

# Isozymes – The First Molecular Marker System

## Equipment

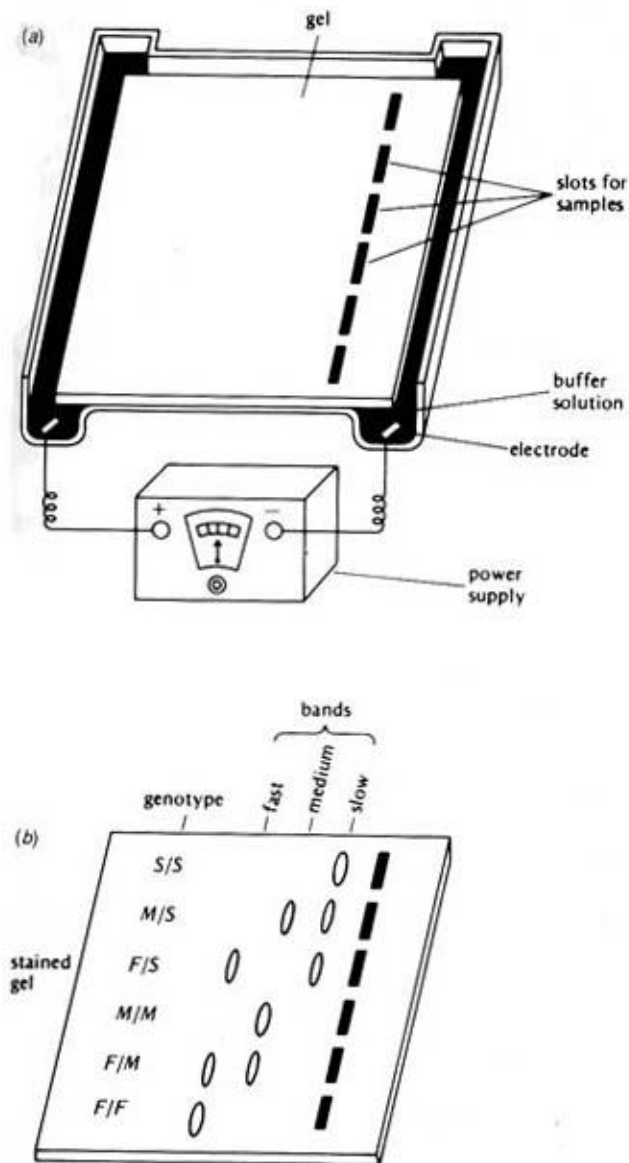


Fig. 6.10. (a) Apparatus for gel electrophoresis. (b) A zymogram produced by staining a gel (see text for a discussion of the interpretation of banding patterns). (From Strickberger, 1985.)

[http://www.plantbiology.siu.edu/PLB479/images/Fig6\\_10.jpg](http://www.plantbiology.siu.edu/PLB479/images/Fig6_10.jpg)

Image of an isozyme starch gel



[http://wheat.pw.usda.gov/ggpages/oatnewsletter/v48/Isozyme\\_files/image002.jpg](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.

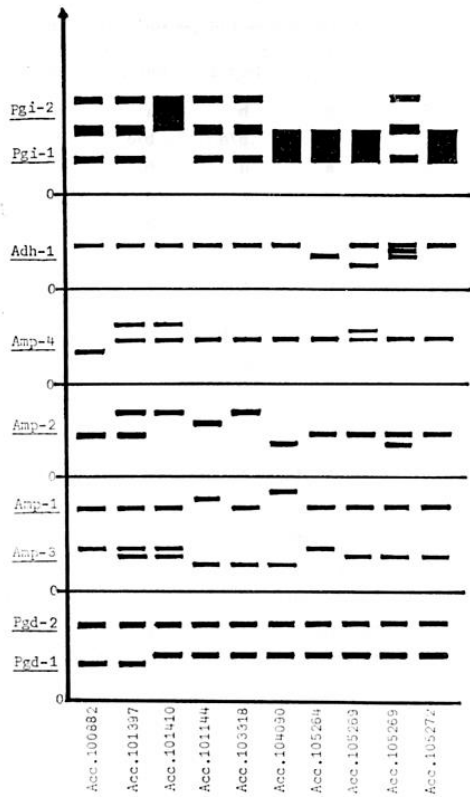
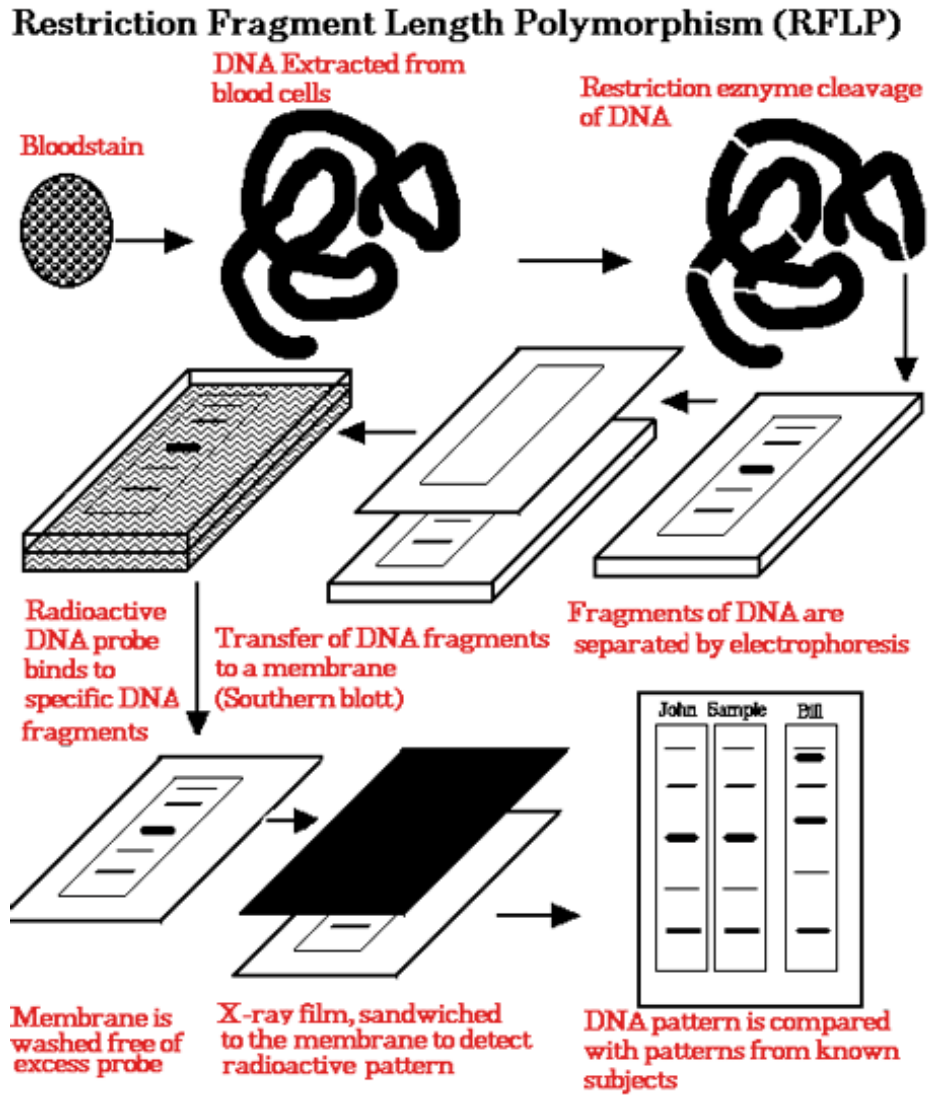


