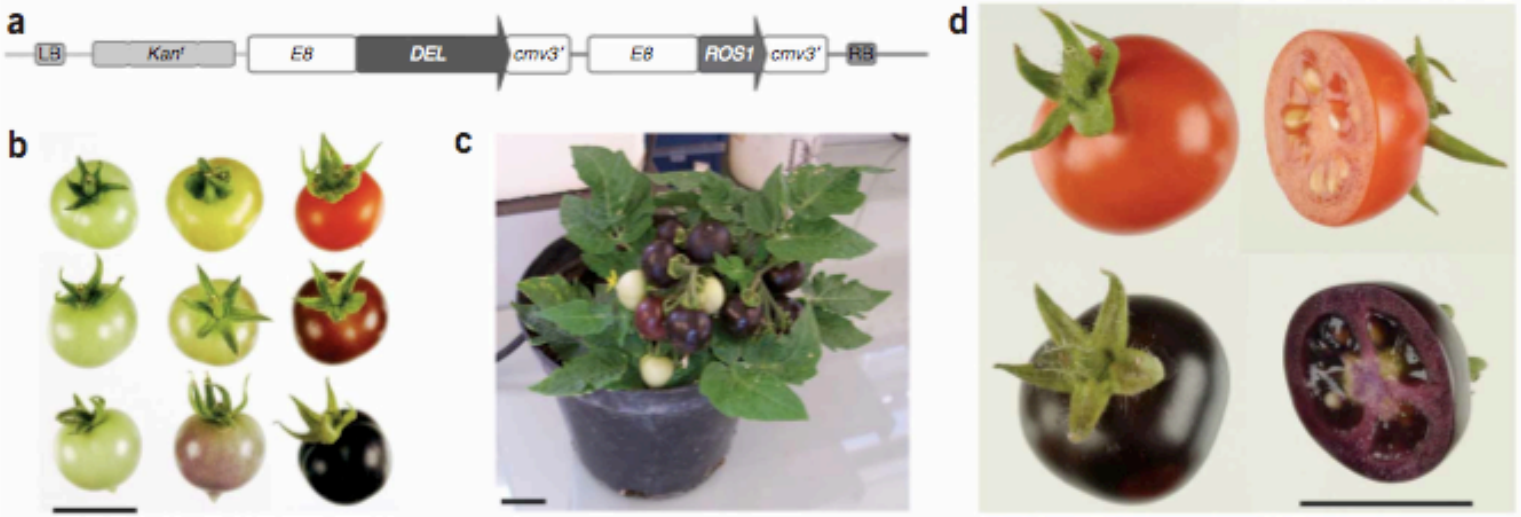

Figure 1 Fruit-specific phenotypes of T1 generation tomatoes (cv. MicroTom) expressing both *Del* and *Ros1* under the control of the *E8* promoter. (**a**) Map of T-DNA region of the binary vector used for transformation. LB, left T-DNA border region; RB, right T-DNA border region; Kan^r, *nptII* gene conferring kanamycin resistance under the control of the *nos* promoter; cmv3′, terminator region of cauliflower mosaic virus. (**b**) Phenotypic analysis of wild-type (upper row), *Del/Ros1*C (middle) and *Del/Ros1*N (lower) tomato fruit harvested at the green (left column), breaker (middle) and red (right) ripening stages. (**c**) *Del/Ros1*N tomato plant showing fruit at different stages of ripening. (**d**) Whole and cross-section of ripe wild-type and *Del/Ros1*N tomato fruit. All scale bars, 2 cm.

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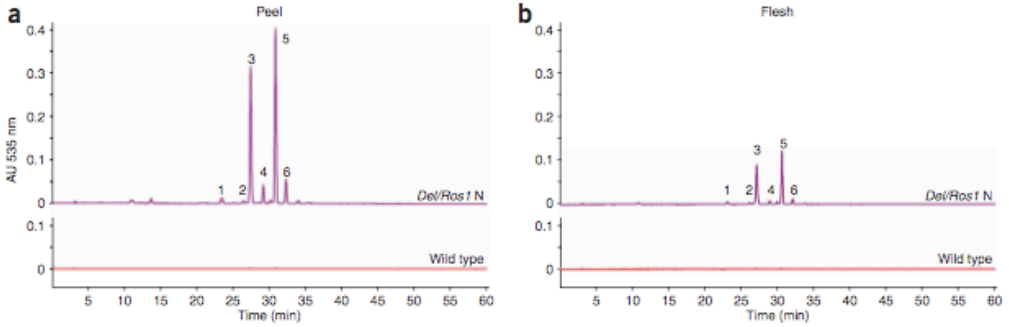
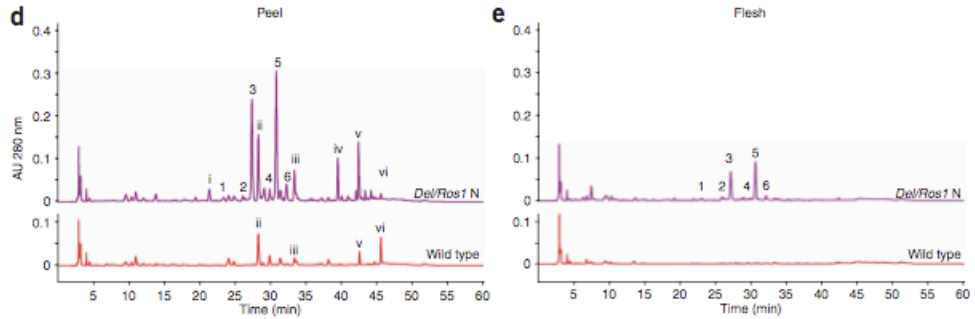


c

	Peak identification	λ_{max} (nm)	$[M+H]^+$ (m/z)	Detected fragments
1	Delphinidin 3-(caffeoyl)-rutinoside-5-glucoside	529	935.24	773.23 (M+Glc), 465.11 (dpd+Glc), 303.05 (dpd)
2	Delphinidin 3-(cis-coumaroyl)-rutinoside-5-glucoside	533	919.27	757.22 (M+Glc), 465.12 (dpd+Glc), 303.06 (dpd)
3	Delphinidin 3-(trans-coumaroyl)-rutinoside-5-glucoside	529	919.26	757.19 (M+Glc), 465.11 (dpd+Glc), 303.05 (dpd)
4	Delphinidin 3-(feruloyl)-rutinoside-5-glucoside	529	949.24	787.21 (M+Glc), 465.10 (dpd+Glc), 303.06 (dpd)
5	Petunidin 3-(trans-coumaroyl)-rutinoside-5-glucoside	531	933.26	771.21 (M+Glc), 479.10 (ptd+Glc), 317.07 (ptd)
6	Petunidin 3-(feruloyl)-rutinoside-5-glucoside	530	963.27	801.23 (M+Glc), 479.13 (ptd+Glc), 317.07 (ptd)



f

	Peak identification	λ_{max} (nm)	$[M+H]^+$ (m/z)	Detected fragments
i	Myricetin-rhamnoglucoside	263/356	627.18	481.12 (M+Rha), 319.06 (M)
ii	Quercetin-rutinoside (rutin)	264/354	611.52	465.10 (Q+Glc), 303.04 (Q)
iii	Kaempferol-rhamnoglucoside	265/345	595.19	449.13 (K+Glc), 287.07 (K)
iv	Myricetin derivate	316	773.13	481.14 (M+Rha), 319.06 (M)
v	Quercetin derivate	315	757.23	465.12 (Q+Glc), 303.06 (Q)
vi	Naringenin chalcone	369	273.32	

Figure 2 Comparative analysis of phenylpropanoid content and composition. HPLC chromatogram of methanol extracts from *Del/Ros1*N (purple line) and wild-type (red line) tomato fruit. HPLC analysis, recorded at 535 nm (**a** and **b**) or 280 nm (**d** and **e**) of extracts from peel (**a** and **d**) or flesh (**b** and **e**) of ripe fruit. Peaks marked with numbers represent anthocyanins and peaks marked with roman numerals represent other flavonoids. Classification and identification of methanol soluble compounds was performed based on PDA absorbance and ESI-Q-TOF mass spectrometry (**c** and **f**). The purified compounds were analyzed by HPLC and ESI-MS/MS. Spectral characteristics, molecular ions and fragments obtained are tabulated. Identification was confirmed by hydrolysis and HPLC analysis of the respective acyl and sugar moieties. M, myricetin; Q, quercetin; K, kaempferol; Glc, glucose; Rha, rhamnose; dpd, delphinidin; ptd, petunidin.

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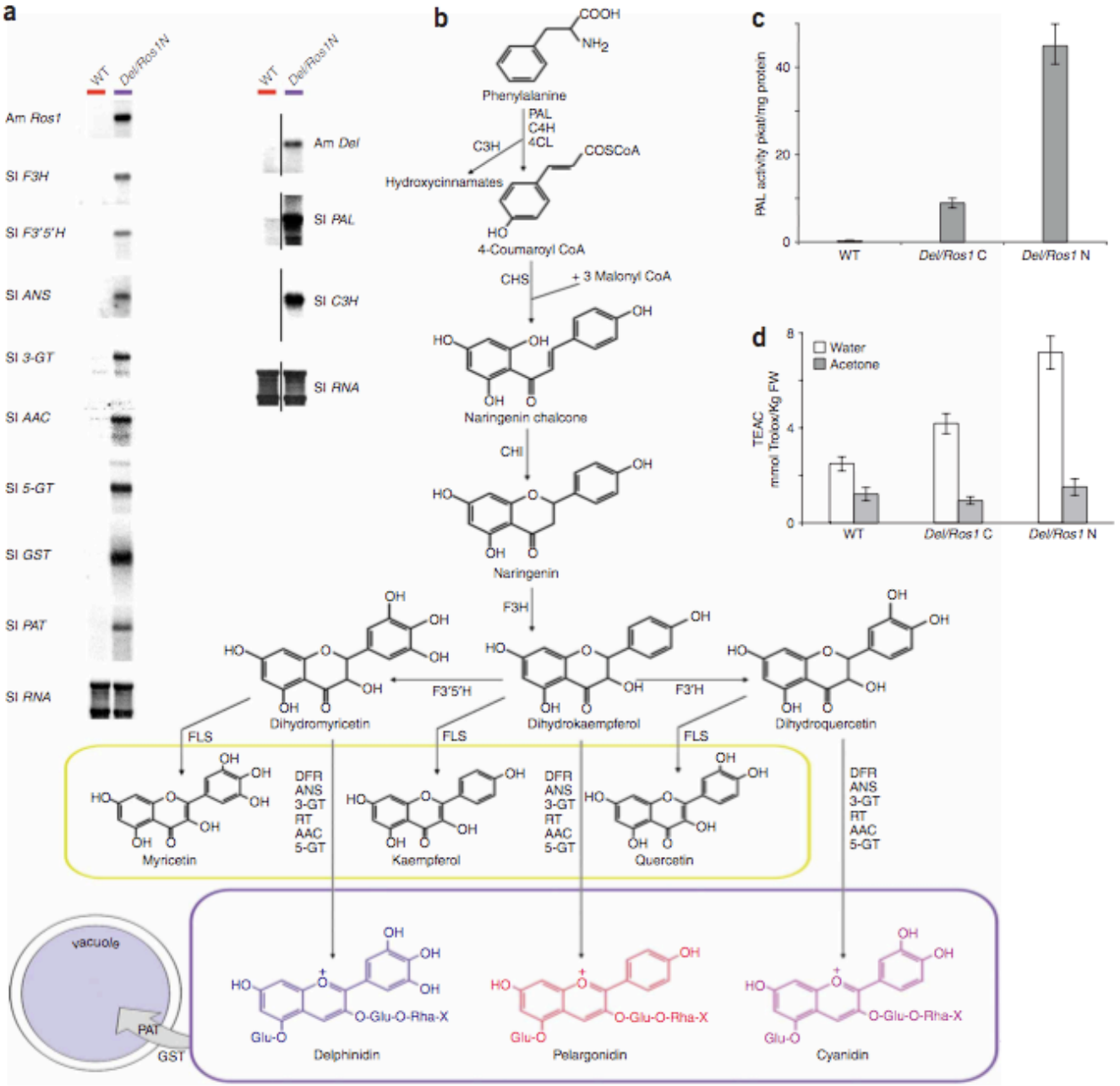

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MOLECULAR PLANT PATHOLOGY (2008) 9(1), 73–83 DOI: 10.1111/J.1364-3703.2007.00447.X

Review

Strategies for antiviral resistance in transgenic plants

MARCEL PRINS[1,*†], MARGIT LAIMER[2], EMANUELA NORIS[3], JÖRG SCHUBERT[4], MICHAEL WASSENEGGER[5] AND MARK TEPFER[6]

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[6]Plant Virology Laboratory, ICGEB Biosafety Outstation, Via Piovega 23, 31056 Ca' Tron di Roncade, Italy

SUMMARY

Genetic engineering offers a means of incorporating new virus resistance traits into existing desirable plant cultivars. The initial attempts to create transgenes conferring virus resistance were based on the pathogen-derived resistance concept. The expression of the viral coat protein gene in transgenic plants was shown to induce protective effects similar to classical cross protection, and was therefore distinguished as 'coat-protein-mediated' protection. Since then, a large variety of viral sequences encoding structural and non-structural proteins were shown to confer resistance. Subsequently, non-coding viral RNA was shown to be a potential trigger for virus resistance in transgenic plants, which led to the discovery of a novel innate resistance in plants, RNA silencing. Apart from the majority of pathogen-derived resistance strategies, alternative strategies involving virus-specific antibodies have been successfully applied. In a separate section, efforts to combat viroids in transgenic plants are highlighted. In a final summarizing section, the potential risks involved in the introduction of transgenic crops and the specifics of the approaches used will be discussed.

http://www.resistvir-db.org/docs/deliverables/WP3/WP3_D22_Review_EG3.pdf

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Review

Techniques for the removal of marker genes from transgenic plants

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[b] *Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT, UK*

Received 30 August 2002; accepted 24 October 2002

Abstract

The presence of marker genes encoding antibiotic or herbicide resistances in genetically modified plants poses a number of problems. Various techniques are under development for the removal of unwanted marker genes, while leaving required transgenes in place. The aim of this brief review is to describe the principal methods used for marker gene removal, concentrating on the most recent and promising innovations in this technology.

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Keywords: Marker-gene; Antibiotic; Herbicide; Genetically modified organism

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http://www.uniroma2.it/didattica/Biotec._vegetali/deposito/Marker_removal_.Scutt_2002_Biochemie..pdf

MOLECULAR PLANT PATHOLOGY (2007) **8**(2), 223–231 DOI: 10.1111/J.1364-3703.2007.00386.X

Review

Sources of natural resistance to plant viruses: status and prospects

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[1]*John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK*
[2]*INRA, Genetics and Breeding of Fruits and Vegetables, Dom. St Maurice, BP94, F-84143, Montfavet Cedex, France*

SUMMARY

Globally, virus diseases are common in agricultural crops and have a major agronomic impact. They are countered through the deployment of genetic resistance against the virus, or through the use of a range of farming practices based upon the propagation of virus-free plant material and the exclusion of the virus vectors from the growing crop. We review here the current status of our knowledge of natural virus resistance genes, and consider the future prospects for the deployment of these genes against virus infection.

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http://www.resistvir-db.org/docs/deliverables/WP3/WP3_D22_Review_EG2.pdf

Comparative mapping and marker-assisted selection in Rosaceae fruit crops

Elisabeth Dirlewanger*, Enrique Graziano[†‡], Tarek Joobeur[†§], Francesc Garriga-Calderé[†¶], Patrick Cosson*, Werner Howad[†], and Pere Arús[†‖]

*Institut National de la Recherche Agronomique, Centre de Bordeaux, Unité de Recherches sur les Espèces Fruitières et la Vigne, B.P. 81, 33883 Villenave d'Ornon Cedex, France; and [†]Institut de Recerca i Tecnologia Agroalimentàries, Departament de Genètica Vegetal, Carretera de Cabrils s/n, 08348 Cabrils (Barcelona), Spain

Edited by Ronald L. Phillips, University of Minnesota, St. Paul, MN, and approved April 5, 2004 (received for review November 30, 2003)

The development of saturated linkage maps using transferable markers, restriction fragment length polymorphisms, and microsatellites has provided a foundation for fruit tree genetics and breeding. A *Prunus* reference map with 562 such markers is available, and a further set of 13 maps constructed with a subset of these markers has allowed genome comparison among seven *Prunus* diploid ($x = 8$) species (almond, peach, apricot, cherry, *Prunus ferganensis*, *Prunus davidiana*, and *Prunus cerasifera*); marker colinearity was the rule with all of them. Preliminary results of the comparison between apple and *Prunus* maps suggest a high level of synteny between these two genera. Conserved genomic regions have also been detected between *Prunus* and *Arabidopsis*. By using the data from different linkage maps anchored with the reference *Prunus* map, it has been possible to establish, in a general map, the position of 28 major genes affecting agronomic characters found in different species. Markers tightly linked to the major genes responsible for the expression of important traits (disease/pest resistances, fruit/nut quality, self-incompatibility, etc.) have been developed in apple and *Prunus* and are currently in use for marker-assisted selection in breeding programs. Quantitative character dissection using linkage maps and candidate gene approaches has already started. Genomic tools such as the *Prunus* physical map, large EST collections in both *Prunus* and *Malus*, and the establishment of the map position of high numbers of ESTs are required for a better understanding of the Rosaceae genome and to foster additional research and applications on fruit tree genetics.

approaches to cultivar improvement. Early selection with molecular markers allows an accurate screening of seedlings several years before the characters can be evaluated in the field, makes possible the accumulation of different resistance factors in a genotype of interest, or shortens the number of generations needed to recover the genotype of the cultivated species after a cross with an exotic genotype or wild species. Overall, these methods result in savings of space, and even more importantly, of time, factors already crucial in herbaceous species, and still more important in woody perennials. Given the poor level of genetic knowledge of these species, the research effort needed to bring them to the level of other important crops has been considerable, particularly in the last decade. Results are now emerging, some important tools (markers, maps, DNA sequences, and quantitative trait loci, QTLs) have been developed and made available to researchers, and applications at the breeding program level have been started. In this paper, we summarize the main results obtained for this group of crops with special emphasis on the members of the *Prunus* genus, which have experienced a particularly fast growth.

