Existing potato markers and marker conversions

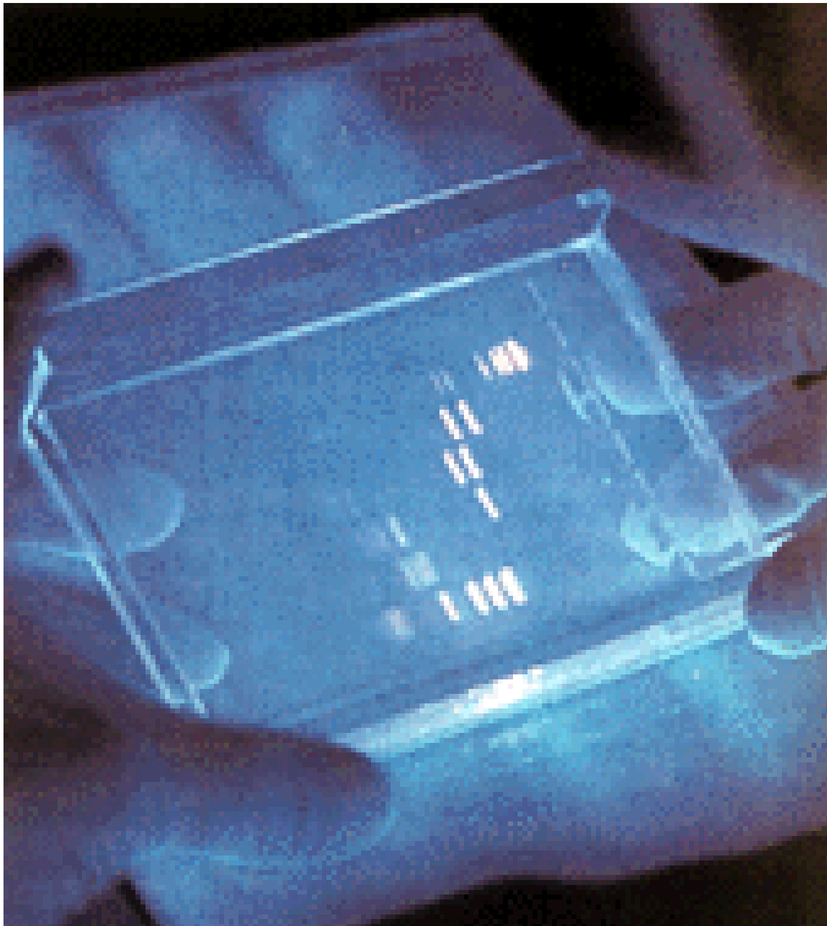
Walter De Jong
PAA Workshop
August 2009

What makes for a good marker?

- diagnostic for trait of interest
- robust – works even with DNA of poor quality or low quantity
- cheap
- easy to use
- co-dominant
- dosage sensitive

Agarose Gel Electrophoresis

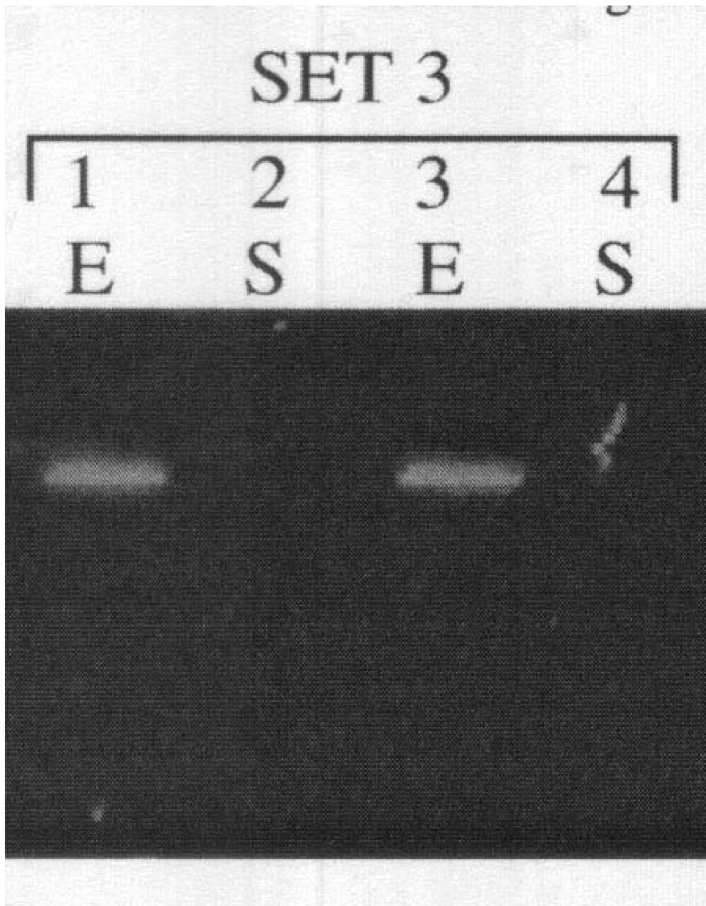
Key assumption of this presentation: most of us will continue to use agarose gels for years to come



- ☺ Easy
- ☺ Universal
- ☹ Expensive per data point
- ☹ Low throughput

Example of a potato marker

PVY (Ry_{adg}) – Kasai et al. 2000 Genome 43:1-8



allele specific amplification of a diagnostic product

- potatoes with the product are resistant
- potatoes without the product are susceptible**

** or the PCR failed for some reason

This may be the most useful potato marker developed to date

How are allele specific markers made?

