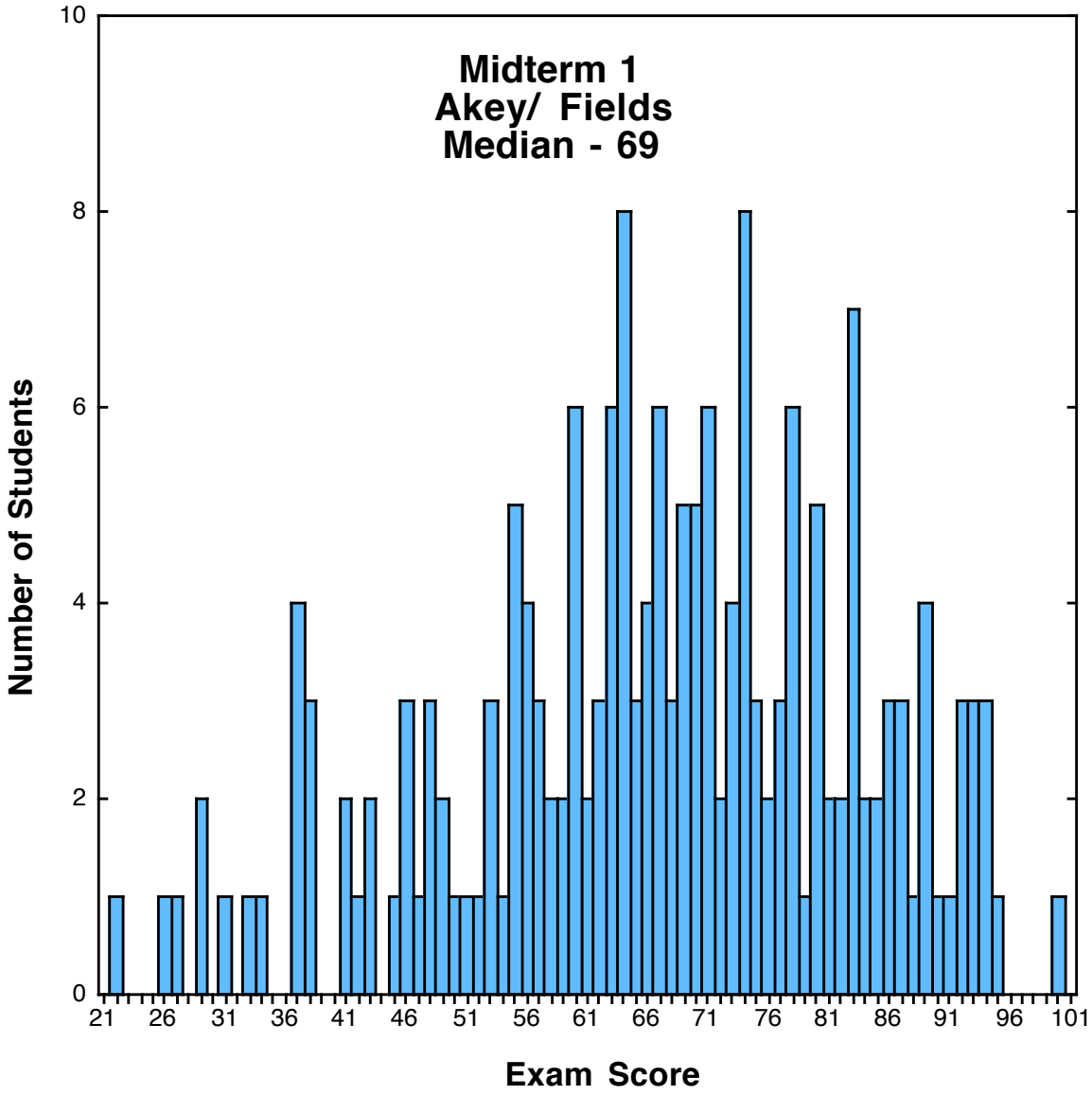
Midterm 1 Results…



Quick review of where we left off

Parental type: the arrangement of alleles on the parental chromosomes

We can identify parental types either by:

