Isozymes – The First Molecular Marker System

Equipment

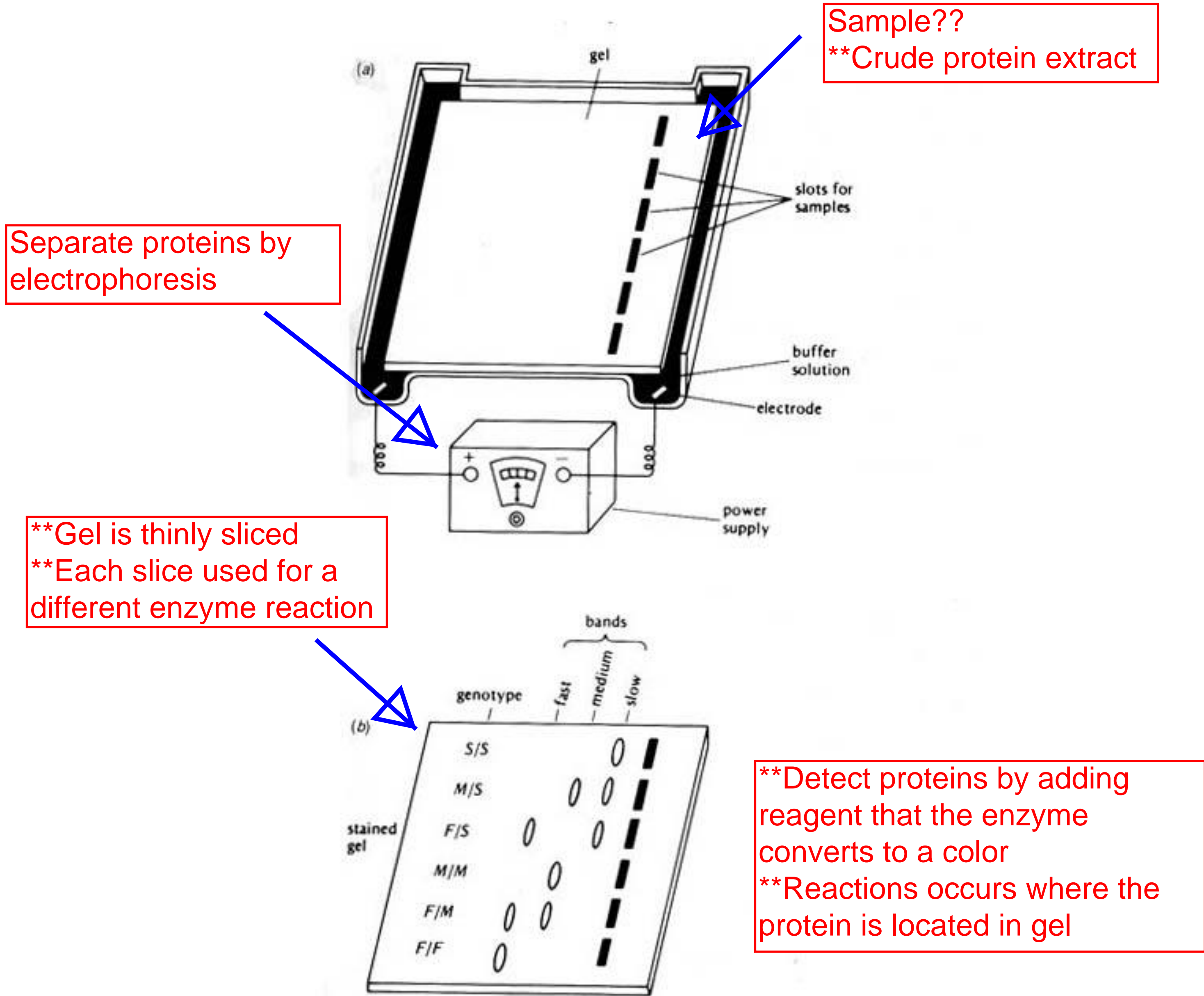


Fig. 6.10. (a) Apparatus for gel electrophoresis. (b) A zymogram produced by staining a gel (see text for a discussion of the interpretation of banding patterns). (From Strickberger, 1985.)

http://www.plantbiology.siu.edu/PLB479/images/Fig6_10.jpg

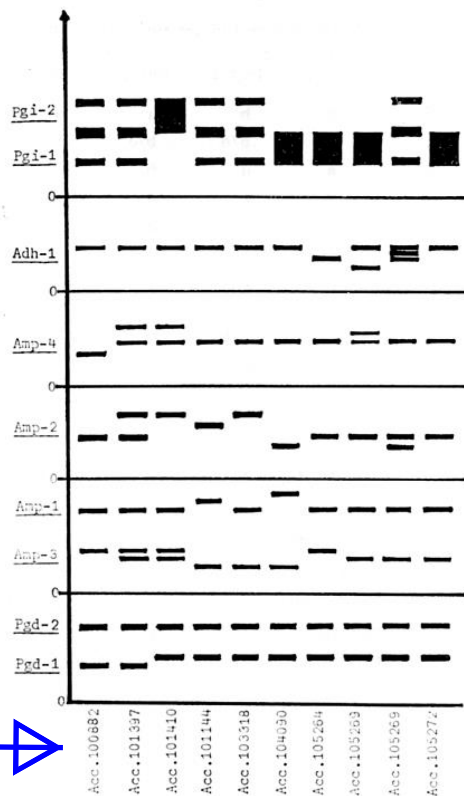
Image of an isozyme starch gel

Polymorphic isozyme

http://wheat.pw.usda.gov/ggpages/oatnewsletter/v48/Isozyme_files/image002.jpg

Schematic of multiple isozyme systems; each was based on a single dye for a single family of proteins. ***Note the segregation pattern*.**