Methods: Linkage Map Construction

The *Prunus* Reference Map. As a result of a European project, a saturated linkage map of 246 markers (235 RFLPs and 11 isozymes) in an almond (cv. Texas) × peach (cv. Earlygold) F_2 progeny was constructed (5). This map (the TxE map) detected

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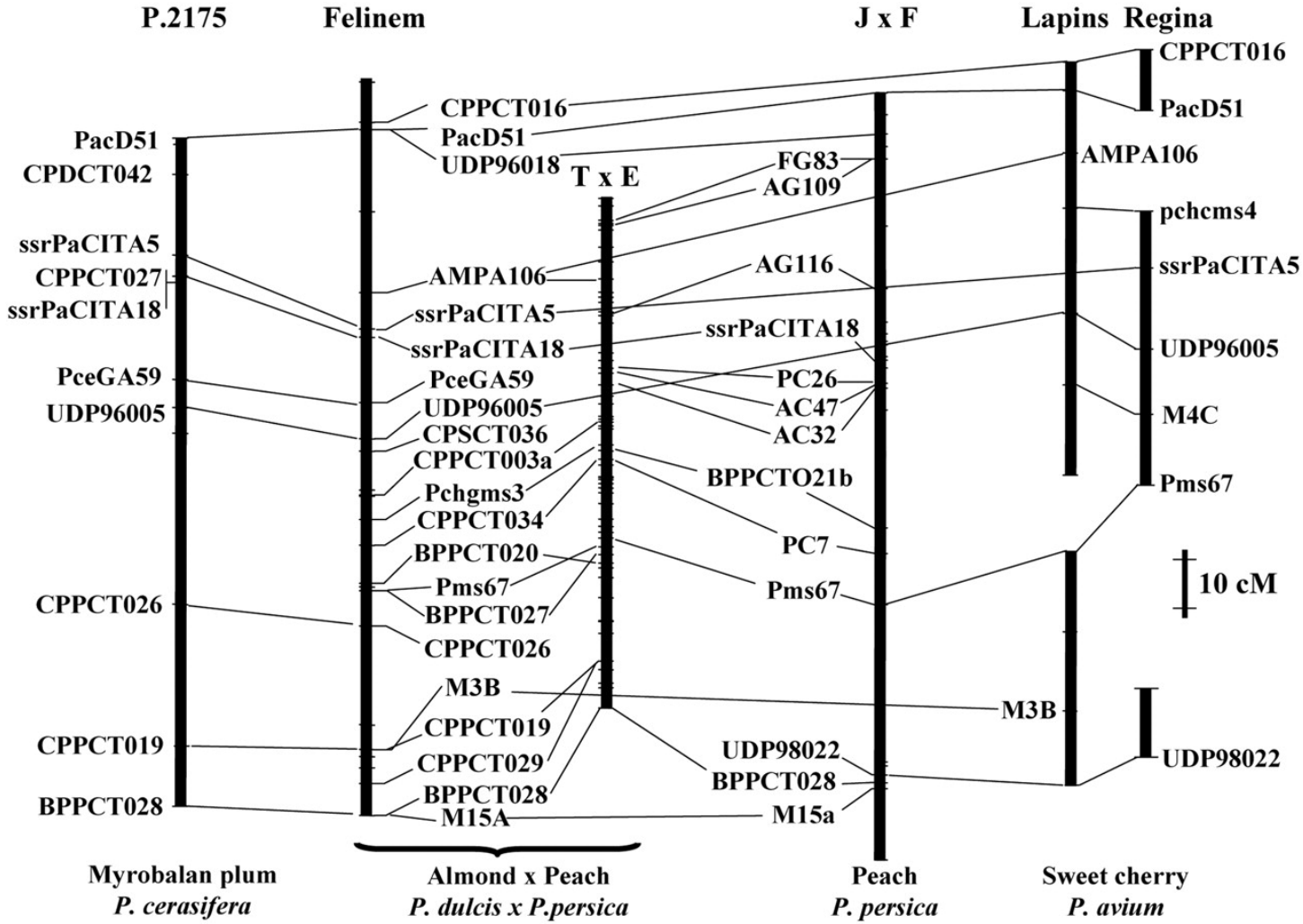
http://www.pnas.org/content/101/26/9891.full.pdf

Comparison among the SSR maps of linkage group 1 in the almond × peach Prunus reference map (TxE), peach (cv



Dirlewanger E. et.al. PNAS 2004;101:9891-9896

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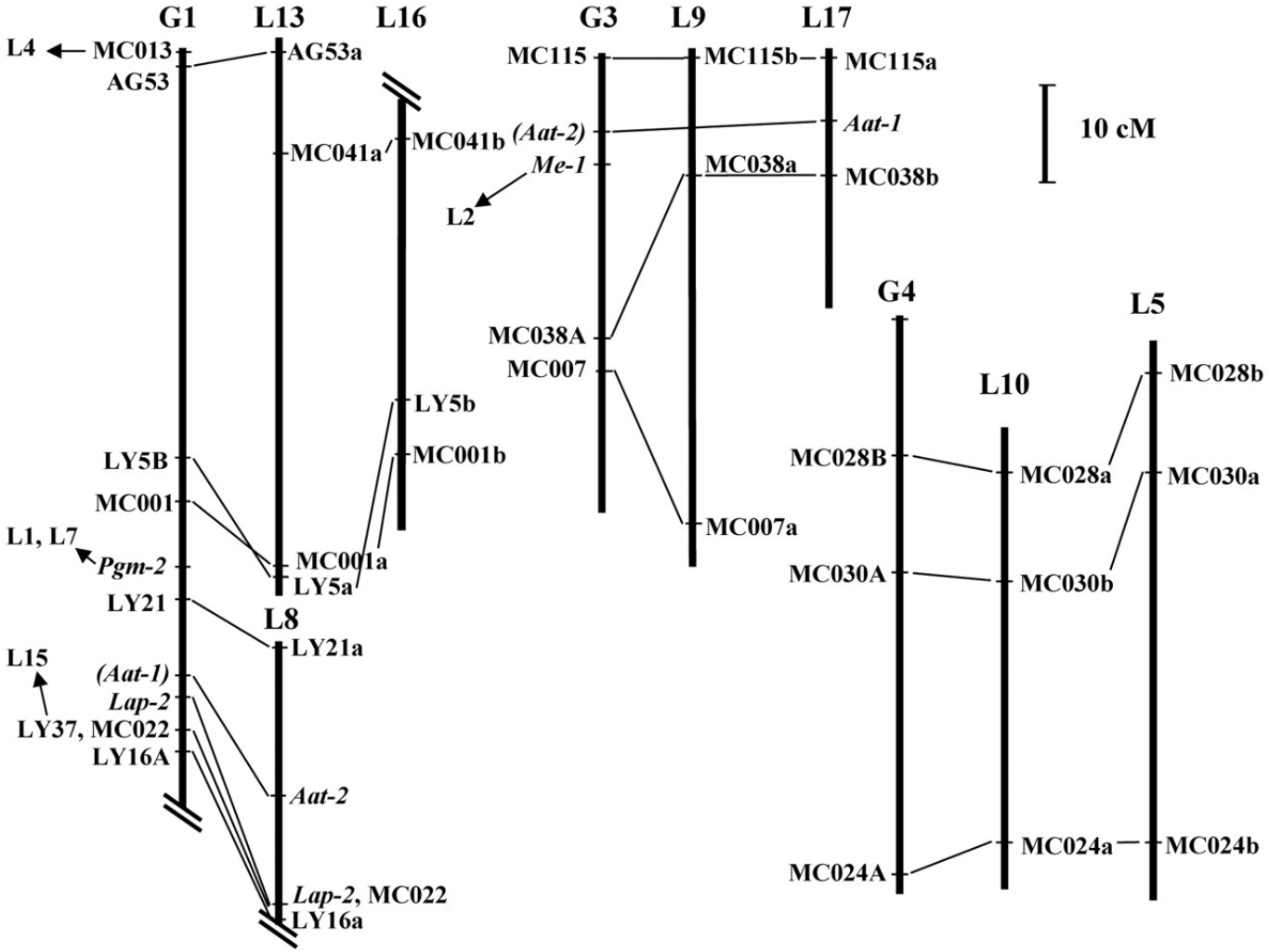
Comparison between Prunus (5) and apple (21) linkage maps



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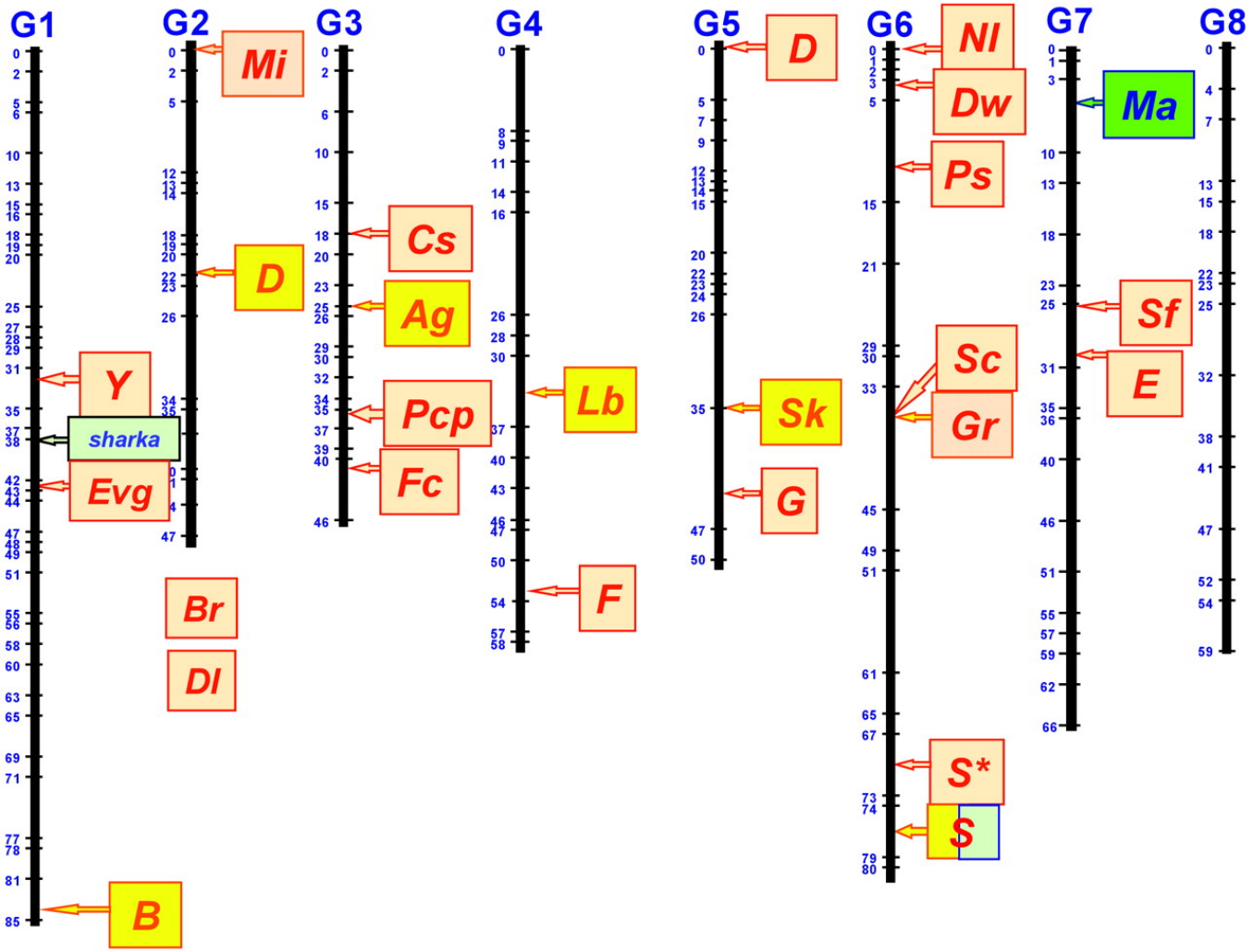
Approximate position of 28 major genes mapped in different populations of apricot (blue background), peach (orange background), almond or almond × peach (yellow background), and Myrobalan plum (green background) on the framework of the Prunus reference map (5)



Dirlewanger E. et.al. PNAS 2004;101:9891-9896

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Linkage disequilibrium (LD)

Il **linkage disequilibrium** (LD) è un termine dell'ambito genetico che indica la presenza di associazione **statistica** tra specifici **alleli** relativi a due o più loci, non necessariamente presenti sullo stesso cromosoma, che costituiscono di solito un particolare aplotipo ancestrale, diffuso nella popolazione in cui è rilevato perché trasmesso lungo la discendenza da un comune progenitore. Per questo motivo il linkage disequilibrium è maggiore in popolazioni omogenee, cioè originate da un nucleo di individui fondatori come le popolazioni sarda o finlandese. Infatti le migrazioni recenti generano substruttura e eterogeneità genetica ed aplotipi differenti associati allo stesso tratto tendono ad abbassare i valori di significatività.
Il linkage disequilibrium è un importante strumento per individuare regioni cromosomiche di limitata ampiezza in cui si collocano i geni per una data malattia (mappaggio ad alta risoluzione) e si avvale dell'analisi molecolare di varianti alleliche (per lo più di SNPs o STRs) che costituiscono aplotipi in pazienti tra loro apparentemente non imparentati. Infatti è prevedibile che pazienti che hanno ereditato lo stesso segmento cromosomico, definito dal medesimo aplotipo, abbiano ereditato anche la stessa mutazione in esso contenuto. Risale agli anni '80, il primo gene mappato con questo approccio: quello della fibrosi cistica in 7q31.2.

Bibliografia

Strachan T. e Read A.P. Human Molecular Genetics 3 Garland Publishing 2004

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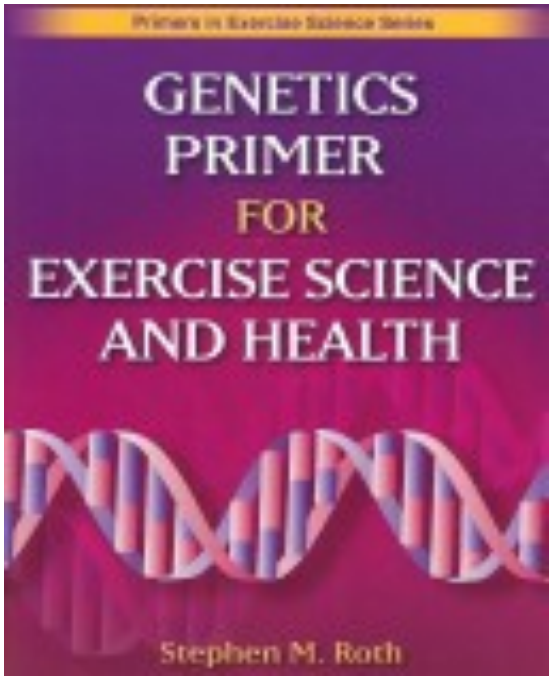
http://it.wikipedia.org/wiki/Linkage_disequilibrium



http://books.google.com/books?id=KzjR8_JSDxgC&pg=PA63&lpg=PA63&dq=linkage+disequilibrium+exercise&source=bl&ots=w8VHLo4UKm&sig=5fgh8HIwo1-B-E0Q1ddoaZislKg&hl=it&sa=X&oi=book_result&resnum=6&ct=result#PPA61,M1

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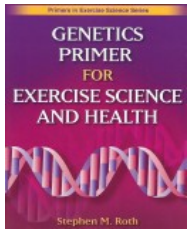

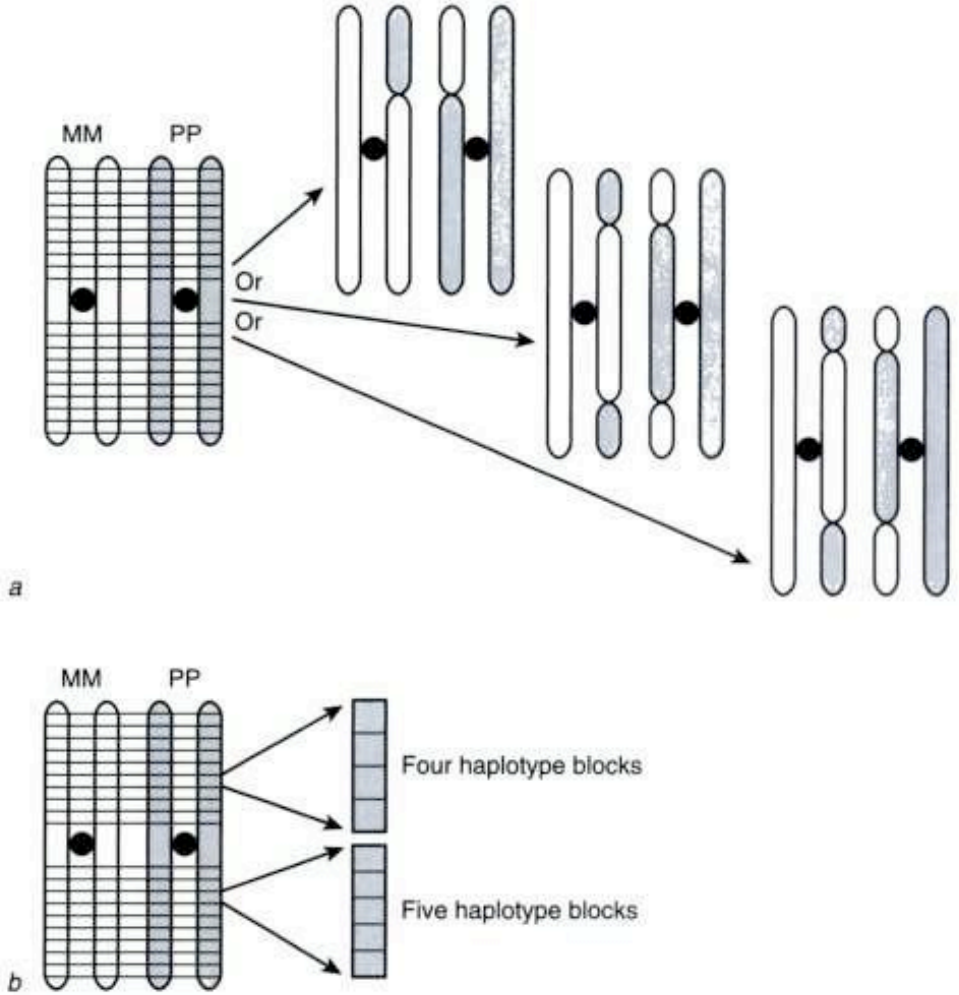

Figure 7.1 While crossover allows the exchange of genetic material between homologous chromosomes (recall figure 4.3), those crossover breakpoints actually occur at specific locations along the chromosomes, known as recombination hotspots. *(a)* Recombination hotspots are shown as lines on the parental chromosomes. During crossover, blocks of DNA sequence can be exchanged at any of those recombination hotspots, meaning that many combinations of DNA exchange are possible (three possibilities are shown; there are many more). *(b)* What is not clear from figure 7.1*a* is that these larger recombination blocks are actually built of many smaller haplotype blocks. In families, this means that DNA is exchanged as large segments of sequence known as *recombination blocks* (each containing many haplotype blocks). Across the human population, hotspot locations are identified by linkage between different haplotype blocks, many of which would be exchanged in a single recombination event as a recombination block.

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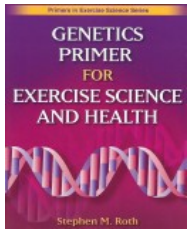

KEY POINT

In recombination of DNA during crossover, DNA sequence is exchanged across homologous chromosomes in large blocks, and the block boundaries, or hotspots, are shared across a population. These blocks are known as recombination blocks in families or haplotype blocks in populations.

KEY POINT

Linkage disequilibrium refers to the extent to which two nearby polymorphisms (or alleles within polymorphisms) travel together during recombination events. Because the alleles travel together over generations, one polymorphism acts as a "marker" for the other polymorphism (i.e., knowing the allele present at one polymorphism provides information about the other polymorphism when they are in linkage disequilibrium).