1. Knowing the gametes that made the individual we are interested in

2. Infer parental types by crossing - two most abundant progeny types define the parental type

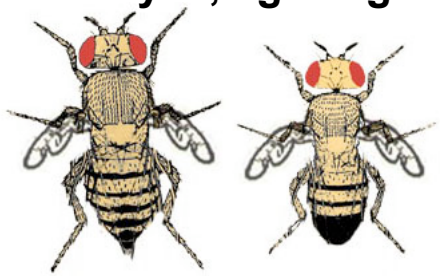
Identifying the Parental Type

Option 2. The two most abundant progeny types

Cross: pr^+ pr vg^+ vg x pr pr vg vg

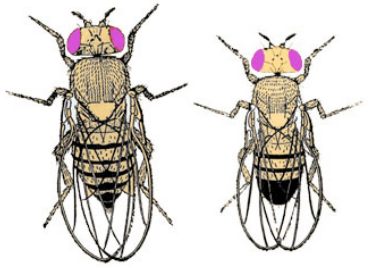
Progeny: $\frac{pr\ vg^+}{pr^+\ vg}$

red eyes, vg wings

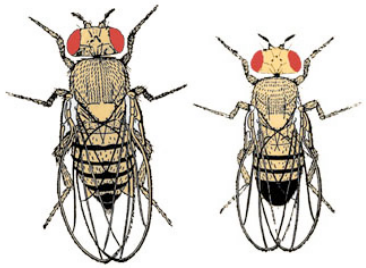


1287 $\frac{pr\ vg}{pr^+\ vg}$

purple eyes, wt wings

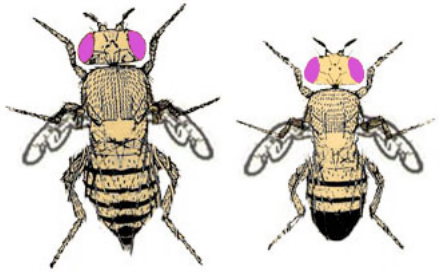


1204 $\frac{pr\ vg}{pr\ vg^+}$



170 $\frac{pr\ vg}{pr^+\ vg^+}$

red eyes, wt wings

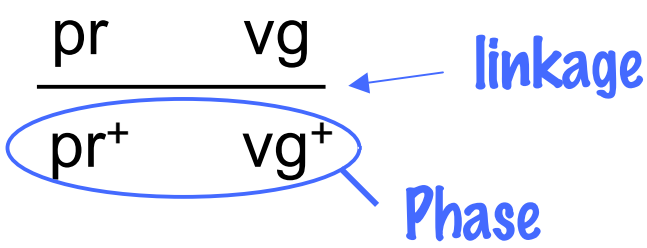


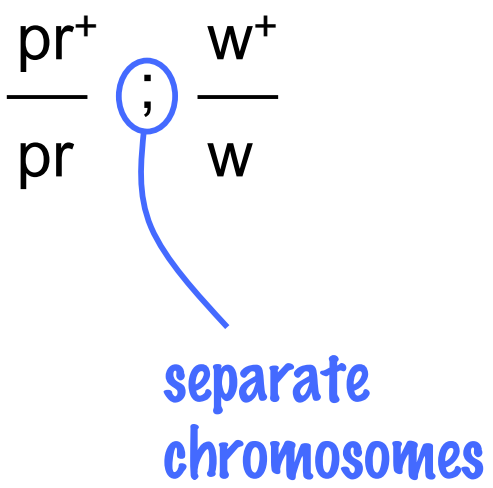
154 $\frac{pr\ vg}{pr\ vg}$

purple eyes, vg wings

What were the gametes that made the heterozygous parent?

A note on notation…





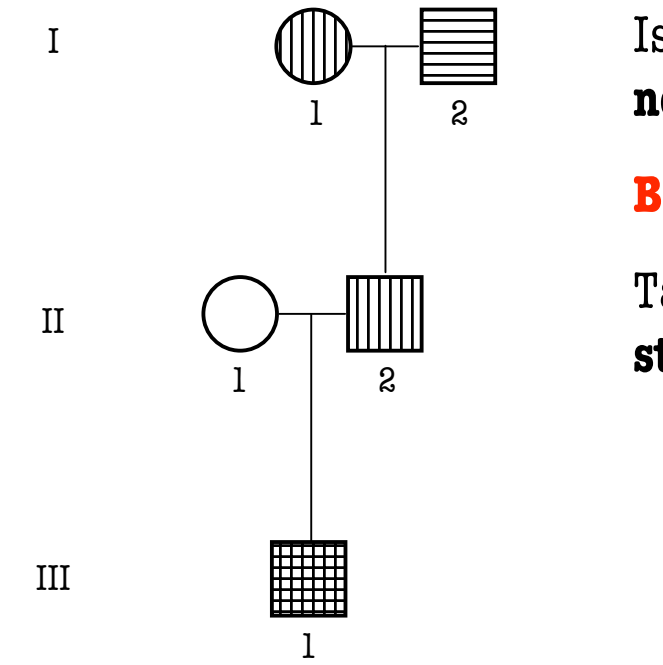
And… What is the difference between genotypes and haplotypes?

Genotypes: pr^+ pr vg^+ vg

Haplotypes: $\frac{pr \quad vg}{pr^+ \quad vg^+}$

Practice question

The pedigree shows segregation of two disorders... one is autosomal dominant (**A**= disease, **a** = not) and one is autosomal recessive (**b** = disease, **B** = not).



Is the gamete that III-1 received from II-2 **parental** or **non-parental**?

BUT FIRST... break down the question:

Talk to your neighbors and come up with a **systematic, step-by-step strategy** to solve the problem

Step 1.

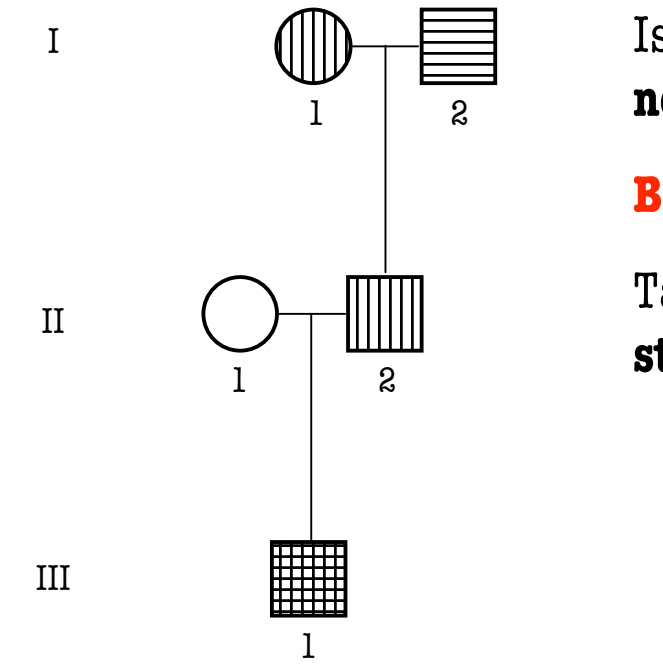
Step 2.

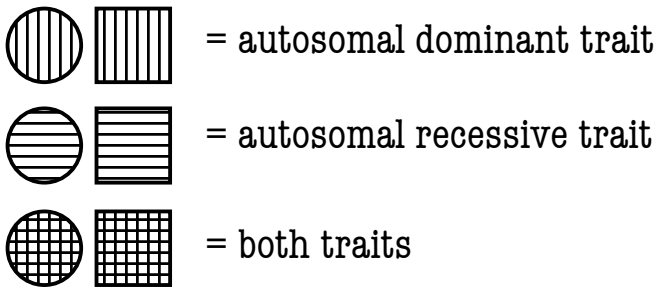
Step 3.

etc.