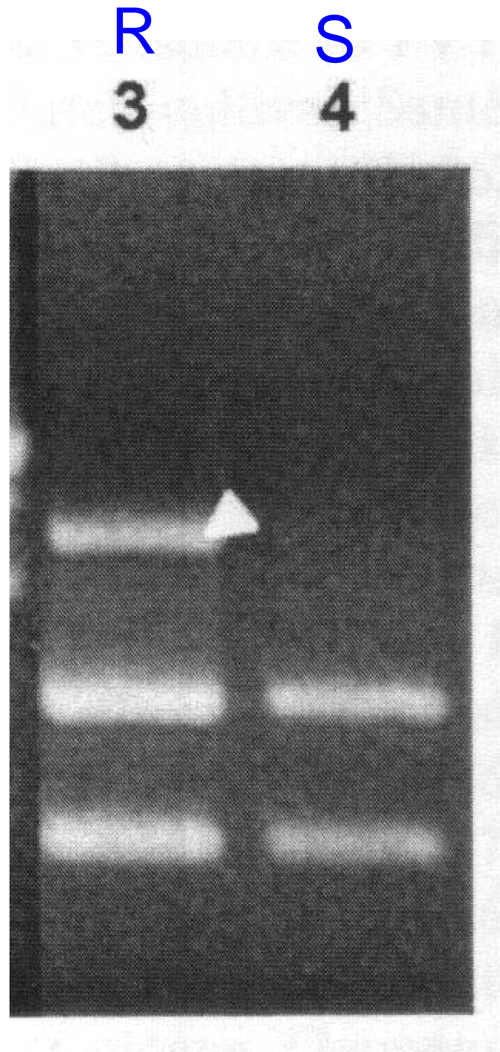
```
                                           ATAAAAC TCTTGGTTAT AGCCTA->
haplotype 1          AATGAGAGAA GACATAAAAC TCTTGGTTAT AGCCTATAGG AGCCAGCTAC 100
haplotype 2          AATGAGAGAA GACACAAAAC TCTTGGT--- ----TATAGG AGCCAGATAC  93
haplotype 3          AATGAGAGAA GACACAAAAC TCTTGGT--- ----TATAGG AGCCAGCTAC  93

haplotype 1          TTTACCGGAA AGTTT-CCCA GAATACTTAT AGGATATCTT ATGTCAAATT 149
haplotype 2          TTTACCGGAG AGTT-CCCCA TAATACTTAT GTCAA----- -----AATTT 132
haplotype 3          TTTACCGGAA AATTTCCCAG AATACTT-AT AGGATATCTT ATGTCAAATT 142

haplotype 1          TCATTGAAGC AGTATTATTT TTTTTCTTGG AATTGGTGAA ACAACACATT 199
haplotype 2          TCA-TGATGC AGTATTATTT TTTC--TTGG AATTGGTGAA ACAACACATT 179
haplotype 3          TCATTGATGC AGTATTTTTT TTTC--TTGG AATTGGTGAA ACAACACATT 190
                                                      <-TAACCACTT TGTTGTGTA
```

One or both amplification primers anneal only to allele of interest

PVY (Ry_{sto}) – Flis et al. 2005 Molecular Breeding 15: 95-101



A co-dominant CAPS marker:

presence of upper band diagnostic for resistance

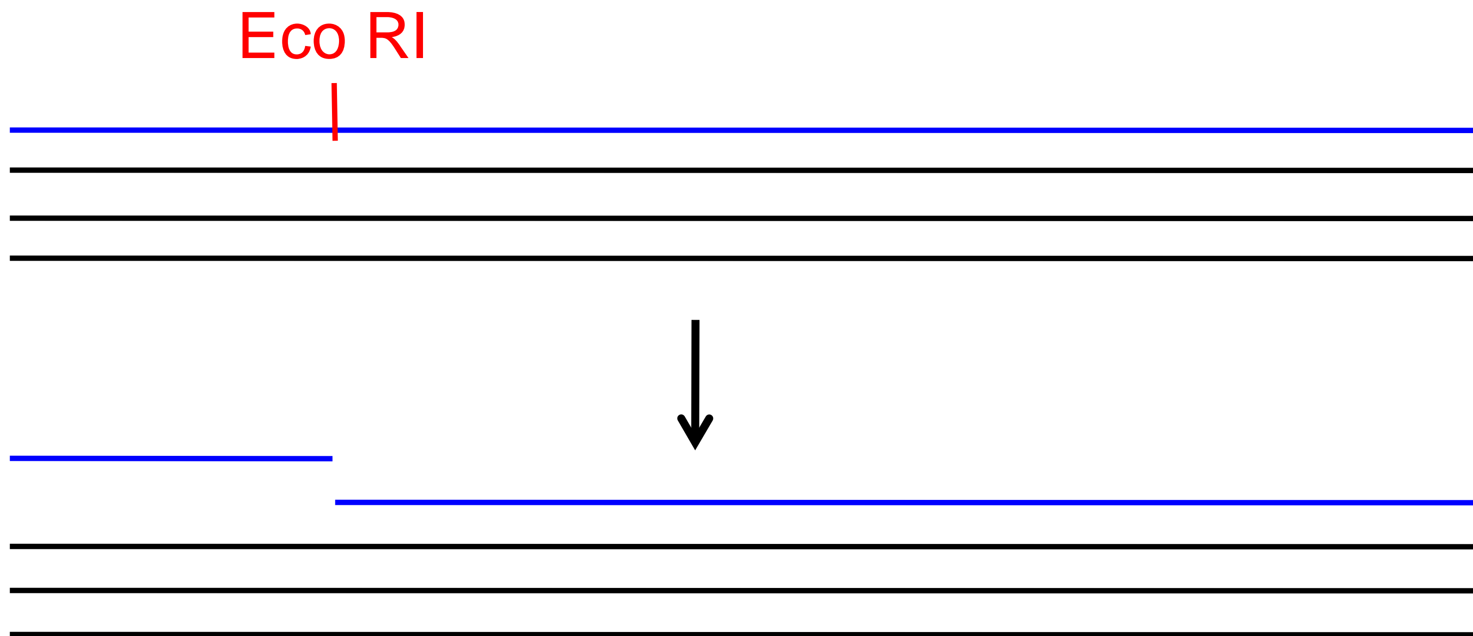
absence of upper band diagnostic for susceptibility

lower bands represent one or more additional alleles, and are present in most progeny

How do CAPS markers work?