Population screen for isozyme variation



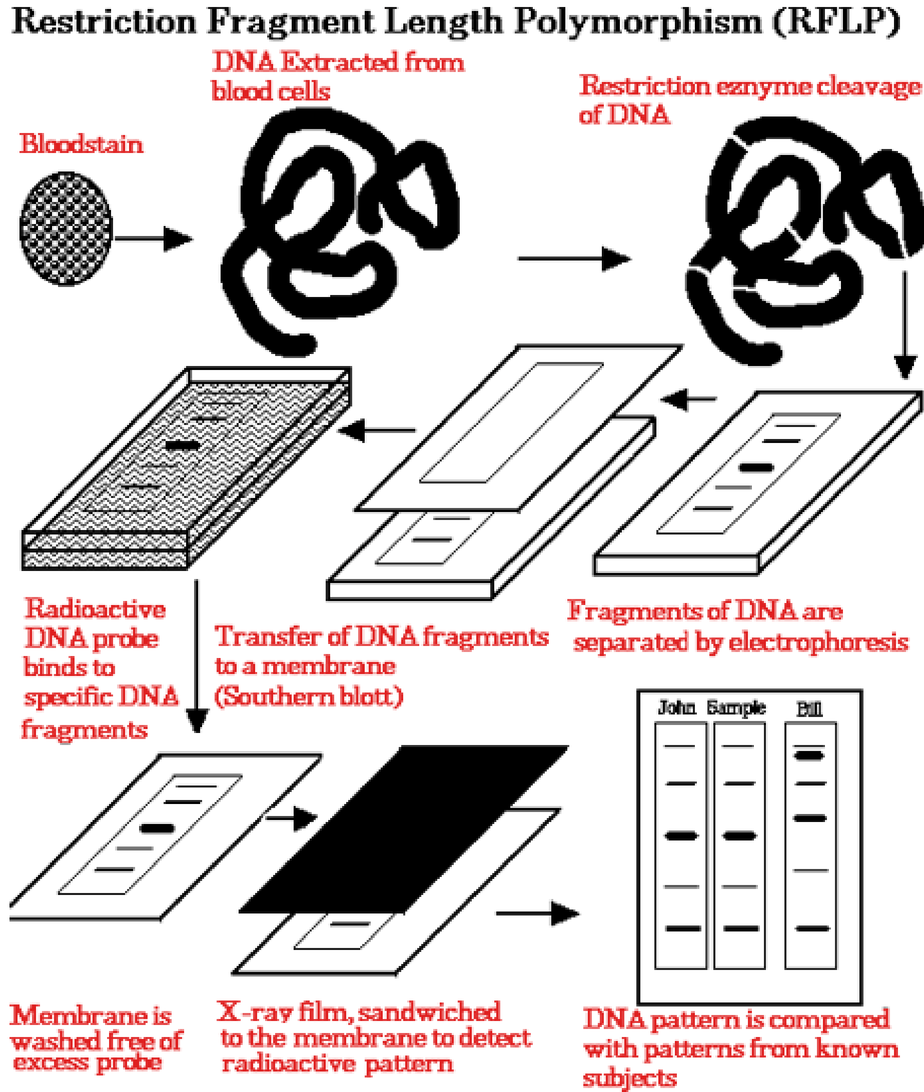
Accessions

Fig. 1.

http://www.shigen.nig.ac.jp/rice/rgn/vol8/v8p83F1.jpg

Restriction Fragment Polymorphisms

Technology Approach



Restriction enzyme and PROBE combination

http://homepage.smc.edu/hgp/images/rflp.gif

Original plant RFLP paper figure (autoradiography image)

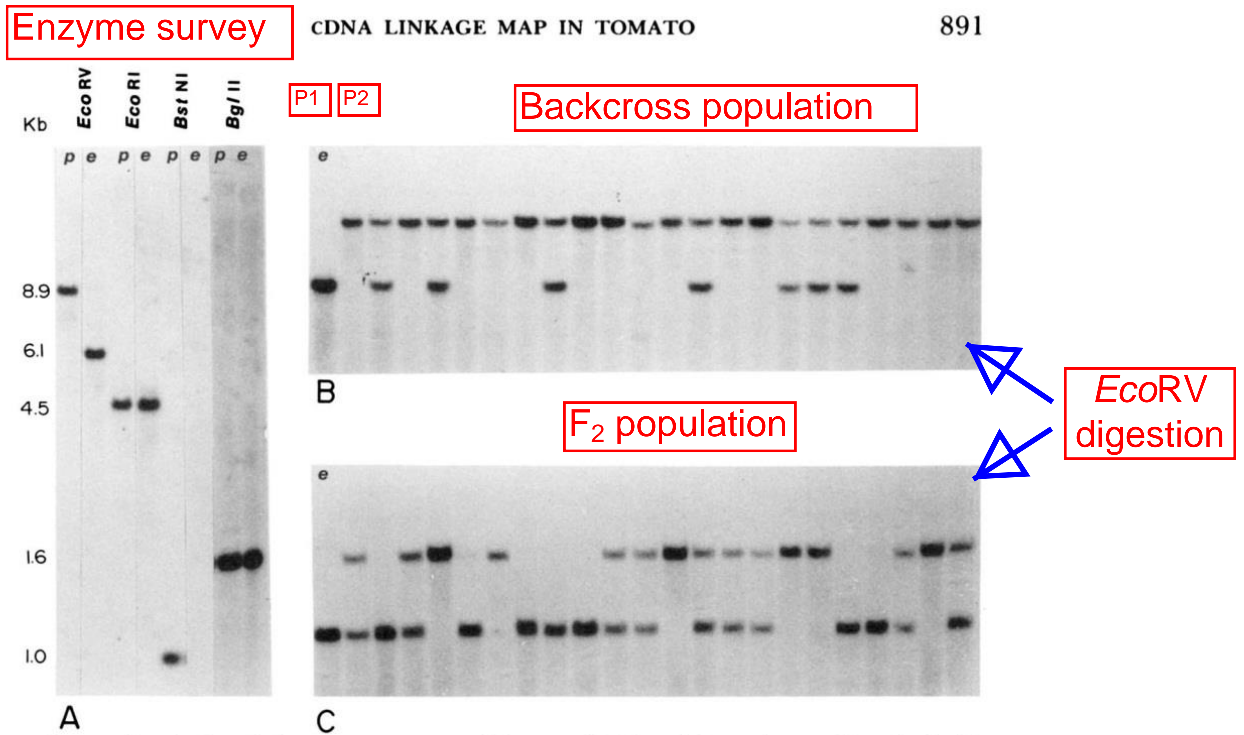


FIGURE 2.—A, Restriction enzyme survey of *L. pennellii* (p) and *L. esculentum* (e) probed with clone 3-41 (*CD14*). The values at left are the fragment sizes in kilobases. B, Backcross progeny DNA (*L. pennellii* as the recurrent parent) digested with *Eco*RV and probed with 3-41. C, F_2 progeny DNA digested with *Eco*RV and probed with 3-41.

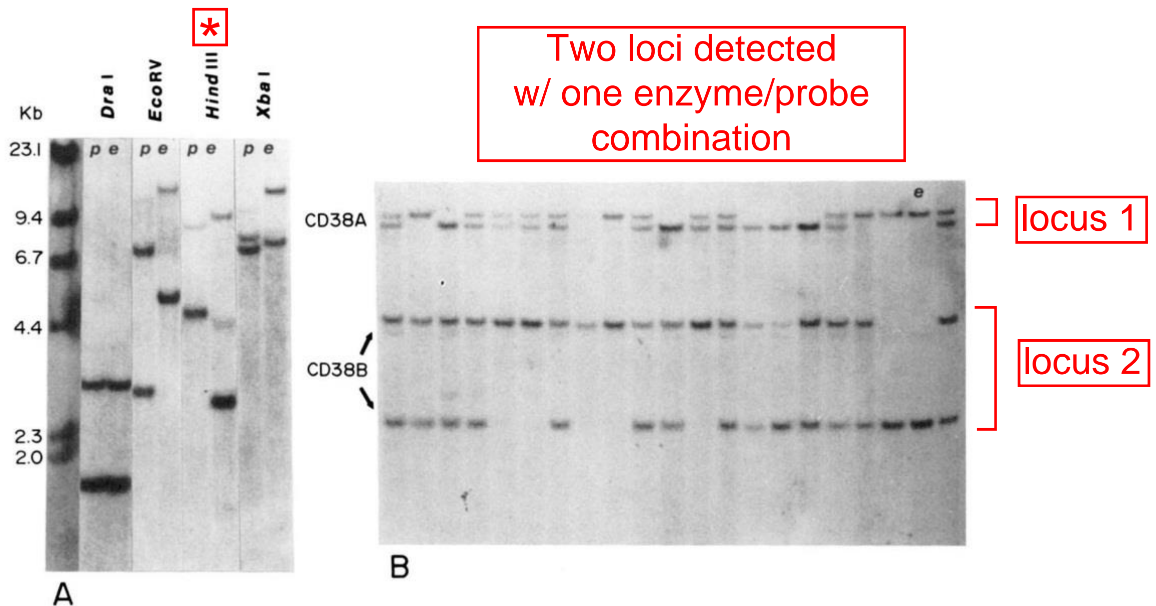


FIGURE 3.—A, Restriction enzyme survey of *L. pennellii* (p) and *L. esculentum* (e) probed with clone 3-275 (*CD38A* and *B*). The first lane is DNA digested with *Hind*III, and the fragment sizes are indicated at left. B, F_2 progeny DNA digested with *Hind*III and probed with 3-275.