Practice question

The pedigree shows segregation of two disorders... one is autosomal dominant (**A**= disease, **a** = not) and one is autosomal recessive (**b** = disease, **B** = not).





Is the gamete that III-1 received from II-2 **parental** or **non-parental**?

BUT FIRST... break down the question:

Talk to your neighbors and come up with a **systematic, step-by-step strategy** to solve the problem

Step 1. Figure out all the genotypes!

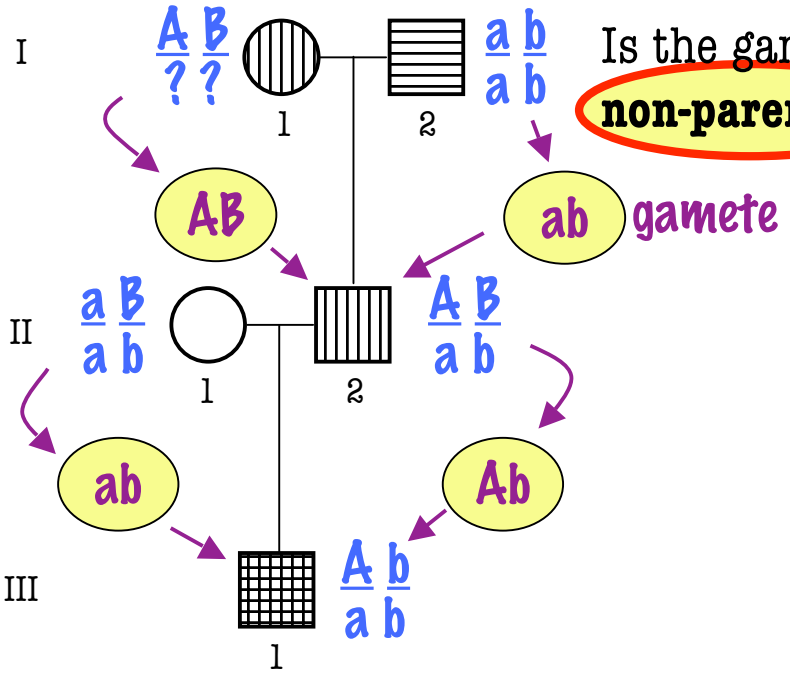
Step 2. What are the gametes that **made** II-2?

Step 3. What is the gamete that II-2 made?

Step 4. Does the gamete that II-2 made have a different genotype than the gamete(s) that made him?

Practice question

The pedigree shows segregation of two disorders... one is autosomal dominant (**A**= disease, **a** = not) and one is autosomal recessive (**b** = disease, **B** = not).



Is the gamete that III-1 received from II-2 **parental** or **non-parental**?

Gametes that made II-2=

AB and ab

Gamete that II-2 gave to III-1 =

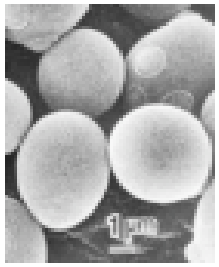
Ab

= autosomal dominant trait

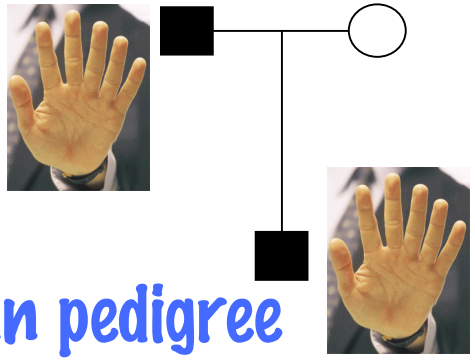
= autosomal recessive trait

= both traits

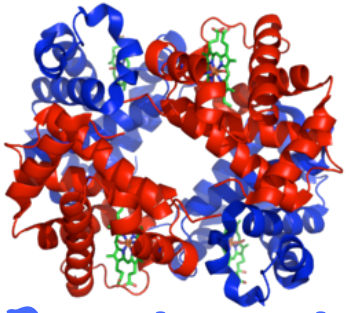
Common theme: linking genotype & phenotype