(CAPS = cleaved amplified polymorphic sequence)

1. Amplify all alleles by PCR
2. Digest with a restriction enzyme that generates unique pattern with allele of interest



reference: Konieczny A, Ausubel FM. Plant J 1993 4(2):403-410

an unpublished marker linked to H1 (golden nematode resistance gene)

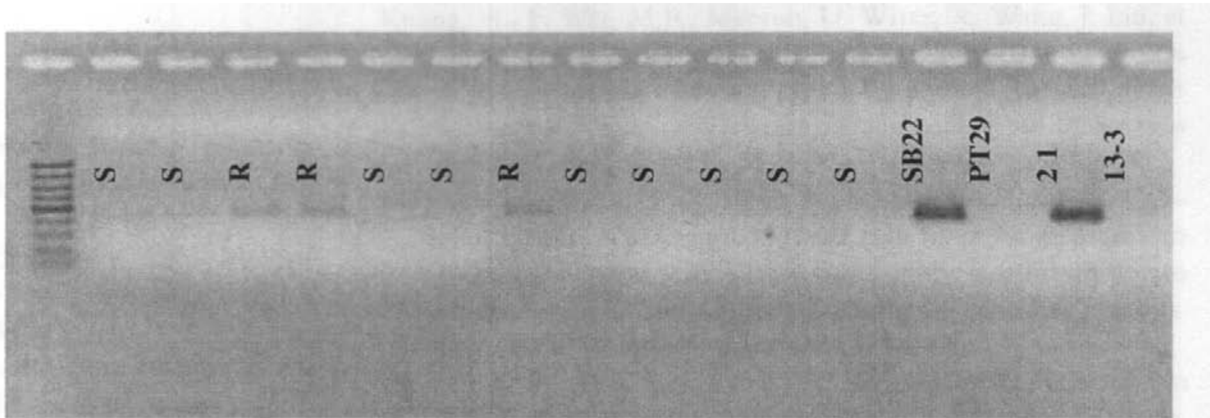
100 bp ladder
Andover (R)
Atlantic (R)
Eva (R)
Maris Piper (R)
Pentland Dell (S)
Pike (R)
Reba (R)
Sante (R)
Saturna (R)
Yagana (R)
Chieftain (S)
Estima (S)
Jacqueline Lee (S)
Kennebec (S)
Nordonna (S)
Russet Burbank (S)
Serrana Inta (S)
Superior (S)
Yukon Gold (S)

300-
200-
100-

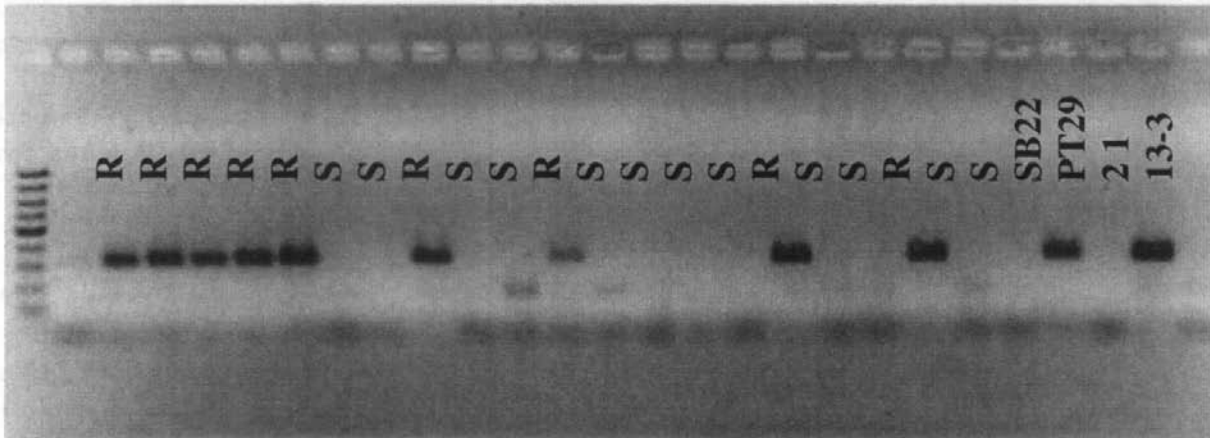
- *bch* internal control
- TG689 allele specific

-- an allele-specific band is diagnostic for resistance
-- an unrelated band is used as a control for success of PCR

Is the marker robust?



a



two markers linked to root-knot resistance:

which would you rather use?

Not all DNA isolation methods yield pristine DNA

Cheap, inexpensive methods like Edwards quickprep result in DNA of variable quality

Many commercial kits cost 1$/prep and yield exceptionally clean DNA

In a breeding program, more likely to use quick-and-dirty methods – and primers that amplify well with clean DNA won't necessarily amplify dirty DNA very well

Try many primer pairs when designing assay you will use a lot – some primer pairs are more robust than others

markers for resistance to potato pathogens

- PVY (Ry_{adg}) – Kasai et al. 2000 Genome 43:1-8
- PVY (Ry_{sto}) – Flis et al. 2005 Molecular Breeding 15: 95-101
- PVX resistance (*Rx1*) – Gebhardt et al. 2006 Theor Appl Genet 112:1458-1464
- Golden nematode (*Gro1-4*) – Gebhardt et al. 2006 Theor Appl Genet 112:1458-1464
- Golden nematode (*H1*) – ask Walter De Jong for a protocol (unpublished)
- White cyst nematode race Pa2/Pa3 – Sattarzadeh et al. 2006 Mol Breeding 18:301-312
- Root-knot nematode ($R_{Mc1(blb)}$) – Zhang et al. 2007 Crop Science 47:2021-2026
- Wart (*Sen1*) – Gebhardt et al. 2006 Theor Appl Genet 112:1458-1464
- Late blight (*RB*) – Colton et al. 2006 Crop Sci 46:589-594

some markers for potato quality traits

- Dominant allele at the red (*R*) locus – De Jong et al 2003 Theor Appl Genet 107:1375-1383
- A dominant allele at the developer (*D*) locus – Zhang et al 2009 Theor Appl Genet doi:10.1007/s00122-009-1100-8
- Dominant allele at the yellow (*Y*) locus – Brown et al 2006 Am J Potato Research 83:365-372
- Allele of invertase associated with chip color – Li et al 2005 Genetics 170:813-821
- Allele of sucrose synthase associated with chip color – Kawchuk et al 2008 Am J Pot Res 85: 227-231

Useful website for markers in 19 crops

Generation Challenge Program Toolbox

http://s2.generationcp.org/gcp-tmm/web/

Many potato markers are described here

Why marker conversion?

The markers used to map traits are generally not the same markers that breeders are willing/able to use for MAS

In potato, RFLP, AFLP and SSR markers have most often been used for mapping. SolCAP will be using Infinium to detect SNPs.

- none of these are simple PCR markers well-suited for agarose gels

In the future, most of us will likely move to SNP-based platforms. We're just not there yet.