Genetics 112:887 (1986)

RAPD (Randomly Amplified Polymorphic) Markers

Operon: company developed primers

Gel image (*Phaseolus vulgaris*)

F_2 population

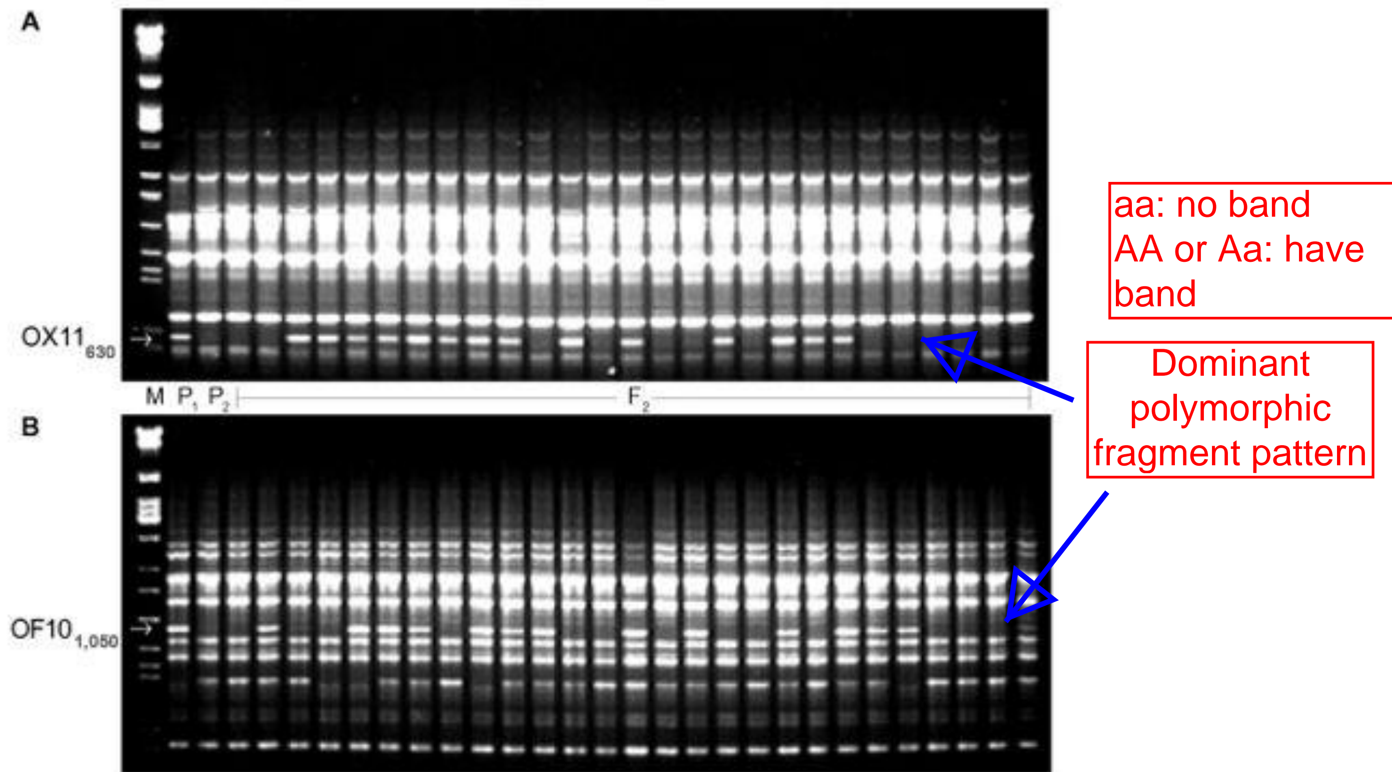
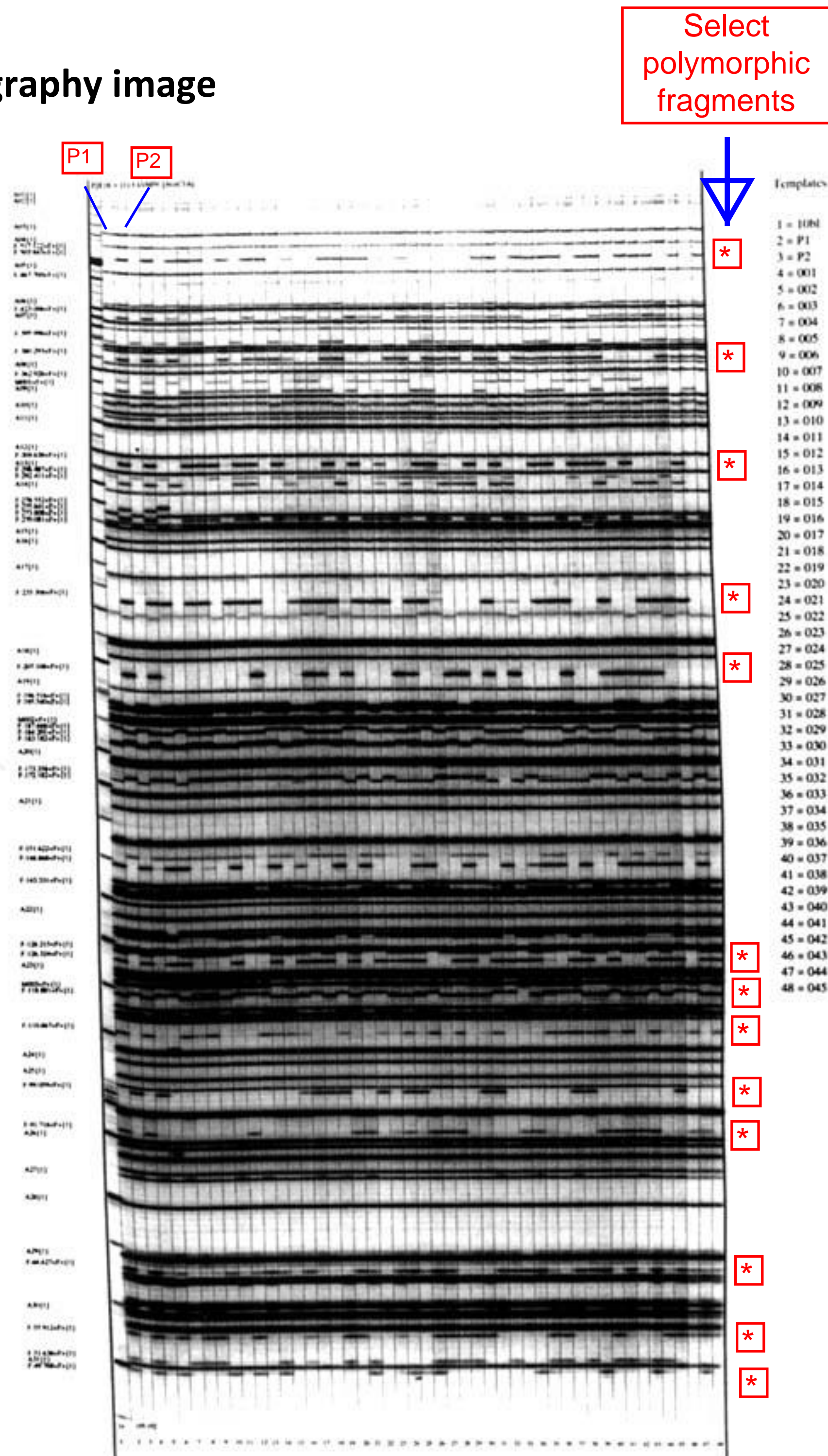


