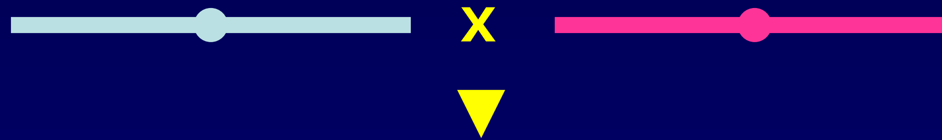
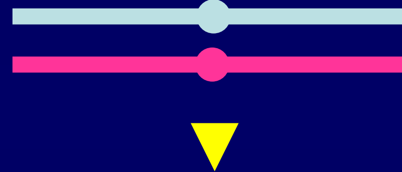
Towards a genetic linkage map for *Physcomitrella*

Meiotic recombination In *Physcomitrella*

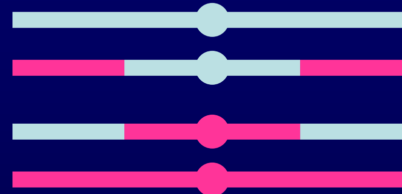
Parental generation (n)



Sporophyte (2n)

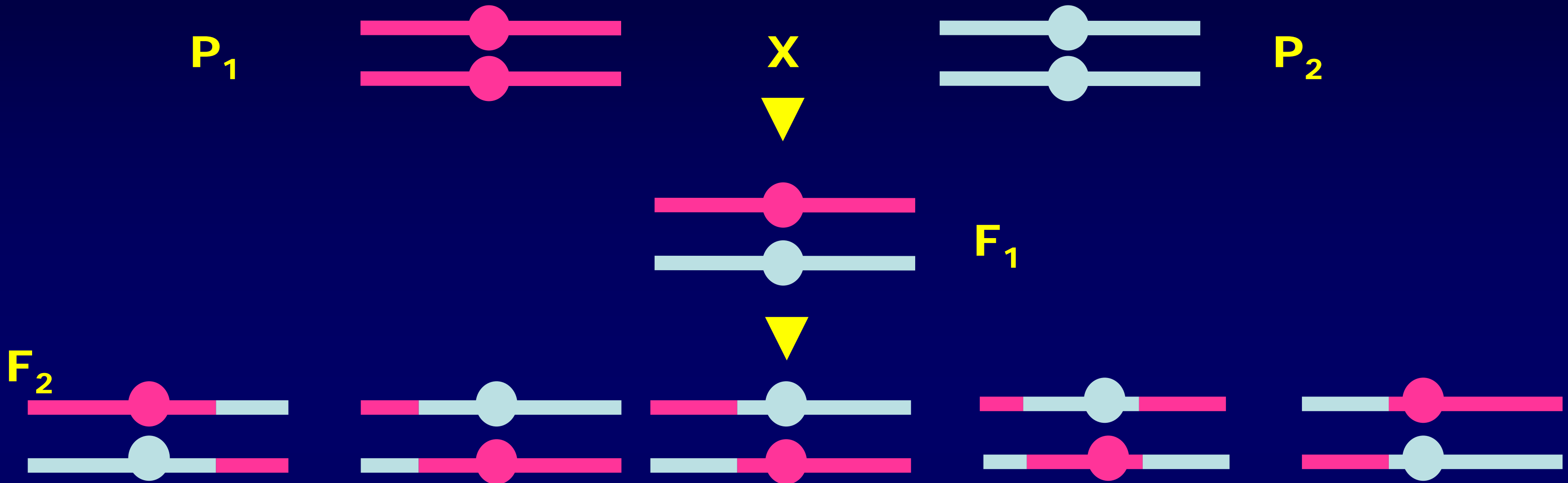


Crossing over