Fig. 1.

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

# Restriction Fragment Polymorphisms

## Technology Approach



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

# Original plant RFLP paper figure (autoradiography image)

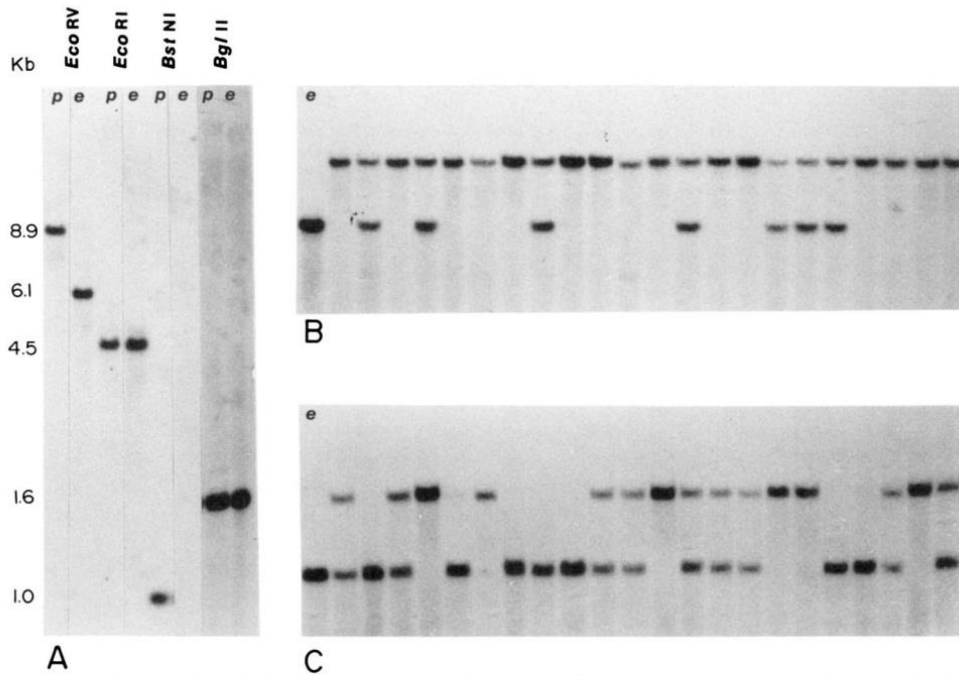


FIGURE 2.—A, Restriction enzyme survey of *L. pennellii* (p) and *L. esculentum* (e) probed with clone 3-41 (*CD14*). The values at left are the fragment sizes in kilobases. B, Backcross progeny DNA (*L. pennellii* as the recurrent parent) digested with *EcoRV* and probed with 3-41. C,  $F_2$  progeny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