SNP to PCR marker conversion

SolCAP will be testing 7600 SNP markers

Example of a SNP:

AAGTCTGTGT
AAGACTGTGT

SolCAP will convert SNP markers to simple PCR markers (small grants program)

Following slides discuss marker conversions in detail

- SNP
- RFLP
- AFLP

Creating a CAPS marker from a SNP requires:

- 25 bp of DNA sequence on each side of the SNP (for PCR primers to anneal to)
  - For each SolCAP SNP, a lot of flanking sequence will generally be known
- a computer with internet access

Identifying a restriction enzyme for a CAPS assay

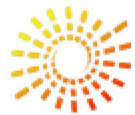
- Several applications automatically identify restriction enzymes that can be used to detect a SNP with a CAPS assay
  - Blastdigester
    - http://bar.utoronto.ca/ntools/cgi-bin/ntools_blast_digester.cgi
    - Ilic et al, 2004. Trends in Genetics 20(7):280-283
  - SNPS2CAPS
    - http://pgrc.ipk-gatersleben.de/snp2caps/
    - Thiel et al, 2004 Nucl. Acids Res. 32: e5.
  - SGN CAPS designer
    - http://sgn.cornell.edu/tools/caps_designer/caps_input.pl

Software comparison

- SGN CAPS is the easiest and simplest to use.
- However, it does not evaluate all RE’s commercially available.
- SNPS2CAPS is more comprehensive and flexible, but not as user friendly.
- Blastdigester finds SNPs in BLAST output

SGN CAPS designer

- This web-based tool accepts sequence input as aligned sequences (clustal format) or individual FASTA sequences



sol genomics network

home | forum | contact | help

search maps sequencing tools sol search

log in

CAPS Designer

Introduction

This tool designes CAPS assays for two sequences. Two types of nucleotide inputs are accepted: fasta sequences and clustal aligment. It generates a list of polymorphic enzymes that cut the sequences into different length products. The user is allowed to input up to three sequences, however each time only two are analyzed.

Suggestions:

1. Low quality nucleotides and "n"s at both ends of a sequence generate ambiguity. Please remove them from the input sequences.
2. Polymorphic digested fragments of too small sizes are hard to visualize on 1-4% agarose gel, thus not suitable for CAPS experiment. Please exclude some nucleotides (for example 20) at both ends to avoid the problem.

http://sgn.cornell.edu/tools/caps_designer/caps_input.pl

SGN CAPS designer

First step: organize two sequences (one for each allele) in FASTA format

>allele1

AATAGCAGCATGGTGGGCATCCTCCTGTCTCCACTGCTCGAATCCTTTCCTCCGGCAATGCACCATGTTTTATTAAGAACTCTCCATCTTCTTTCGTGAATATATCATTTGTTACCTACAAAGAAATGCATCTTAACATGGACATTAATTC
ATTCTACATCTTAGAACAAAAACGTGAAGATTTCAAGATCATAGACTTGGAAAGTGATGAAAGAGCG**C**CGGAATTCCAGAACACCAAATACATGCTTGCACATAACGTATTTCCTGTCAAACTAACAAACGTCAAGTAAACTCCCA
AAACCTCGGTTTGAAGTTTTGGCAGATGCCAATATGTGATGTTTAGGAAGGAGTCCTTAATGATTAAAAAACAAGAACATGACCTATTGAGATAATCTCCAAAGGCAATTGTGTATAAAGAATGTTGTTATTCCACTTGAAATGACTC
AATCAAACGGAAAATTGCATGTAACACCCATCTTCATCTAGAATTTCAAATGATGAGGCAGAGAAAGATATGAAAACCACCAATAAACTTGAGTTCCATTTTCCAAACCACAAAAGTGAATCCACCAGTTCCAAAAAATTGTGCAGAC
TAAACTTATTCATGAAGTAAGTATGTCACAATGGCAAAAGAAAGAAATCATTTGGTGGTACATACTGCTGCAAGACTGTATTTTTCCCTCAAGATTTTACATAATGCCAGCATTAGAGCTGTTTTCCTGCATATAAAAGAACAGTTAGT
CCCTCAAAATCTCAACATGTTCAACCAAAATTTTACAAGCTAATAAACAAGAAAGAAACTGCGATTGGAGGAAAAGCAAAAGCAAACACTAATACTACAAAAACAATAACATACCCTGTGAAGTCACTCCAGTACATTATGAACCTTG
AACTGAAAAGGAAAATGCTCTAGCACACTCATACACACATTCACACAGTCAGATATGTGTCTAATGGAACAATTGTGTTCCTATATGCAGAGTTCTAAAGATTCAATTTTTTTATCAATAAAAATGGTCCCTTTTGTTTCTTACTTGGGT
TGCTGCAGCTAAAAGAAAATCCTACTTACAACAGATACCAAAAGCTACTAAATATCATCCTCCCCCTTCTACTTTCATTTCTCAAAGATTGAATTTTTCTTCTCAAATACTGAAAACCCTTTCACTTGAACACACATCCCAAGACATAAAT
TTAAGAAAAATTGAGGGAAAAGAAGAAATACCCAGTACCAACAGGGCCACCAATTCCAATAGTAAAGGCTCTTTCACTGAAATTCCTGTCATTAAGTGGAGGTGCCCTTCTGCTAAAGTAGCCCAGGTGAATAAATAGG

