Mapping a gene defined by the mutation

Lecture 4, 5, and 6:

A. Classical genetics
B. Molecular mapping
   I. Molecular techniques: Restriction mapping
      Library construction and screen
      PCR (polymerase chain reaction)
      DNA sequencing (lecture 7)
      Southern blot
   II. Molecular markers: RFLP, CAPS, SSLP, SNP
   III, Molecular mapping
C. Map-based cloning

Read chapter 7, p320-322, 326-327 (box 7.2), 329-330
Fig. 7.17; 7.18, 7.29, 7.30
\[
\begin{array}{cc}
A & B \\
\hline
A & B \\
\hline
a & b \\
\hline
\end{array}
\times
\begin{array}{cc}
a & b \\
\hline
a & b \\
\hline
\end{array}
\]

\[
\begin{array}{cc}
A & B \\
\hline
A & B \\
\hline
a & b \\
\hline
\end{array}
\times
\begin{array}{cc}
a & b \\
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\end{array}
\]

\[
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\hline
\hline
a & b \\
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\end{array}
\]

\[
\begin{array}{cc}
A & B \\
\hline
A & b \\
\hline
a & B \\
\hline
\end{array}
\]

\[
\begin{array}{c}
90 \\
90 \\
10 \\
10 \\
\hline
200 \\
\end{array}
\]

\[
\frac{10 + 10}{200} = 0.1 = 10\%
\]

\[
\frac{Ab + aB}{\text{total}} = \text{rec, freq.}
\]
Restriction enzymes

(a) Blunt ends (Rsal)

Sugar-phosphate backbone

(b) Sticky 5' ends (EcoRI)

5' overhangs

(c) Sticky 3' ends (KpnI)
Restriction mapping
Cloned linear DNA segment

Sample 1  Sample 2  Sample 3
Cut with EcoRI  Cut with Bam  Cut with EcoRI and Bam

Load each sample into gel. Run gel.

Restriction map
Library construction and screen (Chapter 7, box 7.2)

1. Isolate DNA.
2. Isolate vector DNA.
4. Inserted plant DNA.
5. Gene for color selection.
6. Inserted plant DNA containing gene of interest.
7. Amp^R (gene for antibiotic resistance).
8. Transform into bacteria.
The polymerase chain reaction, part 1

1. 94°C for 5 minutes
2. 50–60°C for 2 minutes
3. 72°C for 2–5 minutes

Elongation of primers with Taq polymerase

Denatures genomic DNA

Primers base pair at sites flanking target sequence of genomic DNA

Target sequence

3' Mutant
5' β-globin allele
The polymerase chain reaction, part 2

1 copy

2 copies

4 copies

8 copies

16 copies

32 copies

64 copies

animation
Southern blot analysis, part 1

Buffer solution
Sponge
Gel
Nitrocellulose paper
Stacked paper towels

Size markers
Target fragment

animation
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RFLP: Restriction Fragment Length Polymorphism

HindIII digestion
Electrophoresis
Southern blotting
Hybridization with the probe
autoradiography
Example of RFLP

Restriction digestion
GE
Ethyedium bromide stain

Blotting
Hybridization
X-ray radiography
CAPS (Cleaved Amplified Polymorphic Sequences)

```
Ler
\[\text{HindIII}\quad 2\ \text{kb}\quad \text{HindIII}\quad 5.7\ \text{kb}\quad \text{HindIII}\]

Col
```

PCR
HindIII digestion
GE

http://www.arabidopsis.org/index.jsp
Mapping with a CAPS marker

E/e: CAPS marker
F/f: a mutation causing abnormal flowers
Ler: solid line
Col: dotted line

No. of plants  No. of recombinant Chr.
11  0
6  1
0  2

\[
\frac{6}{17 \times 2} = 0.17
\]
Mapping with a SSLP marker

Col

$\text{(AT)}_{20}$

150 bp

Ler

$\text{(AT)}_{15}$

140 bp

http://www.arabidopsis.org/index.jsp
SNPs (Single Nucleotide Polymorphisms)

http://www.arabidopsis.org/index.jsp
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Fig. 7.17; 7.18, 7.29, 7.30
Yeast Artificial Chromosomes YAC (Fig. 7.30)

<table>
<thead>
<tr>
<th>Vector</th>
<th>insert size</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>YAC:</td>
<td>100-1000kb</td>
<td>yeast</td>
</tr>
<tr>
<td>BAC:</td>
<td>80-300kb</td>
<td>bacterium</td>
</tr>
<tr>
<td>Cosmid:</td>
<td>20-50 kb</td>
<td>bacterium</td>
</tr>
<tr>
<td>Lamda:</td>
<td>10-20kb</td>
<td>bacterium</td>
</tr>
<tr>
<td>Plasmid:</td>
<td>0.2-15kb</td>
<td>bacterium</td>
</tr>
</tbody>
</table>
In Arabidopsis: 1% recombination = about 200 kb
0.1% = 20 kb
Mapping with a CAPS marker

E/e: CAPS marker
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No. of plants

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\[
\frac{6}{17 \times 2} = 0.17
\]
The larger the mapping population
The more recombinants between two markers
The higher the resolution of the map
The closer the gene f is from the nearest molecular marker
*lsn-1* is flower mutant

*Wild type*  
*lsn-1*
Map-based cloning of LSN

WT: TACCGA

Lsn-1: TACTCGAA
Create F2 mapping population
↓
establish linkage
↓
identify nearby PCR markers
↓
Assay a large number (1,000) of F2 plants with flanking markers
↓
Establish physical map & contig
↓
More fine mapping
↓
Transformation rescue (complementation) and/or sequence mutant alleles
↓
Identify the gene

Steps in map-based cloning

Download Problem set #1 from Bsci 411 website
Due next Tuesday (2/24/04)