Figure 1 - Electrophoretic analyses of DNA amplification products obtained with primers OX11 (A) and OF10 (B). In both gels, P_1 corresponds to cultivar Ouro Negro, P_2 to cultivar US Pinto 111 and F_2 to 27 individuals from the segregating population. Lane M contains lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The arrows indicate markers $OX11_{630}$(A) and $OF10_{1,050}$(B).

http://www.scielo.br/img/fbpe/gmb/v23n2/2758f1.jpg

AFLP (Amplified Fragment Length Polymorphism) Markers

Autoradiography image

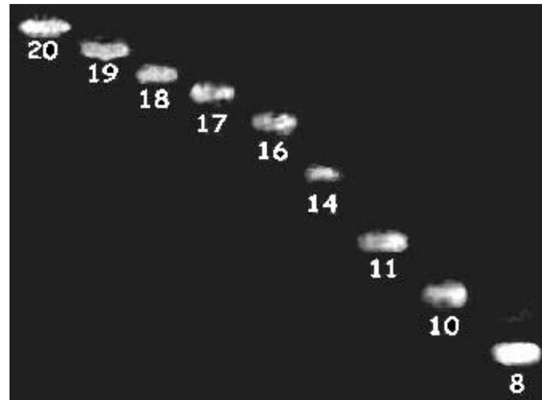


http://www.shigen.nig.ac.jp/rice/rgn/vol14/p106Fig.1.jpg

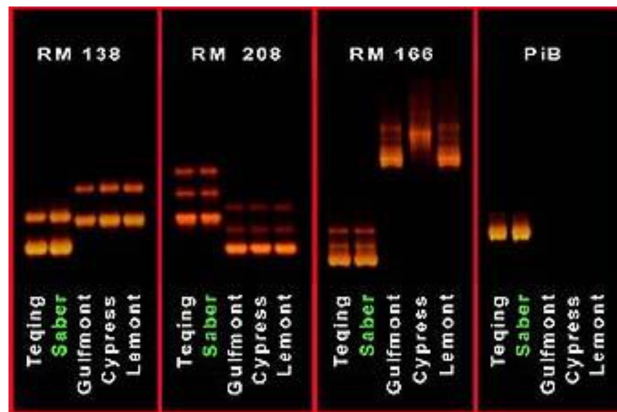
Microsatellite (=SSR) Markers

Gel images

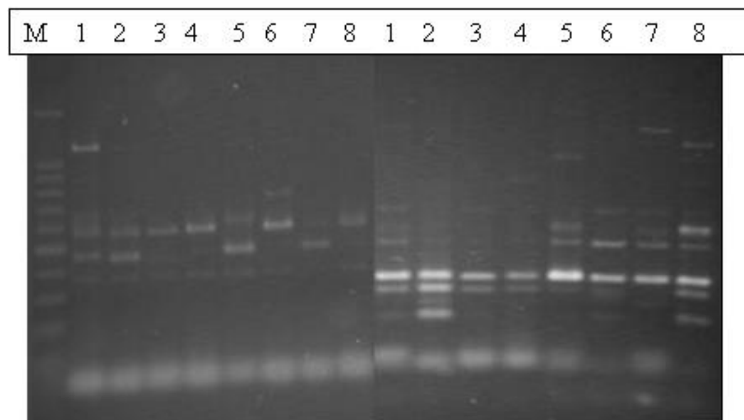
Dinucleotide: $(AG)_n$
Trinucleotide: $(ACT)_n$



CT repeat size differences (http://www.ars.usda.gov/images/docs/7082_7276/bobpic2.jpg)



Rice SSRs (http://www.ars.usda.gov/images/docs/7082_7276/bobpic1.jpg)



Peanut SSRs (http://www.cropscience.org.au/icsc2004/poster/3/1/1341_puppalan-1.gif)

KASP: **K**ompetitive **A**llele **S**pecific **PCR**
**Biosearch Technologies (former owner: LGC)
PACE: **P**CR **A**llelic **C**ompetitive **E**xtension
**3cr Bioscience

Single Nucleotide Polymorphism (=SNP) Markers

Five Primer PCR Systems
**Allele A forward
**Allele A reverse
**Allele B forward
**Allele B reverse
**Common reverse

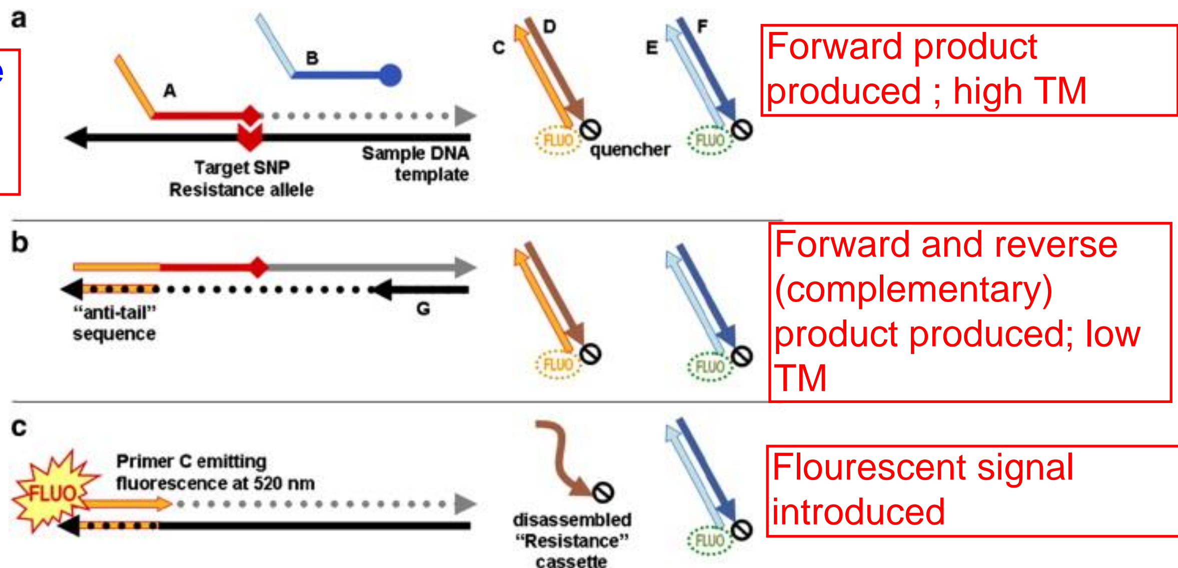
PACE or KASP Markers

- **Single Locus Detection System**
- End Point PCR Detection
    - Read results on Real-time PCR Machine
    - Measures fluorescence difference depending on allele