>allele2

AATAGCAGCATGGTGGGCATCCTCCTGTCTCCACTGCTCGAATCCTTTCCTCCGGCAATGCACCATGTTTTATTAAGAACTCTCCATCTTCTTTCGTGAATATATCATTTGTTACCTACAAAGAAATGCATCTTAACATGGACATTAATTC
ATTCTACATCTTAGAACAAAAACGTGAAGATTTCAAGATCATAGACTTGGAAAGTGATGAAAGAGCG**T**CGGAATTCCAGAACACCAAATACATGCTTGCACATAACGTATTTCCTGTCAAACTAACAAACGTCAAGTAAACTCCCA
AAACCTCGGTTTGAAGTTTTGGCAGATGCCAATATGTGATGTTTAGGAAGGAGTCCTTAATGATTAAAAAACAAGAACATGACCTATTGAGATAATCTCCAAAGGCAATTGTGTATAAAGAATGTTGTTATTCCACTTGAAATGACTC
AATCAAACGGAAAATTGCATGTAACACCCATCTTCATCTAGAATTTCAAATGATGAGGCAGAGAAAGATATGAAAACCACCAATAAACTTGAGTTCCATTTTCCAAACCACAAAAGTGAATCCACCAGTTCCAAAAAATTGTGCAGAC
TAAACTTATTCATGAAGTAAGTATGTCACAATGGCAAAAGAAAGAAATCATTTGGTGGTACATACTGCTGCAAGACTGTATTTTTCCCTCAAGATTTTACATAATGCCAGCATTAGAGCTGTTTTCCTGCATATAAAAGAACAGTTAGT
CCCTCAAAATCTCAACATGTTCAACCAAAATTTTACAAGCTAATAAACAAGAAAGAAACTGCGATTGGAGGAAAAGCAAAAGCAAACACTAATACTACAAAAACAATAACATACCCTGTGAAGTCACTCCAGTACATTATGAACCTTG
AACTGAAAAGGAAAATGCTCTAGCACACTCATACACACATTCACACAGTCAGATATGTGTCTAATGGAACAATTGTGTTCCTATATGCAGAGTTCTAAAGATTCAATTTTTTTATCAATAAAAATGGTCCCTTTTGTTTCTTACTTGGGT
TGCTGCAGCTAAAAGAAAATCCTACTTACAACAGATACCAAAAGCTACTAAATATCATCCTCCCCCTTCTACTTTCATTTCTCAAAGATTGAATTTTTCTTCTCAAATACTGAAAACCCTTTCACTTGAACACACATCCCAAGACATAAAT
TTAAGAAAAATTGAGGGAAAAGAAGAAATACCCAGTACCAACAGGGCCACCAATTCCAATAGTAAAGGCTCTTTCACTGAAATTCCTGTCATTAAGTGGAGGTGCCCTTCTGCTAAAGTAGCCCAGGTGAATAAATAGG

SGN CAPS designer

- Second step: paste FASTA sequences into SGN CAPS designer, select desired options, then click “Find Caps”

Query Input

Input format

- ( ) clustal alignment [What is this?]
- (•) unaligned fasta sequences

Sequence pair to analyze

Please enter **at least two sequences.** Please choose two from the first three sequences to analyze.

- (•) sequence 1 and 2 (default)
- ( ) sequence 1 and 3
- ( ) sequence 2 and 3

Input sequences

```
>CT10649_C
AATAGCAGCATGGTGGGCATCCTCCTGTCTCCACTGCTCGAATCCTTTCCTCCGGCAATGCACCATGTTTTATTAAGAACTCTCCATCTTCTTTCGTGAATA

>CT10649_T
AATAGCAGCATGGTGGGCATCCTCCTGTCTCCACTGCTCGAATCCTTTCCTCCGGCAATGCACCATGTTTTATTAAGAACTCTCCATCTTCTTTCGTGAATA
```

Options

- [ ] Find enzymes priced less than $65/1000u.

Exclude [0] nucleotides at both ends

Don't show enzymes that cut both parents more than [4] times

[Find Caps]

[Reset]

SGN CAPS designer

If the first option is checked, the output will only display *inexpensive* RE's that can be used for a CAPS marker.

Entering the default of 20 bp insures that the RE site is not within 20 bp of the end of the fragment. so the marker can be resolved by agarose gel electrophoresis.

Options

☐ Find enzymes priced less than $65/1000u.

Exclude [0] nucleotides at both ends

Don't show enzymes that cut both parents more than [4] times

If there are to many sites for an enzyme, the fragment will be cut into several small pieces, which will be difficult to resolve and score on agarose gels.

SGN CAPS designer

Output (truncated): This is the top of the output after clicking on “Find Caps”

It is a good idea to click on the link to view the clustal alignment to make sure results are reasonable.

CAPS Designer Result

For experienced users

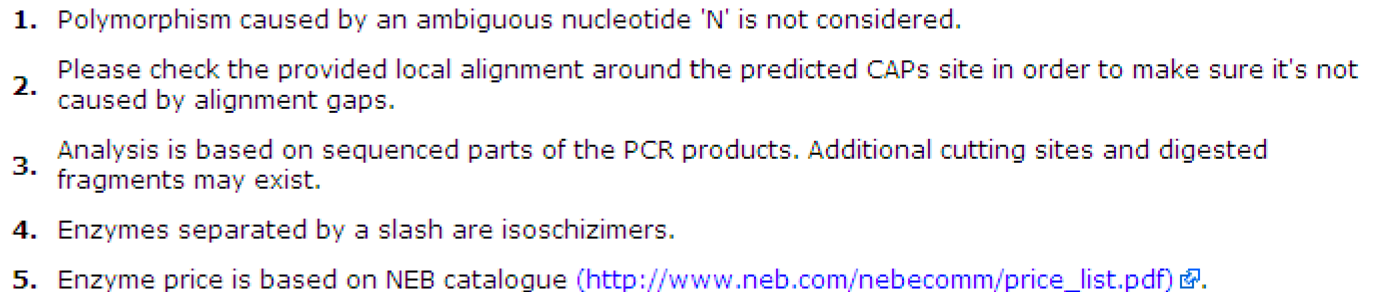
View/download plain text result file

View/download alignment in clustal format

View/dowload alignment in fasta format

Back to input

Notes



1. Polymorphism caused by an ambiguous nucleotide 'N' is not considered.
2. Please check the provided local alignment around the predicted CAPs site in order to make sure it's not caused by alignment gaps.
3. Analysis is based on sequenced parts of the PCR products. Additional cutting sites and digested fragments may exist.
4. Enzymes separated by a slash are isoschizimers.
5. Enzyme price is based on NEB catalogue (http://www.neb.com/nebecomm/price_list.pdf).