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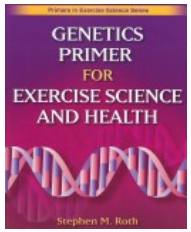

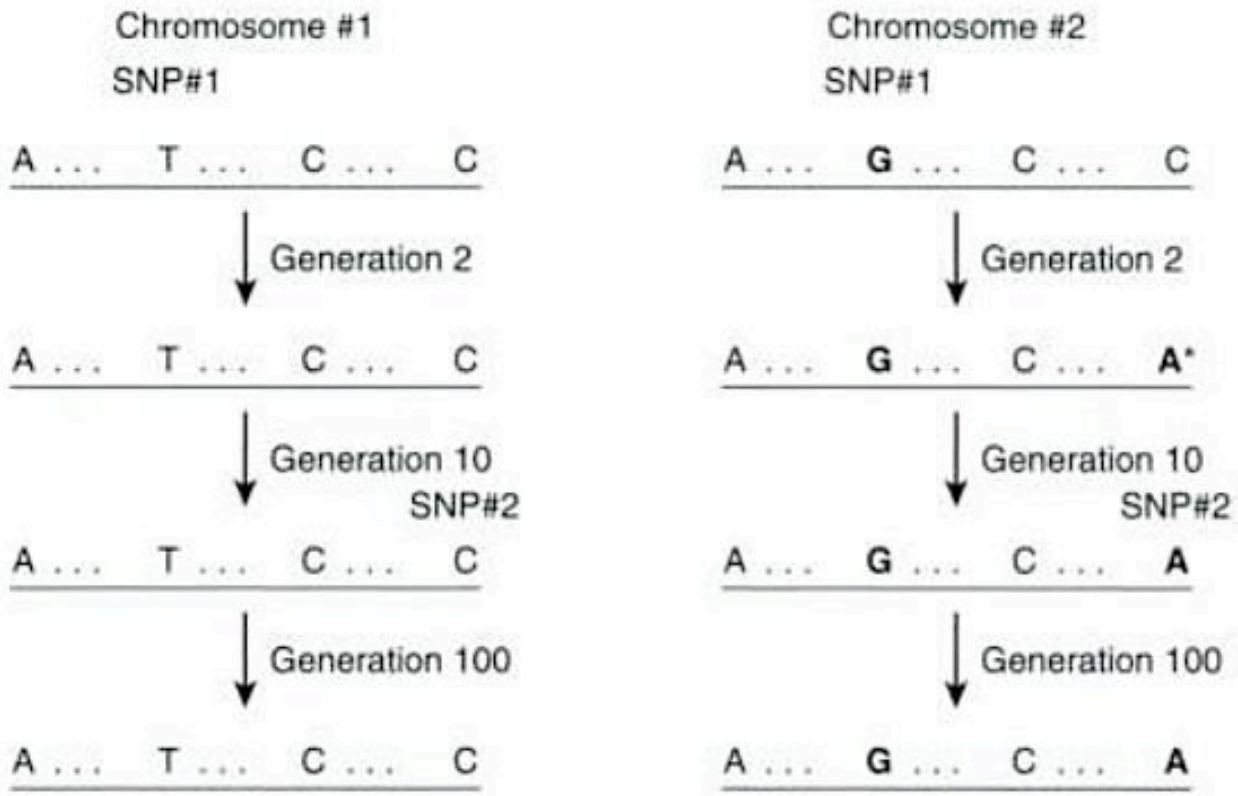

Figure 7.2 Two different DNA sequence segments from a specific region of the same chromosome. This DNA region contains one single-nucleotide polymorphism (SNP) in generation 1, with the chromosome sequence on the left carrying the T allele and the chromosome on the right carrying the G allele. Notice in generation 2 that a novel mutation exists on one of the chromosomes. Over many generations, this is passed along to other members of the population, thus becoming a polymorphism (labeled as SNP #2). If we look at the alleles carried at each of the two polymorphisms, notice that over time the A allele of SNP #2 is always found with the G allele of SNP #1; there is no evidence of recombination occurring between the location of SNP #1 and SNP #2 on the gene. In other words, within the haplotype block that carries SNPs #1 and #2, the alleles T and G tend to travel together over generations, and the alleles G and A tend to travel together—they are not exchanged within the block. Notice also that the other nearby nucleotides are also traveling together; as these are not polymorphisms, there is no variation at the position. Thus, SNP #1 and SNP #2 (or alleles G and A or T and C within those SNPs) are said to be in *linkage disequilibrium*, and knowing one allele of the pair can be used to predict the other allele.

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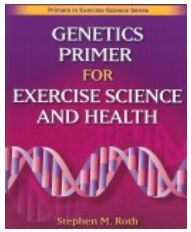

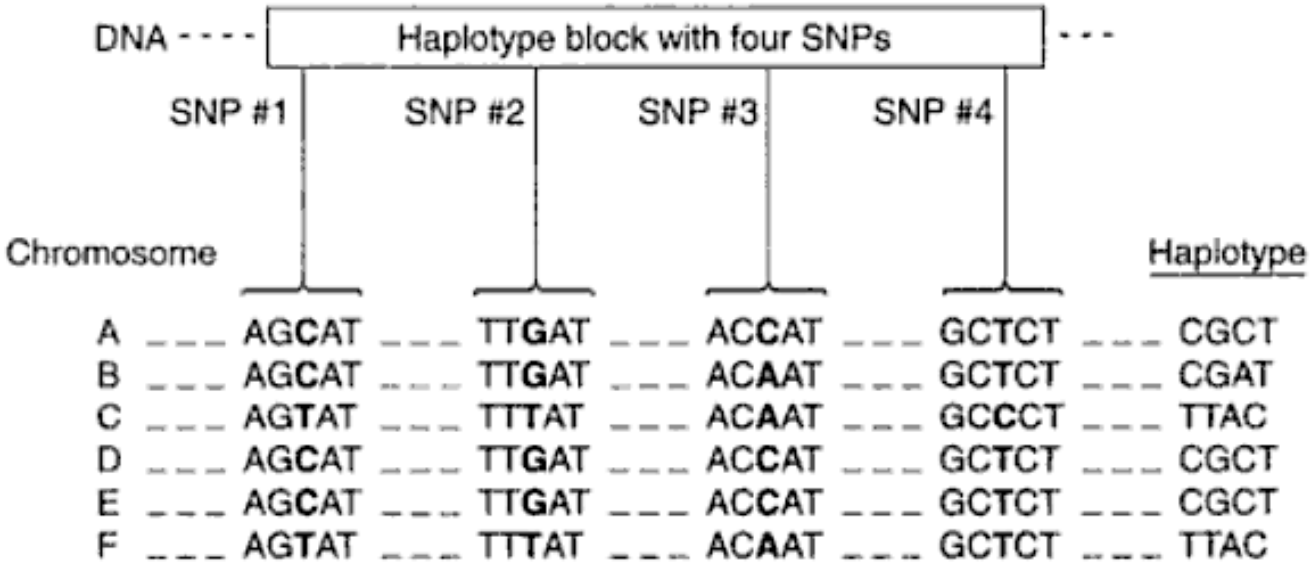

Figure 7.4 A haplotype block in which four SNPs are present. The alleles within these four SNPs are thus in linkage disequilibrium and tend to be carried together during recombination events. The particular pattern of alleles carried within a haplotype block determines the haplotype for that chromosome. In this example, the combination of C, G, C, and T alleles carried for chromosome A at the four SNPs can be called haplotype "CGCT." Similarly, different allele combinations at the four SNPs can result in other haplotype spellings. In this example, six different chromosome sequences were examined, and three different haplotypes were identified: CGCT (carried by three chromosomes), TTAC (carried by two chromosomes), and CGAT (carried by one chromosome).

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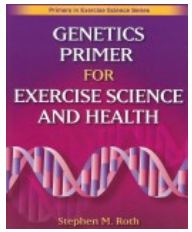

with "common" defined as being present in at least 5% of individuals in a population. Just as there are very rare alleles present in as few as one person in a population, very rare haplotypes also exist for each haplotype block, but our focus is on those haplotypes that are common in a population and thus suitable for study from a genetic association perspective.

The other unique attribute of alleles within haplotypes is that very often some alleles are in complete linkage disequilibrium with other alleles, making one allele completely informative for one or more other alleles in that block. Thus, when we are genotyping alleles to determine haplotype in an individual, complete linkage disequilibrium makes it unnecessary to genotype all of the 30+ SNPs in the block. In fact, most data suggest that only 10% to 25% of all SNPs in a haplotype block will need to be genotyped in order to accurately define the specific haplotype spelling for that block. The SNPs used to define haplotype are thus known as **tag SNPs,** in that they act as tags or markers for other alleles within the block. An example of tag SNPs is shown in figure 7.5. Researchers are able to genotype this smaller subset of tag SNPs and thus determine the full haplotype spelling for any particular block because of the principle of linkage disequilibrium.

How are haplotypes potentially more important than single polymorphisms in genetic association studies? The simple answer is that more of the genome is being studied when haplotypes versus single polymorphisms are used; thus the opportunity for identifying important genome regions is improved. In fact, the major goal of haplotype-based analysis in genetic association studies is very similar to the goals of linkage analysis in families. In linkage analysis, marker polymorphisms are used to identify large genome regions correlated with the trait of interest in family members, which share large blocks of DNA sequence. In haplotype analysis, tag SNPs are used to identify smaller genome regions associated with the trait of interest in unrelated individuals, which share smaller (haplotype-size) blocks of DNA sequence. The similarity between the two approaches is that the causal allele (i.e., functional allele) is not necessarily expected to be one of the marker polymorphisms that is genotyped; rather the markers will be in linkage disequilibrium with the nearby functional or causal allele. In identifying the smaller block region by relying on linkage disequilibrium,

KEY POINT

Tag SNPs can be used to identify a specific haplotype without the need for all polymorphisms within that haplotype to be genotyped individually. This is possible because some SNPs within a haplotype will be in complete linkage disequilibrium and will be completely redundant for each other.

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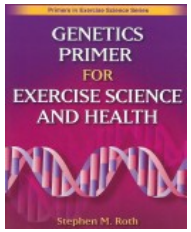

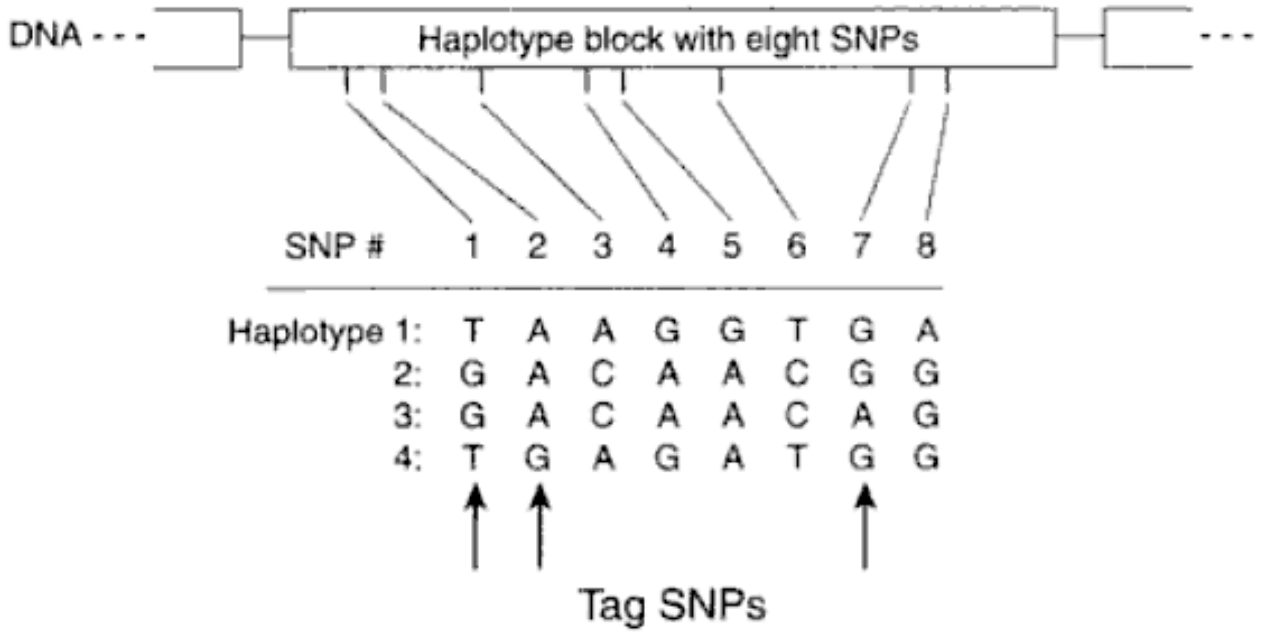

Figure 7.5 A haplotype block with eight SNPs. Across those eight SNPs, four different haplotype spellings are seen in a population (haplotype 1 is most frequent, with haplotypes 2-4 having lower frequencies). Not all eight SNPs must be genotyped in order to determine the particular haplotype carried by an individual. In fact, only three of the SNPs are needed to determine the specific haplotype (indicated by arrows; note that other combinations of SNPs could also be chosen as tag SNPs). These three SNPs are known as *tag SNPs*. For example, notice that the alleles carried in SNP #1 (T and G) match perfectly the alleles carried in SNP #3 (A and C), SNP #4 (G and A), and SNP #6 (T and C), thus making the genotyping of all four redundant and inefficient. Similarly, SNP #5 and SNP #8 are completely redundant for each other. Different combinations of SNPs can be identified as tag SNPs, but the goal is to identify the lowest number of tag SNPs that can identify all haplotype sequences. Various software programs are available to do this.

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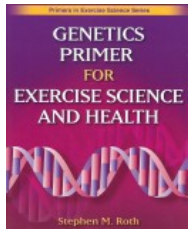

SPECIAL FOCUS

(continued)

Begun in 2002, a first draft of the **HapMap** was published in late 2005, containing more than 1 million SNPs genotyped in 269 individuals across the four populations. An expanded HapMap database of over 3 million SNPs was made available at www.hapmap.org in 2006, and the project researchers will continue by performing more genotyping in the four populations, as well as examining tag SNPs in other populations to confirm their usefulness for genetic association studies. Ultimately, the HapMap gives researchers a tool with which genetic association studies can be performed across larger areas of the genome. Rather than focusing on a small number of polymorphisms in a single gene, researchers will be allowed with use of the tag SNPs to examine (via linkage disequilibrium) much larger segments of genome sequence with fewer genotypes. And with the continued development of high-throughput genotyping methods, the ability to perform genome-wide association studies in unrelated individuals is no longer science fiction.

The HapMap is not without its detractors, who argue that haplotype boundaries are not easily defined and that the size and location of blocks are likely to be different if different tag SNPs are genotyped. Ultimately, even knowing these limitations about using haplotypes for genetic association studies is meaningful, so few doubt that the HapMap will provide important information for human geneticists. In fact, even by the time this section was written, the draft version of the HapMap was used successfully for the identification of a gene polymorphism important for the development of age-related macular degeneration.

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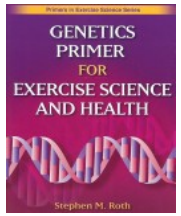

DIPLOTYPE: COMBINATIONS OF HAPLOTYPES

Thus far, the use of haplotypes in genetic association analysis has been presented as a fairly straightforward identification of tag SNPs, from which haplotype can be determined and used in genetic studies. Unfortunately, the use of haplotypes in such studies is more complex than the discussion so far has indicated.

Recall that for any gene in the genome (excepting the X and Y chromosomes in men), every individual carries two copies of that gene, one from each parent. Thus, for any single polymorphism, we don't simply report the allele carried in a particular gene sequence (e.g., A or G); we report the combination of alleles present in both gene copies, which we call the *genotype* (e.g., A/A, A/G, or G/G). The same holds true for haplotype. Each gene copy will contain a particular haplotype spelling within a haplotype block, and any individual will thus carry a combination of *two* haplotypes, one from each parent, in his or her genome. The pair of haplotypes that each individual carries for a particular block is known as a **diplotype.** Identifying diplotype in an individual is much more challenging than determining a single-polymorphism genotype, and the genetic analysis can become more complex.

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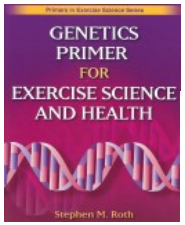
Why is diplotype so difficult to measure? Figure 7.7 illustrates the problem. Any particular person will carry two gene copies for any autosomal gene, which means that he or she carries two different haplotype sequences for any particular haplotype block. In the three examples shown, we see that the maternal autosome and the paternal autosome each have a unique haplotype, the combination of which is known as a *diplotype* (this is similar to two alleles making up a genotype). Thus, each of these individuals would be considered heterozygous for haplotype. When one performs any typical genotyping assay for a single polymorphism, both gene copies are present in the assay, such that the final result represents the combination of the two alleles, which is then read as a genotype, or combination of the two alleles carried by the individual. In determining haplotype, the same types of data are obtained for each of the tag SNPs genotyped in the haplotype block, resulting in a genotype for each tag SNP. Thus, for each person, you get one genotype for each of the tag SNPs genotyped.

The problem for determining diplotype is that the specific combination of alleles that are carried *on a particular chromosome sequence* cannot typically be determined by genotype alone. In other words, which gene copy (Mom's or Dad's) contains which combination of alleles? Identifying the source chro-



KEY POINT

Because we each carry two copies of each autosome (and females carry two copies of the X chromosome), we actually carry two haplotypes for any particular haplotype block, one on the maternal and one on the paternal chromosome. This combination of haplotypes is known as a diplotype (this is similar to two alleles making up a genotype).

mosome of particular alleles is known as determining the **phase** of the alleles, or knowing whether or not alleles at two or more linked polymorphisms are on the same or different chromosomes.

If we consider only two SNPs, the issue of phase (sometimes called "phase ambiguity") is problematic only for individuals heterozygous at both tag SNPs. If a person is homozygous at either SNP, we know that the same allele is traveling on both chromosomes, whereas double heterozygotes could have either of two haplotype combinations: For the neighboring SNPs A/T and C/G, a person heterozygous at both sites could have haplotypes A-C and T-G, or haplotypes A-G and T-C. The problem is further compounded with the addition of more SNPs, which is often the case when one is genotyping tag SNPs for haplotypes (three to five tag SNPs may be needed to distinguish the major haplotypes in a block). A detailed example of this is shown in figure 7.7. Thus, defining haplotype and diplotype for an individual can be problematic.

There are two main ways to determine diplotype for any individual: (1) a brute force molecular analysis, in which the two gene copies are physically separated and individually sequenced to determine the exact haplotype sequences and diplotype, or (2) estimation of diplotype from the tag SNP genotype data using statistical procedures. The first technique is very labor-intensive and costly, and is not used frequently. Occasionally, for smaller blocks with fewer tag SNPs, easier laboratory methods may allow direct haplotype and diplotype determination. The vast majority of researchers opt for estimation techniques, which rely on statistical procedures that estimate the sequence of the two haplotypes based on the total combination of genotypes present at each tag SNP. Various statistical programs have been developed to perform this estimation, and all rely on using the measures of linkage disequilibrium among the tag SNPs to help guess at which haplotype combinations are more likely across a population, and therefore more likely

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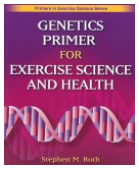

Individual	Diplotype	Genotypes		
#1	Maternal haplotype = G C T + Paternal haplotype = G C A	SNP #1 G/G	#2 C/C	#3 T/A
#2	Maternal haplotype = G C T + Paternal haplotype = A C T	SNP #1 G/A	#2 C/C	#3 T/T
#3	Maternal haplotype = G C T + Paternal haplotype = A T A	SNP #1 G/A	#2 C/T	#3 T/A

Figure 7.7 A person's diplotype can be challenging to determine directly using only genotype information. If we imagine that each of the three SNPs within the haplotype block was genotyped for each individual (shown on the right side of the figure), diplotype can be predicted for individuals #1 and #2 because they are homozygous for no more than one of the three SNPs. In contrast, the haplotypes and thus diplotype of individuals who are heterozygous for two or more SNPs within a haplotype block cannot be determined without additional information (as is the case for individual #3). In other words, with just the genotypes of G/A, C/T, and T/A for individual #3, we cannot be certain which haplotypes that person is carrying without additional information (i.e., GCT, GTT, GTA, GCA, etc., are all possible).