KASP (Kompetitive Allele Specific PCR) Example

- **Features of primers**: 3' end of primers A and B are allele-specific for an SNP (A vs. G)
    - **PCR Cycle 1**: Primer A is complementary to sample (see Fig a)
    - **PCR Cycle 2**: "Anti-tail" sequence product produced (see Fig b)
    - **PCR Cycle 3**: Round 3 and beyond: Here abundant product for primer A is produced (see Fig c)
    - Primer C has fluorescent single specific to allele A and is detected using a fluorescent detection system

This is the competitive step between forward primers!!!!



https://www.researchgate.net/figure/Scheme-of-a-KASP-reaction-for-a-mutant-homozygous-resistant-sample-The-following_fig2_262880486

YouTube Vides from LGC (company that owns the KASP technology)

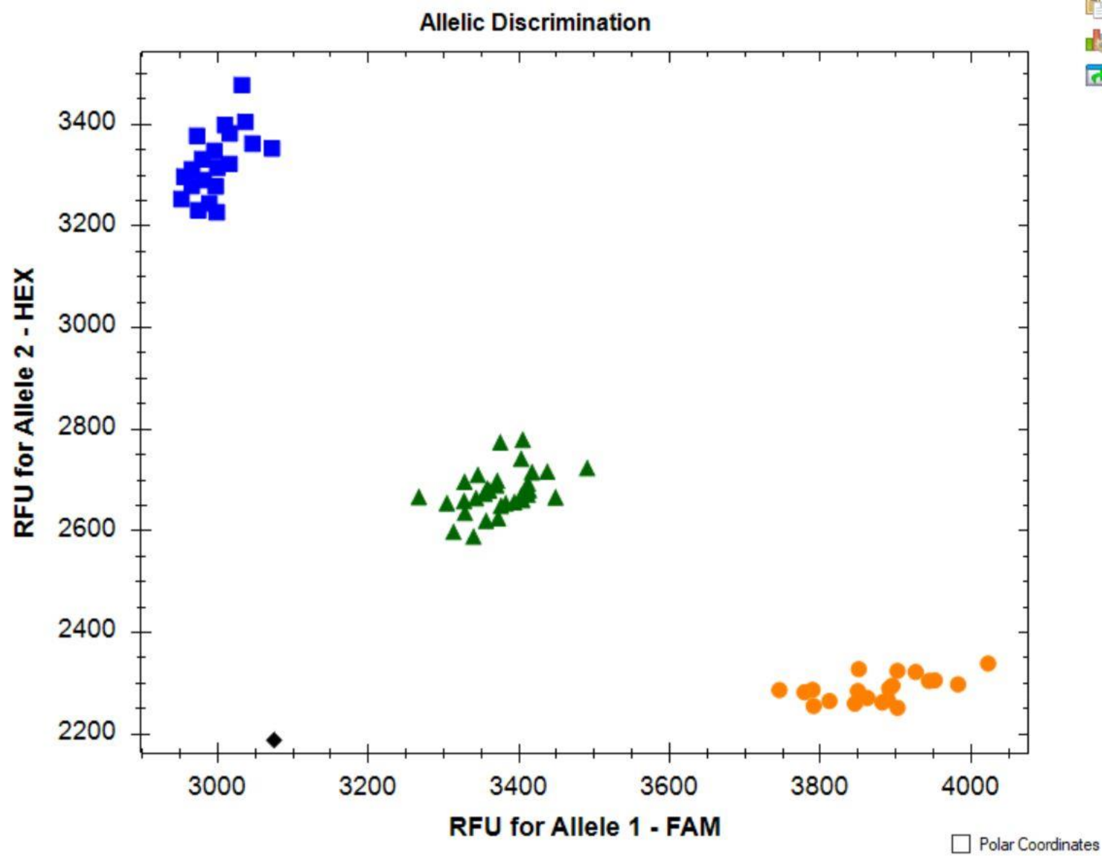
KASP Assay Components: https://www.youtube.com/watch?v=AZYm9g_6cpk
Assaying a single sample: https://www.youtube.com/watch?v=Uq9HhmzOqUQ
Reading the output of a population of samples: https://www.youtube.com/watch?v=GJbM7UbE7ZI
Applications in Plant and Animal Breeding: https://www.youtube.com/watch?v=l8zo9MA4ls0

Detection of SNP Allele

Plant breeding
Seed chip
Keep only seed homozygous for your preferred allele.

- Many samples are screened simultaneously using a 96, 384, or 1536 plate format

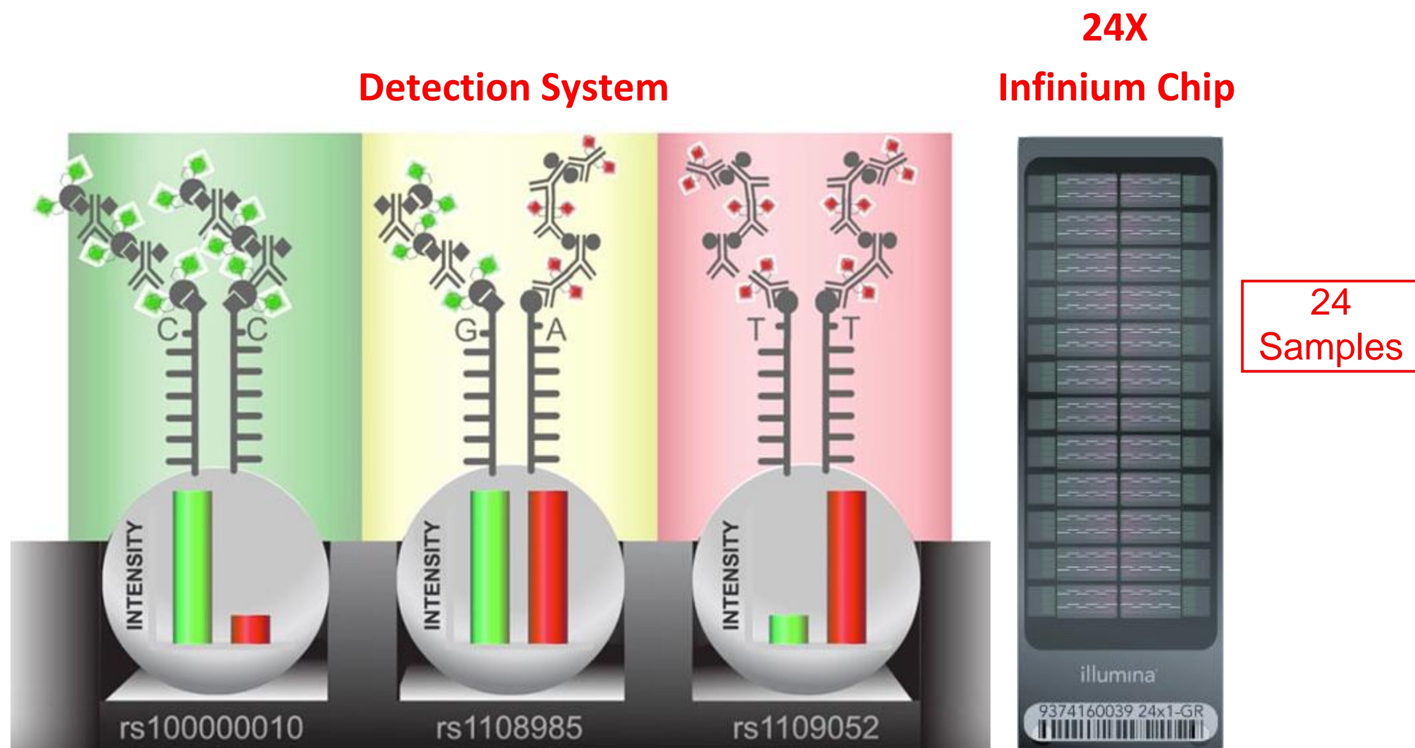
  Plates are read on ***fluorescence detection system***
  - **Upper left**: Homozygous Allele 1 (AA)
  - **Bottom right**: Homozygous Allele 2 (aa)
  - **Center**: Heterozygous (Aa)