Query Summary

Aligned Sequences	CT10649_C, CT10649_T		
Alignment Length(w/ gaps)	1330 bp	**Search Range**	1 - 1330 bp
Cutting Sites Limit	4		
Enzyme Selection	All		

SGN CAPS designer

A reasonable alignment, showing a single SNP

Example is from tomato, where SNPs are relatively uncommon

```
CLUSTAL W (1.83) multiple sequence alignment


CT10649_C       AATAGCAGCATGGTGGGCATCCTCCTGTCTCCACTGCTCGAATCCTTTCCTCCGGCAATG
CT10649_T       AATAGCAGCATGGTGGGCATCCTCCTGTCTCCACTGCTCGAATCCTTTCCTCCGGCAATG
                ************************************************************

CT10649_C       CACCATGTTTTATTAAGAACTCTCCATCTTCTTTCGTGAATATATCATTTGTTACCTACA
CT10649_T       CACCATGTTTTATTAAGAACTCTCCATCTTCTTTCGTGAATATATCATTTGTTACCTACA
                ************************************************************

CT10649_C       AAGAAATGCATCTTAACATGGACATTAATTCATTCTACATCTTAGAACAAAAACGTGAAG
CT10649_T       AAGAAATGCATCTTAACATGGACATTAATTCATTCTACATCTTAGAACAAAAACGTGAAG
                ************************************************************

CT10649_C       ATTTCAAGATCATAGACTTGGAAAGTGATGAAAGAGCGCCGGAATTCCAGAACACCAAAT
CT10649_T       ATTTCAAGATCATAGACTTGGAAAGTGATGAAAGAGCGTCGGAATTCCAGAACACCAAAT
                ************************************** *********************
                                           SNP?

CT10649_C       ACATGCTTGCACATAACGTATTTCCTGTCAAACTAACAAACGTCAAGTAAACTCCCAAAA
CT10649_T       ACATGCTTGCACATAACGTATTTCCTGTCAAACTAACAAACGTCAAGTAAACTCCCAAAA
                ************************************************************

CT10649_C       CCTCGGTTTGAAGTTTTGGCAGATGCCAATATGTGATGTTTAGGAAGGAGTCCTTAATGA
CT10649_T       CCTCGGTTTGAAGTTTTGGCAGATGCCAATATGTGATGTTTAGGAAGGAGTCCTTAATGA
                ************************************************************

CT10649_C       TTAAAAAACAAGAACATGACCTATTGAGATAATCTCCAAAGGCAATTGTGTATAAAGAAT
CT10649_T       TTAAAAAACAAGAACATGACCTATTGAGATAATCTCCAAAGGCAATTGTGTATAAAGAAT
                ************************************************************

CT10649_C       GTTGTTATTCCACTTGAAATGACTCAATCAAACGGAAAATTGCATGTAACACCCATCTTC
CT10649_T       GTTGTTATTCCACTTGAAATGACTCAATCAAACGGAAAATTGCATGTAACACCCATCTTC
                ************************************************************
```

Output (truncated):
This is the bottom of the output

For this example, we have a choice of five RE's that we can use in a CAPS marker.

Factors to consider when choosing a RE:
1. Price
2. The number of fragments produced
3. Is the RE already in use in your lab?
4. Does the enzyme cut OK in PCR buffer?

5. Especially for an autotetraploid – the ideal enzyme cleaves all alleles except the allele you want to track – see next two slides

CAPS Candidates

Enzyme	CfoI/HhaI	Price	HhaI,2000u/$53
Recognition Sequence	GCGC		
CT10649_C Cutting Site(s)	219	CT10649_C Fragments(s),bp	219 1111
CT10649_T Cutting Site(s)	None	CT10649_T Fragments(s),bp	1330
CAPS Site	219	CT10649_C ggaaagtgatgaaagaGCGCcggaattcca CT10649_T ggaaagtgatgaaagaGCGTcggaattcca	

Enzyme	Hpy188I	Price	Hpy188I,1000u/$58
Recognition Sequence	TC.GA		
CT10649_C Cutting Site(s)	943	CT10649_C Fragments(s),bp	943 387
CT10649_T Cutting Site(s)	223 943	CT10649_T Fragments(s),bp	223 720 387
CAPS Site	223	CT10649_C agtgatgaaagagcgCCGGAattccagaac CT10649_T agtgatgaaagagcgTCGGAattccagaac	

Enzyme	Hpy99I	Price	over $65/1000u
Recognition Sequence	CG[T|A]CG		
CT10649_C Cutting Site(s)	None	CT10649_C Fragments(s),bp	1330
CT10649_T Cutting Site(s)	221	CT10649_T Fragments(s),bp	221 1109
CAPS Site	221	CT10649_C aaagtgatgaaagagCGCCGgaattccaga CT10649_T aaagtgatgaaagagCGTCGgaattccaga	

Enzyme	LpnI/Bsp143II/BstH2I/HaeII	Price	over $65/1000u
Recognition Sequence	[A|G]GCGC[C|T]		
CT10649_C Cutting Site(s)	220	CT10649_C Fragments(s),bp	220 1110
CT10649_T Cutting Site(s)	None	CT10649_T Fragments(s),bp	1330
CAPS Site	220	CT10649_C gaaagtgatgaaagAGCGCCggaattccag CT10649_T gaaagtgatgaaagAGCGTCggaattccag	

Enzyme	MspI	Price	MspI,5000u/$53
Recognition Sequence	CCGG		
CT10649_C Cutting Site(s)	55 222	CT10649_C Fragments(s),bp	55 167 1108
CT10649_T Cutting Site(s)	55	CT10649_T Fragments(s),bp	55 1275
CAPS Site	222	CT10649_C aagtgatgaaagagcgCCGGaattccagaa CT10649_T aagtgatgaaagagcgTCGGaattccagaa	

When designing CAPS markers, if possible, choose an enzyme that doesn't cut allele of interest

1. Larger bands are easier to score on an agarose gel (more ethidium binding sites per molecule = brighter)

When designing CAPS markers, if possible, choose an enzyme that doesn't cut allele of interest

2. In a heterodimer, the absence of a restriction site is dominant over the presence of a restriction site

In last cycle of PCR, many heterodimers are formed as strands of different alleles anneal to each other

1/4

1/4

1/4

1/4

Eco RI

1/16

6/16

9/16

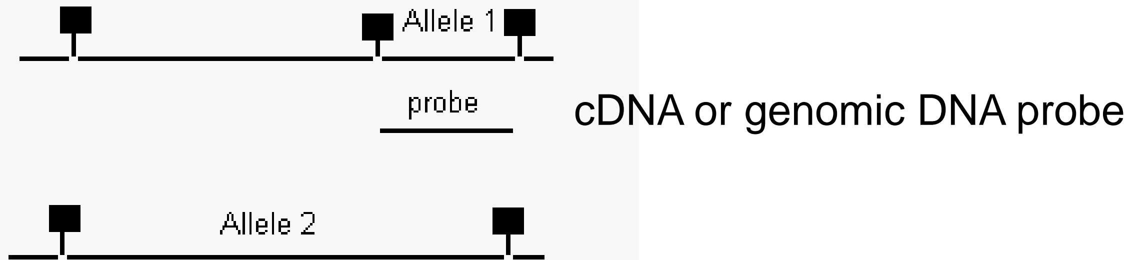
7/16 don't cut

Sometimes the SGN CAPS designer does not identify any useful REs. This might be because there are no RE sites created by any SNPs present.