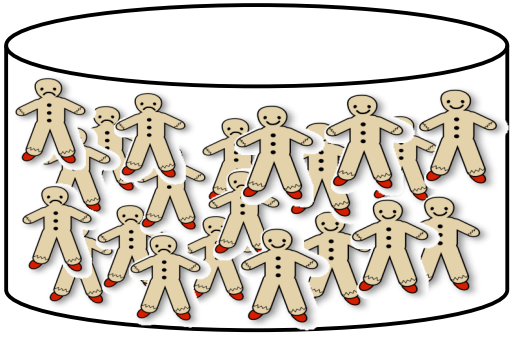
Mutant identified
in a model organism



Human pedigree
segregating a trait



Protein acting in
a biological process



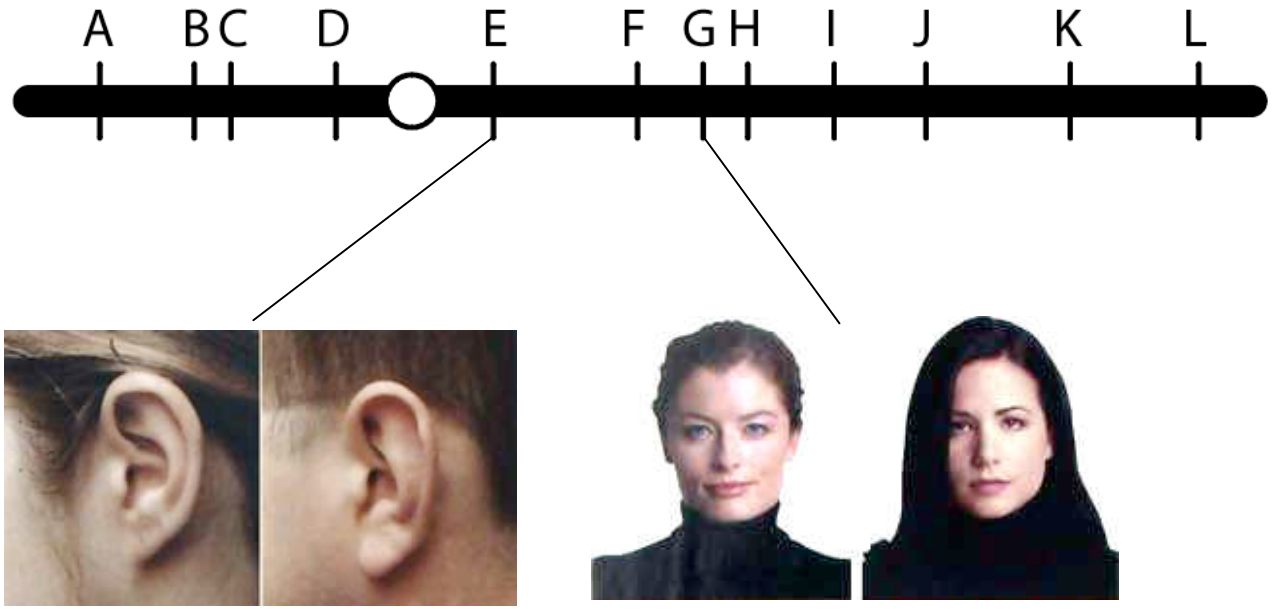
Association study

```
 946  ATT GTC TGT AGC CGA TTG GAG GAG TAC AAC AGC CAT
1009  GGA CCT TTA CGG CGT AAT CCT GGA AAC CAT GAC AAA
1072  GCT GAT GTA GAA TTT TGC CTG AGT TTG ACC CAA TAT
1135  AAT TTC AGC TTT AGA AAT ACA CTG GAA GGA TTT GCT
1198  TCT CAA AGC AGC ATG CAC AAT GCC TTG CAC ATC TAT
1261  GGA TCT GCC AAC GAT CCT ATC TTC CTT CTT CAC CAT
1324  TGG CTC CGA AGG CAC CGT CCT CTT CAA GAA GTT TAT
```

Sequence analysis

Markers

Genetic markers - inherited variations that are used to test genetic hypotheses



Limitations?

Molecular Markers

Rather than using observable traits, why don't we use *molecular markers* - variation in DNA sequence

We DO!

***Polymorphic* molecular markers are the primary types of markers used in contemporary genetics studies**

What Is A Polymorphic Molecular Marker?

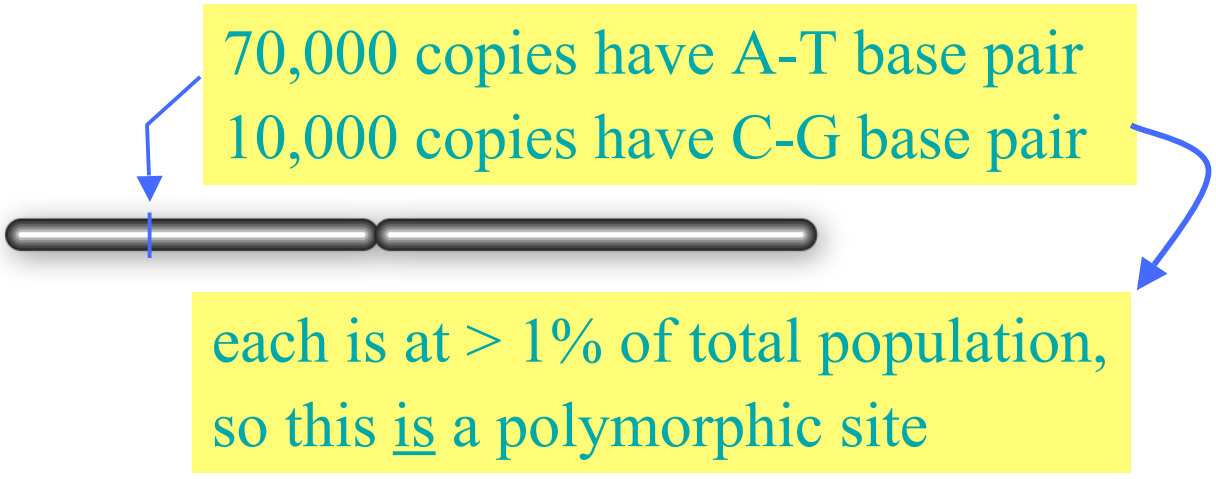
A polymorphic site or locus…

A location in the genome where at least two versions of the sequence exist in the population,

each at a frequency of at least 1%

UW student population - N ~ 40,000

⇒ 2N = 80,000 copies of (e.g.) chromosome 2



Types of Polymorphic Molecular Markers

1. Single Nucleotide Polymorphisms (SNPs)

..TCTTGATC..
..TCTCGATC..

2. Insertion/Deletions (Indels)

..TCTTGATC..
..TCTTTGATC..

3. Variable Number of Tandem Repeats (VNTRs)

..CCGCAGCAGCAGCAGCAGATTC..
..CCGCAGCAGCAGCAGCAGCAGATTC..
..CCGCAGCAGCAGCAGCAGCAGCAGATTC..