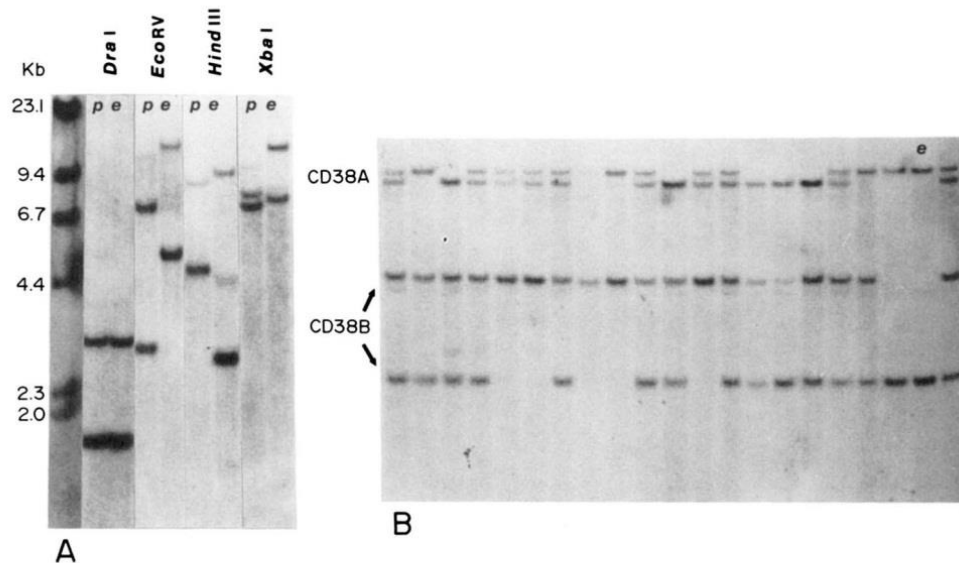
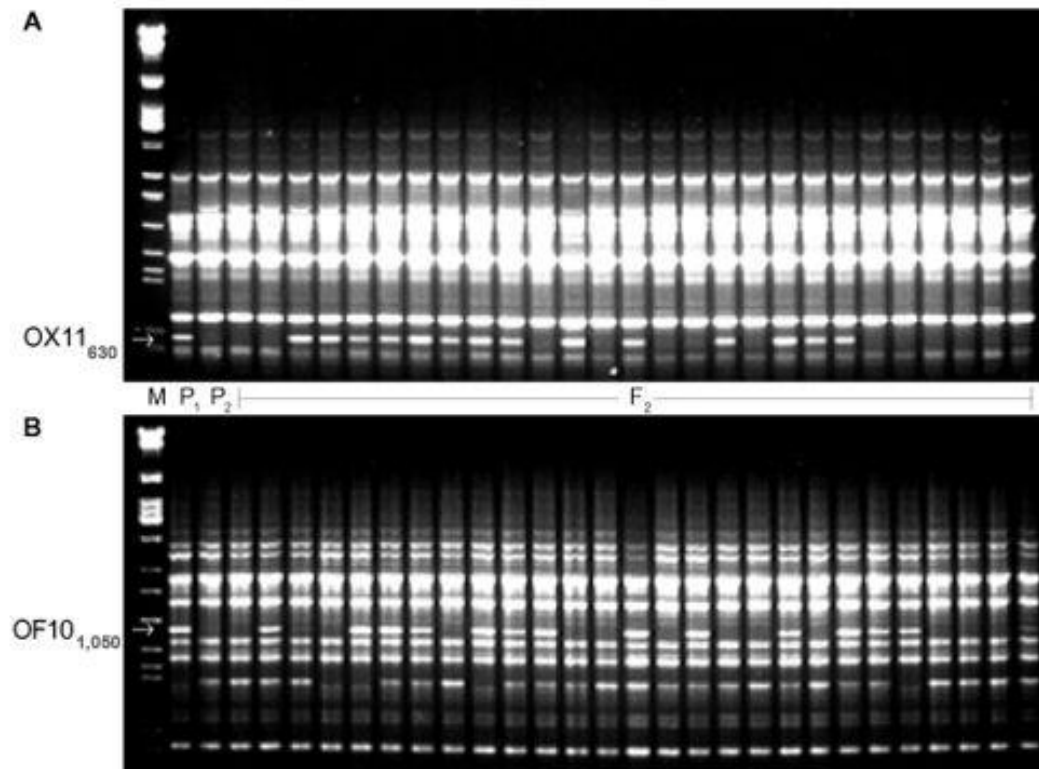


FIGURE 3.—A, Restriction enzyme survey of *L. pennellii* (p) and *L. esculentum* (e) probed with clone 3-275 (*CD38A* and *B*). The first lane is DNA digested with *HindIII*, and the fragment sizes are indicated at left. B,  $F_2$  progeny DNA digested with *HindIII* and probed with 3-275.

# RAPD (Randomly Amplified Polymorphic) Markers

Gel image (*Phaseolus vulgaris*)