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Crossover: Concepts and Applications in Genetics, Evolution, and Breeding : an Interactive Computer-based Laboratory Manual
Di Jack E. Staub, Karl Braunschweig, Claire Rinehart
Collaboratore Karl Braunschweig, Claire Rinehart
Pubblicato da Univ of Wisconsin Press, 1994
ISBN 0299135640, 9780299135645
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Features

A handheld way to prevent adverse drug reactions

Feb. 17, 2009

A handheld device to predict whether patients will respond adversely to medication is one step closer to the market, thanks to a new partnership announced today.

Imperial College London and its spinout company DNA Electronics have developed a prototype healthcare device that assesses whether patients are genetically predisposed to suffering adverse reactions to prescription drugs. They are now carrying out trials to test its effectiveness, thanks to a new partnership with the pharmaceutical company Pfizer.

Each year, the NHS spends £460 million to treat 250,000 patients who are admitted to hospital suffering adverse reactions to prescribed medication. These reactions can vary in severity, from dizziness and nausea to heart palpitations or unconsciousness.

A test to identify people likely to react badly to prescribed medication such as anti depressants or drugs to lower cholesterol could enable doctors to tailor dosages and drugs to the individual needs of each patient.

The device undergoing trials is the Single Nucleotide Polymorphism Doctor, or SNP Dr (pronounced 'snip doctor'). It is a portable technology that gives fast accurate spot test results for specific DNA sequences that indicate how we are likely to respond to certain drugs.

The SNP Dr works by analyzing genetic variations found in DNA called Single Nucleotide Polymorphisms (SNPs). SNPs are the parts of human DNA that make us all respond differently to disease, bacteria, viruses, toxins or medication.

In particular, researchers are exploring how the SNP Dr might detect genetic sequences linked with metabolism. A slow metabolism can make drugs stay in the body longer, causing adverse side effects, while a fast metabolism can process medication too quickly for it to have any effect.

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DNA Tests Confirm Remains of Russian Royals Killed During Revolution

February 24, 2009
By Andrea Anderson

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NEW YORK (GenomeWeb News) – DNA evidence has verified that remains found in two graves near the Russian city of Yekaterinburg belonged to the Romanovs, Russia's last royal family. The research, scheduled to appear online this week in the *Proceedings of the National Academy of Sciences*, confirm that Czar Nicholas II and his entire nuclear family were killed during the Russian Civil War.

Researchers from the Russian Academies of Science and Medical Science, the University of Massachusetts, the Lomonosov Moscow State University, and the Canadian company Molecular World used DNA forensics to identify human remains found at two grave sites discovered more than 15 years apart. By isolating, sequencing, and piecing together mitochondrial and nuclear DNA from bone shards at each site, the team could compare the DNA with the family's living relatives and other sources.

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http://www.floragenex.com/filestore/FGXPAG17poster.pdf

Sequenced RAD Markers: A SNP Discovery and Genotyping Platform for Plants and Animals

FLORAGENEX

Abstract

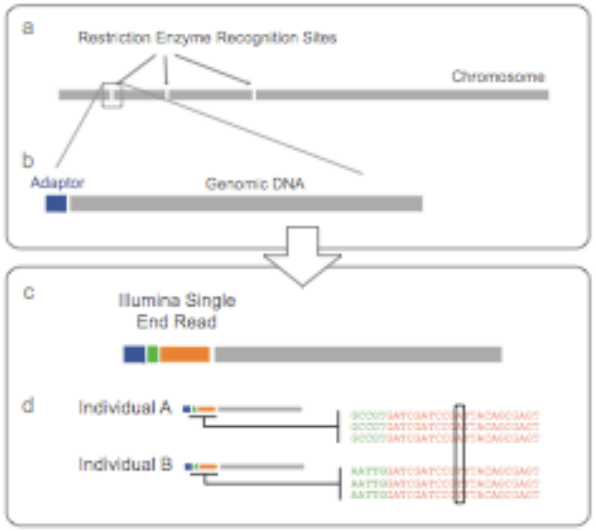
RAD (Restriction-site Associated DNA) markers are short tags of DNA adjacent to restriction enzyme recognition cut sites throughout a genome. Assaying RAD markers by massively parallel sequencing allows the rapid detection of polymorphisms within or near the cut site. The complexity reduction produced by RAD markers allows high coverage of marker sequence for high confidence SNP detection. We have further developed a multiplexing capability to simultaneously assay many samples in a sequencing run. We demonstrate the performance and capabilities of the RAD marker platform by reporting the discovery of hundreds to thousands of SNPs in a variety of plant and animal species, as well as the genotyping of many individuals in a population for trait mapping and population structure analysis.

Introduction

Restriction enzyme based marker systems such as RFLPs and AFLPs have been standard marker systems in plants for decades. Floragenex's Restriction site Associated DNA technology uses a similar "complexity reduction" method coupled to 2nd generation sequencing platforms to enable cost-effective DNA sequencing (Baird, et al. PLoS ONE, 2008). This adaptation permits deep (high depth) sequencing of DNA adjacent to restriction enzyme sites within a genome, allowing identification of single nucleotide polymorphisms (SNPs) for use as genetic markers in molecular assisted selection or breeding.

Figure 1 presents an overview of RAD sequencing. Genomic DNA is digested (a) and an oligonucleotide adaptor with a nucleotide index (barcode) is affixed to the resulting asymmetric overhangs (b). This adaptor directs the sequencing of adjacent DNA on next generation platforms utilizing sequencing by synthesis (c). Sequences from the two individuals are sorted by index (green) and common loci are scanned for SNPs (d).

Figure 1 - Overview of RAD sequencing



1. Resequencing / SNP Discovery

Introduction:
The purpose of this study was to assess the performance and sampling of Restriction site Associated DNA (RAD) markers within the maize genome.To foster sequencing within the gene space of Maize, a species with a large genome, Floragenex used a methylation sensitive restriction enzyme (NotI) to create RAD tags with the expectation of reduced sampling of repetitive retrotransposon elements subject to CpG methylation.

2. Cultivar Identification

Introduction:
Floragenex generated whole genome RAD marker profiles of two reference cultivars of blueberry (*Vaccinium ssp*) to develop a genetic fingerprint which could be used as a diagnostic test for varietal identification. Genomic DNA from elite blueberry lines designated "Emerald" and "Jewel" was digested with SbfI and processed into RAD tags.

Figure 4. Varietal identification Schematic

3. Bulked Segregant Analysis

Introduction:
Floragenex collaborated with an investigator at Oregon State University to identify genetic markers linked to a genetic locus conferring resistance to eastern filbert blight (EFB) in Hazelnut (*Corylus avellana*). EFB resistance is a dominant, qualitative trait. Two F1 populations segregated by resistance and susceptibility contributed 10 seedlings each to the study. The seedlings were selected such that each group had several recombination events flanking the resistance locus (Figure 6).

4. Population Structure

Introduction:
To derive a portrait of genetic diversity within the vertebrate model organism *Danio rerio* (zebrafish), DNA from fourteen individuals representing seven major zebrafish breeding strains (1 male and 1 female fish per strain) was digested by SbfI and converted into RAD tags.

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http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003376

Baird_2008_(SNP-discovery-mapping-RAD-markers).pdf

RESEARCH ARTICLE OPEN ACCESS

Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers

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Abstract

Single nucleotide polymorphism (SNP) discovery and genotyping are essential to genetic mapping. There remains a need for a simple, inexpensive platform that allows high-density SNP discovery and genotyping in large populations. Here we describe the sequencing of restriction-site associated DNA (RAD) tags, which identified more than 13,000 SNPs, and mapped three traits in two model organisms, using less than half the capacity of one Illumina sequencing run. We demonstrated that different marker densities can be attained by choice of restriction enzyme. Furthermore, we developed a barcoding system for sample multiplexing and fine mapped the genetic basis of lateral plate armor loss in threespine stickleback by identifying recombinant breakpoints in F_2 individuals. Barcoding also facilitated mapping of a second trait, a reduction of pelvic structure, by *in silico* re-sorting of individuals. To further demonstrate the ease of the RAD sequencing approach we identified polymorphic markers and mapped an induced mutation in *Neurospora crassa*. Sequencing of RAD markers is an integrated platform for SNP discovery and genotyping. This approach should be widely applicable to genetic mapping in a variety of organisms.

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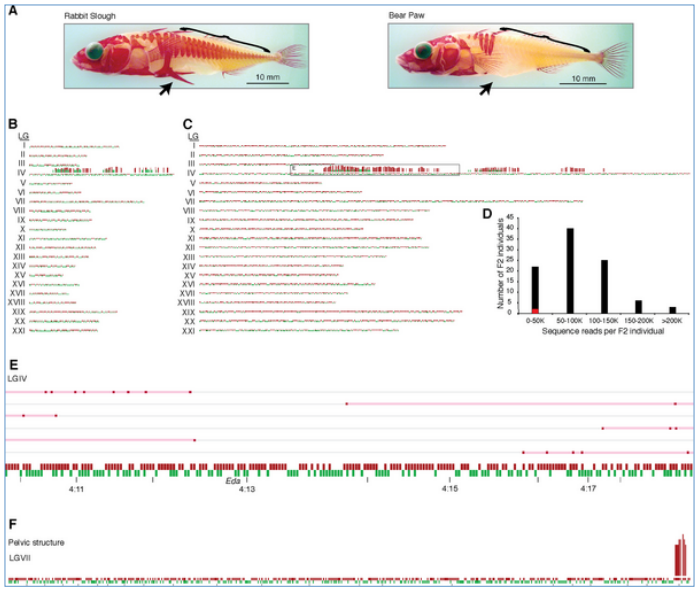
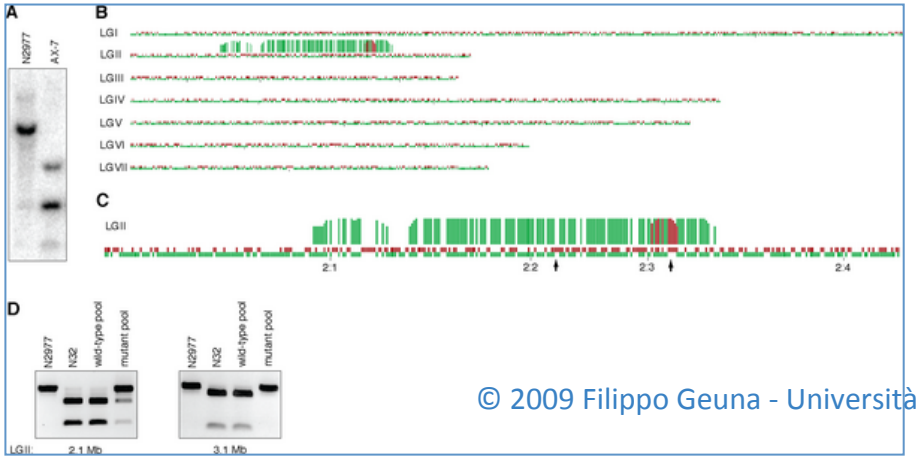
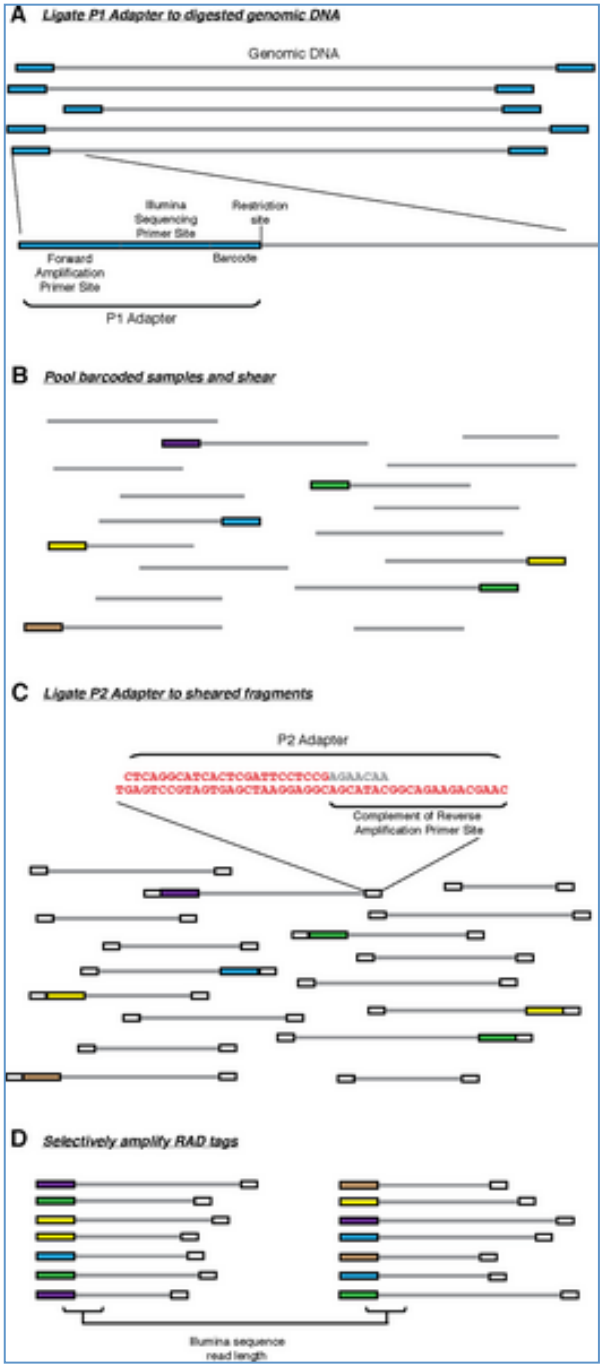
Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers







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NATURE METHODS | VOL.4 NO.11 | NOVEMBER 2007 | 907

Microarray-based genomic selection for high-throughput resequencing

David T Okou[1], Karyn Meltz Steinberg[1,2], Christina Middle[3], David J Cutler[1], Thomas J Albert[3] & Michael E Zwick[1,2]

We developed a general method, microarray-based genomic selection (MGS), capable of selecting and enriching targeted sequences from complex eukaryotic genomes without the repeat blocking steps necessary for bacterial artificial chromosome (BAC)-based genomic selection. We demonstrate that large human genomic regions, on the order of hundreds of kilobases, can be enriched and resequenced with resequencing arrays. MGS, when combined with a next-generation resequencing technology, can enable large-scale resequencing in single-investigator laboratories.

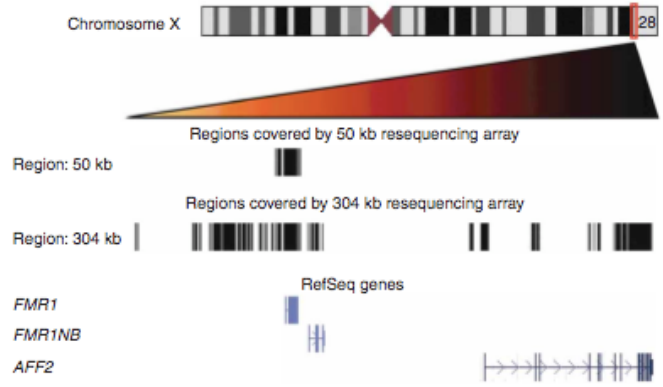

Figure 2 | Genomic regions (50 kb, 304 kb) resequenced in the two MGS validation experiments. Targeted sequences included both coding and unique noncoding genome sequences adjacent to Xq28. The shaded triangle represents an expanded view of regions resequenced.

http://www.nature.com.dl380.lib.unimi.it/nmeth/journal/v4/n11/pdf/nmeth1109.pdf

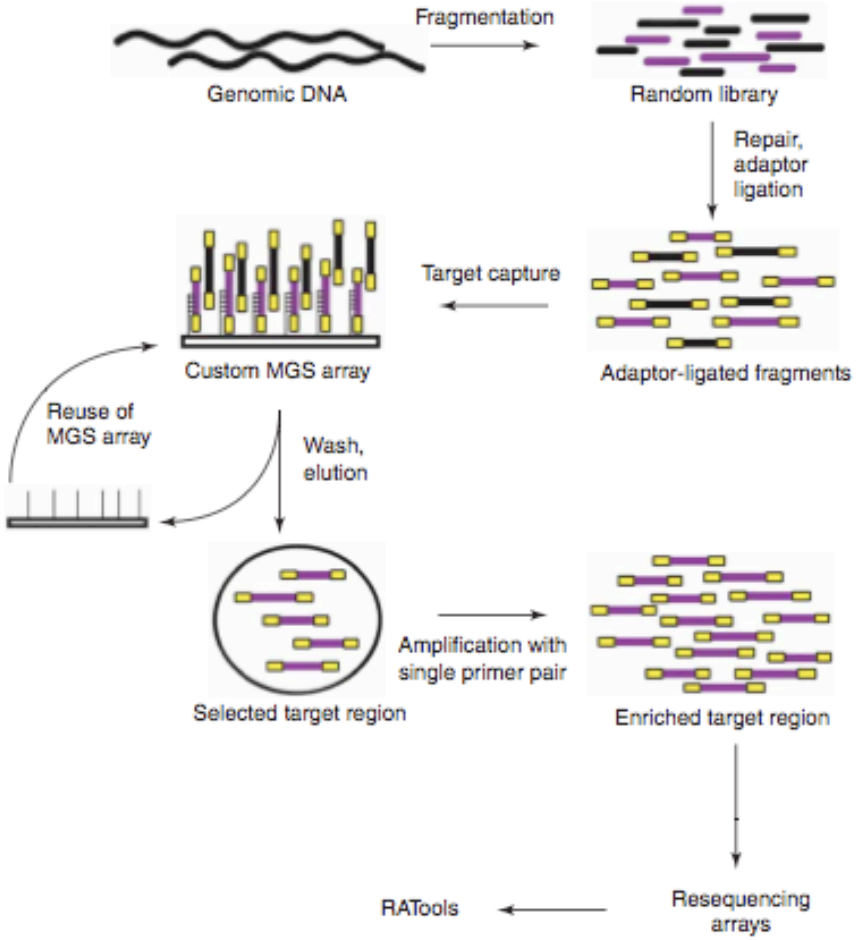

Figure 1 | Microarray-based genomic selection and resequencing of complex genomes. Sheared genomic fragments are repaired and ligated to generic adaptors. Hybridization to a custom designed high-density oligonucleotide microarray allows the capture of the target DNA regions. The selected target is eluted and amplified using a one-step PCR and a single primer pair per template. Amplified targets were resequenced with resequencing arrays and analyzed with RATools.

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http://www.genomeweb.com/node/912027

Articolo sulle metodiche di genotyping

Publications

de Bakker PIW, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. (2005). Efficiency and power in genetic association studies. Nature Genetics. 37: 1217-1223.

Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Hirschhorn JM, Abecasis G, Altshuler D, Bailey-Wilson JE, Brooks LD, Cardon LR, Daly M, Donnelly P, Fraumeni,Jr JF, Freimer NB, Gerhard DS, Gunter C, Guttmacher AE, Guyer MS, Harris EL, Hoh J, Hoover R, Kong CA, Merikangas KR, Morton CC, Palmer LJ, Phimister EG, Rice JP, Roberts J, Rotimi C, Tucker MA, Vogan KJ, Wacholder S, Wijsman EM, Winn DM, Collins FS for NCI-NHGRI Working Group on Replication in Association Studies. (2007). Replicating genotype–phenotype associations. Nature. 447: 655-660.

McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JPA, Hirschhorn JN. (2008). Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nature Reviews Genetics. 9: 356-369.

Packer BR, Yeager M, Burdett L, Welch R, Beerman M, Qi L, Sicotte H, Staats B, Acharya M, Crenshaw A, Eckert A, Puri V, Gerhard DS, Chanock SJ. (2006). SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Research. 34: D617-D621.

