Colorado Potato Breeding and Selection Program: Strategies for the future

Colorado State University

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Additional Strategy: **PCR** [Towards **M**arker **A**ssisted **S**election]

Polymerase Chain Reaction (PCR) The **polymerase chain reaction** is a

- biochemical copy machine
- used to amplify a single copy or a few copies of a piece of **DNA**
- generating thousands to millions of copies of a particular DNA sequence.

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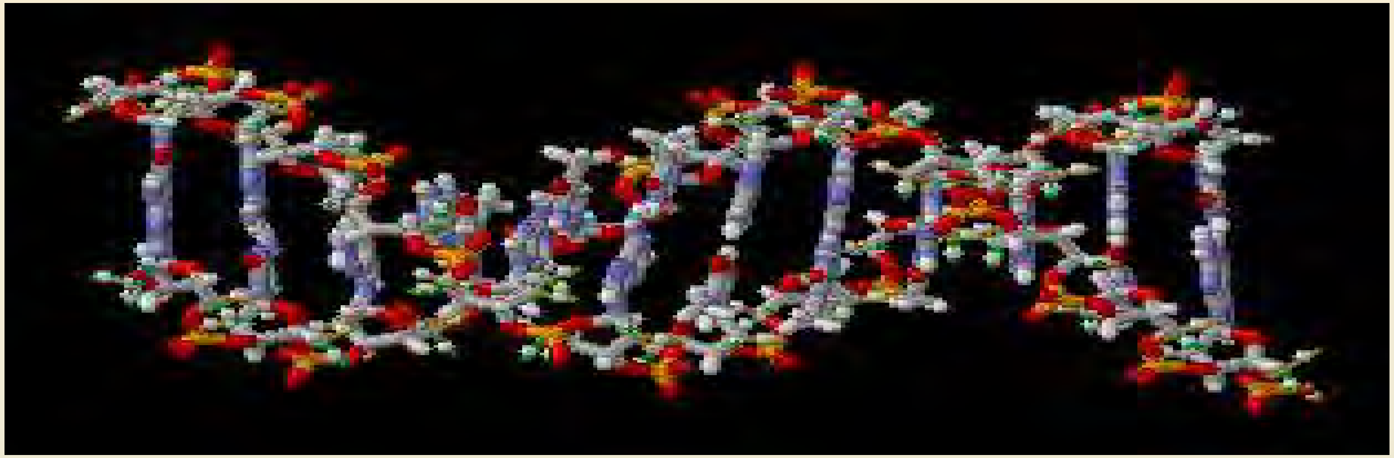
Background: DNA



Organism = Book
Chromosome = Chapter
Cluster = Paragraph
Gene = Sentence

"The cat chased the mouse."

http://upload.wikimedia.org/wikipedia/commons/d/db/DNA_orbit_animated_static_thumb.png

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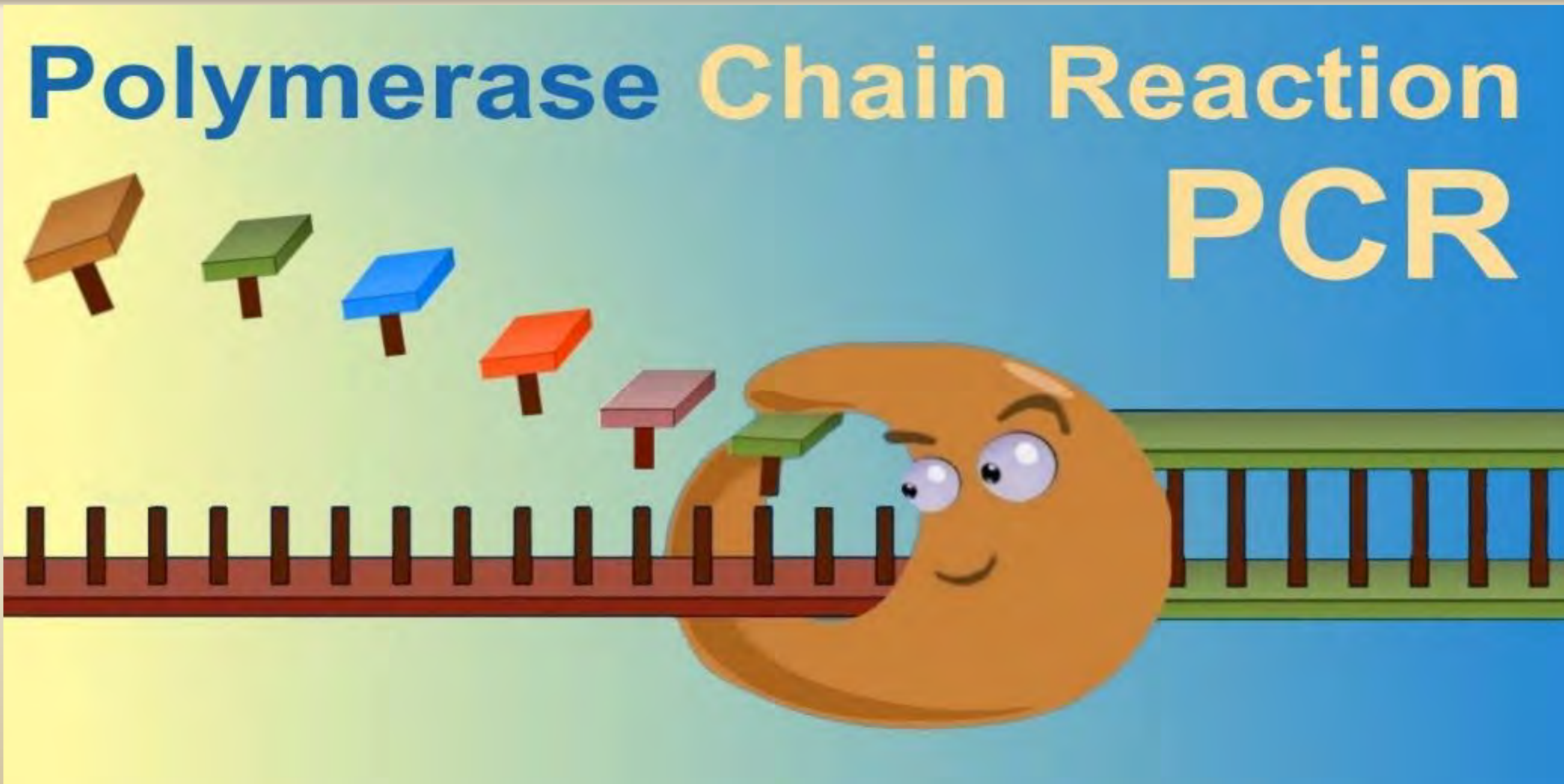