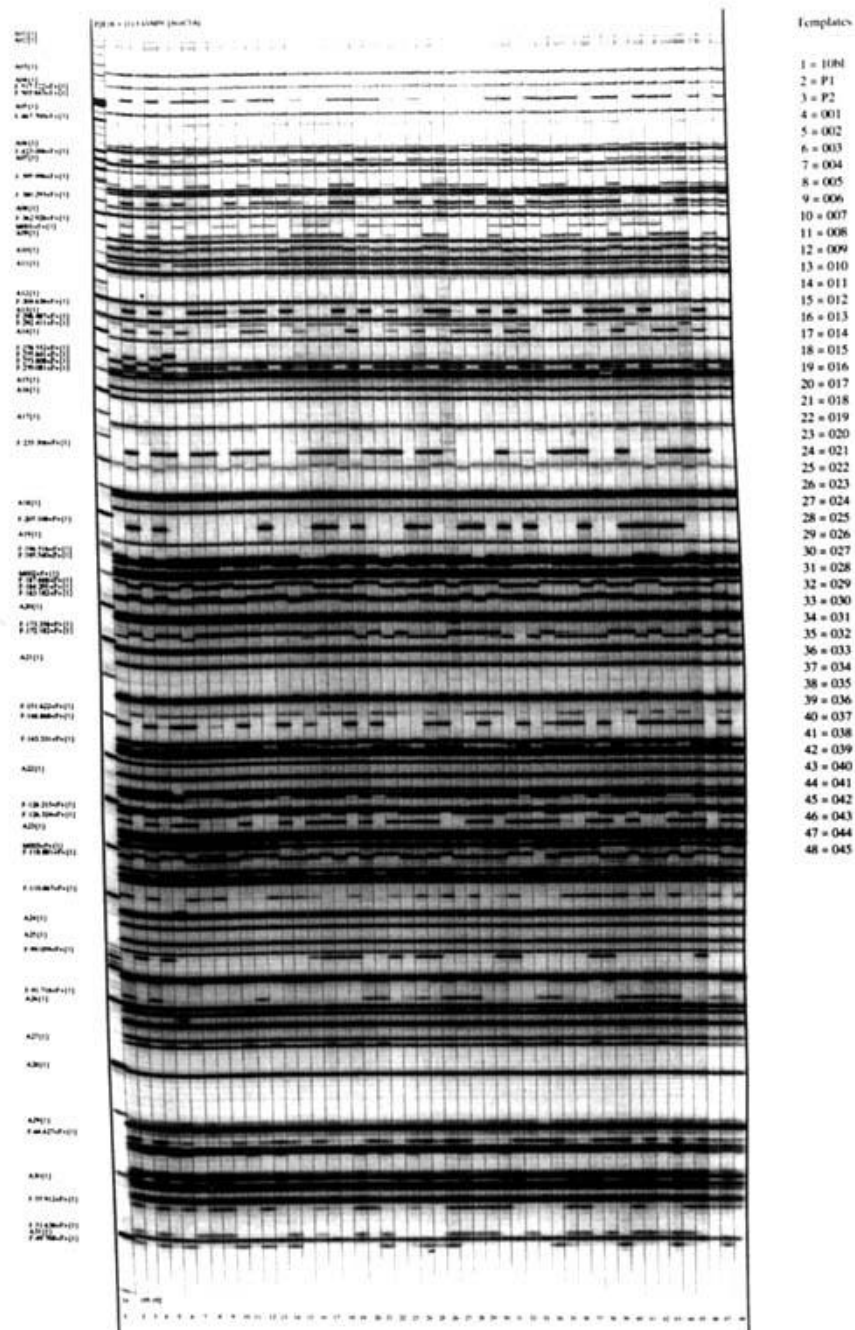


**Figure 1** - Electrophoretic analyses of DNA amplification products obtained with primers OX11 (A) and OF10 (B). In both gels, P<sub>1</sub> corresponds to cultivar Ouro Negro, P<sub>2</sub> to cultivar US Pinto 111 and F<sub>2</sub> to 27 individuals from the segregating population. Lane M contains lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hind*III (size markers). The arrows indicate markers OX11<sub>630</sub>(A) and OF10<sub>1,050</sub>(B).

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

# AFLP (Amplified Fragment Length Polymorphism) Markers

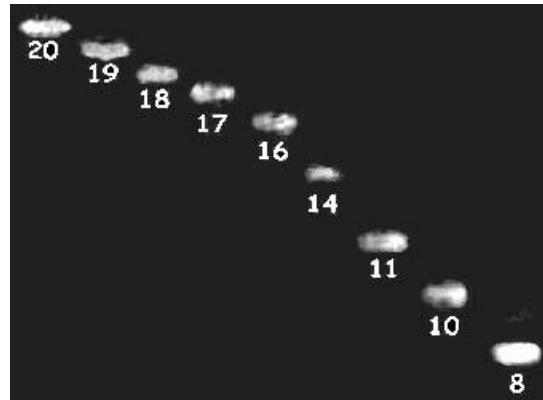
Autoradiography image



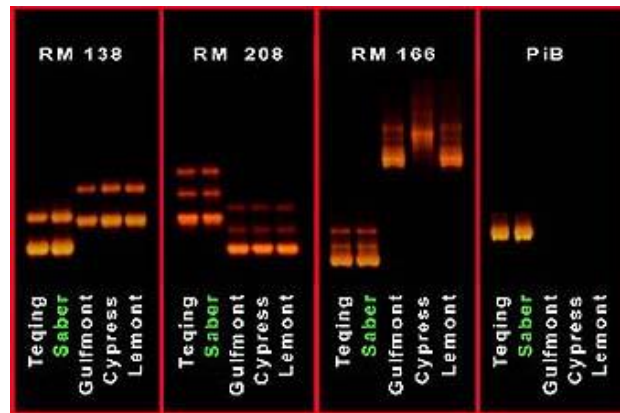
<http://www.shigen.nig.ac.jp/rice/rgn/vol14/p106Fig.1.jpg>

# Microsatellite (=SSR) Markers

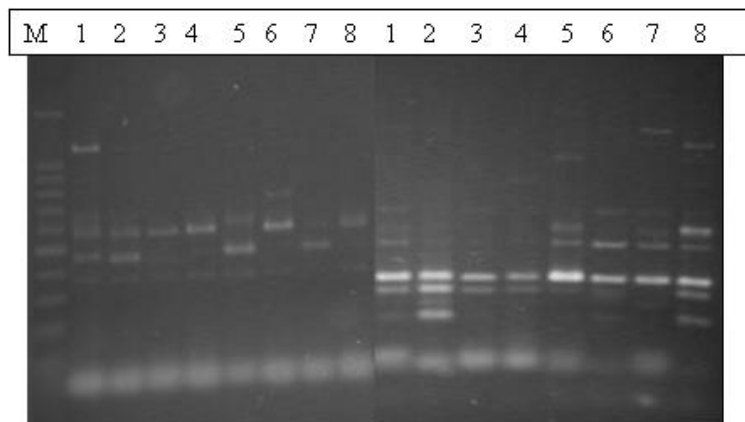
Gel images



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



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



Peanut SSRs ([http://www.cropsscience.org.au/icsc2004/poster/3/1/1341\\_puppalan-1.gif](http://www.cropsscience.org.au/icsc2004/poster/3/1/1341_puppalan-1.gif))