Illumina Infinium Assay System

- **Genome-wide Detection System**
- Bead-based system
- Each bead has a single SNP locus
- **MANY SNP** loci on chip
- Flourescence determines genotype at the locus
- Chip Size (= #SNPs)
    - **Common bean**: ~13,000 SNPs
    - **Soybean**: ~50,000 SNPs
    - **Wheat**: ~90,000 SNPs
    - **Human**: ~750,000 SNPs (one of many chips)

$20-$30 per sample

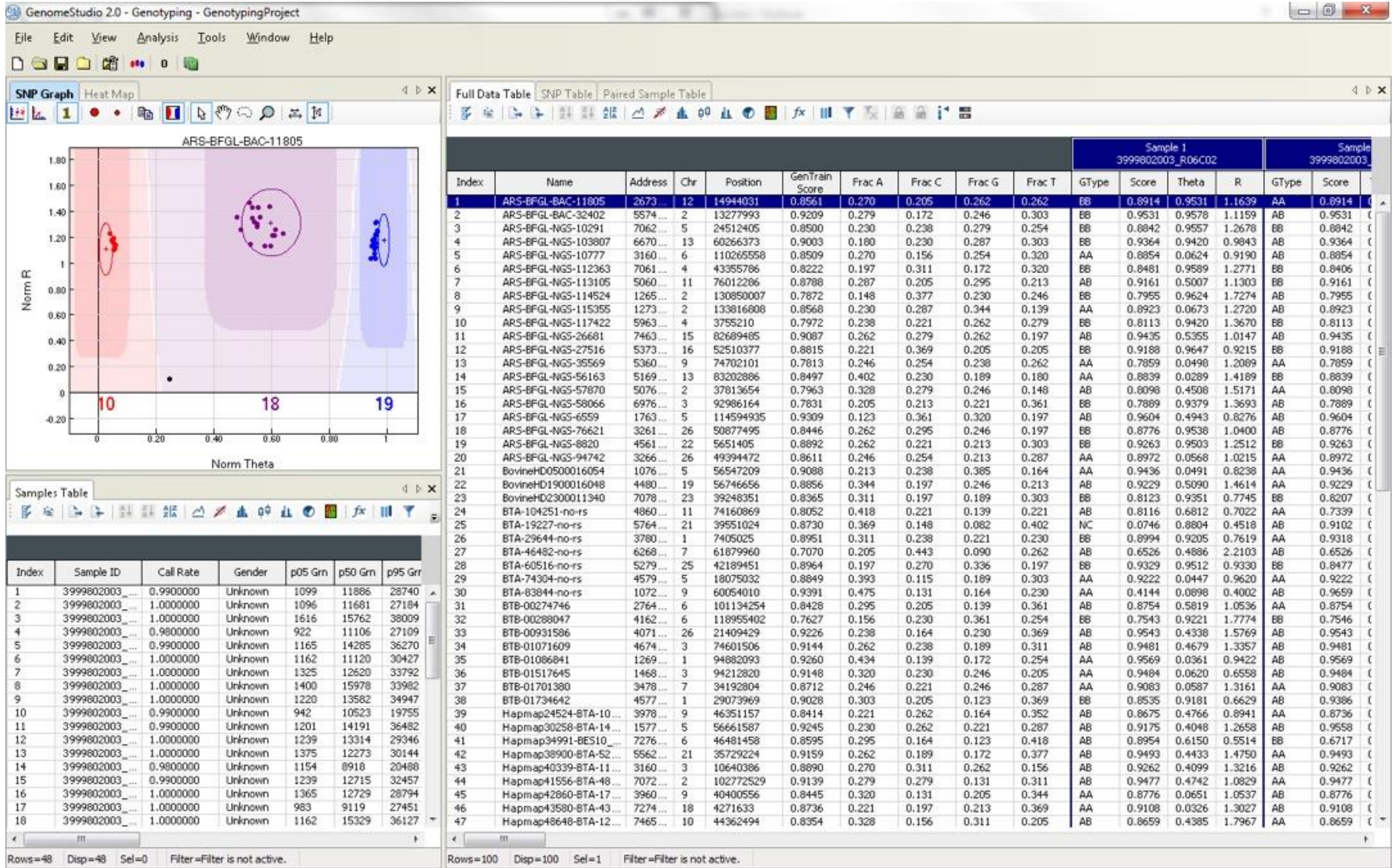


YouTube Video of Principles

https://www.youtube.com/watch?v=lVG04dAAyvY

Infinium Assay Output

Genome Studio Software



Infinium® II Assay Workflow

Illumina's Infinium II Assay provides unlimited multiplexing for whole-genome genotyping applications with manual or automated workflow

INTRODUCTION

The Infinium II Whole-Genome Genotyping Assay (*Figure* 1) is designed to interrogate a large number of SNPs at unlimited levels of loci multiplexing. Using a single bead type and dual color channel approach, the Infinium II Assay scales genotyping from 10,000 to hundreds of thousands of SNPs per sample. Illumina's optional Laboratory Information Management System (LIMS) and automation ensure positive sample tracking while reducing time required and labor costs.

ASSAY PROTOCOL

The DNA sample used for this assay is isothermally amplified in an overnight step (*Figure* 1; *Step* 1 *and Step* 2). This amplification has no appreciable allelic partiality. Additionally, a relatively low DNA sample requirement of 750 ng is sufficient to assay over 500,000 SNP loci. The amplified product is then fragmented by a controlled enzymatic process that does not require gel electrophoresis (*Step* 3). After alcohol precipitation and resuspension of the DNA (*Step* 4), the BeadChip is prepared for hybridization in the capillary flow-through chamber (*Step* 5)*;* samples are applied to BeadChips and incubated overnight. The amplified and fragmented DNA samples anneal to locus-specific 50-mers (covalently linked to one of over 500,000 bead-types) during the hybridization step (*Step* 6). One bead type corresponds