in meiosis

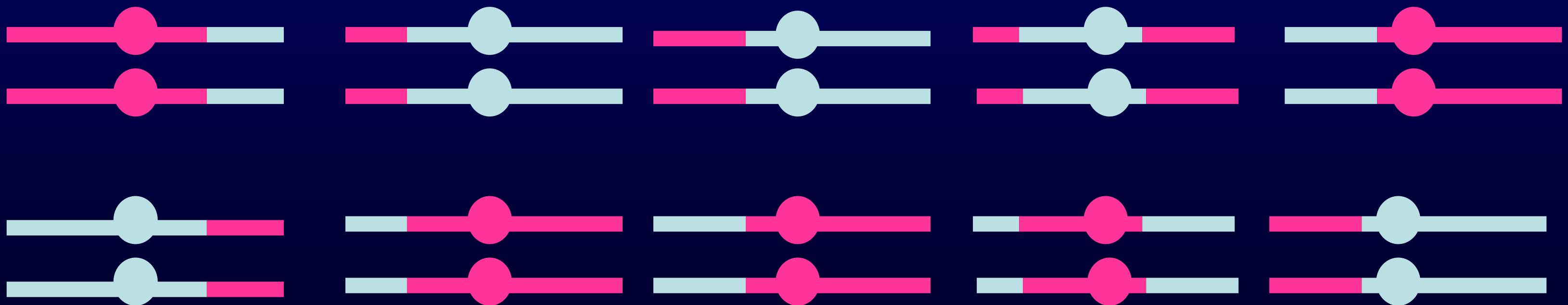


Segregation of spores (n)

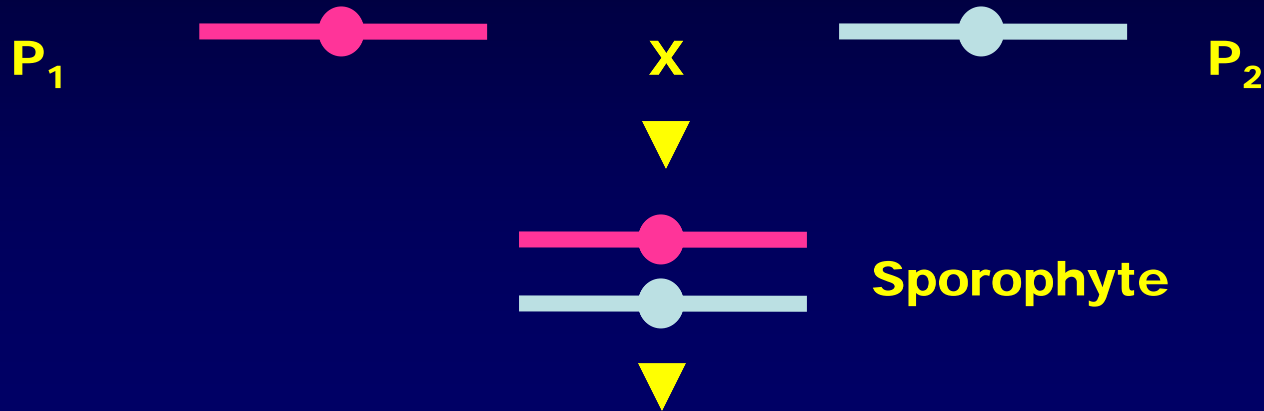
Recombinant Inbred lines
(flowering plants)



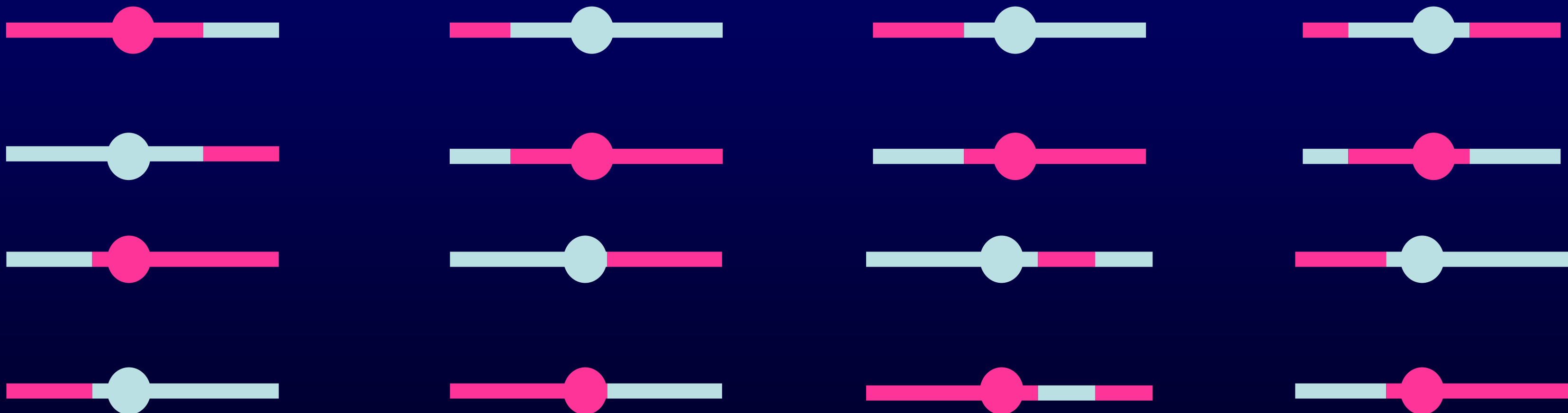
Self individual F_2 progeny to homozygosity to get lines with fixed recombination junctions. (approx. 6 generations)