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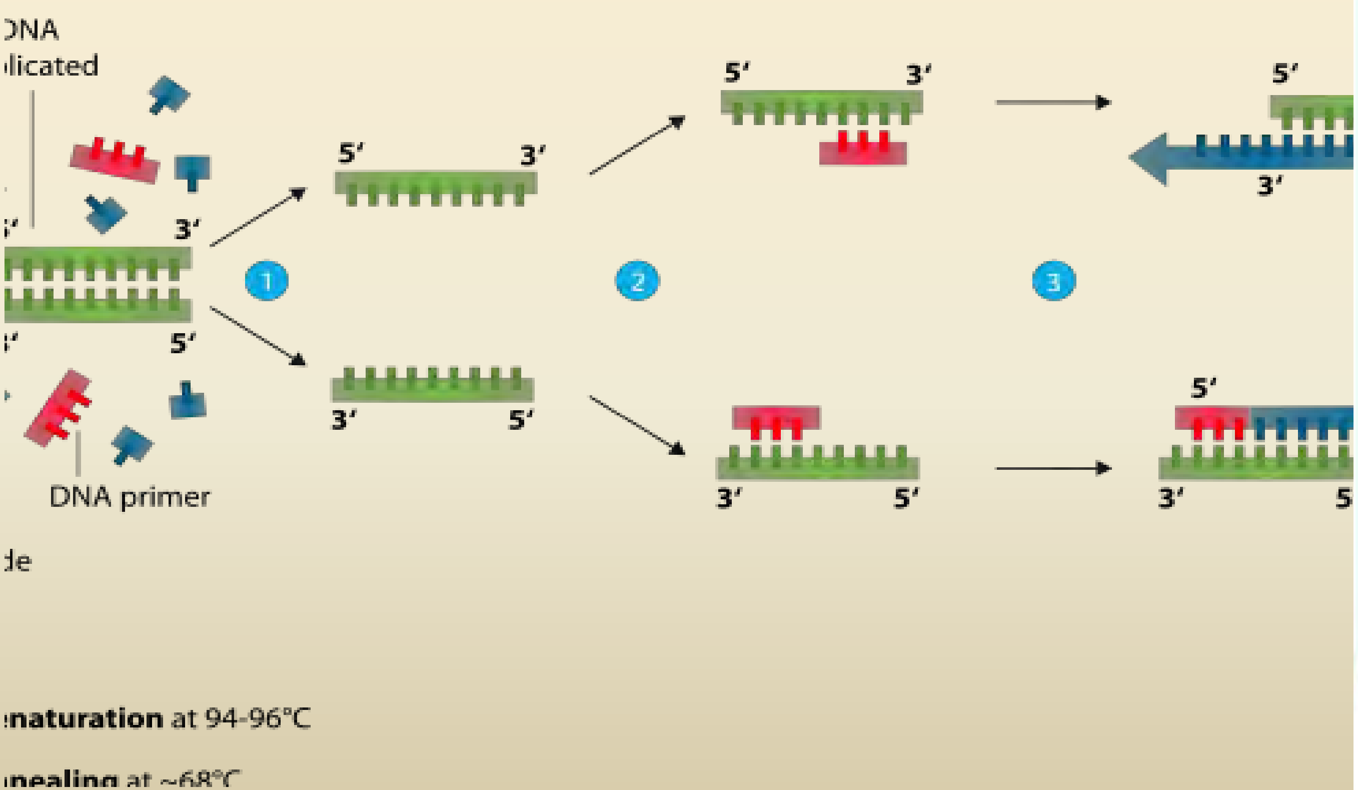

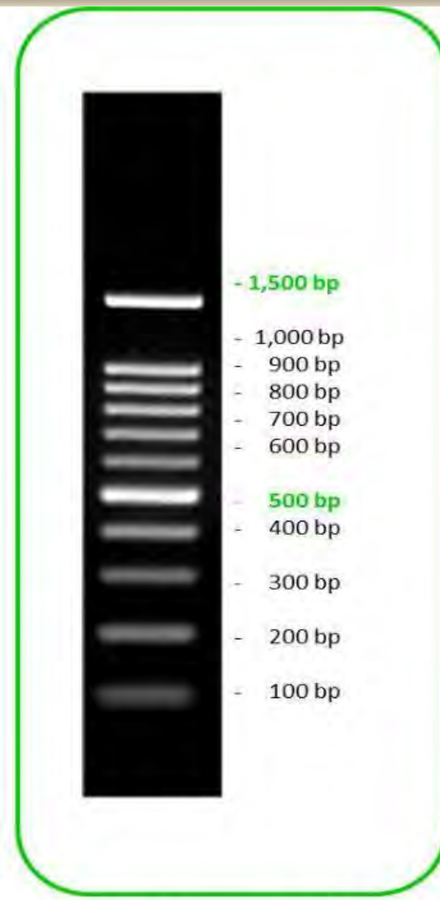

1.5% Agarose Gel 1xTAE
(100-1,500 bp) 11 Fragments

Cycle	Copies
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1,024
11	2,048
12	4,096
13	8,192
14	16,384
15	32,768
16	65,536
17	131,072
18	262,144
19	524,288
20	1,048,576
21	2,097,152
22	4,194,304
23	8,388,608
24	16,777,216
25	33,554,432
26	67,108,864
27	134,217,728
28	268,435,456
29	536,870,912
30	1,073,741,824

https://en.wikipedia.org/wiki/Polymerase_chain_reaction

Colorado Potato Breeding and Selection Program: Strategies for the future

Three Ways How PCR Can Help Breeding Selection

- **Gene Expression** (RT/std-PCR: differential screen for marker development)
- **Gene Insertion** (std-PCR: amplify gene of interest for transfer)
- **Genotype** (std-PCR: qualify presence or absence)

Colorado Potato Breeding and Selection Program: Strategies for the future

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"The cat chased the mouse."
"The **b**at chased the mouse."