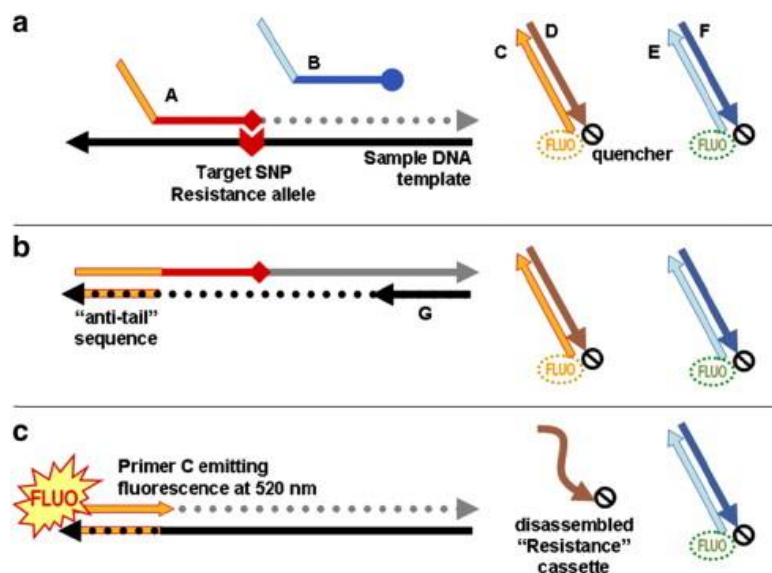
# Single Nucleotide Polymorphism (=SNP) Markers

## 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



[https://www.researchgate.net/figure/Scheme-of-a-KASP-reaction-for-a-mutant-homozygous-resistant-sample-The-following\\_fig2\\_262880486](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](https://www.youtube.com/watch?v=AZYm9g_6cpk)

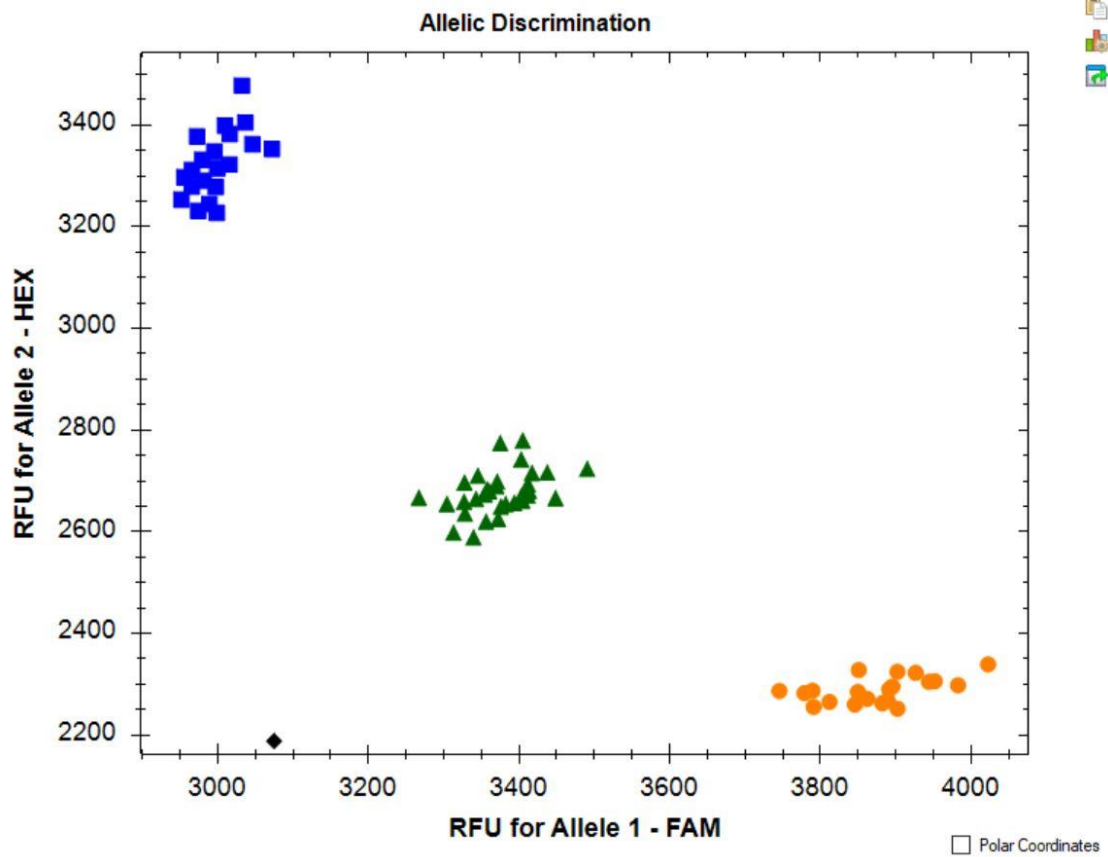
Assaying a single sample: <https://www.youtube.com/watch?v=Uq9HhmzOqUQ>