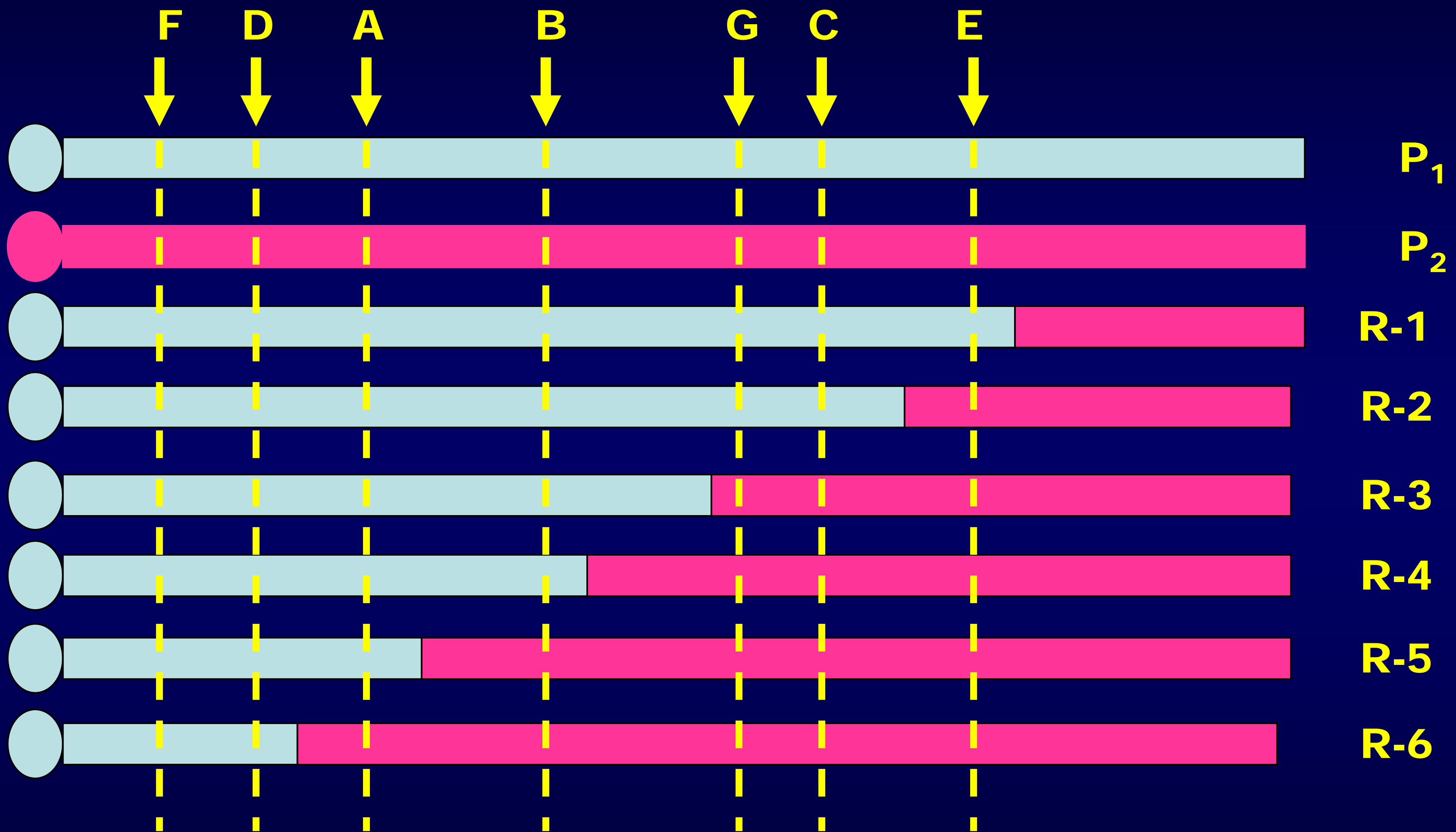
Recombinant Haploid lines
(moss)