4. Restriction Fragment Length Polymorphisms (RFLPs)

Restriction Fragment Length Polymorphisms (RFLPs)

- Differences in DNA fragment lengths after cutting with one or more restriction endonucleases *Dde* I

5′...CTNAG...3′
3′...GANTC...5′

- An example from hemoglobin B

Dde I restriction enzyme site

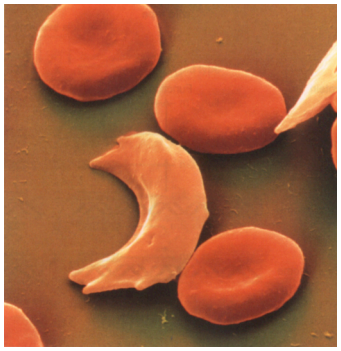
Allele "A"

5′ ... GTG CAC CTG ACT CCT GAG GAG ...3′
3′ ... CAC GTG GAC TGA GGA CTC CTC ...5′

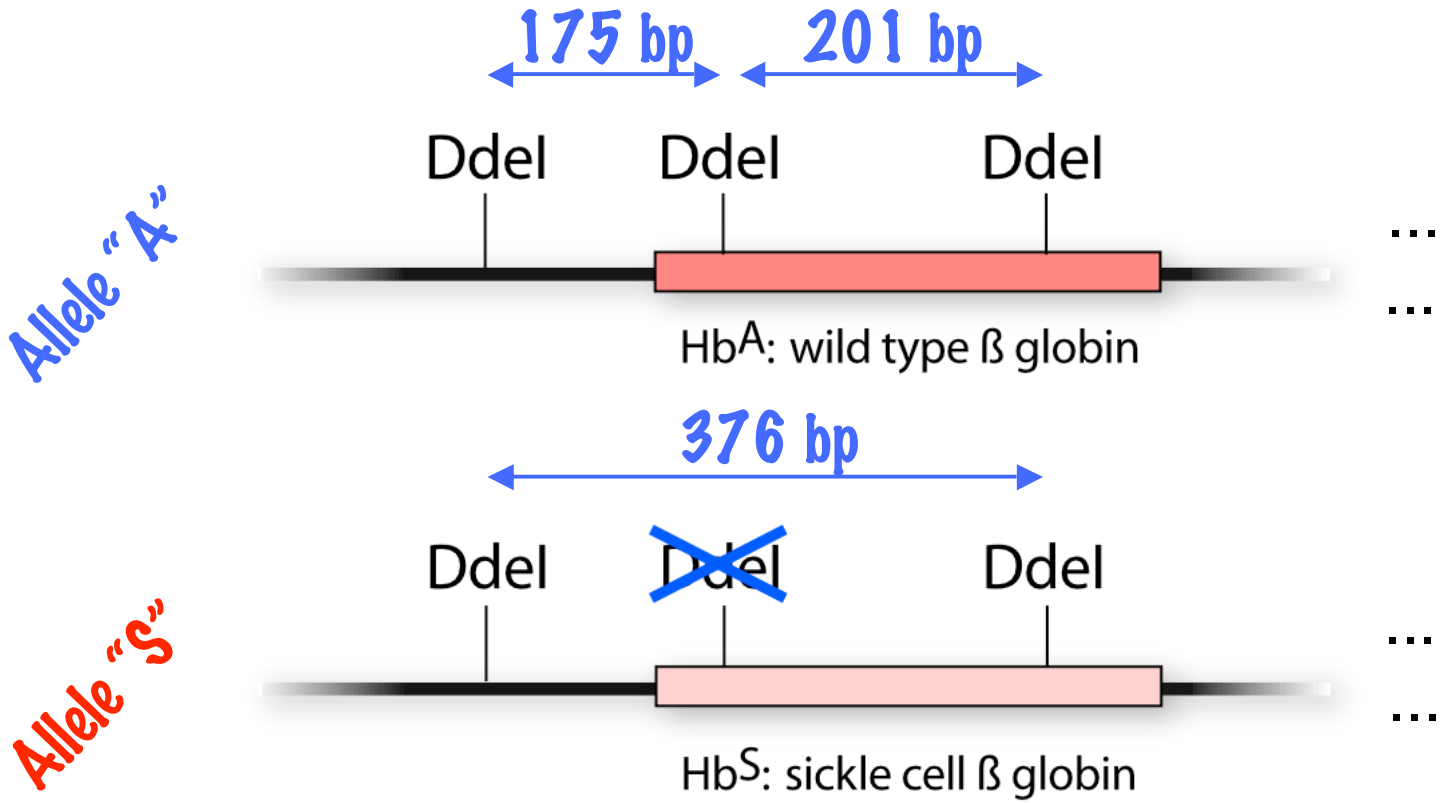
Allele "S"