FIGURE 1: INFINIUM II ASSAY PROTOCOL

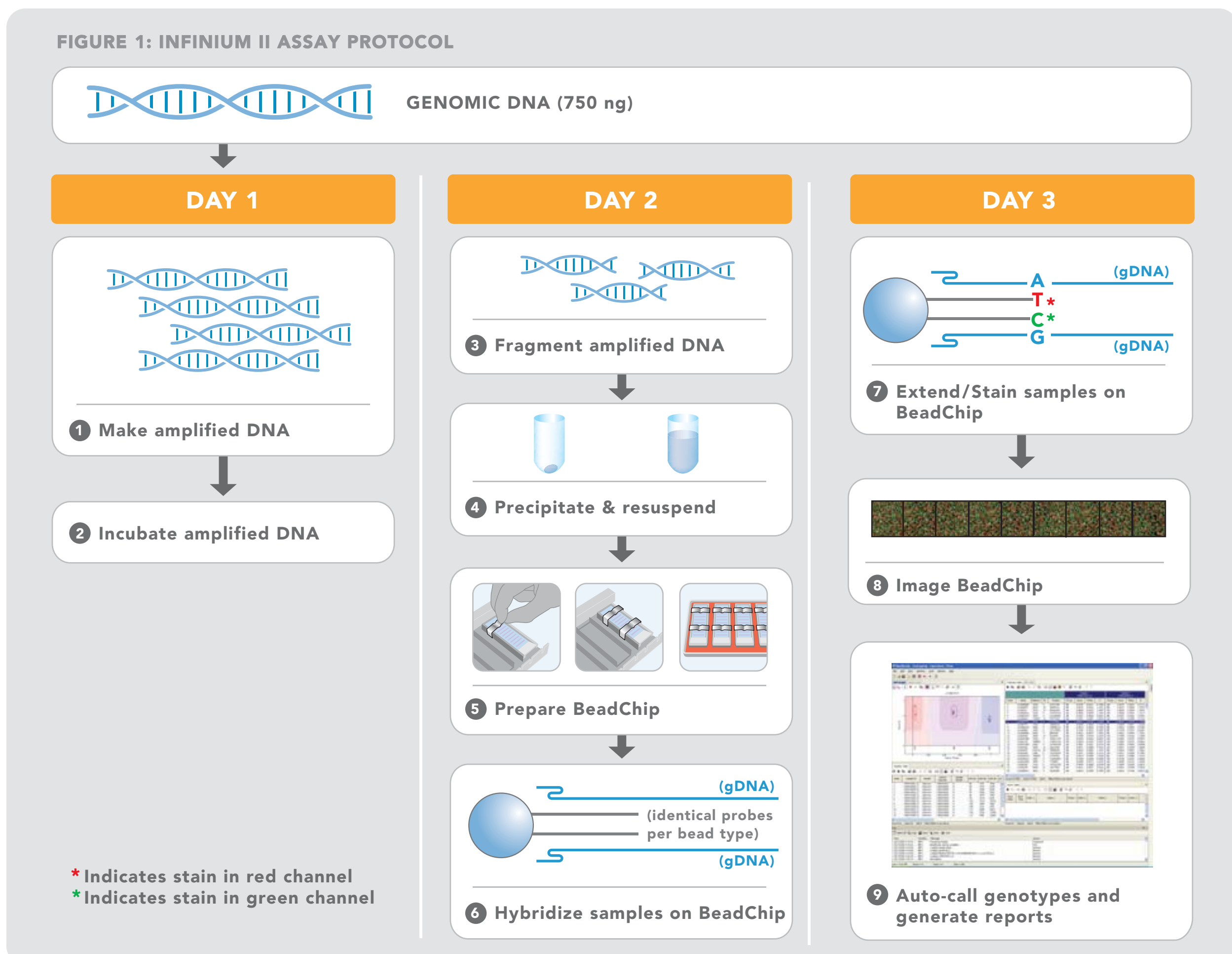


FIGURE 2: INFINIUM II ASSAY WORKFLOW

AUTOMATED

	PROTOCOL STEP	8 BEADCHIPS Hands-on	8 BEADCHIPS Total	16 BEADCHIPS Hands-on	16 BEADCHIPS Total	24 BEADCHIPS Hands-on	24 BEADCHIPS Total	Incubation*
DAY ONE	1 Set up DNA amplification	5min	25min	5min	35min	5min	40min	20h (o/n)
DAY TWO	2 Fragment amplified DNA	5min	1h 15min	5min	1h 20min	5min	1h 25min	1h
	3 Precipitate amplified DNA	5min	2h 15min	5min	2h 25min	5min	2h 30min	1h 50min
	4 Resuspend amplified DNA	5min	1h 10min	5min	1h 15min	5min	1h 20min	1h
	5 Prepare BeadChip	30min	30min	40min	40min	50min	50min	-
	6 Hybridize sample to BeadChip	5min	5min	5min	10min	5min	15min	20m + 16h (o/n)
	DAY 2 TOTALS	**50min**	**5h 15min**	**1h**	**5h 50min**	**1h 10min**	**6h 10min**	
DAY THREE	7 Extend and stain BeadChip	5min	2h 25min	5min	4h 50min	5min	7h 15min	-
	8 Scan BeadChip (1 scanner)	5min	6h	5min x 2	12h	5min x 3	18h	-
	TOTAL GENOTYPES (HUMANHAP550v1.0)	**> 4x10⁶**		**> 8.5x10⁶**		**> 13x10⁶**		

* These are total incubations, but several can be nested within each other

MANUAL

	PROTOCOL STEP	8 BEADCHIPS Hands-on	8 BEADCHIPS Total	16 BEADCHIPS Hands-on	16 BEADCHIPS Total	24 BEADCHIPS Hands-on	24 BEADCHIPS Total	Incubation
DAY 1	1 Set up DNA amplification	5min	15min	5min	15min	5min	15min	10m + 20h (o/n)
DAY 2	2 Fragment amplified DNA	10min	1h 10min	10min	1h 10min	10min	1h 10min	1h
	3 Precipitate amplified DNA	5min	1h 55min	5min	1h 55min	5min	1h 55min	1h 50min
	4 Resuspend amplified DNA	10min	1h 10min	10min	1h 10min	10min	1h 10min	1h
	5 Prepare BeadChip	30min	30min	40min	40min	50min	50min	-
	6 Hybridize sample to BeadChip	30min	1h	40min	1h	40min	1h 5min	20m + 16h (o/n)
	DAY 2 TOTALS	**1h 25min**	**5h 45min**	**1h 45min**	**5h 55min**	**1h 45min**	**6h 10min**	
DAY 3	7 Extend and stain BeadChip	35min	2h 25min	1h 10min	4h 50min	1h 45min	7h 15min	1h 50min
	8 Scan BeadChip (1 scanner)	5min	6h	5min x 2	12h	5min x 3	18h	-
	TOTAL GENOTYPES (HUMANHAP550v1.0)	**> 4x10⁶**		**> 8.5x10⁶**		**> 13x10⁶**		

to each allele per SNP locus. After hybridization, allelic specificity is conferred by enzymatic base extension. Products are subsequently fluorescently stained (*Step* 7). The intensities of the beads' fluorescence are detected by the Illumina BeadArray Reader (*Step* 8), and are in turn analyzed using Illumina's software for automated genotype calling (*Step* 9).

Figure 2 shows the estimated hands-on time required for completing the Infinium II Assay using the Illumina BeadStation 500 system. With Illumina's optional Laboratory Information Management System (LIMS) to ensure positive sample tracking, the Infinium II Assay is a robust protocol with a straight forward workflow that can be automated or processed manually.