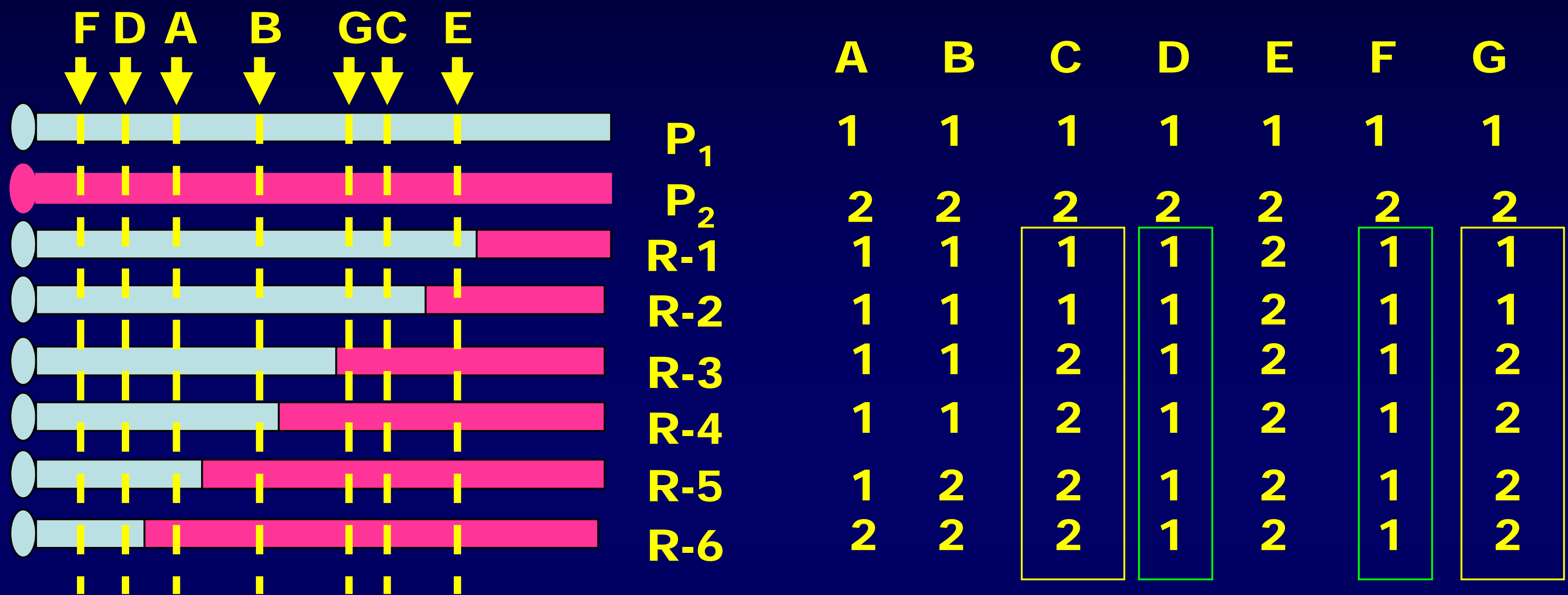
Spores: Each spore produces a recombinant haploid plant in which the recombination position is fixed.