How PCR Can Help Breeding Selection

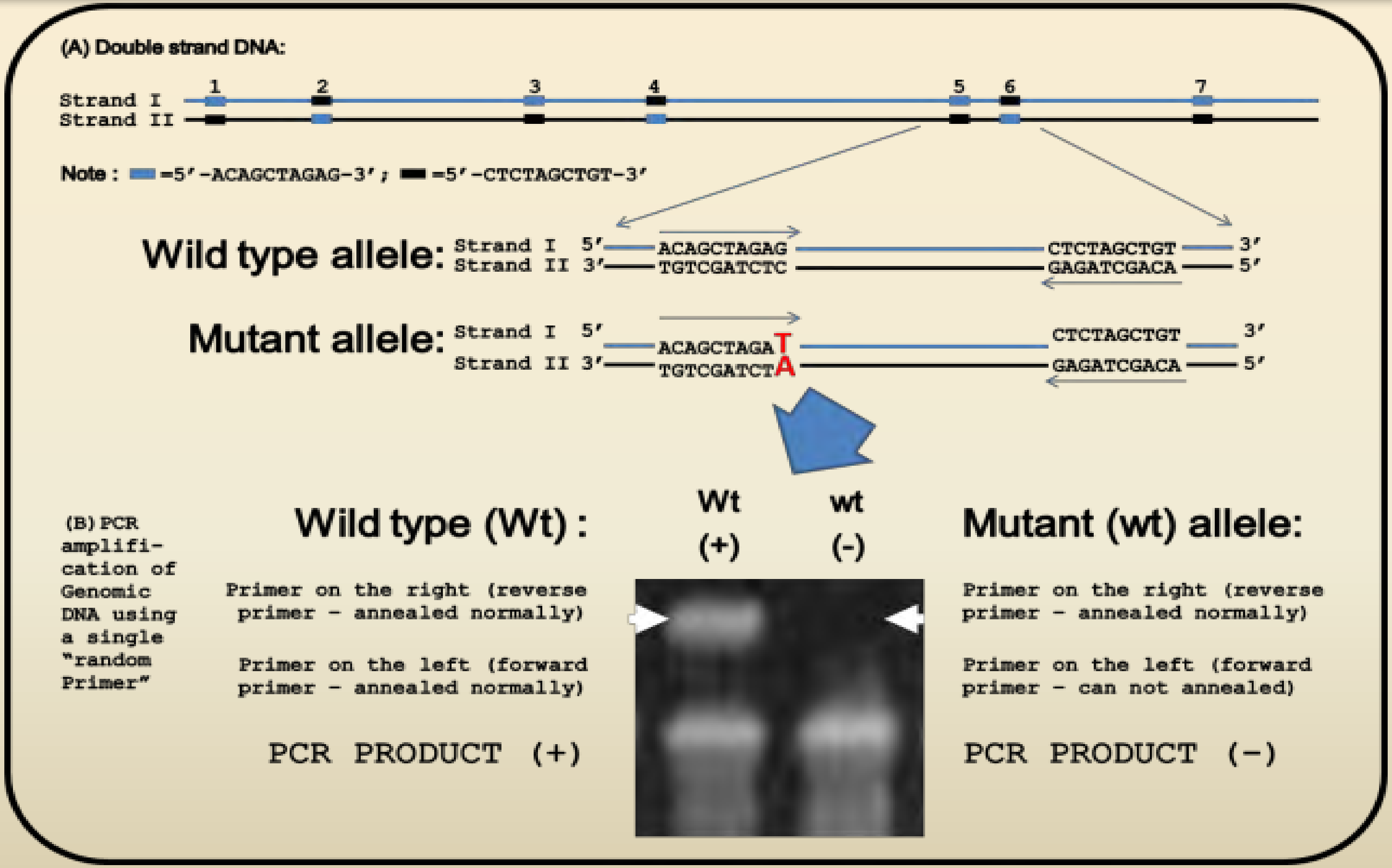


Figure 1. Point mutation at the forward primer annealing site changes the marker (+) into marker (-)

https://pmblab.wordpress.com/2013/09/28/qa-in-agh635-course-what-have-caused-allele-diversities-in-rapd-marker/

How PCR Can Help Breeding Selection

Organism = Book
Chromosome = Chapter
Cluster = Paragraph
Gene = Sentence

"The cat chased the mouse."
"The **baseballb**at chased the mouse."