Walley DC, Tripp BE, Song YC, Walley KR, Tebbutt SJ. (2006). MACGT: multi-dimensional automated clustering genotyping tool for analysis of microarray-based mini-sequencing data. Bioinformatics. 22(9):1147-1149.

Yang Y, Li SS, Chien J, Andriesen J, Zhao LP. (2008). A systematic search for SNPs/haplotypes associated with disease phenotypes using a haplotype-based stepwise procedure. BMC Genetics. 9:90.

Websites

GeneWindow: http://genewindow.nci.nih.gov/

WelcomeSNP500Cancer: http://snp500cancer.nci.nih.gov/

TagZilla: http://tagzilla.nci.nih.gov/

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http://www.iwmi.cgiar.org/Publications/Water_Policy_Briefs/PDF/WPB30.pdf

IWMI International Water Management Institute

Water implications of biofuel crops:
understanding tradeoffs and identifying options

Box 1. Why the interest in biofuels?

- Rising oil prices
- Energy security concerns
- Boosting jobs and incomes in rural areas
- Lowering greenhouse gas emissions

But

- Water is essential to grow biofuel crops. Most biofuel crops are thirsty and compete for already scarce water.
- Clearing land and forests for biofuel crops releases carbon and reduces biodiversity.
- Crops such as maize and wheat used as raw materials for biofuel rather than for food mean less food and higher prices.
- Energy from biofuel isn't cheap. Biofuel production is still mostly subsidized.

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http://www.iwmi.cgiar.org/Publications/Water_Policy_Briefs/PDF/WPB30.pdf

Table 1. A fourfold increase in biofuel crops between 2005 and 2030 raises serious concerns for water supplies. Although the additional irrigation water needed to grow biofuel crops is just a few percent of the global total, the impacts in some countries could be highly significant, with serious implications for water resources. Rapidly growing economies such as China and India are unlikely to be able to meet future biofuel and food demands without greatly aggravating water scarcity, unless alternative feedstocks are used.

Country	*Main biofuel crop*	*% of total crop water used for biofuel*		*% of irrigation water used for biofuel*	
		2005	2030	2005	*2030*
Brazil	Sugarcane	10.7	14	3.5	8
USA	Maize	4	**11	2.7	**20
China	Maize	1.5	4	2.2	7
India	Sugarcane	0.5	3	1.2	5
EU	Rapeseed		17		1
World		1.4	3	1.1	4

** *includes Canada* *Source: de Fraiture et al. 2008*

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Table 2. How much water does it take to produce a liter of ethanol from crops such as maize or sugarcane? In tropical Brazil, where sugarcane is grown for biofuel, under rainfed conditions with limited irrigation, it takes only 90 liters of irrigation water to produce a liter of ethanol. But, in India, where sugarcane depends heavily on irrigation, it takes 3,500 liters of irrigation water.

Country	*Biofuel crop*	*Liters of irrigation water per liter of ethanol*
Brazil	Sugarcane – mostly rainfed	90
USA	Maize – mostly rainfed	400
Northern China	Maize – partly irrigated	2,400
India	Sugarcane – irrigated	3,500

Box 4. Sweet sorghum: a biofuel crop that doesn't need much water

Sorghum is already widely grown in dryland areas—11.7 million hectares in Asia and 23.4 million hectares in Africa. New varieties of sweet sorghum have multiple uses: the grain can be used for food, the leaves to feed animals, and the stalks to make ethanol.

Scientists working in India at ICRISAT have bred sorghum varieties that have lots of sugar-rich juice. These types of sweet sorghum need only one-seventh as much water as sugarcane. So, sweet sorghum has advantages over other biofuel crops because it is not so thirsty and doesn't replace food crops.

Source: ICRISAT pro-poor biofuel initiative
http://www.icrisat.org/Media/2007/media5.htm

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http://www.iwmi.cgiar.org/Publications/Water_Policy_Briefs/PDF/WPB30.pdf

Molecular Ecology Resources (2008) 8, 3–17 doi: 10.1111/j.1471-8286.2007.02019.x

TECHNICAL REVIEW

Sequencing breakthroughs for genomic ecology and evolutionary biology

MATTHEW E. HUDSON
Department of Crop Sciences, University of Illinois, Urbana, 334 NSRC, 1101 W. Peabody Blvd., IL 61801, USA

Abstract

Techniques involving whole-genome sequencing and whole-population sequencing (metagenomics) are beginning to revolutionize the study of ecology and evolution. This revolution is furthest advanced in the Bacteria and Archaea, and more sequence data are required for genomic ecology to be fully applied to the majority of eukaryotes. Recently developed next-generation sequencing technologies provide practical, massively parallel sequencing at lower cost and without the requirement for large, automated facilities, making genome and transcriptome sequencing and resequencing possible for more projects and more species. These sequencing methods include the 454 implementation of pyrosequencing, Solexa/Illumina reversible terminator technologies, polony sequencing and AB SOLiD. All of these methods use nanotechnology to generate hundreds of thousands of small sequence reads at one time. These technologies have the potential to bring the genomics revolution to whole populations, and to organisms such as endangered species or species of ecological and evolutionary interest. A future is now foreseeable where ecologists may resequence entire genomes from wild populations and perform population genetic studies at a genome, rather than gene, level. The new technologies for high throughput sequencing, their limitations and their applicability to evolutionary and environmental studies, are discussed in this review.

Keywords: DNA sequencing, ecology, evolution, genomic, metagenomics

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Hudson_2008_(Sequencing-breakthroughs-genomic-ecology).pdf

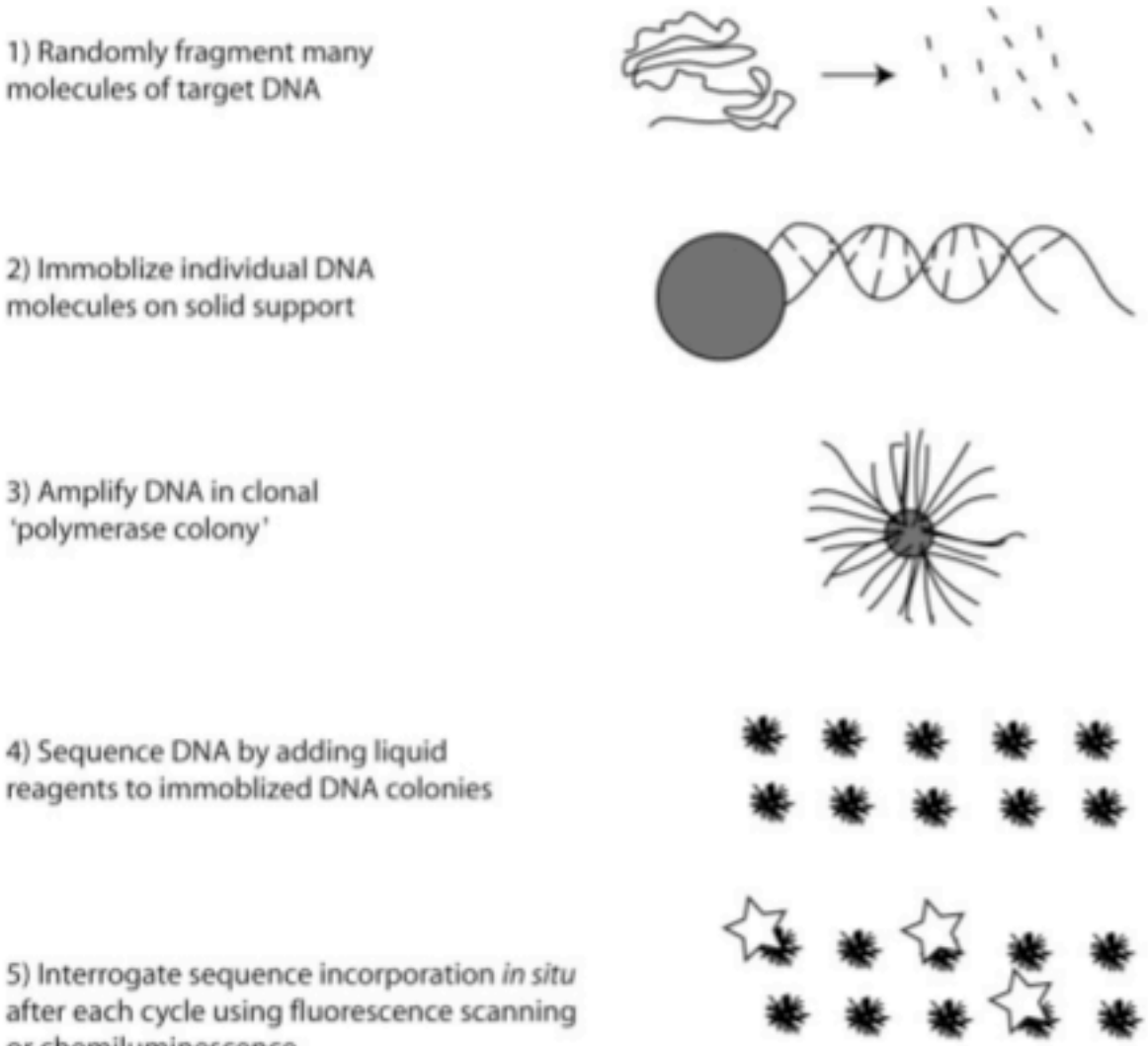

Fig. 2 A generalized description of the steps common to next-generation genome sequencing technologies. All these technologies involve genomic DNA random fragmentation, immobilization of single molecules on a solid support (a bead or planar solid surface), amplification by PCR, and subsequent *in situ* biochemical interrogation of the template DNA at each base in turn.

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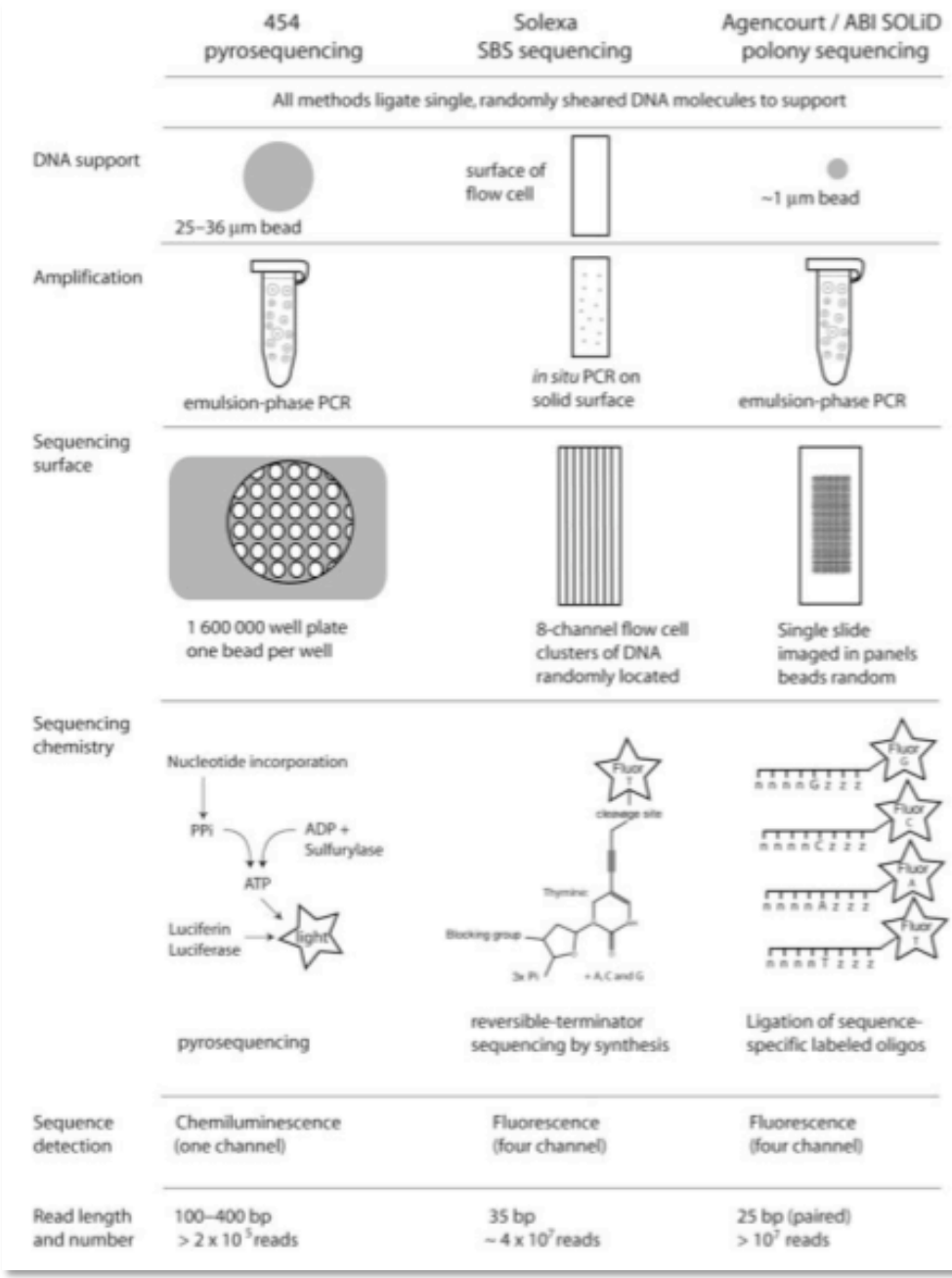

Fig. 3 A description of the key features of, and differences between, the three commercially available next-generation sequencing methods. The major steps in each procedure are arranged in the order in which they are performed by the operator or sequencing instrument. All three technologies share a common workflow, but differ greatly in the type of solid support used and the chemistry used to interrogate the DNA base pairs.

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http://www.genomenewsnetwork.org/articles/06_00/sequence_primer.shtml

SEQUENCING THE GENOME

By Bijal P. Trivedi

June 2, 2000

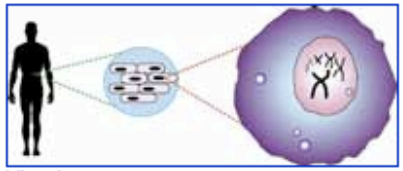
There are essentially two ways to sequence a genome. The BAC-to-BAC method, the first to be employed in human genome studies, is slow but sure. The BAC-to-BAC approach, also referred to as the map-based method, evolved from procedures developed by a number of researchers during the late 1980s and 90s and that continues to develop and change.*

The other technique, known as whole genome shotgun sequencing, brings speed into the picture, enabling researchers to do the job in months to a year. The shotgun method was developed by GNN president J. Craig Venter in 1996 when he was at the Institute for Genomic Research (TIGR).*

Now that the human genome sequence is nearing completion, the next phase—understanding the meaning and function of genes—can begin.

A primer on the two approaches to sequencing follows.

* References to the scientific literature are at the end of this primer.



View larger

The human body has about 100 trillion cells. Inside each of those cells is the nucleus that contains the genome—46 human chromosomes—which govern human development.

Each chromosome is one long string of DNA that is tightly coiled in a compact bundle. Chromosomes are comprised of millions of copies of the four letters of the genetic code—the DNA bases A, C, G, T—that are arranged into genes and non-coding sections. Finding the order, or sequence, of these four letters is the goal of genomics. The entire human genome is made up of about 3.5 billion bases.

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Trends in Biotechnology
Volume 26, Issue 11, November 2008, Pages 602-611

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doi:10.1016/j.tibtech.2008.07.003
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Review

Single-molecule DNA sequencing technologies for future genomics research

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Available online 21 August 2008.

During the current genomics revolution, the genomes of a large number of living organisms have been fully sequenced. However, with the advent of new sequencing technologies, genomics research is now at the threshold of a second revolution. Several second-generation sequencing platforms became available in 2007, but a further revolution in DNA resequencing technologies is being witnessed in 2008, with the launch of the first single-molecule DNA sequencer (Helicos Biosciences), which has already been used to resequence the genome of the M13 virus. This review discusses several single-molecule sequencing technologies that are expected to become available during the next few years and explains how they might impact on genomics research.

Article Outline

Introduction
'State of the art' of single-molecule sequencing

Sequencing by synthesis
Nanopore sequencing
Transmission electron microscopy for DNA sequencing

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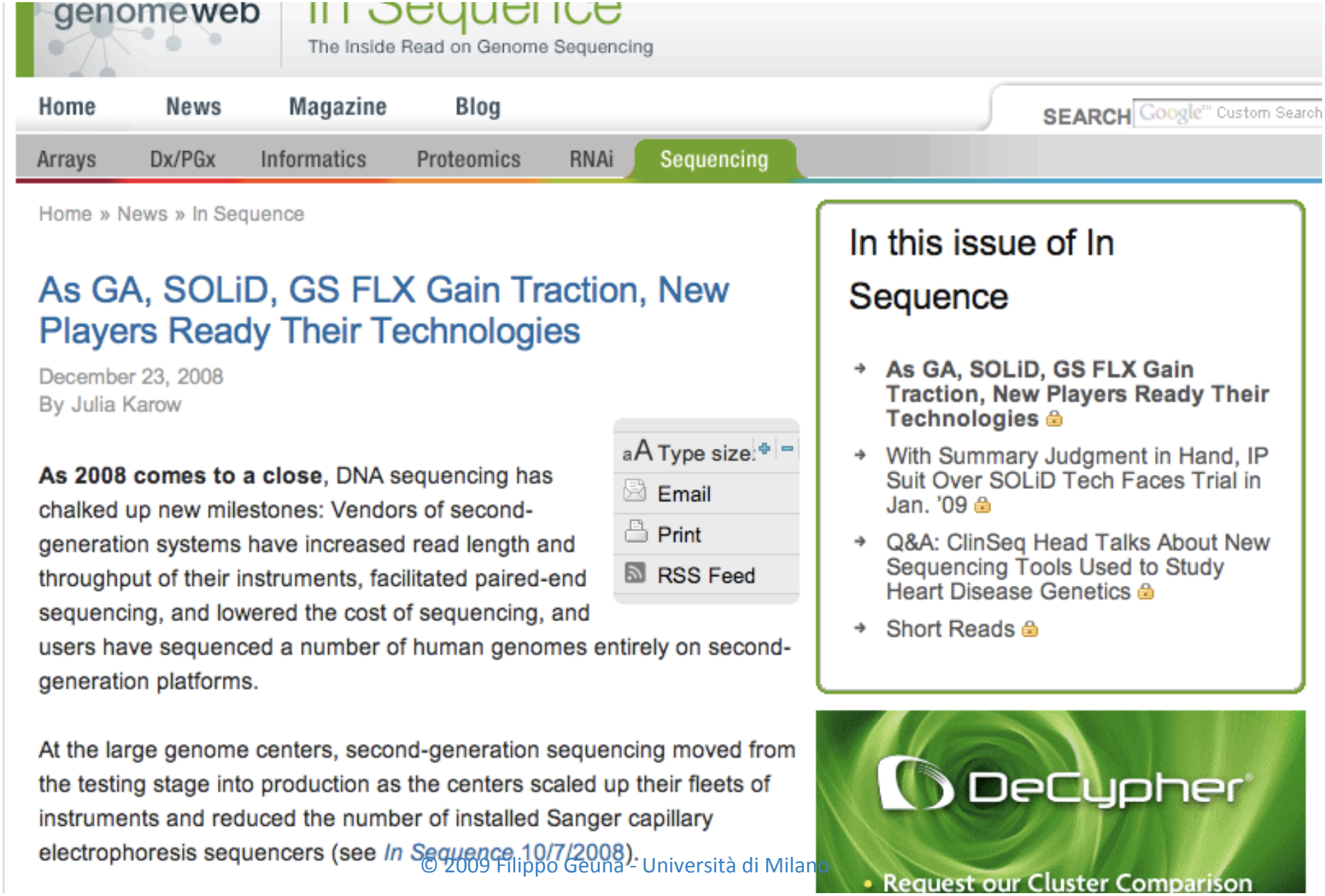
http://www.genomeweb.com/sequencing/ga-solid-gs-flx-gain-traction-new-players-ready-their-technologies



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As GA, SOLiD, GS FLX Gain Traction, New Players Ready Their Technologies

December 23, 2008
By Julia Karow

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As 2008 comes to a close, DNA sequencing has chalked up new milestones: Vendors of second-generation systems have increased read length and throughput of their instruments, facilitated paired-end sequencing, and lowered the cost of sequencing, and users have sequenced a number of human genomes entirely on second-generation platforms.