Gene mapping with Recombinant Haploid Lines



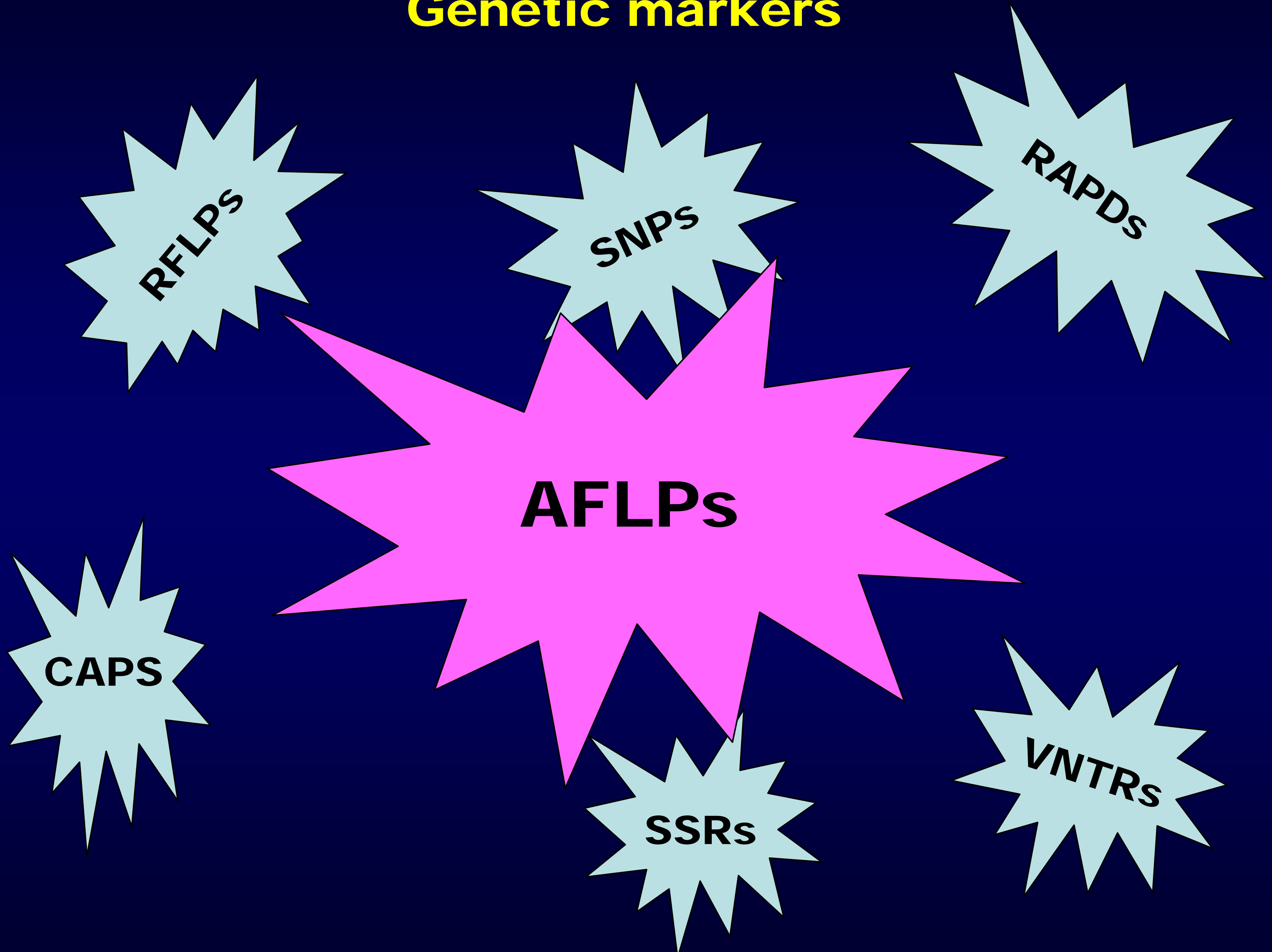
Any marker which is polymorphic between line P_1 and P_2 can have only one of the two states, in any recombinant haploid line.



	A	B	C	D	E	F	G
P_1	1	1	1	1	1	1	1
P_2	2	2	2	2	2	2	2
R-1	1	1	1	1	2	1	1
R-2	1	1	1	1	2	1	1
R-3	1	1	2	1	2	1	2
R-4	1	1	2	1	2	1	2
R-5	1	2	2	1	2	1	2
R-6	2	2	2	1	2	1	2

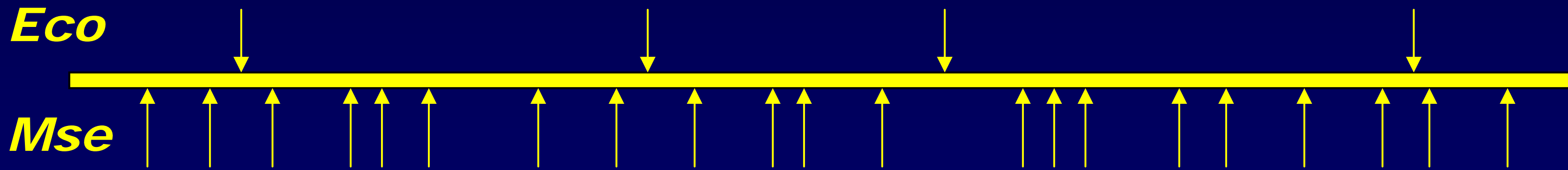
Each character is scored in each line, for whether it is in “state 1” or “state 2”. For every pair of characters, the recombination frequency is plotted. In this example, markers F and D, and G and C always co-segregate, indicating that these pairs of markers are closely linked.