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=l8zo9MA4Is0>

### Detection of SNP Allele

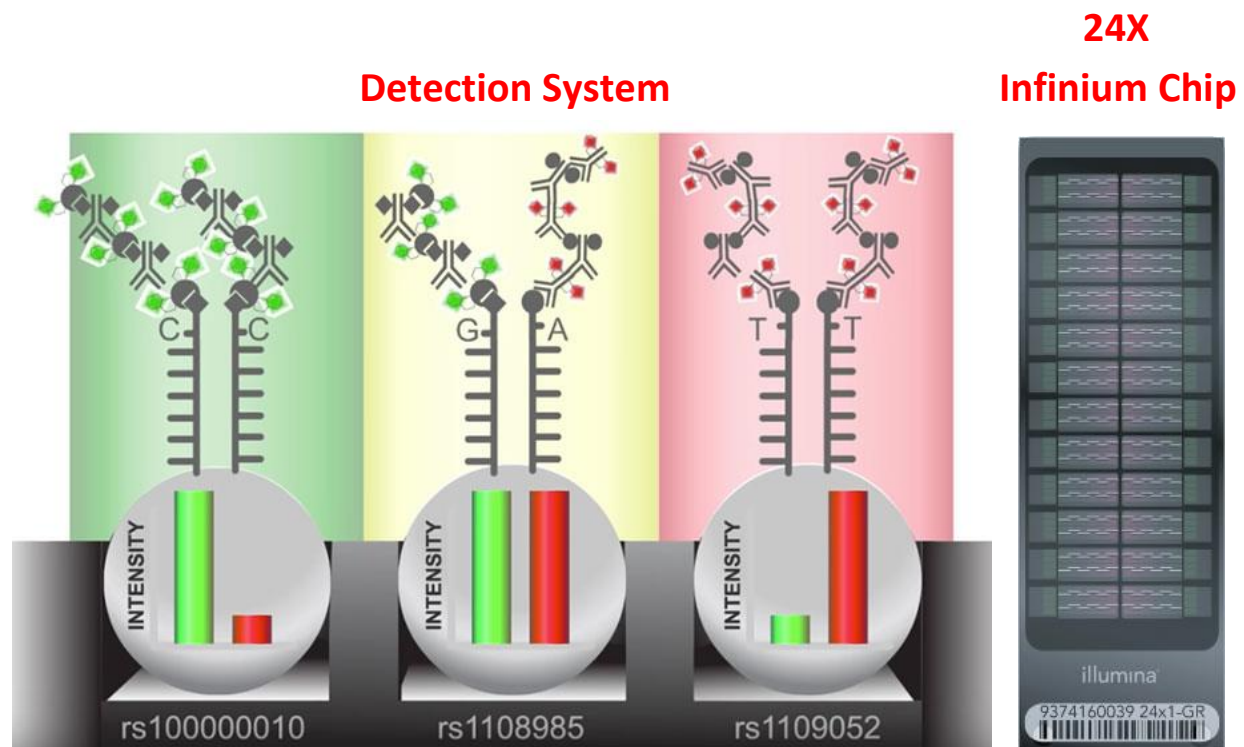
- Many samples are screened simultaneously using a 96, 384, or 1536 plate format
- Plates are read on *fluorescence detection system*
  - **Upper left:** Homozygous Allele 1 (AA)
  - **Bottom right:** Homozygous Allele 2 (aa)
  - **Center:** Heterozygous (Aa)



# Illumina Infinium Assay System

- **Genome-wide Detection System**

- Bean-based system
- Each bead has a single SNP locus
- **MANY SNP** loci on chip
- Fluorescence 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)