How PCR Can Help Breeding Selection

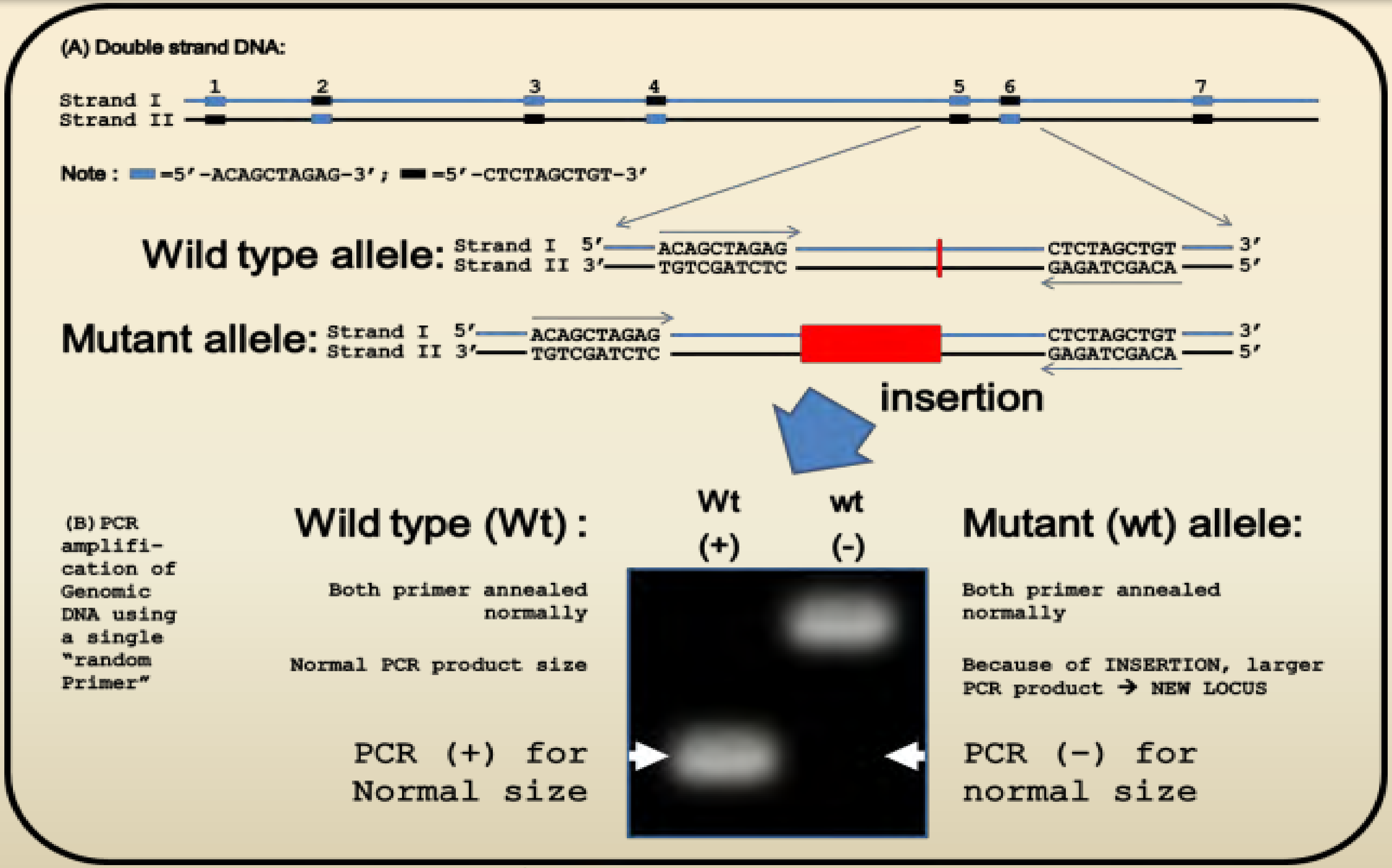


Figure 4. Insertion mutation between the forward and reverse primer annealing sites, in addition to changing the marker (+) into marker (-), it also created a new RAPD marker loci at the size larger than the original site

How PCR Can Help Breeding Selection

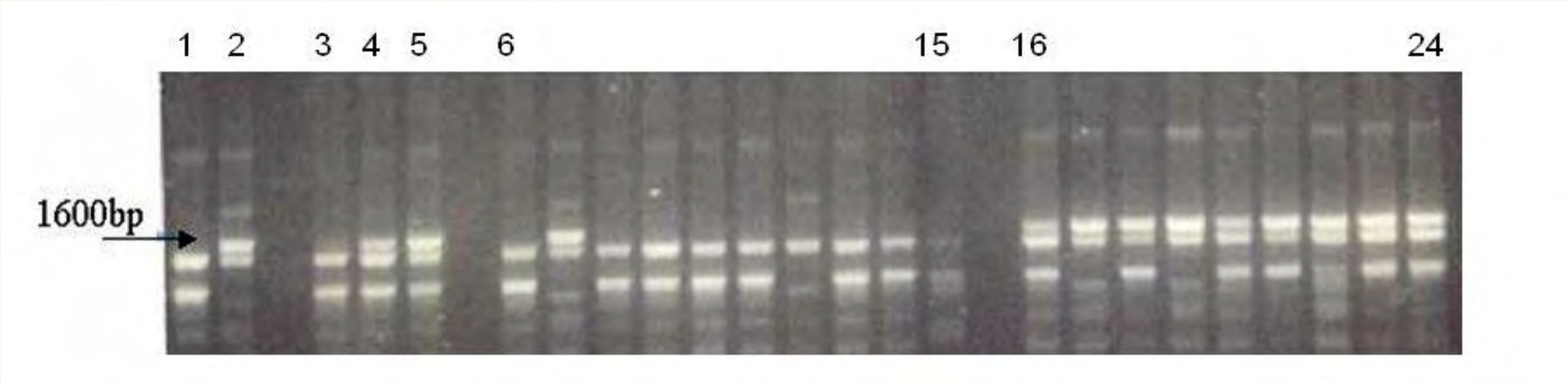


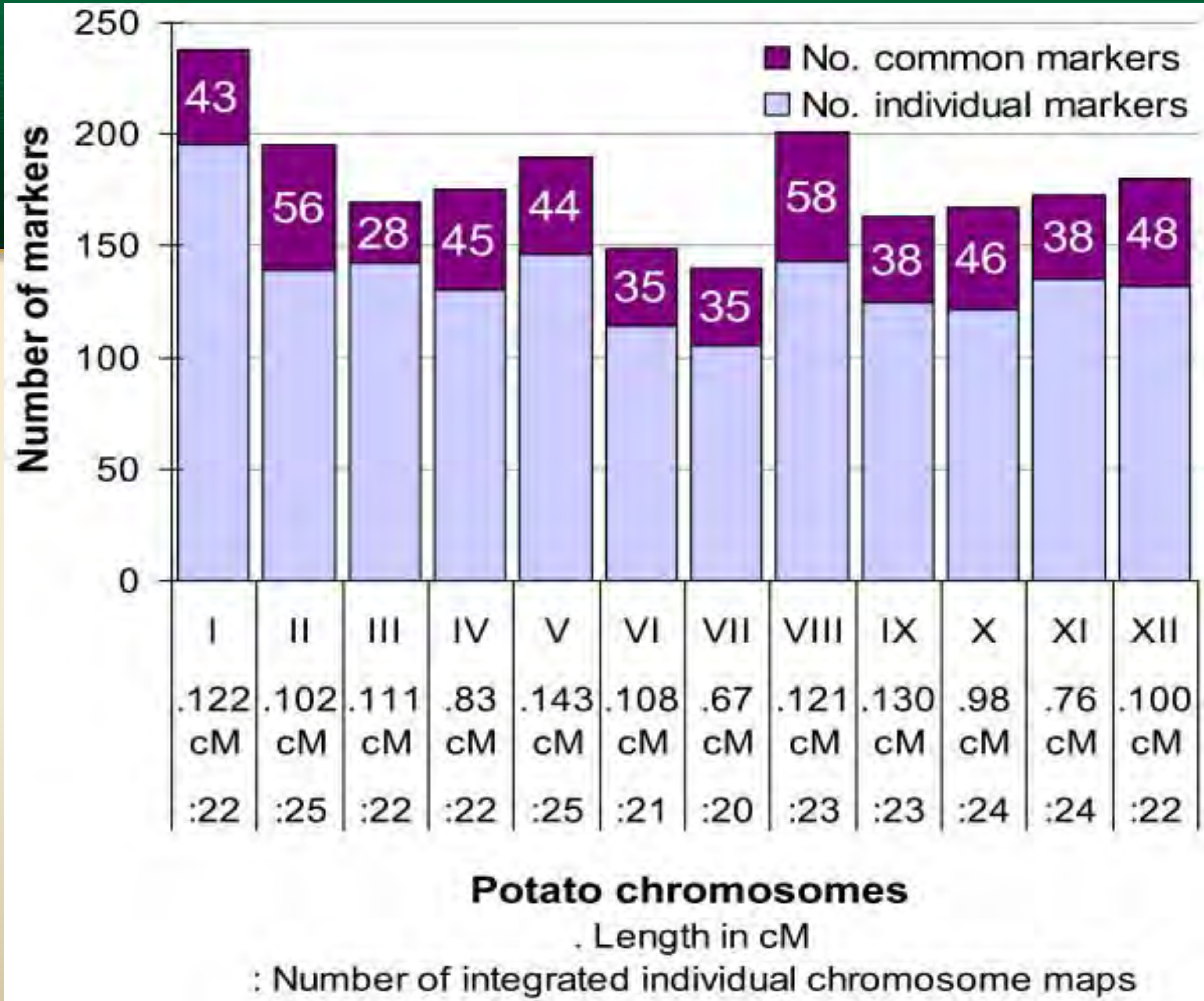
Fig. 2. RAPD profile of primer OPAE-14 in parents, bulks and individuals of the bulks linked to low grain yield in rice. Lanes 1; IR64, 2; Azucena, 3; high yielding bulk, 4; medium yielding bulk, 5; low yielding bulk, 6-15; individual genotypes of high yielding bulk, 16-24; individual genotypes of low yielding bulk.