5′ ... GTG CAC CTG ACT CCT GTG GAG ...3′
3′ ... CAC GTG GAC TGA GGA CAC CTC ...5′

~~Dde I restriction enzyme site~~



Identifying Hb Genotype



Presence or absence of restriction site ⇒ RFLP

Restriction fragment length polymorphism

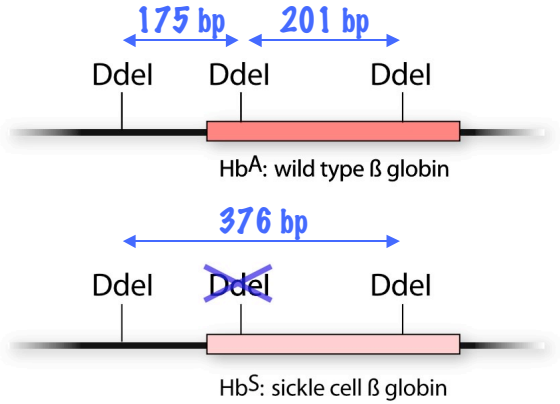
multiple forms

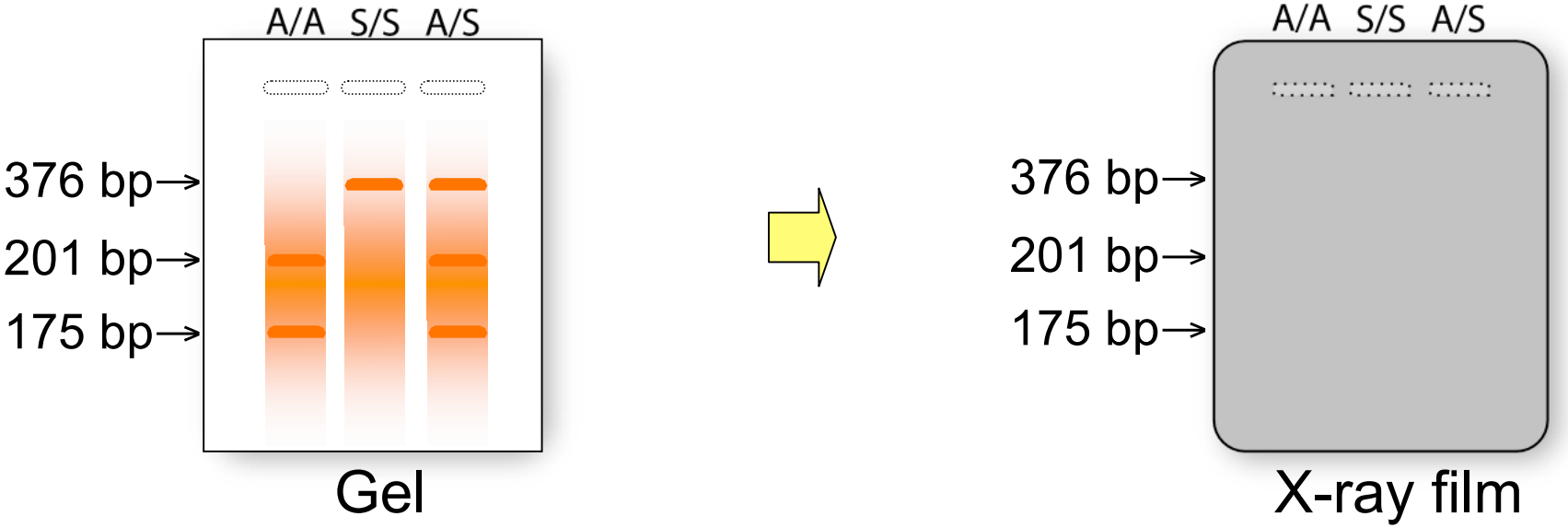
How Can We Genotype RFLPs?

1. Based on hybridization with a labeled probe
   "Southern blot"
2. Based on PCR

Identifying Hb Genotype by a Southern Blot

1. Digest human DNA sample with DdeI
2. Run gel
3. Blot to filter, hybridize with probe
4. Wash off excess probe, expose film





SNPs

..TCTTGAATCGGACGTATGCTCAATTACGATC..
..TCTCGATTCGGACGTATACTCAATTACGATC..

- If it was possible to sequence your genome, how many SNPs would we expect to find?

  ~ 1 SNP per 1000 bp => 3 million

- Stable genetic markers: mutation rate ~ 2 x 10^{-8}/site/gen
  How many new SNPs do you carry?

  ~ 3 x 10^{9} x 2 x 10^{-8} x 2 = 120 new SNPs

You're a Mutant

Genotyping methods—

- sequencing
- hybridization

Distinguishing Between SNP Alleles by Hybridization

Hybridization with **Allele-Specific Oligonucleotides**

= "few"

okay?

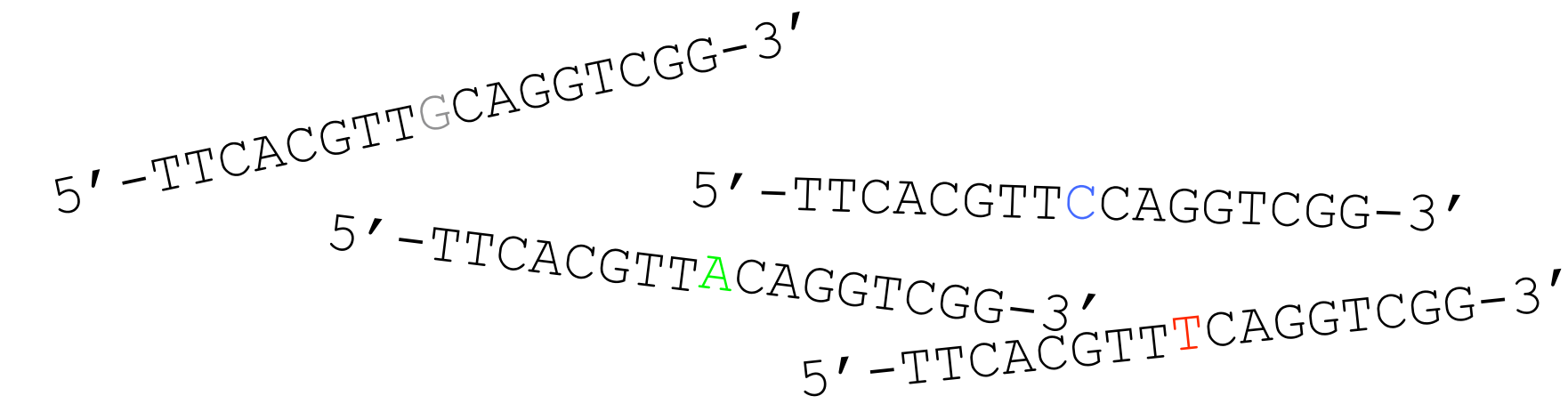
Not for small oligos!

- Small probes (25-30 bases) can work if conditions (salt, temperature) are adjusted. Mismatches much more significant for small probes.