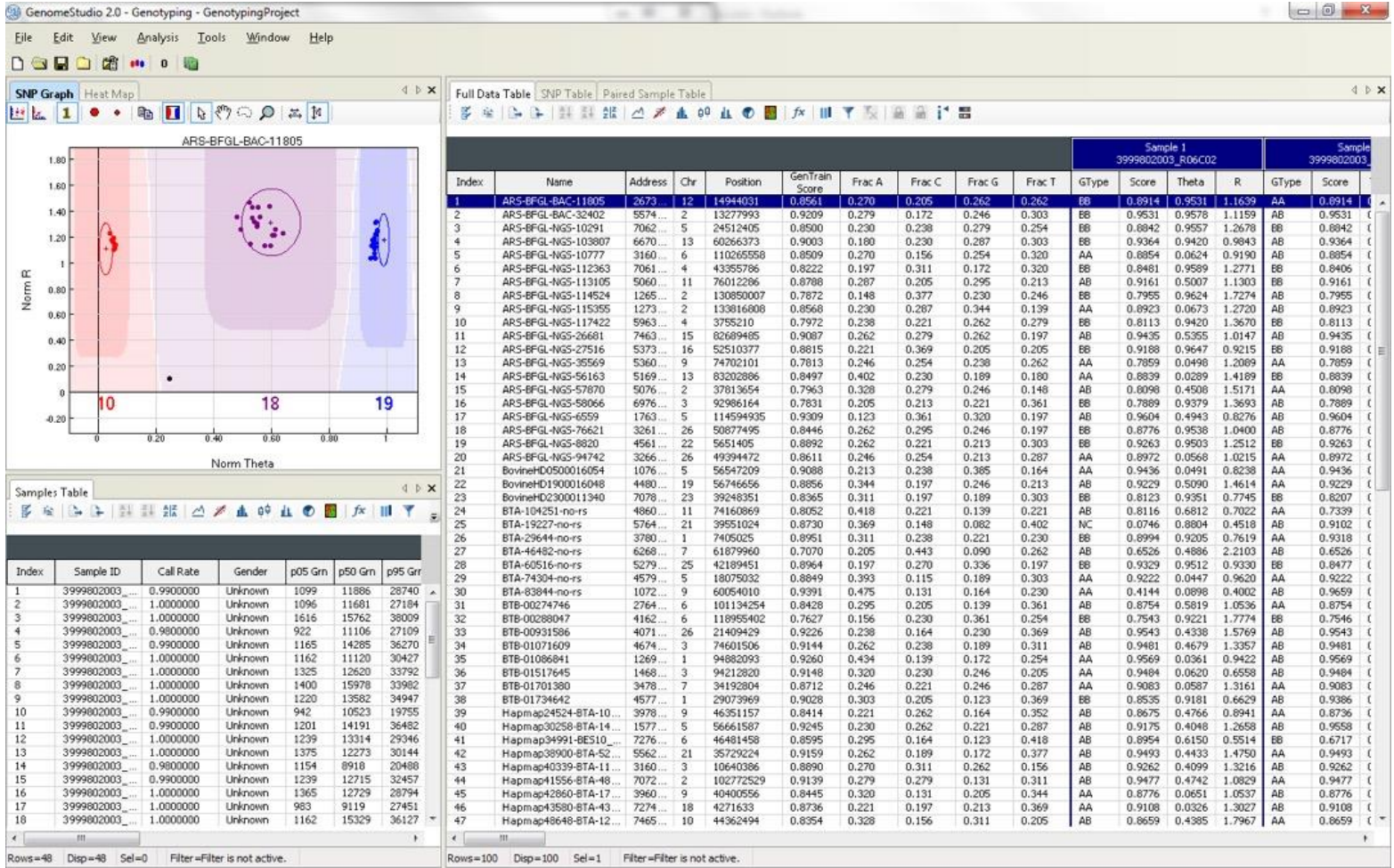


## YouTube Video of Principles

<https://www.youtube.com/watch?v=IVG04dAAyY>

# 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 enzy-

matic 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

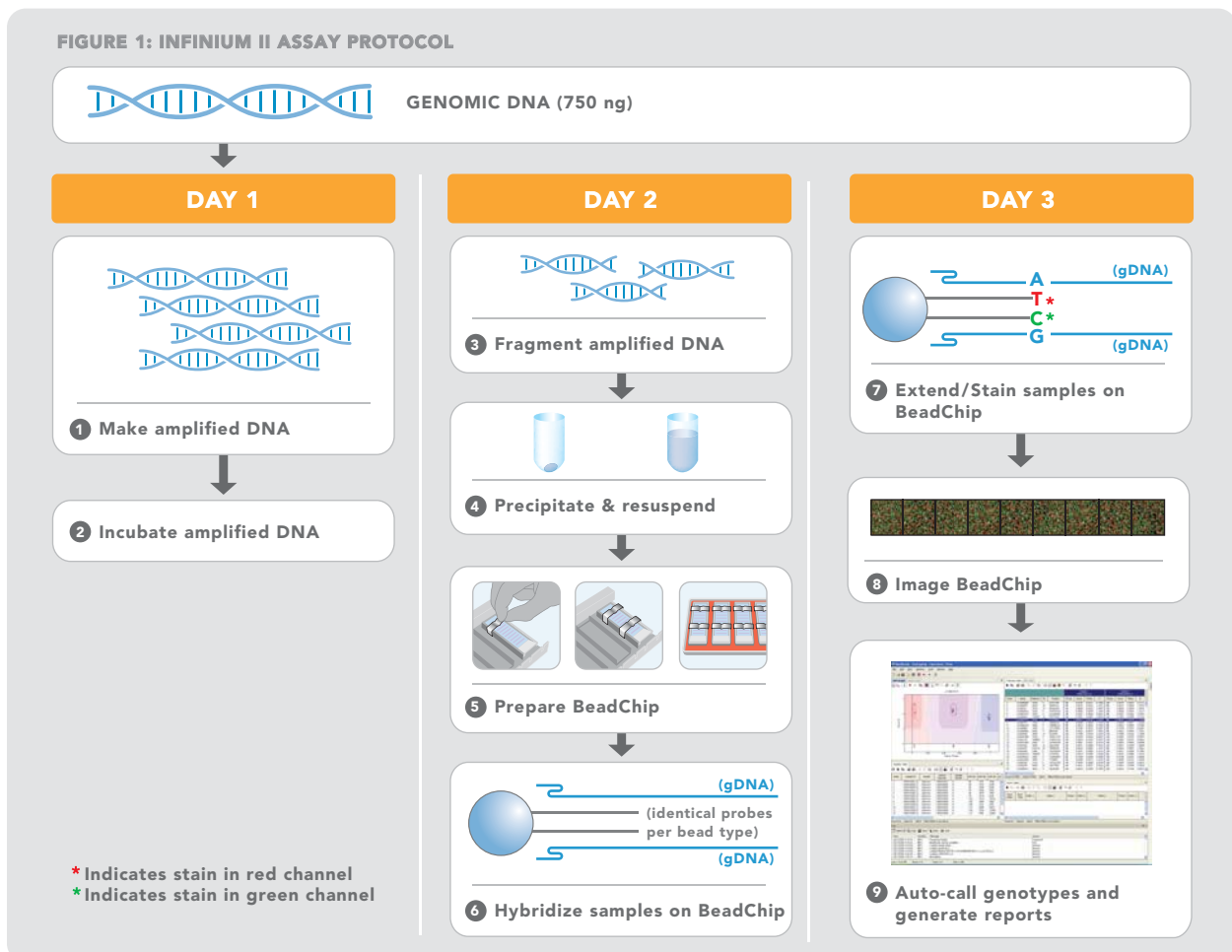


FIGURE 2: INFINIUM II ASSAY WORKFLOW

**AUTOMATED**

DAY	PROTOCOL STEP	8 BEADCHIPS		16 BEADCHIPS		24 BEADCHIPS		Incubation*
		Hands-on	Total	Hands-on	Total	Hands-on	Total	
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)
	<b>DAY 2 TOTALS</b>		<b>50min</b>	<b>5h 15min</b>	<b>1h</b>	<b>5h 50min</b>	<b>1h 10min</b>	<b>6h 10min</b>
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	-
<b>TOTAL GENOTYPES (HUMANHAP550v1.0)</b>		<b>&gt; 4x10<sup>6</sup></b>		<b>&gt; 8.5x10<sup>6</sup></b>		<b>&gt; 13x10<sup>6</sup></b>		

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

**MANUAL**

DAY	PROTOCOL STEP	8 BEADCHIPS		16 BEADCHIPS		24 BEADCHIPS		Incubation
		Hands-on	Total	Hands-on	Total	Hands-on	Total	
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)
	<b>DAY 2 TOTALS</b>		<b>1h 25min</b>	<b>5h 45min</b>	<b>1h 45min</b>	<b>5h 55min</b>	<b>1h 45min</b>	<b>6h 10min</b>
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	-
<b>TOTAL GENOTYPES (HUMANHAP550v1.0)</b>		<b>&gt; 4x10<sup>6</sup></b>		<b>&gt; 8.5x10<sup>6</sup></b>		<b>&gt; 13x10<sup>6</sup></b>		

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.