At the large genome centers, second-generation sequencing moved from the testing stage into production as the centers scaled up their fleets of instruments and reduced the number of installed Sanger capillary electrophoresis sequencers (see *In Sequence* 10/7/2008).

In this issue of In Sequence

- **As GA, SOLiD, GS FLX Gain Traction, New Players Ready Their Technologies**
- With Summary Judgment in Hand, IP Suit Over SOLiD Tech Faces Trial in Jan. '09
- Q&A: ClinSeq Head Talks About New Sequencing Tools Used to Study Heart Disease Genetics
- Short Reads

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http://www.plantphysiol.org/cgi/reprint/149/1/111.pdf

Poaceae Genomes: Going from Unattainable to Becoming a Model Clade for Comparative Plant Genomics

C. Robin Buell*

Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824

Genomics has an immense potential for improving our understanding of critical issues in plant growth and development, some of which can be applied to improvement of crop production. Midway into the second decade of genomics, genome and transcriptome sequencing efforts with the Poaceae are impressive given the technical and fiscal challenges presented by the typically large, repetitive genomes found within the Poaceae (Smith and Flavell, 1975; Arumuganathan and Earle, 1991; SanMiguel et al., 1996). Indeed, as of October 30, 2008, there are 10,847,522 Poaceae sequences representing 11,142 Mb (11.1 Gb) in GenBank, confirming the fast pace of sequence generation for the Poaceae. With respect to representation of genera and species within the Poaceae, 2,740 of the approximately 10,000 species reported within the Poaceae (http://www.kew.org/scihort/poaceae.html) have at least one sequence in GenBank. Genome-scale datasets represent a more narrow phylogenetic base and of the 47 Poaceae species with genome or transcriptome sequences (Table I; discussed below), 32 are derived from the BEP clade (Bambusoideae, Ehrhartoideae

portion of a genome and provide a rapid, economic approach to sampling the gene space of an organism. As early access sequence datasets, ESTs can be used for (1) development of genetic markers (for example, see Harushima et al., 1998), (2) electronic gene expression analyses (for example, see Ewing et al., 1999), (3) improvement of structural annotation (Haas et al., 2003), and (4) functional genomic resources for use in overexpression, in vitro expression, and gene-silencing studies. In 1997, the first set of ESTs for a Poaceae species was reported for rice (*Oryza sativa*; Yamamoto and Sasaki, 1997). Now, 11 years later, there are 36 Poaceae species with EST collections greater than 1,000 sequences (Table I); of these, >1 million ESTs are available for three Poaceae species (maize [*Zea mays*], rice, wheat [*Triticum aestivum*]). Collectively, as of October 30, 2008, within the dbEST division of GenBank, there were 5,491,939 Poaceae EST sequences totaling 2,764 Mb (2.76 Gb). With the high degree of repetitive sequences within the majority of Poaceae species, coupled with the availability of high-throughput next-generation sequencing platforms for transcrip-

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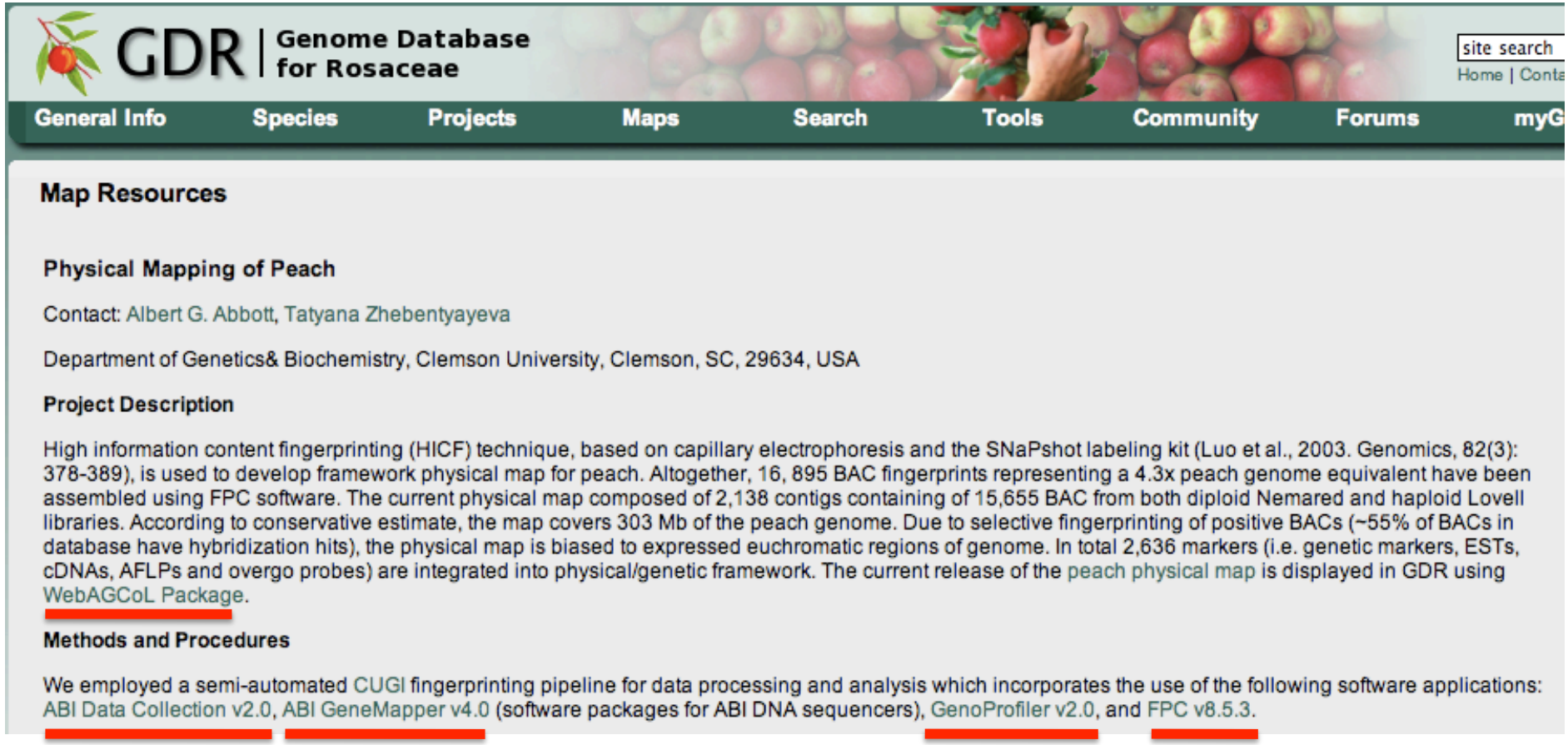
Un progetto di mappatura fisica (dal sito web GDR della Clemson University)
http://www.bioinfo.wsu.edu/gdr/physical_map.php



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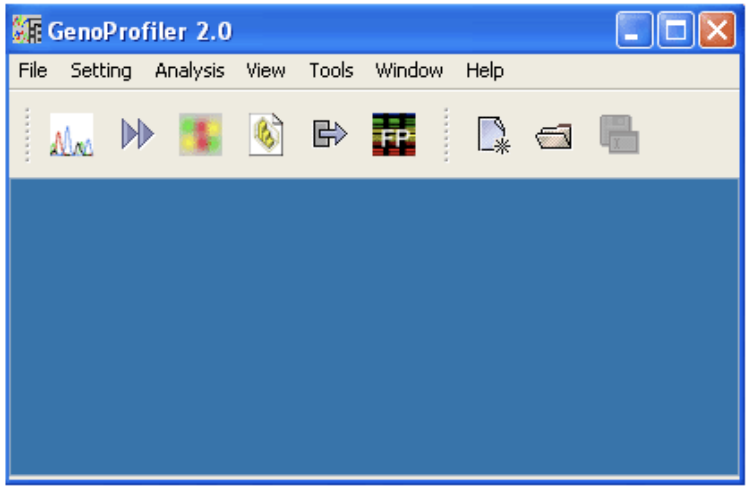
Uno degli strumenti software utilizzati per assemblare la mappa fisica
http://wheat.pw.usda.gov/PhysicalMapping/tools/genoprofiler/genoprofiler.html

GenoProfiler 2.0

The automated procedure of physical mapping is crucial to large scale genome sequencing and genome research. This automation procedure involves three important steps: (1) high-throughput fingerprinting of (BI)BAC clones, (2) accurate and efficient high-throughput data processing, and (3) efficient contig assembly grouping clones into different contigs.

In the NSF-supported Wheat D genome physical mapping project , a protocol of high-throughput fingerprinting of BAC clones with SNaPshotTM Kit using ABI 3100 capillary genetic analyzer has been developed and utilized (Luo et al. 2003). Other high-throughput fingerprinting methods are also available (Ding et al. 1999, 2001). For contig assembly, FPC (FingerPrint Contig) software has been developed by Soderlund et al. (1997) and widely used to build contigs in many physical mapping projects. However, manually handling of the size editing from raw sample files, and pre-analysis of large amount of fragment size data are time-consuming and error-prone process for a large-scale physical mapping project. As the application of high-throughput fingerprinting protocols in the large-scale physical mapping projects, high-throughput fingerprinting data processing becomes a bottleneck of automation of physical mapping.



GenoProfiler is a cross-platform software package for the fully automated processing of fingerprinting data from ABI 3100/3700/3730 Genetic Analyzer. This software provides the algorithms to accurately extract the traces and peak data (sizes, peak height, peak area, and scan number or data point) directly from sample files obtained from ABI 3100/3700 Genetic Analyzer without using ABI GeneScan, GeneMapper, or other software, and automatically remove the background (noise) bands and "false" bands, and detect and split double bands or triple bands, and create edited size files with FPC size format which can be directly used for contig assembly using FPC software and for other purposes (such as genotyping for phynogenetic analysis). GenoProfiler also provides some very useful tools to make fingerprinting data processing fully automatic. Those tools includes detecting cross-contaminations of clones within 384-well plates and 96-well plates and chloroplast DNA contaminations of clones, fragment frequency analysis of produced fragment sizes, renaming clones, sub-setting clone sizes from edited size files, sub-setting sample files from the sample file directories, sub-setting clones from contigs in the fpc files (*.fpc), and fingerprints viewer.

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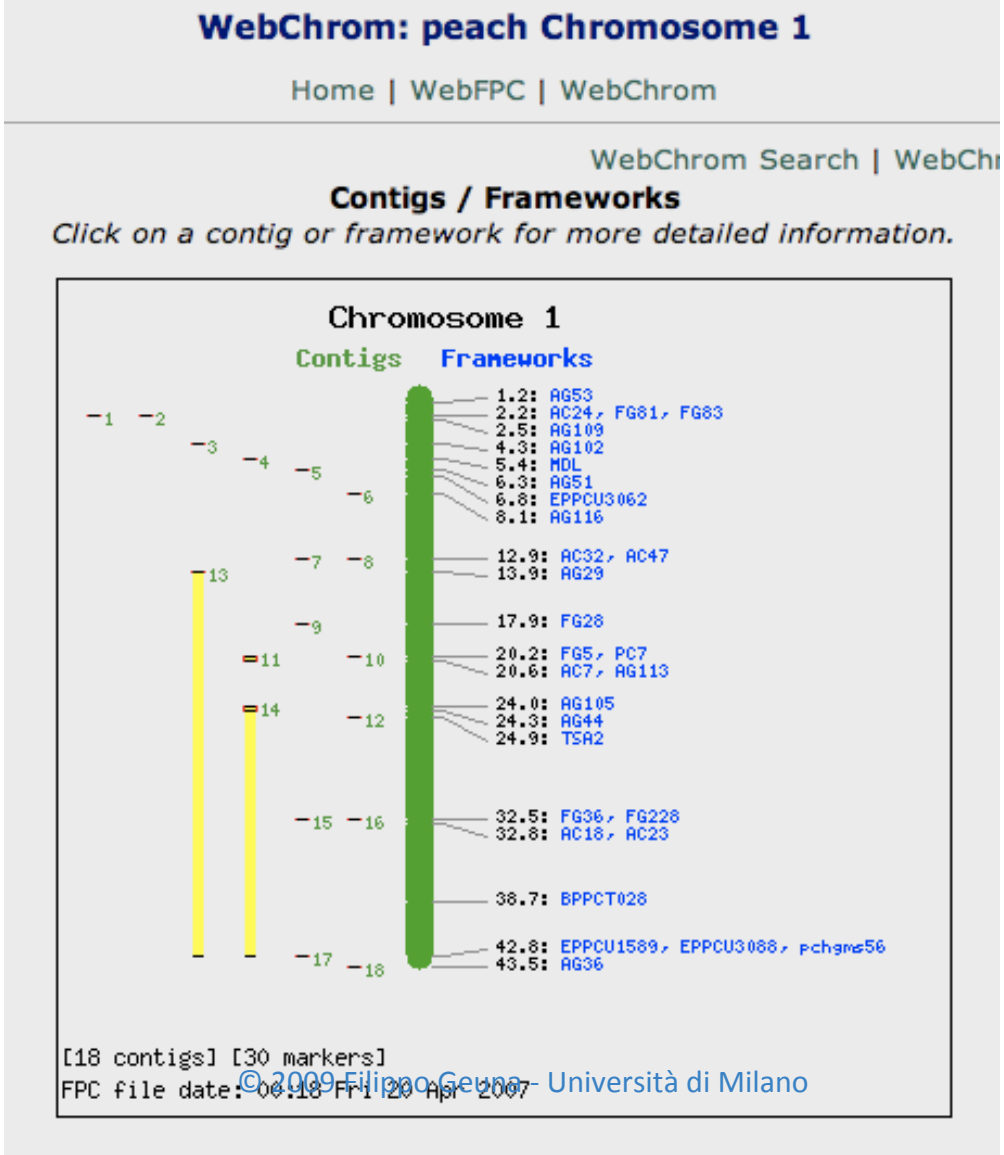
GenoProfiler is a Java-based window application which should work on all operating systems with JDK 1.4 or later.

Esempio di allineamento di cloni BAC rispetto al cromosoma 1 (tratto dal sito web GDR della Clemson University)
http://www.bioinfo.wsu.edu/gdr/WebAGCoL/peach/WebChrom/wcpeach1.php



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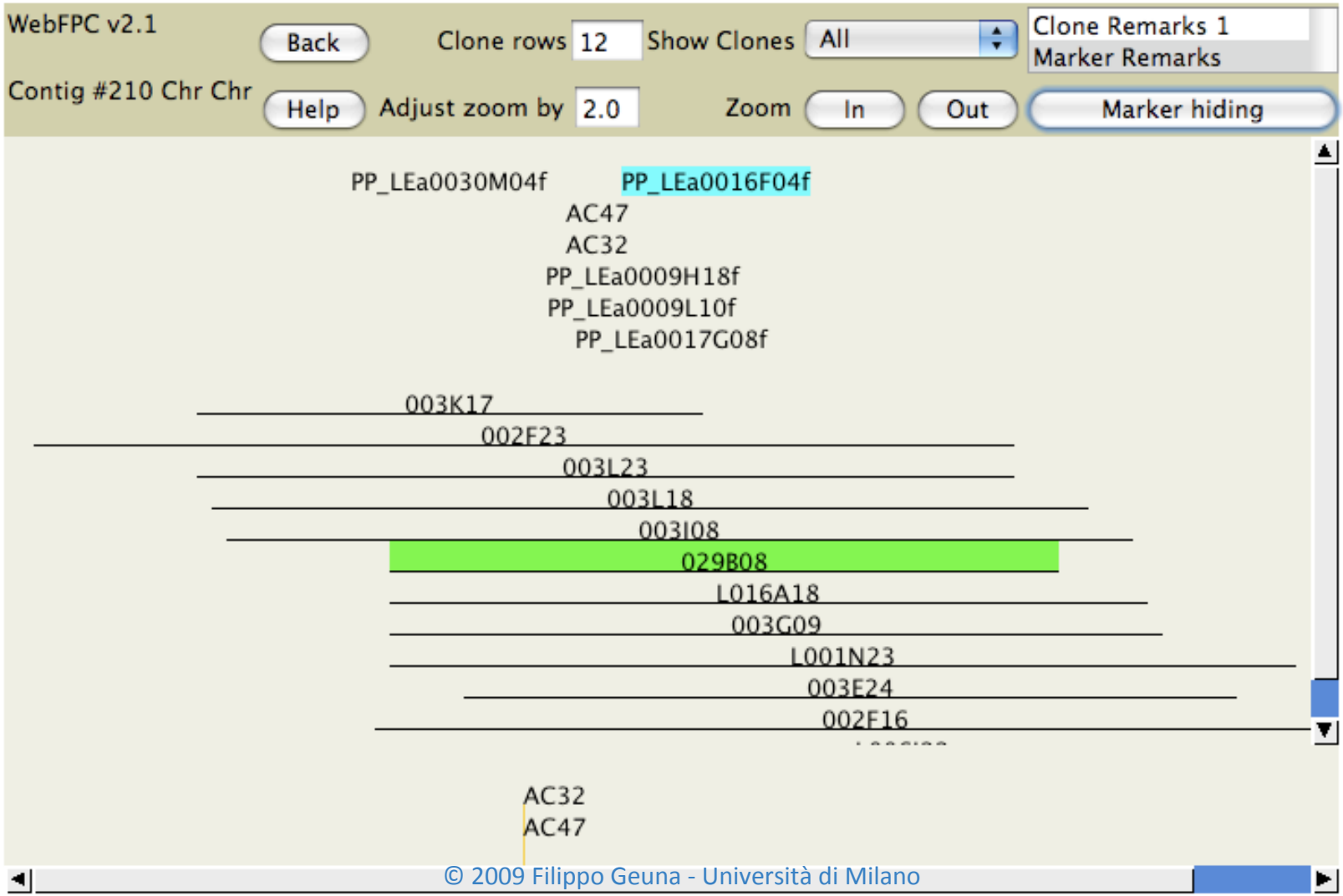

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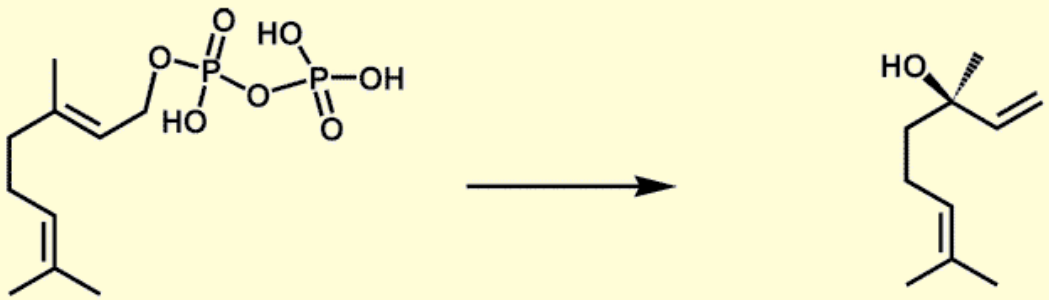
Reaction: Linalool synthesis



geranyl diphosphate **(3R)-linalool**

E.C.: Not Assigned	Updated: 2005-01-24

External Links:

This reaction may produce (3R)-linalool or (3S)-linalool. It's unclear from the literature which of the two products are formed by this particular enzyme. The partial reactions displayed for this overall reaction show the pathway for the production of (3R)-linalool.