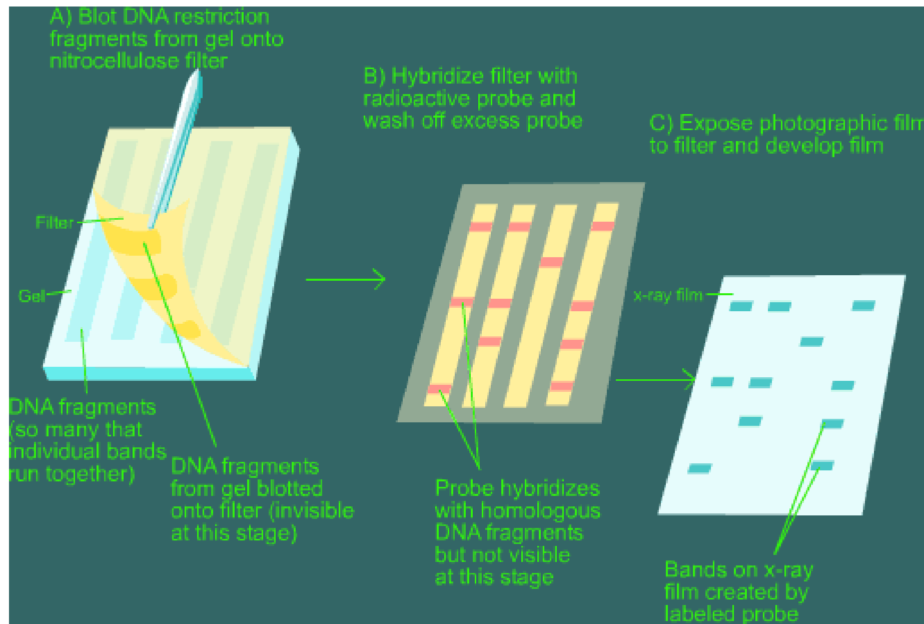
However, note that the SGN CAPS designer does not test all commercially available RE’s. For a more comprehensive analysis, you can use SNPS2CAPS.

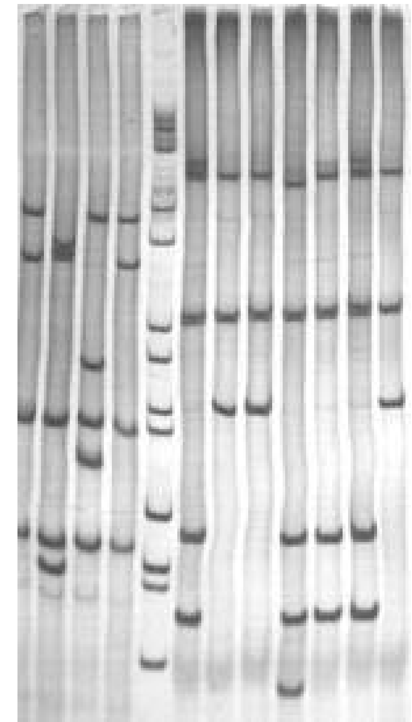
Restriction sites can also be created by designing primers with one or two mismatches that anneal in the vicinity of the SNP. This is the “dCAPs” approach; for a reference see Neff et al 2002 Trends in Genetics 18:613-615

How RFLP markers work



cDNA or genomic DNA probe



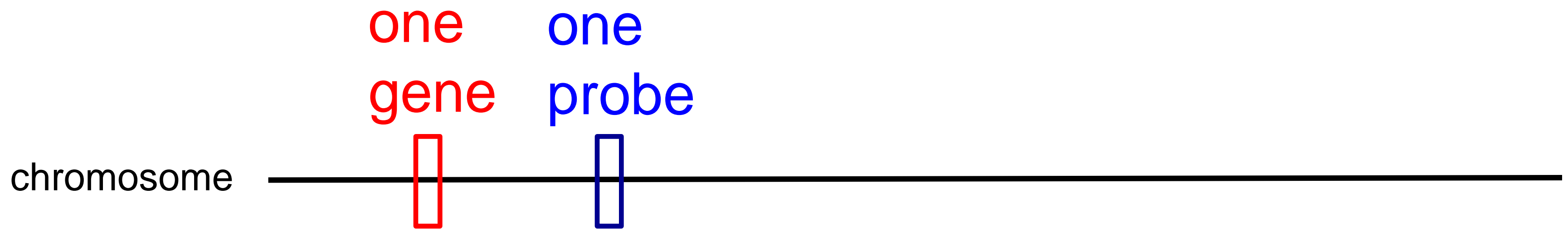


RFLP to PCR marker conversion

- Two sources of RFLP marker polymorphism:
  - polymorphic restriction sites
  - large deletions/insertions in sequence between restriction sites
- If you sequence an RFLP probe, you can't tell what the polymorphism was that gave rise to the polymorphic band
  - all you have is the sequence of a small part of the genome, one allele of which happens to be (tightly) linked to trait of interest

alleles, not genes, are key in breeding

How a molecular biologist thinks about the relationship between an RFLP probe and a gene:



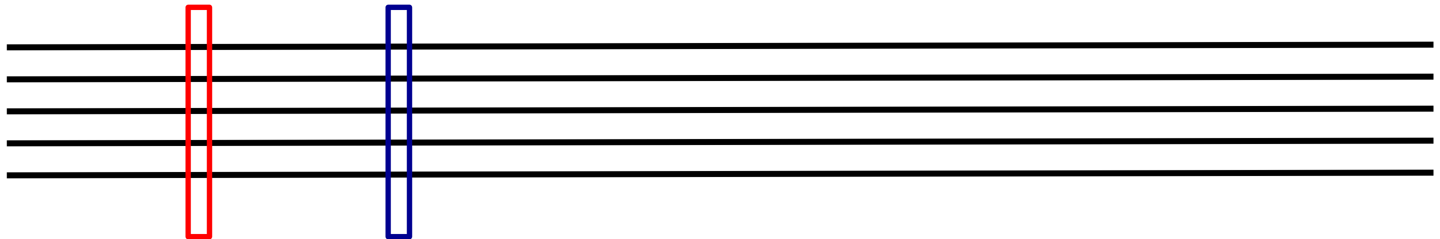
alleles, not genes, are key in breeding

How a breeder thinks about the relationship between an RFLP probe and a gene:

many alleles of the gene

many alleles of the probe

chromosome diversity across species



important issue in marker conversion: re-establishing the relationship between PCR marker and trait alleles