Strategy… hybridize with small oligo (17 - 20 bases long)

Hybridization seen only if target and probe match perfectly

SNP allele identification by allele-specific oligonucleotides



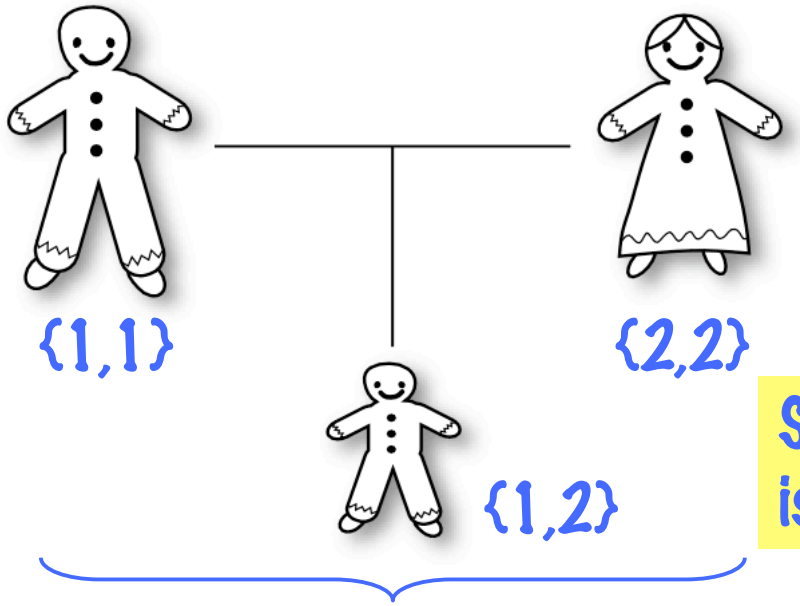
5′-CTATCCAATAGTGTTTCACGTT?CAGGTCGGTCCCTCATA-3′
3′-GATAGGTTATCACAAAGTGCAA?GTCCAGCCAGGGAGTAT-5′

which allele?

...which oligo shows stable hybridization to the target?

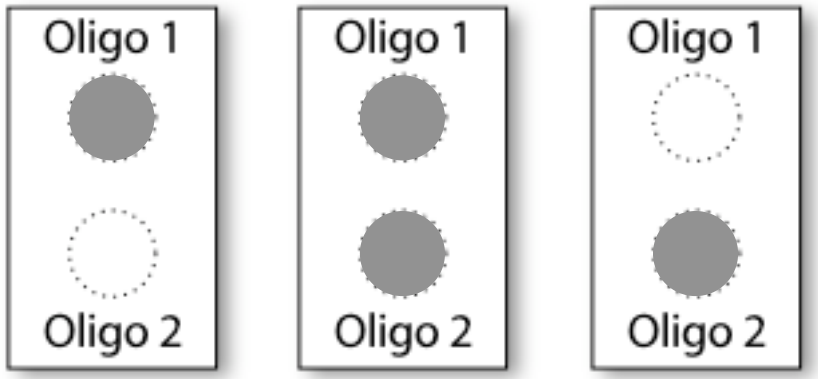
SNP Genotype Identification

Oligo is on membrane (or slide); hyb with labeled DNA sample from person



SNP inheritance is Mendelian

Cell sample→label DNA→hyb to oligos

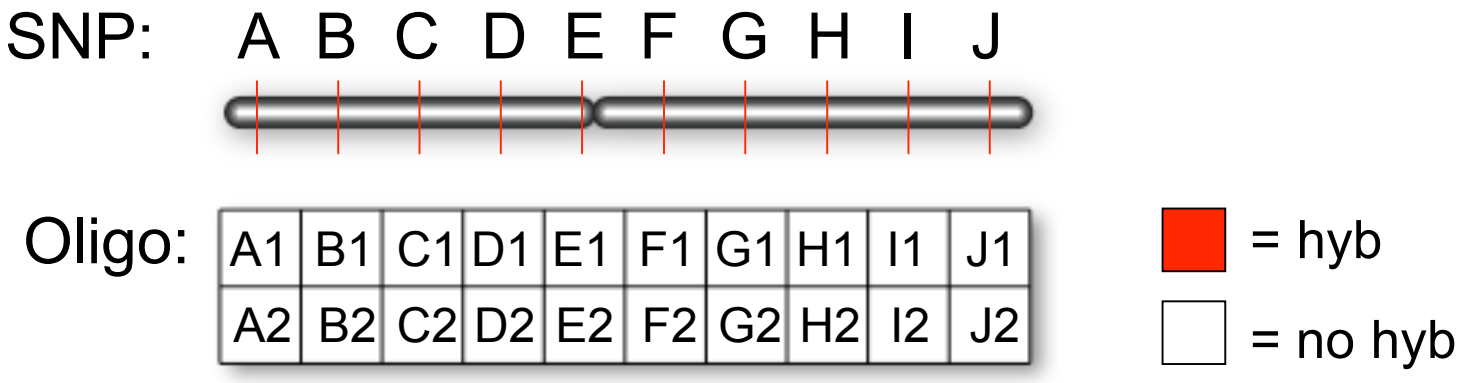


-Allows high throughput analysis

High Throughput SNP Genotyping

Label genomic DNA, hybridize to oligos for a set of SNPs

→simultaneous identification of genotype at all those SNPs!



Genotype?