Enzyme Functional Domains Performing This Reaction

Name	Species	Superfamily	Subgroup	Family	X-Ray Struct.	Model Struct.
Linalool Synthase	Mentha aquatica	terpene cyclase	Monoterpenes (10 carbons)	Linalool		1
Linalool Synthase	Clarkia breweri	terpene cyclase	Monoterpenes (10 carbons)	Linalool		1
Linalool Synthase	Clarkia concinna	terpene cyclase	Monoterpenes (10 carbons)	Linalool		1
Linalool Synthase	Perilla frutescens	terpene cyclase	Monoterpenes (10 carbons)	Linalool		
Linalool Synthase	Arabidopsis thaliana	terpene cyclase	Monoterpenes (10 carbons)	Linalool		1
Linalool Synthase	Oryza sativa	terpene cyclase	Monoterpenes (10 carbons)	Linalool		

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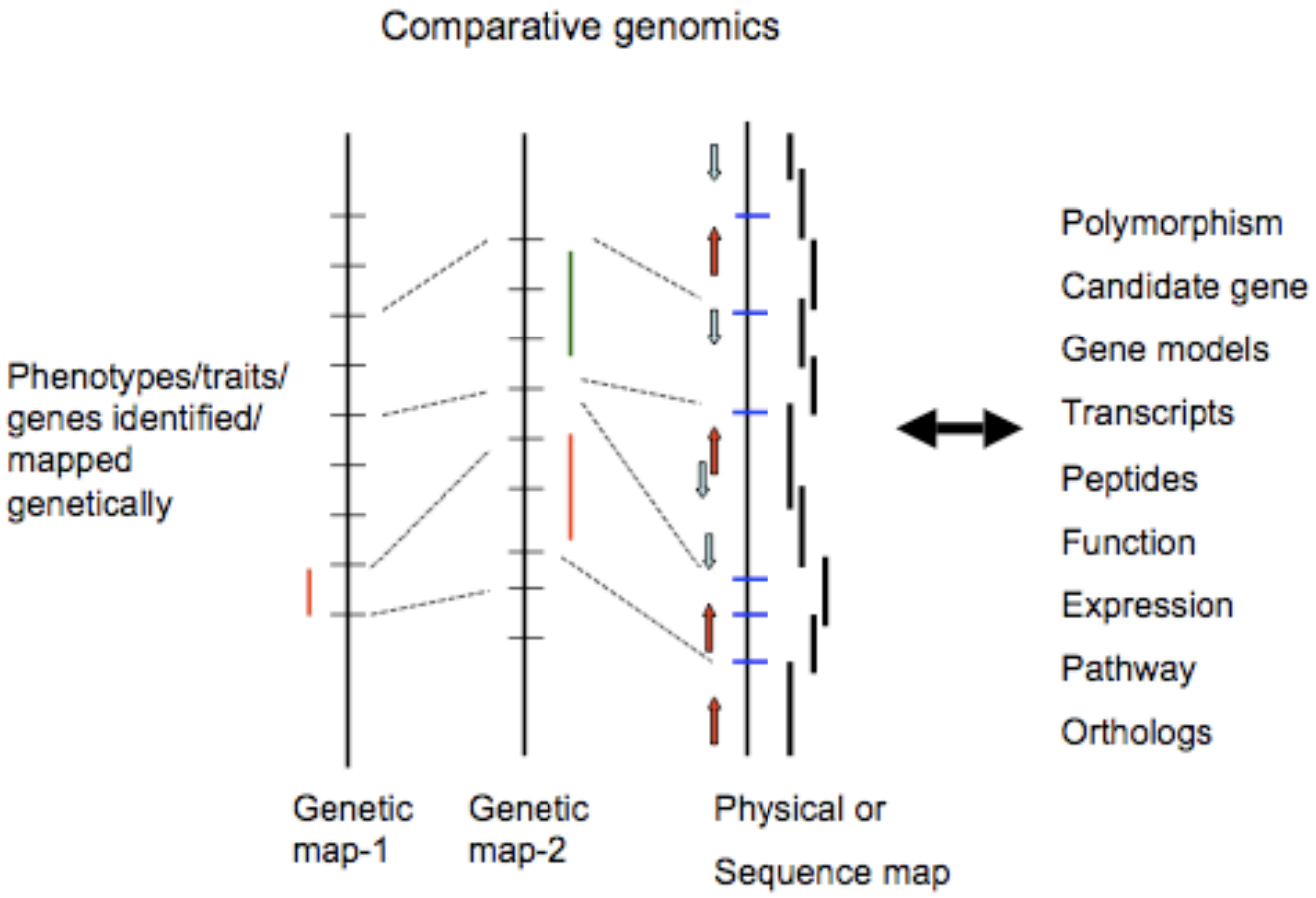
http://www.gramene.org/workshop/EPD/02pjaiswal.pdf

Tutorial @ Gramene



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http://hpgl.stanford.edu/projects/microsat/microdist.html

A review of mutation processes and methods of phylogenetic inference

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Contents

1. Introduction
2. Molecular details
3. Genetic distance measures
4. Discussion
5. Acknowledgements

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http://tomato.bio.trinity.edu/search.php?q=Festuca&field_name=species&id=&page=1

About the Database

This is the sister database for **Molecular Ecology Resources** (formerly known as Molecular Ecology Notes), containing the details for reported loci (*i.e.*, primer sequences, amplification conditions, polymorphism levels, cross-species amplification, and literature citations) in a searchable format.

The database contains all Primer Note submissions to Molecular Ecology and Molecular Ecology Notes and Resources. It is hoped that this database will become the on-line resource for molecular markers developed for "non-commercial" and non-model species.

The database may be searched using either the **search page** (searches based on family, genus or species names) or, in the future, an advanced search page, which will allow more flexible and detailed queries as the database grows.

Authors whose data have been accepted for publication in the database should familiarize themselves with the **database submission instructions** and then use the **database submission form** to add their data.

For your convenience, links to the search page, this page, and the Molecular Ecology Resources homepage at the Blackwell site are positioned at the top and bottom of each page.

If you have any questions or comments about this site, please email the database/website **administrator**.

Please direct comments and questions about this database to the **administrator**.

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http://tomato.bio.trinity.edu/search.php?q=Festuca&field_name=species&id=&page=1

Blackwell Publishing

MOLECULAR ECOLOGY RESOURCES

Home | Search | New Submission | Recent Submissions | MER Journal

Search the Database

Your search for **Festuca** in **Species** returned 95 records
Pages: 1 | **2** | **3** | **4** | **5**

Details	Submitted By	Options
Species: *Festuca arundinacea* Locus: *TeaSSR1* Marker: *microsatellite*	Susanne Barth **sbarth@oakpark.teagasc.ie** 30 November, 1999	**View** **Use as template**
Species: *Festuca gigantea* Locus: *TeaSSR1* Marker: *microsatellite*	Susanne Barth **sbarth@oakpark.teagasc.ie** 30 November, 1999	**View** **Use as template**
Species: *Festuca ovina* Locus: *TeaSSR1* Marker: *microsatellite*	Susanne Barth **sbarth@oakpark.teagasc.ie** 30 November, 1999	**View** **Use as template**
Species: *Festuca pratensis* Locus: *TeaSSR1* Marker: *microsatellite*	Susanne Barth **sbarth@oakpark.teagasc.ie** 30 November, 1999	**View** **Use as template**
Species: *Festuca vivipara* Locus: *TeaSSR1* Marker: *microsatellite*	Susanne Barth **sbarth@oakpark.teagasc.ie** 30 November, 1999	**View** **Use as template**

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Il Database della settimana

"…perché produciamo più informazione di quanto siamo in grado di utilizzarne…"

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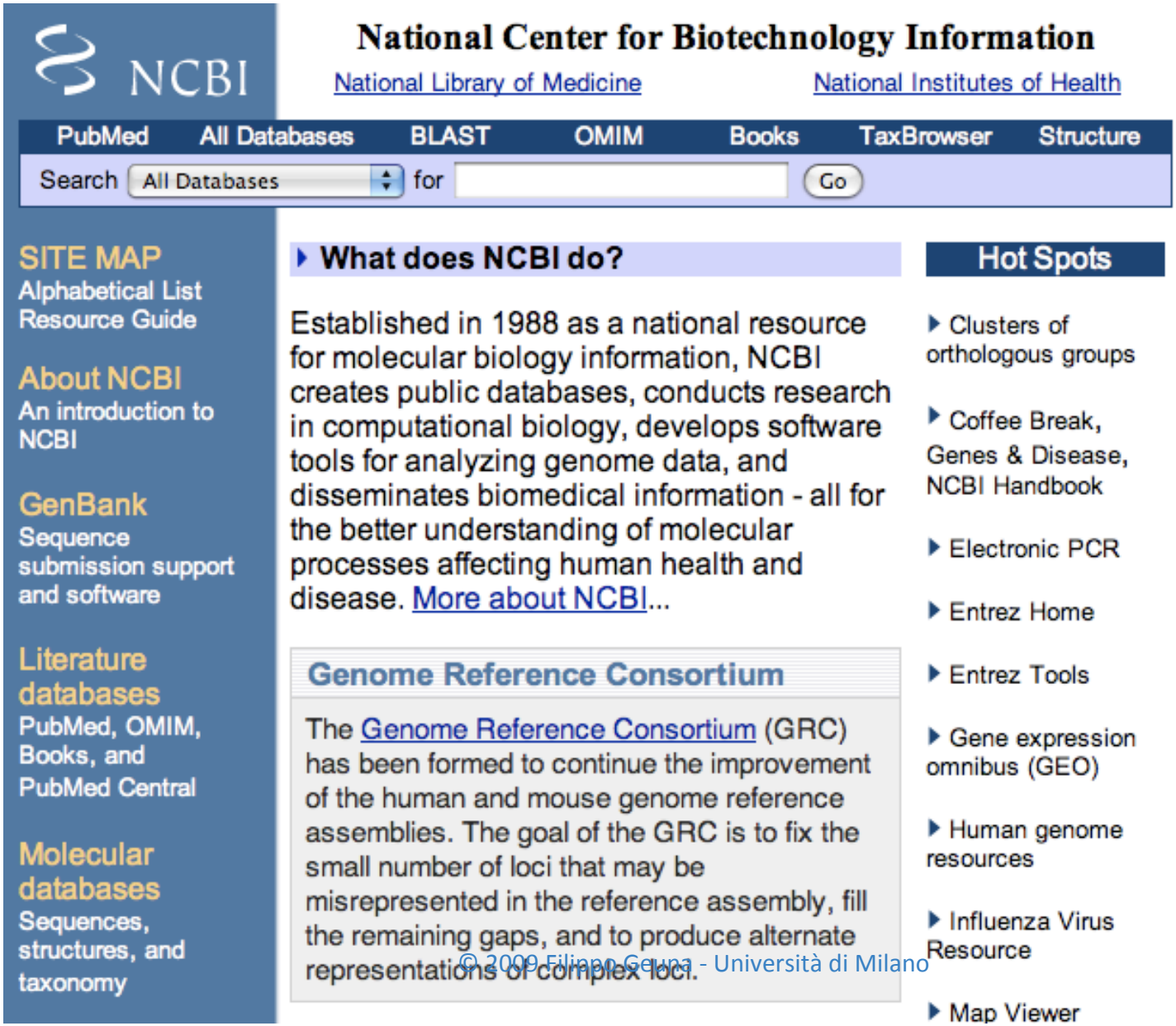
Il Database della settimana

http://www.ncbi.nlm.nih.gov



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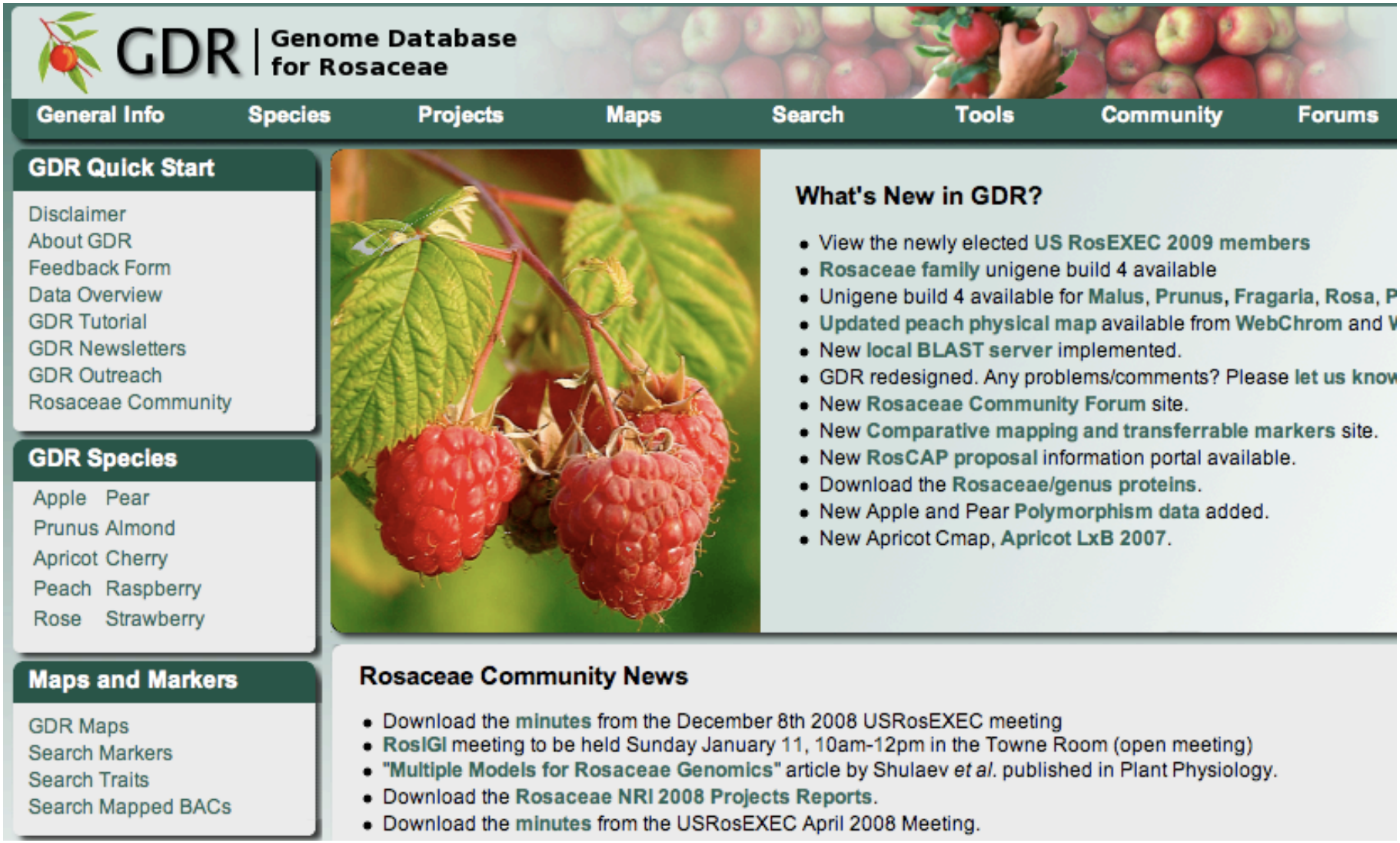
Il Database della settimana

http://www.bioinfo.wsu.edu/gdr/



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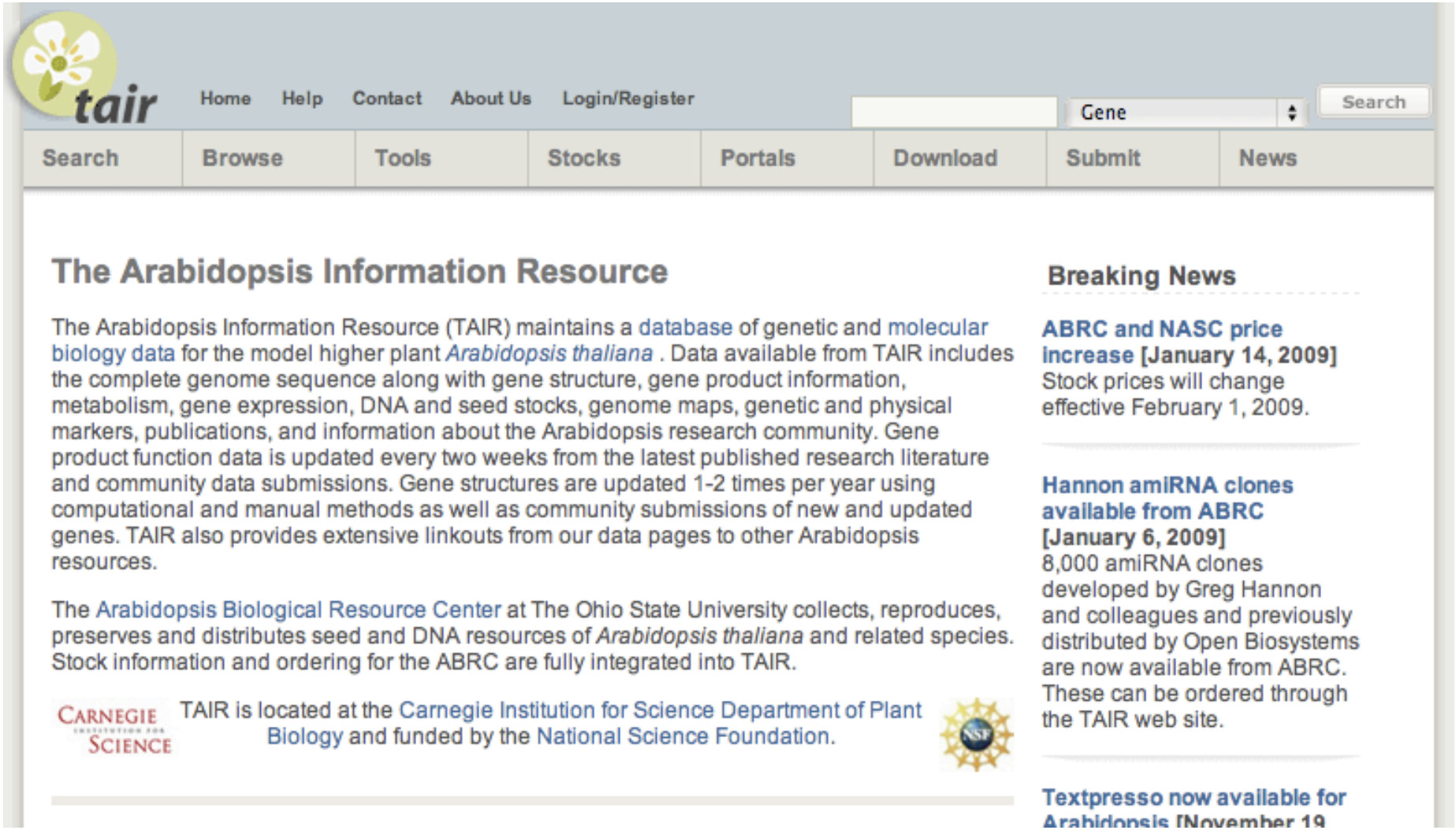
http://www.arabidopsis.org/