- a simple way to do this:
  - identify all alleles of new PCR marker
  - test each allele in turn, to see which (if any) correlates with desired trait allele
- sometimes no correlation is found
  - can occur if primers don't amplify necessary marker allele (lots of SNPs in potato; can occur at primer binding site)
  - can also occur if primers amplify a different part of the genome entirely (problem in repetitive DNA)

typical sequence variation / haplotype structure in potato (observed in pooled DNA, 10 cultivars)

COSII_ At3g54470; 19 clones sequenced; first 215 bp; orotate phosphoribosyltransferase

haplotype	polymorphic positions											%
	28	**68**	125	132	134	141	151	157	183	207	214	
a	G	A	C	T	G	A	-	-	A	-	G	37
b	G	A	C	C	A	G	-	-	A	G	G	26
c	A	G	C	C	G	A	-	-	A	-	G	16
d	G	G	C	C	A	A	-	-	T	-	G	11
e	G	G	A	C	G	A	A	G	C	-	A	11

COSII_At4g26750; 17 clones sequenced; 367 bp
unknown protein; matches tomato EST DB722697 over entire length

haplotype	polymorphic positions											%
	47	**48**	**76**	**101**	**111**	**128**	**188**	**229**	**308**	**309**	**310**	
a	C	A	A	G	A	A	T	T	-	-	-	35
b	T	T	A	G	G	C	A	G	G	T	G	35
c	T	T	G	A	G	A	T	T	G	T	G	30

How AFLP markers work

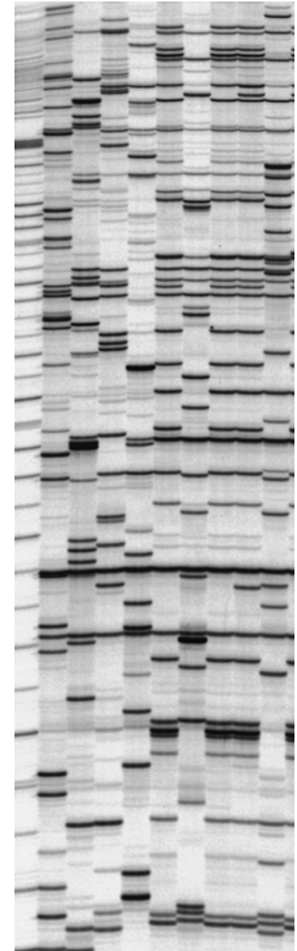
genomic DNA

Restriction enzyme digestion

Adaptor ligation

Selective PCR amplification

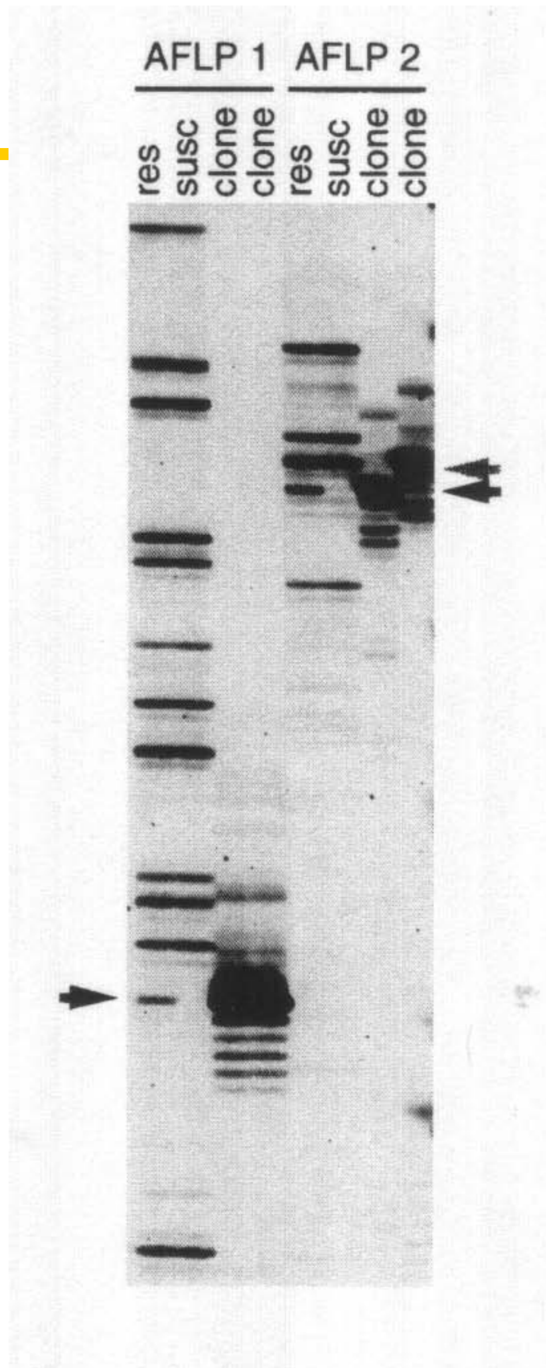
AFLP fingerprint



AFLP to PCR marker conversion

- Two sources of AFLP marker polymorphism:
  - polymorphic restriction sites
  - polymorphic SNPs in sequence adjacent to adaptors (affect primer binding)

- Just as for an RFLP probe - if you clone and sequence an AFLP band, you can't immediately ID the polymorphism that gave rise to the band
  - Unlike an RFLP probe, AFLP bands are generally small – this provides less sequence to design primers against, and less sequence polymorphism to work with
  - It is often necessary to clone and sequence flanking sequence to obtain useful polymorphism (e.g. by inverse PCR)



If you want to clone and sequence an AFLP band, make sure the clone is the correct size before sequencing it

once potato genome sequence becomes available...

...there will be huge amounts of flanking sequence around each SNP, providing essentially infinite ways to design primers for useful PCR assays

assessing allele dosage

- for the more advanced – two references that describe general approaches for measuring dosage of specific alleles in potato:
  - TaqMan (De Jong et al 2003 Theor Appl Genetics 107:1384-1390)
  - High Resolution Melting (De Koeyer et al 2009 Molecular Breeding DOI 10.1007/s11032-009-9309-4)