Genetic markers



AFLPs

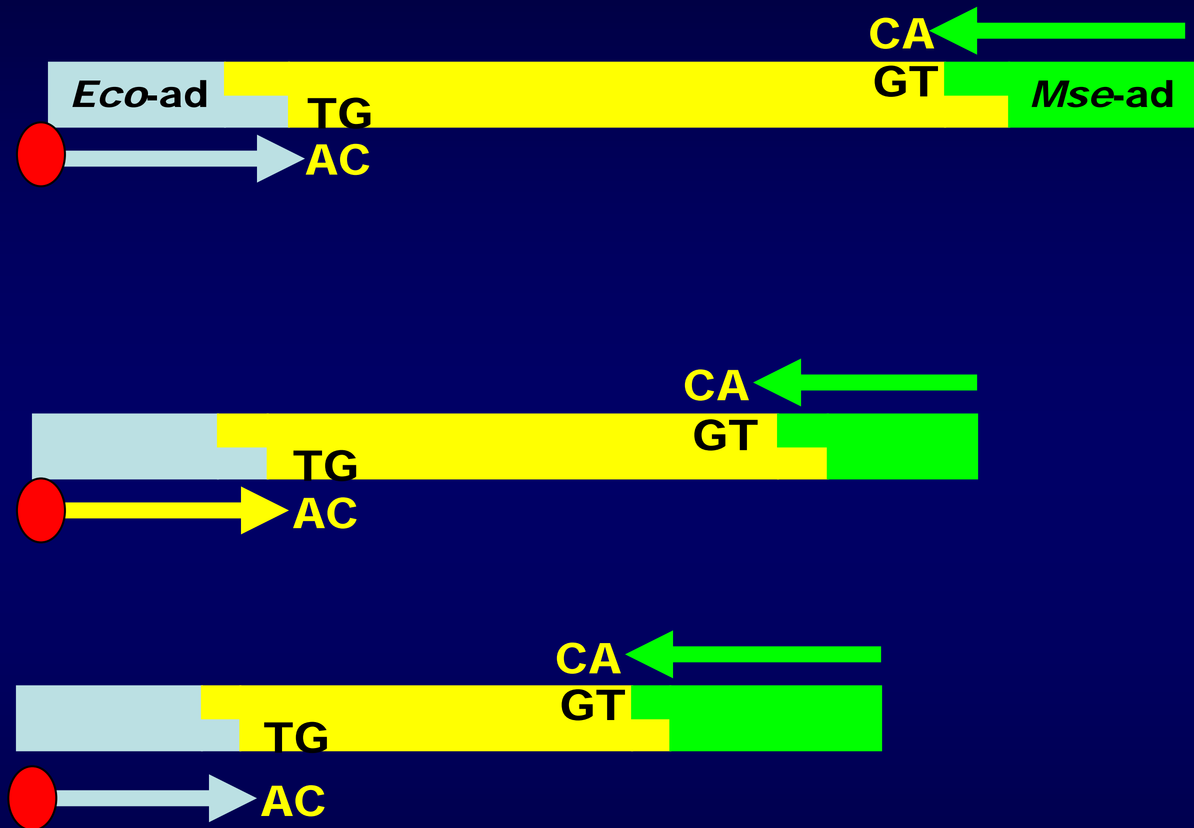
1. Cut genomic DNA with a 6-cutter (*e.g. EcoRI*) and a 4-cutter (*e.g. MseI*)