Commercially available: "SNP-chips" to detect ~1 million SNPs

Microsatellite/VNTR Genotyping

1. Make lots of copies of the DNA between the invariant sequences
   —Polymerase chain reaction (PCR)

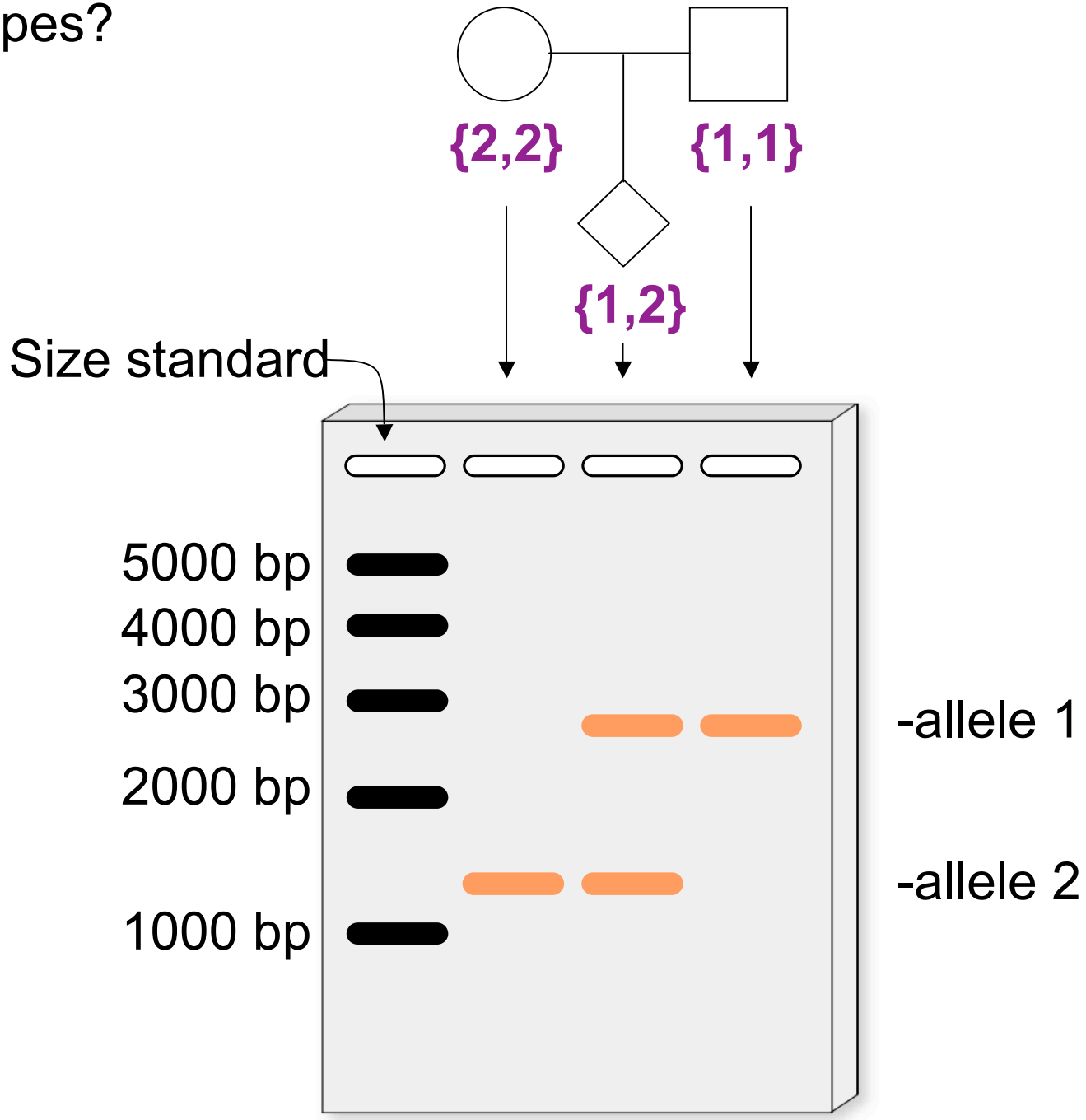
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGATTCACTG
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGATTCACTG
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGATTCACTG

TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGCAGATTCACTG
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGCAGATTCACTG
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGCAGATTCACTG

TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGATTCACTG
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGATTCACTG
TCCAAGCGCACCGGCCGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGATTCACTG

2. Measure the size of the DNA you've made
   —gel electrophoresis

Genotypes?


{2,2}
{1,1}
{1,2}
Size standard
5000 bp
4000 bp
3000 bp
2000 bp
1000 bp
-allele 1
-allele 2

Summary

- Variant forms of DNA sequence (polymoprhisms) can be used to map gene locations
- Polymorphisms include single nucleotide polymorphisms and length polymorphisms
- Alleles of polymorphic sites show Mendelian inheritance
- Alleles of polymorphic sites can be detected using methods including DNA hybridization, PCR, and gel electrophoresis

Genome 371, 1 Feb 2010, Lecture 7

Genomic Maps and Linkage Analysis

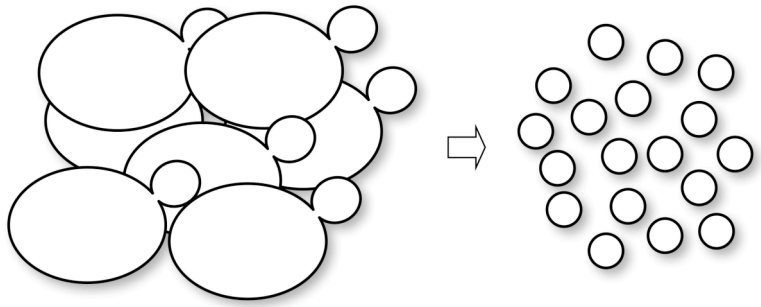
- Genomic maps
  - Linkage maps
  - Physical maps
- Using molecular markers for linkage analysis

Making a Genetic Map in Yeast - QS5

» What % of gametes are recombinant? “random spore” analysis

Let lots of diploid cells undergo meiosis...

for the two loci of interest, how many parental vs non-parental spores?



Random Spore Analysis

An example from QS3: Are *ADE* and *HIS* genes linked?