[Markers linked to grain yield using bulked segregant analysis approach in rice (Oryza sativa L.)(Shashidar)]

TABLE 1—*Distribution on the potato map of QTLs controlling yield and tuber traits.*

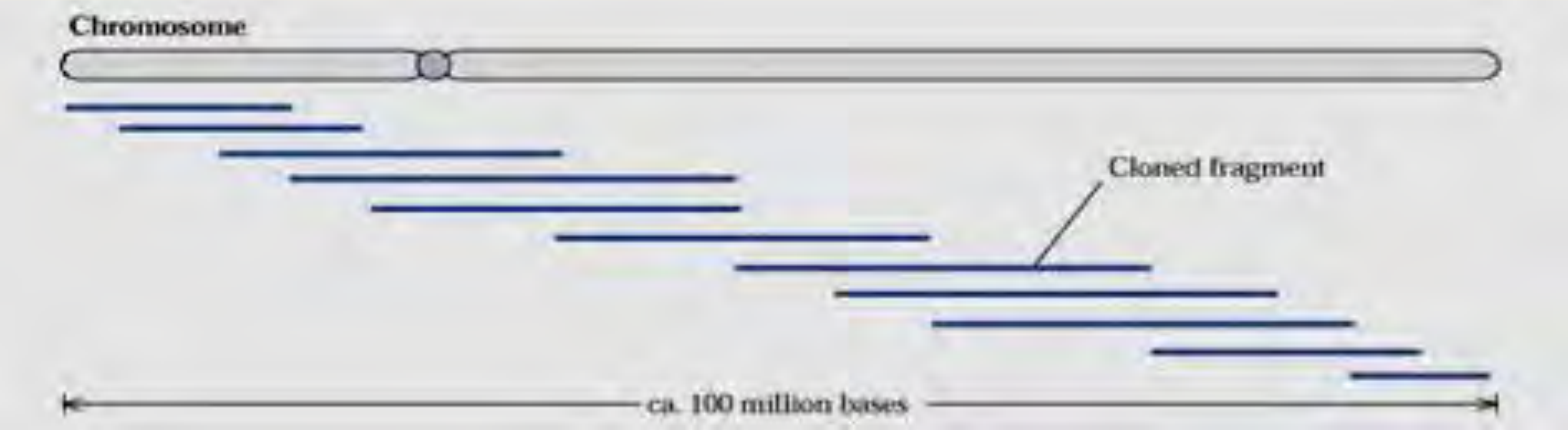
Trait	Chromosome	Reference
Yield	I, II, V, VI, VII, VIII, X, XII	Schafer-Pregl et al. 1998
Tuber number	II, IV, VII, IX	Bonierbale et al. 1993
Tuber formation	I, II, III, IV, V, VI, VIII	van der Berg et al. 1996a
Tuber weight	I, II, IV, VII, IX	Bonierbale et al. 1993
Tuber dormancy	II, III, IV, V, VIII	Freyre et al. 1994; van der Berg et al. 1996b
Specific gravity	I, II, III, IV, V, VII, IX, XI, XII	Bonierbale et al. 1993; Freyre and Douches 1994
Chip color	II, IV, V, X	Douches and Freyre 1994
Starch content	All	Schafer-Pregl et al. 1998
Glycoalkaloid content	I, IV, VI, VIII, XI, XII	Yencho et al. 1998; Bouarte-Medina et al. 2002

Molecular Marker-assisted Selection for Potato Breeding (Barone 2004)



Construction of a potato consensus map and QTL meta-analysis . . .genetic architecture of late blight resistance and plant maturity traits (Danan et.al.2011)

How PCR Can Help Breeding Selection: Molecular Mapping



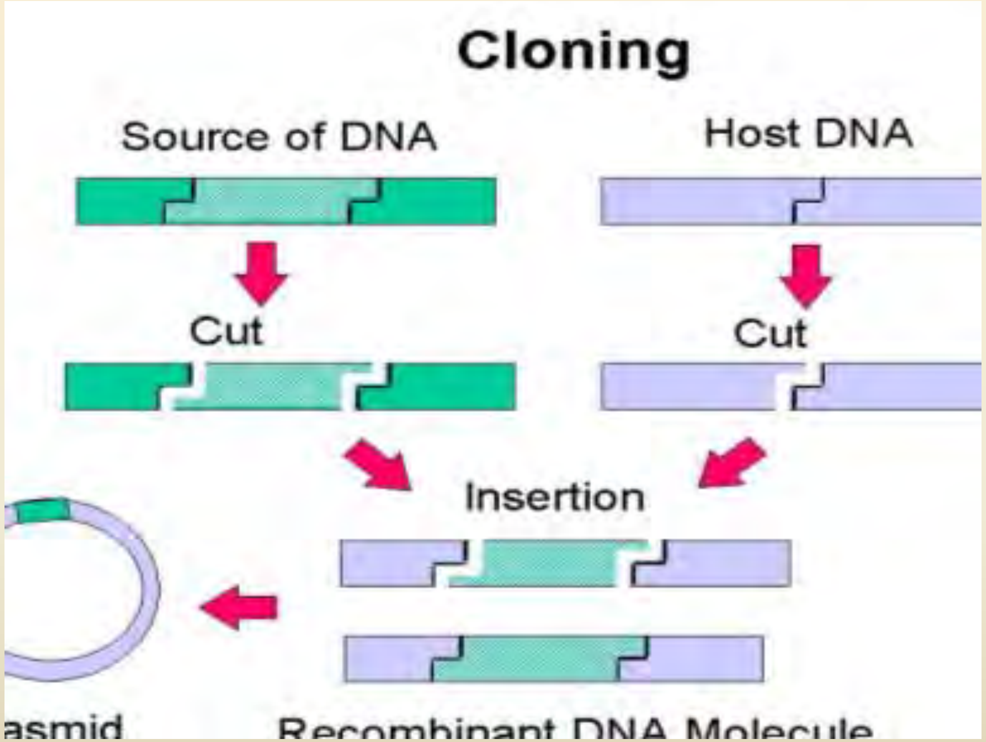
http://www.web-books.com/MoBio/Free/Ch8D1.htm