2. Ligate with "adapter oligonucleotides" containing a "target sequence" and a compatible sticky end for each site.

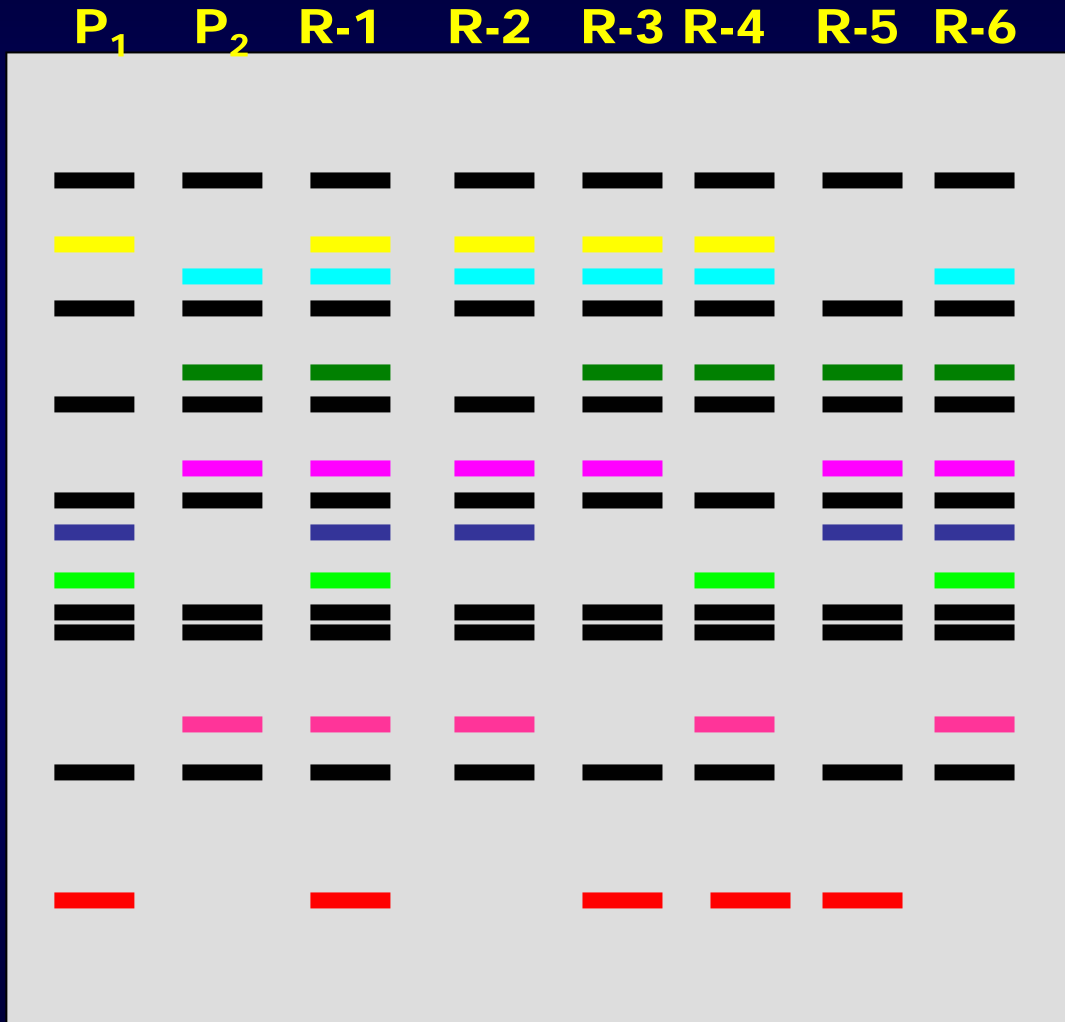
3. PCR-amplify with *Eco* and *Mse* adapter-specific primers to amplify fragments with one *EcoRI* end and one *MseI* end.

PCR-amplify using primers comprising target+RE site + 2 or 3 additional bases at 3′-end to amplify a subset of fragments



Note: Primers at the *EcoRI* end carry a fluorescent tag.

If the *Eco* primer is labelled at its 5′-end with tag, then the amplified fragments can be displayed on a sequencing gel:



The products visualised will be:

1. Fragments with specific base sequences following the site.

2. Non-polymorphic fragments (same in P_1 & P_2)

3. Polymorphic fragments

Strategy:

1. Screen all Physcotypes by AFLP analysis to identify suitably polymorphic parental lines.

2. Cross selected parents and harvest spore capsules.

3. Propagate individual spores to generate a population of recombinant haploid lines (to be made generally available).

4. Generate AFLP profiles from RH lines for linkage mapping.

Details:

Parental strains should be genetically marked to facilitate identification of spore capsules derived from a cross-, rather than a self-fertilization event

One parental strain should be the one selected for genome sequence analysis (?Gransden strain?)

Because AFLP markers are bands on gels, they can be isolated and cloned for use as hybridisation probes: this will enable integration of the genetic and physical map

Thanks to

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