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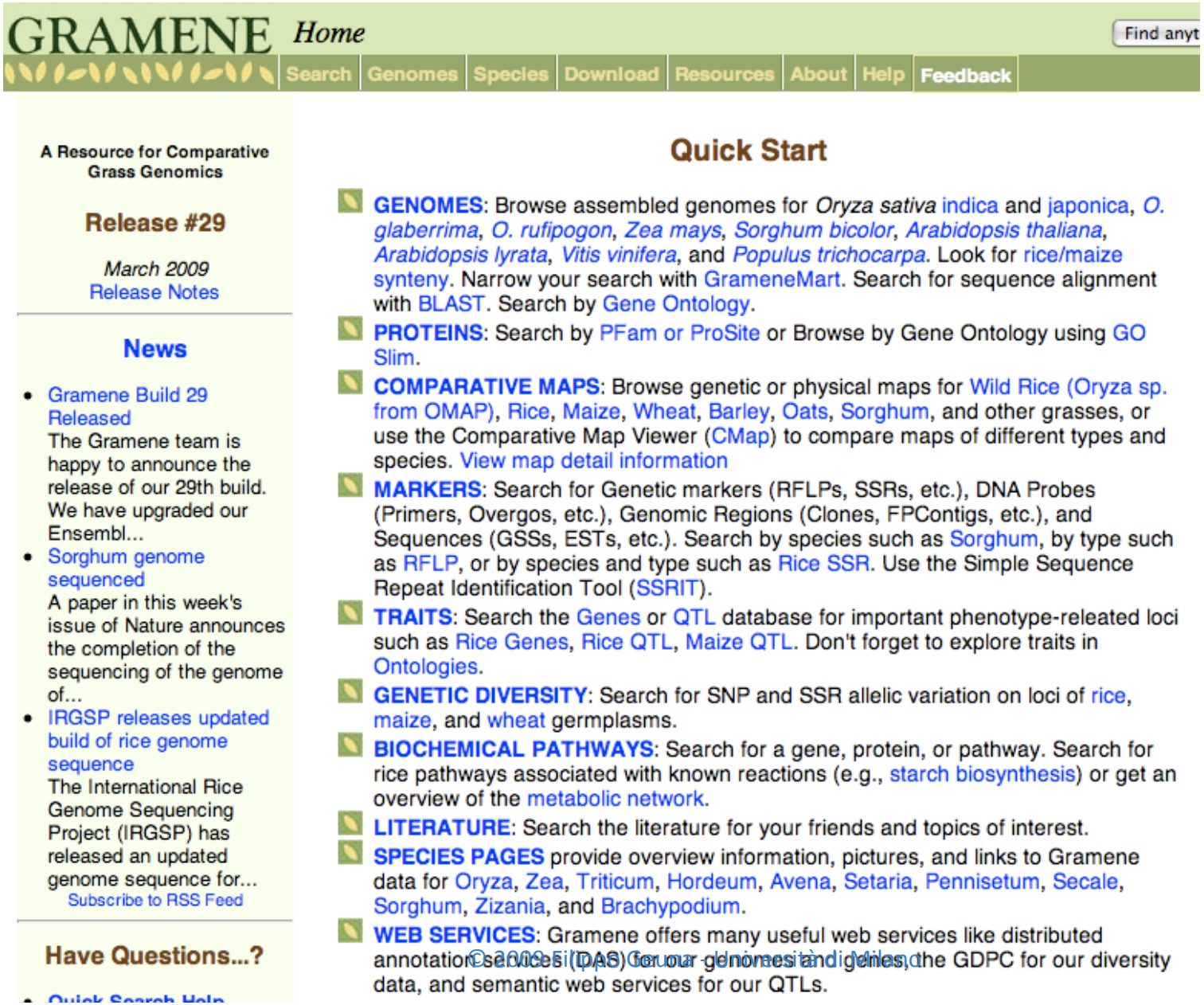
http://www.gramene.org/



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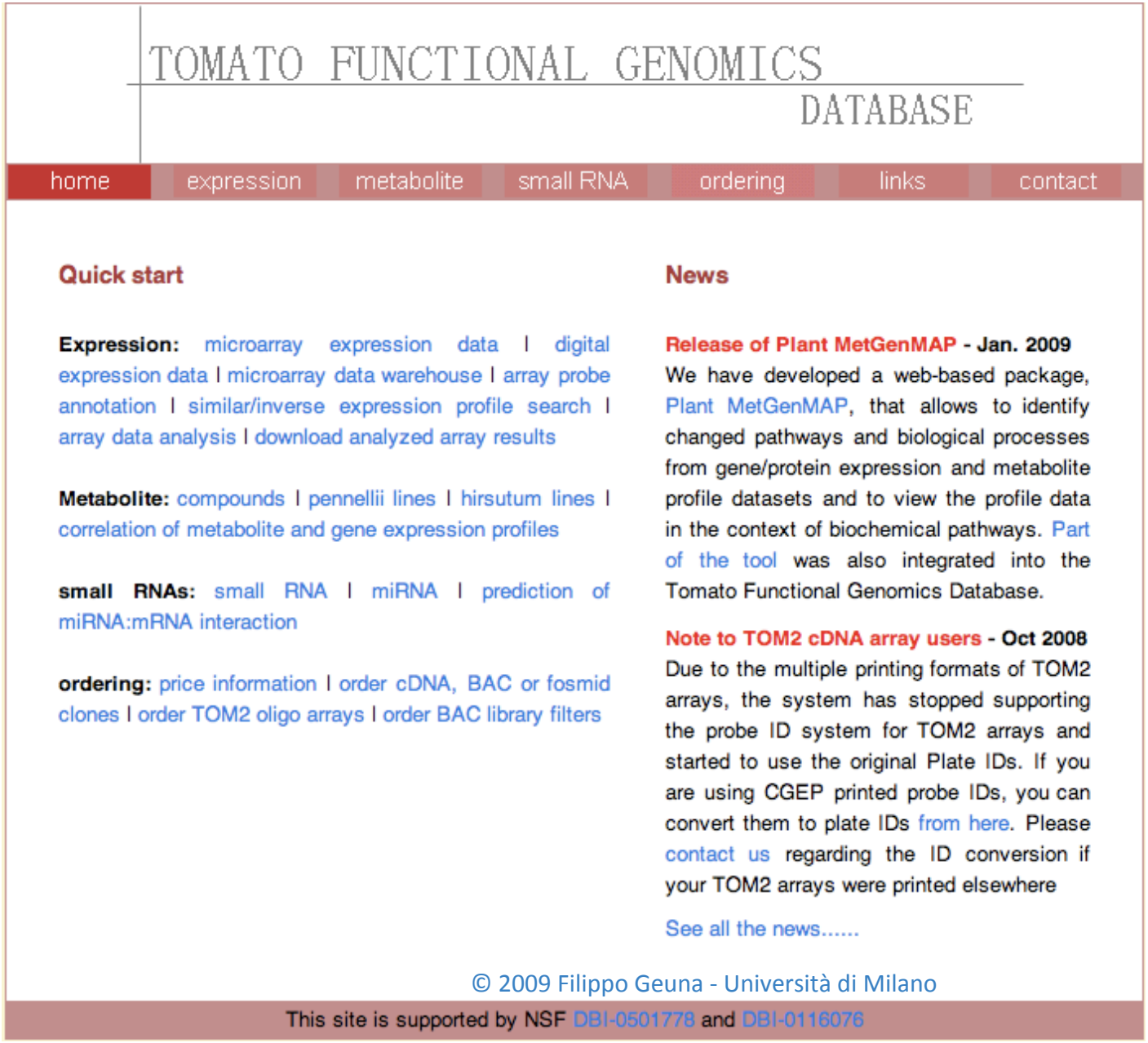
http://ted.bti.cornell.edu/



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Il Database della settimana

http://www.oleadb.it/

OLEA databases

Germplasm Info

Cultivars, Synonyms, Cultivation areas, Collections, Descriptors

Marker Info

Search Markers

NEW SSR Database

General Info

- Contact us

The **OLEA databases** is a comprehensive olive (*Olea europaea* L.) science portal established in 2007 by olive researchers in Europe.

The intent of the publicly accessible **OLEA databases** is to deliver updated and integrated information ranging from classical olive genetic resource information to recent genomics. The **OLEA databases** consists, at the moment, of two parts:

- (1) genetic resource stock information: **Germplasm Info**
- (2) molecular (SSR and AFLP) data: **Marker Info**

We are calling for additional information to incorporate into the **OLEA databases**. Markers, maps, ESTs and BAC sequences, newly identified genes and mapped traits will be welcome to integrate into the **OLEA databases**. A more extensive cross-referencing of **OLEA databases** to the major DNA sequence, literature and other databases is planned, in order to provide the wealth of information to olive researchers, breeders and producers.

IVALSA

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Proteomica e “virtual electrophoresis”

.....-04-2009

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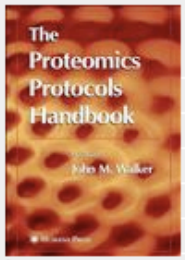

large version

Virtual 2-D Gel Electrophoresis by MALDI Mass Spectrometry

Book	The Proteomics Protocols Handbook
Publisher	Humana Press
DOI	10.1385/1592598900
Copyright	2005
ISBN	978-1-58829-343-5 (Print) 978-1-59259-890-8 (Online)
DOI	10.1385/1-59259-890-0:417
Pages	417-429
Subject Collection	Biomedical and Life Sciences
SpringerLink Date	Tuesday, October 09, 2007

PDF (402.4 KB) HTML

The Proteomics Protocols Handbook

10.1385/1-59259-890-0:417

John M. Walker

Angela K. Walker[2], Gary Rymar[2] and Philip C. Andrews[2]

(2) Michigan Proteome Consortium, University of Michigan, Ann Arbor, MI

Abstract

Two-dimensional (2-D) gel electrophoresis (2-DE) is capable of separating several hundred to thousands of proteins on a single gel, depending on the format (*1*,*2*). The most commonly used methods for visualization of proteins in gels include Coomassie Brilliant Blue (CBB), silver stains, or fluorescent stains (*1*–*3*). Each staining method exhibits different levels of overall sensitivity, and they vary widely in their relative sensitivities for specific proteins. Two-dimensional gels also allow only the approximate sizes of proteins to be determined. It is desirable to develop methods that combine the high resolution of electrophoretic separations with the

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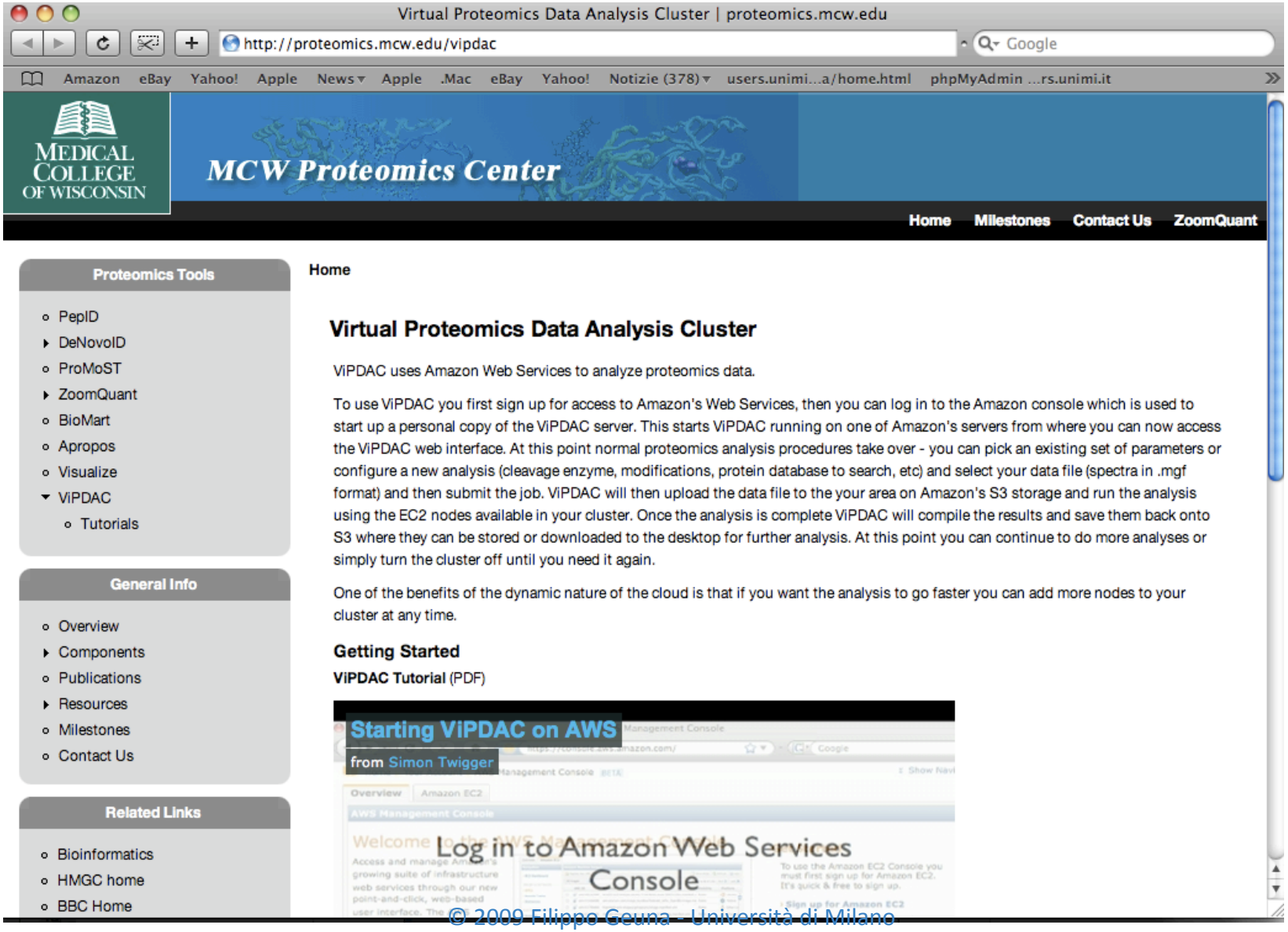
http://www.springerlink.com/content/u26u86216201p730/

Proteomica computazionale distribuita (cloud computing)

VE 10-04-2009



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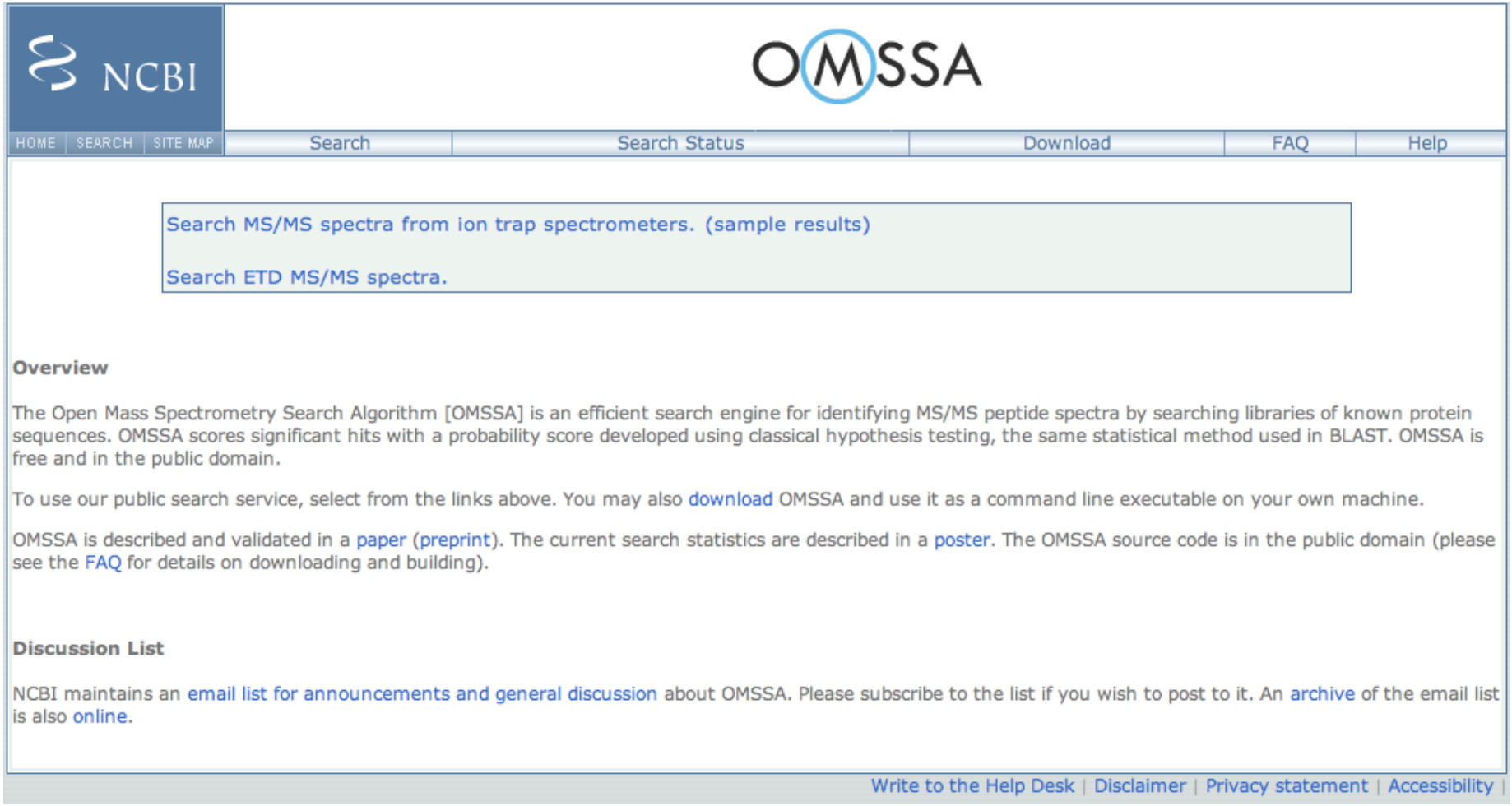
http://proteomics.mcw.edu/vipdac

Proteomica computazionale (OMSSA @ NCBI)

VE 10-04-2009



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http://pubchem.ncbi.nlm.nih.gov/omssa/

Proteomica computazionale (X!Tandem)

The Global Proteome Machine Organization
www.thegpm.org
TANDEM project

| Home | FAQ | Mutations | Release Notes | Instructions | API |

Databases

- GPMDB

Search sites

- Genomes
- Plant UniGene
- Fish
- Human
- Prokaryotes
- Xenopus
- Cow
- Mouse
- Rat

Other algorithms

- X! P3
- X! Hunter

Info

- FAQ
- References
- Site map

Projects

X! TANDEM Spectrum Modeler

X! Tandem open source is software that can match tandem mass spectra with peptide sequences, in a process that has come to be known as protein identification.

This software has a very simple, sophisticated application programming interface (API): it simply takes an XML file of instructions on its command line, and outputs the results into an XML file, which has been specified in the input XML file. The output format is described here (PDF). This format is used for all of the X! series search engines, as well as the GPM and GPMDB.

Unlike some earlier generation search engines, all of the X! Series search engines calculate statistical confidence (expectation values) for all of the individual spectrum-to-sequence assignments. They also reassemble all of the peptide assignments in a data set onto the known protein sequences and assign the statistical confidence that this assembly and alignment is non-random. The formula for which can be found here. Therefore, separate assembly and statistical analysis software, e.g. PeptideProphet and ProteinProphet, do not need to be used.

Latest release: 2008.12.01

This is a maintenance release of X! Tandem TORNADO. It includes an improvement in the threading mechanism that should improve overall performance for analyzing large datasets on multiprocessor/multicore computers and preliminary support for mzML data files.

System level changes

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http://www.thegpm.org/tandem/