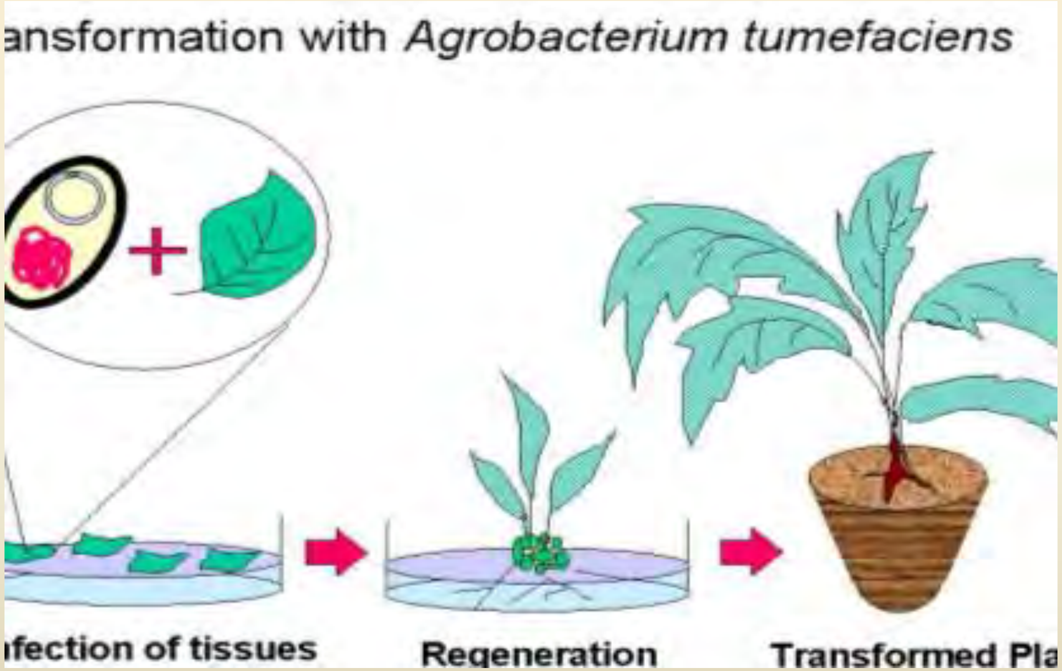
TABLE 1 *R* genes and QTL mapped in potato

Chromosome	Gene	Anchor markers	Pathogen	Reference
I	*Eca_QTL*	*CP108, St3.3.13(d)*	*E. carotovora* ssp. *atroseptica*	119
	Pi_QTL	*CP11*	*P. infestans*	78
II	*Eca_QTL*	*GP23*	*E. carotovora* ssp. *atroseptica*	119
	Pi_QTL	*GP23*	*P. infestans*	78
	Pi_QTL	*GP26*	*P. infestans*	78
III	*Eca_QTL*	*St1.2.1(b)*	*E. carotovora* ssp. *atroseptica*	119
	Pi_QTL	*GP1(a), GP25, CP6*	*P. infestans*	61, 78
	Pi_QTL	*TG135*	*P. infestans*	30
	Gro1.4_QTL	*Ssp8*	*G. rostochiensis*	58
	Pi_QTL	*GP276*	*P. infestans*	78
	Pi_QTL	*4CL*	*P. infestans*	61, 106
IV	*Pi_QTL*	*GP180, MBF, TG62*	*P. infestans*	61, 78
	Eca_QTL	*St1.2.4(e)*	*E. carotovora* ssp. *atroseptica*	119
	Gpa4_QTL	*Stm3016*	*G. pallida*	10
	Pi_QTL	*Stm3016*	*P. infestans*	73
	R2	—	*P. infestans*	64
V	*Pi_QTL*	*Gp21, GP179*	*P. infestans*	61, 78
	R1	*GP21, GP179*	*P. infestans*	60
	Rx2	*GP21*	*Potato Virus X*	87
	Nb	*GP21*	*Potato Virus X*	20
	Gpa_QTL	*Ssp72*	*G. pallida*	57
	Gpa5_QTL	*GP21, GP179*	*G. pallida*	92
	Grp1_QTL	*GP21, GP179*	*G. pallida, G. rostochiensis*	91
	Pi_QTL	*CP113*	*P. infestans*	78
	H1	*CP113, CD78*	*G. rostochiensis*	34, 83
	GroV1	*TG69*	*G. rostochiensis*	49
VI	*Pi_QTL*	*GP79*	*P. infestans*	78
	Eca_QTL	*St3.3.13(c)*	*E. carotovora* ssp. *atroseptica*	119
	Pi_QTL	*GP76*	*P. infestans*	61, 78
VII	*Eca_QTL*	*GP219*	*E. carotovora* ssp. *atroseptica*	119
	Gro1	*CP56, St3.3.2*	*G. rostochiensis*	4, 59
VIII	*Pi_QTL*	*GbssI(wx), Osm*	*P. infestans*	78, 106
	Eca_QTL	*GbssI*	*E. carotovora* ssp. *atroseptica*	119
	Eca_QTL	—	*E. carotovora* ssp. *atroseptica*	119
	R_{blc}	*CP53*	*P. infestans*	76
IX	*Pi_QTL*	*Prp1, PAL(a,c)*	*P. infestans*	61, 78
	Eca_QTL	*GP129*	*E. carotovora* ssp. *atroseptica*	119
	Nx_{phu}	*TG424, CT220*	*Potato Virus X*	104
	Gpa6_QTL	*CT220*	*G. pallida*	92
X	*Eca_QTL*	—	*E. carotovora* ssp. *atroseptica*	119
	R_{ber}	*TG63*	*P. infestans*	30
	Gro1.2_QTL	*TG63*	*G. rostochiensis*	56
XI	Ry_{adg}	*CP58, GP125*	*Potato Virus Y*	42
	Ry_{sto}	*CP58, TG523*	*Potato Virus Y*	12
	Na_{adg}	*TG523*	*Potato Virus A*	40
	R_{Mc1}	*TG523*	*M. chitwodii*	13
	Pi_QTL	*GP125*	*P. infestans*	61, 78
	Eca_QTL	*GP125, St3.3.13(a)*	*E. carotovora* ssp. *atroseptica*	119
	Sen1	*CP58, GP125*	*S. endobioticum*	45
	Gro1.3_QTL	*Ssp75, TG30*	*G. rostochiensis*	56
	R3	*TG105(a), GP185, GP250*	*P. infestans*	23
	R6	*GP185, GP250*	*P. infestans*	25
	R7	*GP185, GP250*	*P. infestans*	25
	Pi_QTL	*GP185, GP250*	*P. infestans*	78
	Eca_QTL	*GP185, St1.2.1(a)*	*E. carotovora* ssp. *atroseptica*	119
XII	*Eca_QTL*	—	*E. carotovora* ssp. *atroseptica*	119
	Pi_QTL	—	*P. infestans*	38
	Pi_QTL	*GP34, CP60*	*P. infestans*	78
	Rx1	*GP34, CP60*	*Potato Virus X*	87
	Gpa2	*GP34*	*G. pallida*	93