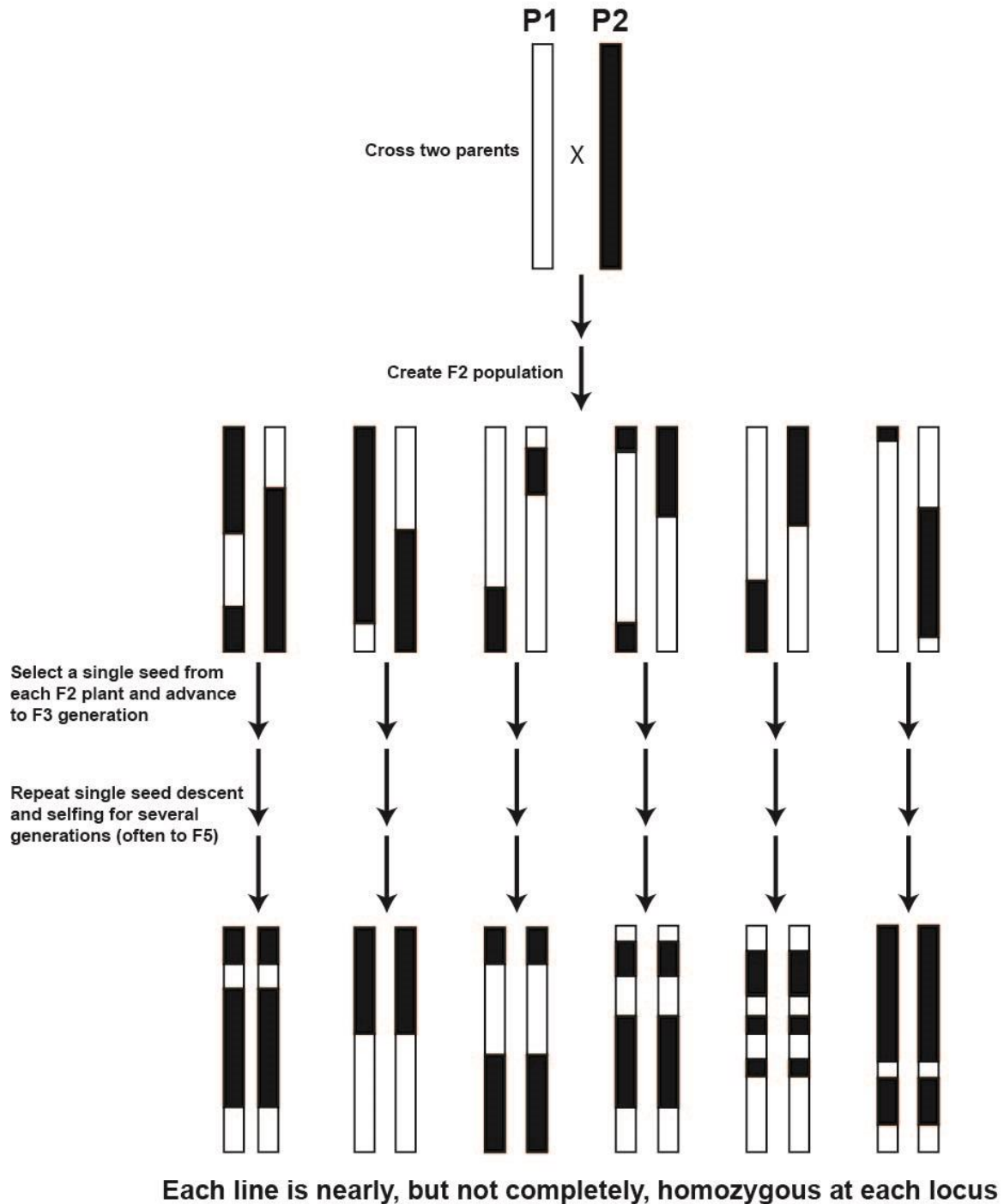
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 9885 Towne Centre Drive  
 San Diego, CA 92121-1975  
 1.800.809.4566 (toll free)  
 1.858.202.4566 (outside the U.S.)  
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 www.illumina.com

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 Pub. No. 370-2006-027 07Dec06



# Developing a Recombinant Inbred Population



# Power of Different Mapping Populations

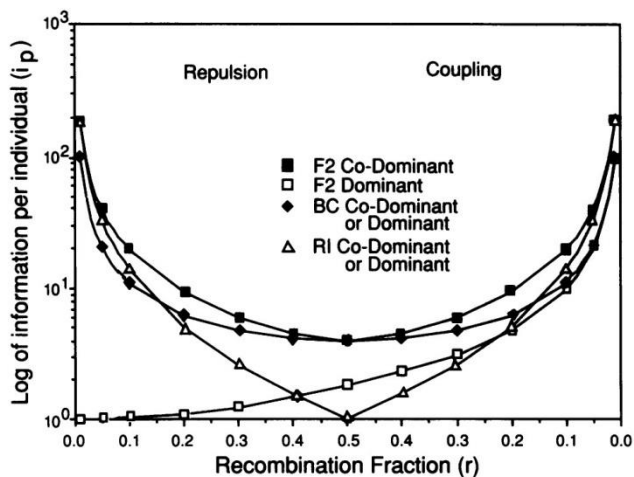


FIG. 5. Theoretical efficiency with which RI, F<sub>2</sub>, and backcross (BC) populations can detect recombinants by using either codominant or dominant markers. The amount of information per individual ( $i_p$ ) in a mapping population is the inverse of the variance divided by population size (28). 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)

# Bulk Segregant Screening

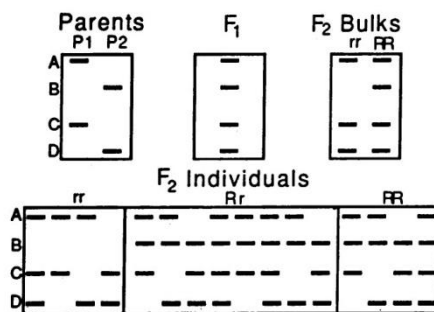


FIG. 1. Genetic basis of bulked segregant analysis. The schematic shows genotypes of four RAPD loci (A–D) detected by a single primer in two parents (P1 and P2), their F<sub>1</sub> and F<sub>2</sub> progeny, and bulks derived from F<sub>2</sub> individuals homozygous for resistance or susceptibility. 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)