ADDITIONAL INFORMATION

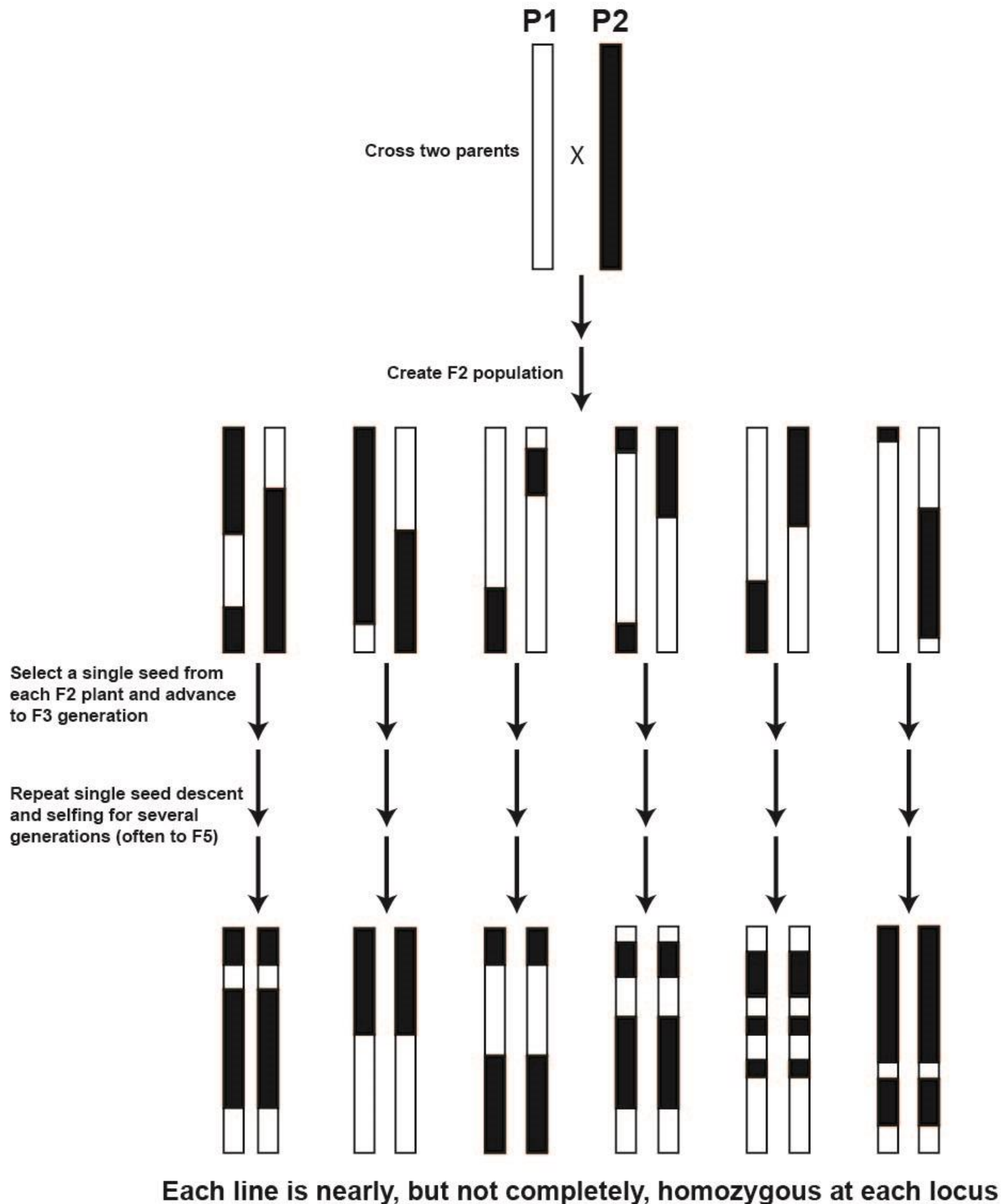
For more information about Infinium II or other products and services from Illumina, visit our website or contact technical support at the address below.

Illumina, Inc.
Customer Solutions
9885 Towne Centre Drive
San Diego, CA 92121-1975
1.800.809.4566 (toll free)
1.858.202.4566 (outside the U.S.)
techsupport@illumina.com
www.illumina.com

FOR RESEARCH USE ONLY

© 2006 Illumina, Inc.
Illumina, BeadArray, BeadXpress, CSPro, Sentrix, Array of Arrays, GoldenGate, Infinium, iSelect, DASL, Sentrix, VeraCode, and Making Sense Out of Life, are trademarks or registered trademarks of Illumina. All other names and marks are the property of their respective owners.
Pub. No. 370-2006-027 07Dec06

Developing a Recombinant Inbred Population



Each line is nearly, but not completely, homozygous at each locus

Power of Different Mapping Populations

At closer distance,
***&All populations work equally well with co-dominant markers.
***SNP mapping will work fine with any of the populations.

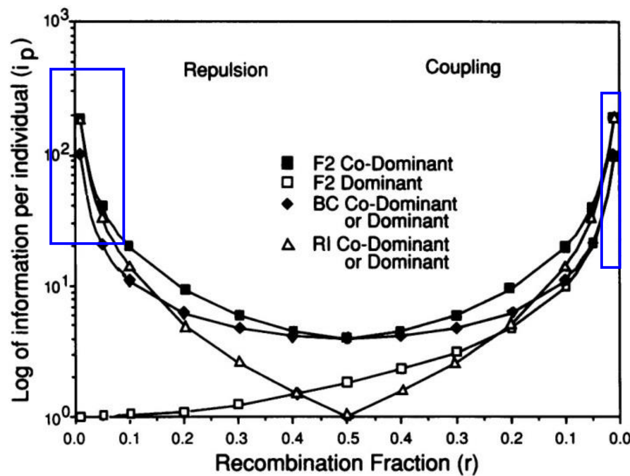


FIG. 5. Theoretical efficiency with which RI, F_2, and backcross (BC) populations can detect recombinants by using either codominant or dominant markers. The amount of information per individual (i_p) in a mapping population is the inverse of the variance divided by population size (28). For an RI population i_p is approximately equal to $2/r(1 + 2r)^2$. Allard (28) previously derived i_p for the other populations shown. The amount of information per individual is represented by the logarithm of i_p and is plotted against the recombination fraction (r) for repulsion- and coupling-phase linkage. An RI population is equally efficient with either codominant or dominant markers and is very efficient for closely linked markers.

PNAS 89:1477 (1992)

Predominant Populations Today
***F2
***RILs
NILs
Double-haploid

Bulk Segregant Screening

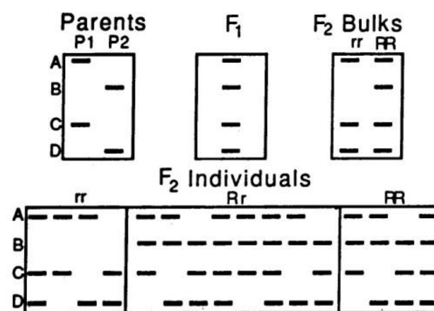


FIG. 1. Genetic basis of bulked segregant analysis. The schematic shows genotypes of four RAPD loci (A–D) detected by a single primer in two parents (P1 and P2), their F_1 and F_2 progeny, and bulks derived from F_2 individuals homozygous for resistance or susceptibility. The dominant allele at locus B is linked in cis to the *R* allele and therefore is polymorphic between the bulks. The other three loci that are polymorphic between the parents are unlinked to the resistance locus and therefore appear monomorphic between the bulks. This is an interpretation of the pattern obtained with primer OPF12 in Fig. 4.

PNAS 88:9828 (1991)