Organization of genes controlling disease resistance in the potato genome (Gebhardt et.al. 2001)

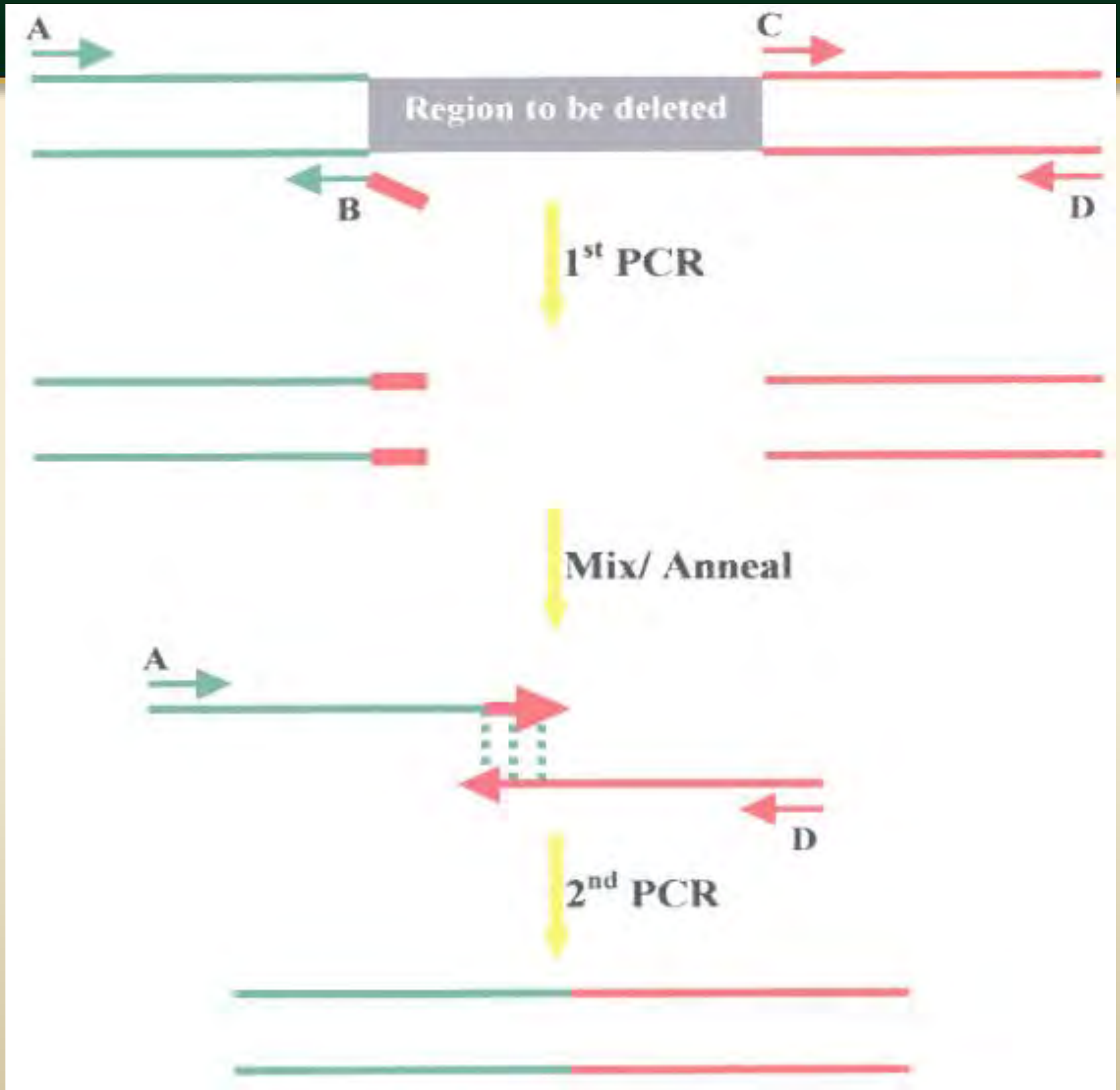
How PCR Can Help Breeding Selection: Introgression: Insertion





http://inspection.gc.ca/plants/potatoes/guidance-documents/chapter-10/

How PCR Can Help Breeding Selection: Introgression: Repair



http://www.molecularinfo.com/images/B2-1.jpg

How PCR Can Help Breeding Selection: Genotype Screening

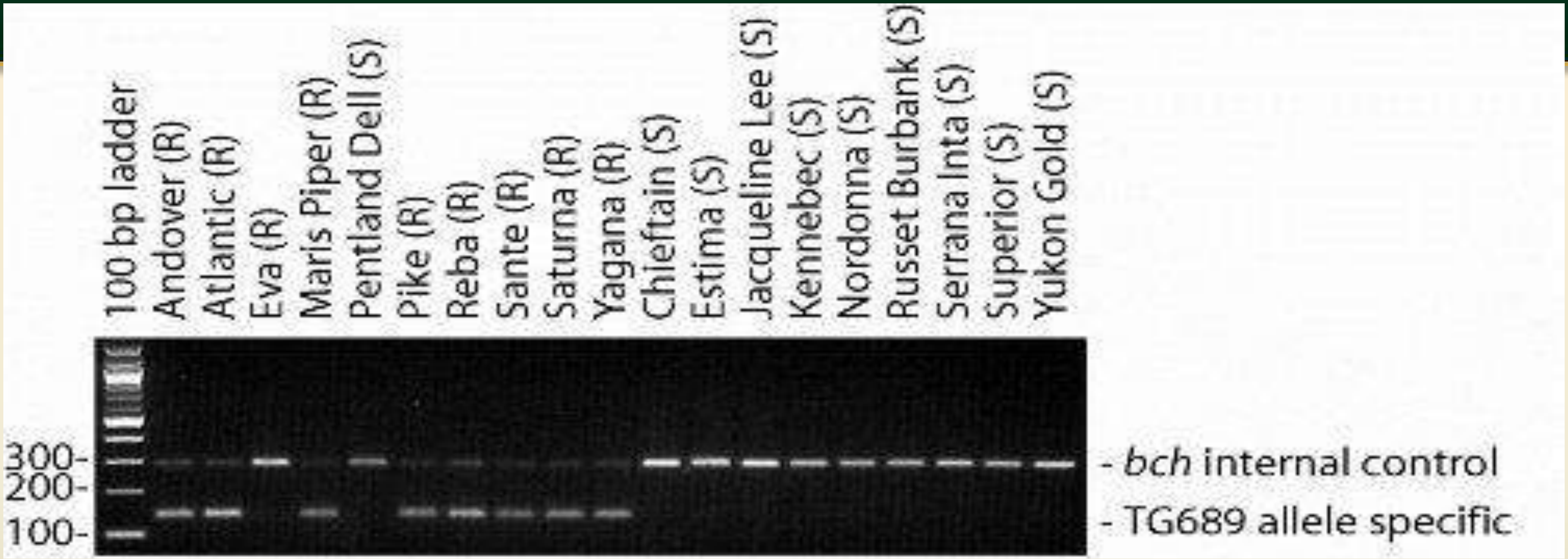


Figure 1. Results of PCR amplification of the *H1* locus from several commercial potato varieties. Varieties followed by (R) are GN resistant based on phenotypic evidence, those followed by (S) are susceptible. Almost all resistant varieties exhibit the TG689 allele-specific band (141 bp); known exceptions include the resistant varieties 'Eva', 'Salem', and 'Sunrise'. The 290 bp band is an

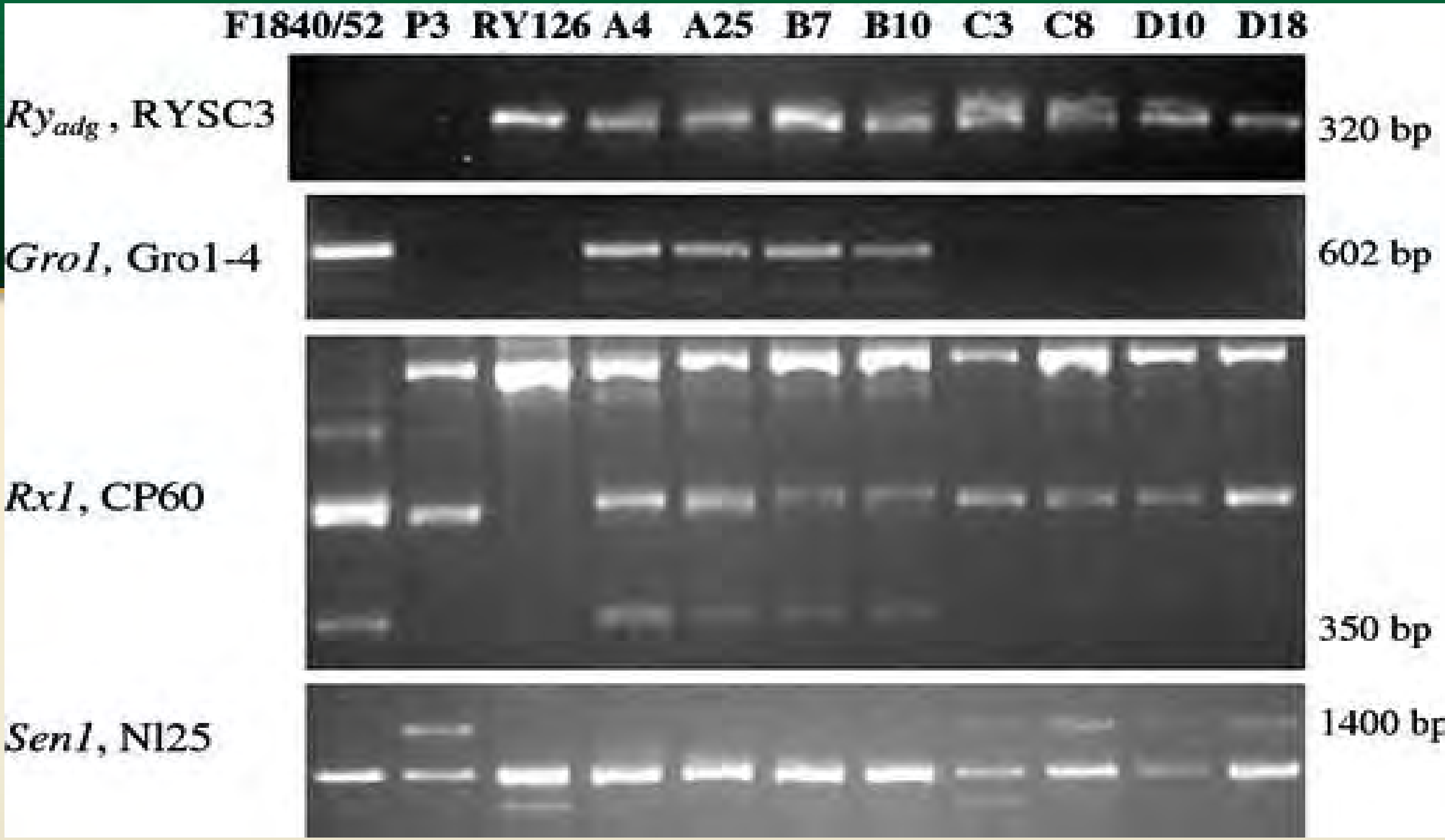


Fig. 1 PCR-based marker phenotypes used for MAS. The resistance locus and the linked marker are shown on the left of each panel. The diagnostic DNA fragment for each resistance locus is indicated by its approximate size in base pairs shown on the right. The first three lanes from the left in each panel show the parental marker phenotypes. The remaining lanes show the marker phenotypes of two individuals, each of the H98A, H98B, H98C and H98D family

Marker-assisted combination of major genes for pathogen resistance in potato (Gebhardt et.al.2016)

How PCR Can Help Breeding Selection Summary

Three Ways How PCR Can Help Breeding Selection

- **Marker development**: $$$$
- **Amplify gene and introgression**: $$$
- **Qualify presence or absence**: $

PCR, as one of the recent tools in breeding, offers screening of more progeny in 4-5 years vs. phenotype selection in 6+ years in a potato breeding program.


Thank You!