



Biology 475 - Molecular Biology Lab

Restriction Mapping Using Mystery DNA

Adapted from Morgan/Carter's Investigating Biology



Goals

Understand what restriction enzymes are, how to use them, and why they are useful
Understand agarose gel electrophoresis and how it works in relation to DNA structure
Map your mystery DNA, determining its size and the positions of several restriction sites

Restriction Enzymes

Unique bacterial "protection" enzymes that cut specific sequences of DNA (hundreds available)
Named after organism, strain, enzyme number (e.g. many bacteria make more than 1 enzyme)
Each has optimal buffer/environmental conditions that match bacterial source - see Buffers Below
Cut DNA fragments can be analyzed for patterns (identification, forensics, diagnostics), cloned...

Common Enzymes Used In This Class

Name	Bacterial Source	Recognizes	BL	BM	BH	BK
EcoRI	Escherichia coli	GAATTC	20	100	100	100
Avall	Anabaena variabilis	GGCC	80	100	20	20
HindIII	Haemophilus influenzae	AAGCTT	60	100	20	100
PstI	Providencia stuartii	CTGCAG	20	60	100	80
PvuII	Proteus vulgaris	CAGCTG	80	100	40	20
HhaI	Haemophilus haemolyticus	GCGC	80	100	100	100
HaeIII	Haemophilus aegyptius	(Pu)GCGC(Py)	60	100	100	100

B columns refer to common buffers that match different bacterial sources. The numbers below these columns refer to the performance (100% = best) if this enzyme is used in this particular buffer. This was taken from the Takara Website: http://bio.takara.co.jp/BIO_EN/catalog_d.asp?C_ID=C0003

Probability Theory and Restriction Enzymes

For enzymes that recognize 6 bp: $(1/4)^6 \times$ target DNA size = number of times it is predicted to cut
The predicted fragment size = the size of the target being cut divided by the number above
Be able to calculate this for a variety of enzymes and targets

Based on probability theory, will agarose resolve your predicted fragments?

Agarose Gels - Low Resolution

DNA moves mostly by size and conformation - distinguishes 50-100 bp differences
We will use today to separate cut fragments of mystery DNA for mapping
Later, we will run plasmid isolations to assess for size and quality
Later, we will blot our gel-separated PCR product, probing for certain DNA sequences

RESTRICTION MAPPING ACTIVITIES

Restriction Digests Contain

1 ug target DNA to be cut (in your case, provided as 0.5 ug/ul)
3 units enzyme per ug DNA to be cut (for this course, all enzymes are 10 units/ul)
1X APPROPRIATE buffer (all buffers supplied in 10X concentrate, choose best buffer - see above)
Water to a volume that dilutes the total enzyme volume at least 10-fold
In general, the order is: water, buffer, DNA – with enzyme ALWAYS last

For this exercise, your total volume should be 15 and you will use 0.3 ul of enzyme in all cases. YOU NEED TO MAKE LOTS OF DECISIONS AND CALCULATIONS, however.

Must Be Handwritten in Pre-Lab	DNA	Buffer (name & amount)	Enzyme(s) (name /amount)	Water to 15 ul
Uncut Control				
AvaII				
PvuII				
EcoRI				
AvaII/PvuII				
AvaII/EcoRI				
PvuII/EcoRI				
AvaII/PvuII/EcoRI				

In Lab Procedures - You Should Be Set-Up/Digesting No Later Than 1:00

All the materials above will be set out on ice; keep everything on ice as much as physically possible
 You need to set up ALL appropriate tubes, digest 60 minutes (for this course, at 37°C)
 During wait, proceed to pouring your gel and problems and/or pre-lab for next week

AGAROSE GEL ACTIVITIES

Agarose Gel Pouring

Prepare 50 ml of 1% agarose per gel box in TAE running buffer
 After boiling (heat on high and watch like a hawk), cool 10 minutes at 50°C before pouring
 Tape ends of a tray and place ONE 12-lane comb in upper holder
 Pour the cooled agarose into the tray; it will take 10-15 minutes to harden
 Place hardened gel/tray in box such that, when the power is on, the DNA will run to the positive end
 Cover the gel completely with 1X TAE running buffer. CAREFULLY pull the comb out of the gel

Make sure to remove any visible air bubbles that are trapped in the wells after comb removal.

Loading Samples - You Should Be Doing This No Later Than 2:15

Mix your samples with loading dye by adding 3 ul dye to the top walls of each tube and tapping down
 I will do a quick demonstration load of the standard markers (10 ul - very expensive - see below)
 Load your samples, setting the pipetteman to 17 ul
 Run your gels at 120 V for 60-90 minutes (check with me every 20 minutes for advice)
DO NOT let the yellow dye come within 1 inch of the end of the gel!!!
 Make ABSOLUTELY CERTAIN you have recorded EXACTLY what you loaded where
 I recommend drawing a picture of the gel as you loaded it

While gels are running, work on template and pre-labs for next week!!!!

Staining Gels - I Will Demonstrate 3:15-3:30

The following things cannot be touched because they are dangerous
 All are handled as hazardous waste; NS201 hood provides only area these materials stored/discarded
 Stain gels with ethidium bromide, a DNA stain (and, consequently, a mutagen) 10-20 minutes
 Photograph gels using UV light, making sure to include wells - visualized ethidium bromide/DNA
 Retrieve pictures and use information to map your mystery DNA

Why So Many Uncut Bands?

Uncut DNA = YOUR CONTROL

Lowest band = supercoiled, truly uncut

Upper Bands = nicked accidentally (poor handling) any number of times (each = higher band)

BEWARE of "partials" in your theoretically cut lanes - partials are not fully cut

Determining Band Sizes - ALL WORK Must Be Shown in Lab Notebook Results

PRECISELY measure distance between wells and each band

Using semi-log graph paper, plot Y = fragment size (log Kb) vs. X = plot distance (cm)

Use the markers to make a standard line and "solve" your unknowns from this

Loading Marker

Pre-cut DNA that will be used to "size" your DNA

Top to Bottom (kb): 23.1, 9.4, 6.5, 4.4, 2.3, 2.0, 0.56, 0.13

Mapping - SOME SUGGESTIONS

Determine size of mystery DNA; keep in mind - it is circular

Draw the linear fragments generated by each enzyme separately, always keeping track of sizes

Analyze double digest data, deducing relative positions of both enzymes

Assemble all data, drawing on a circle

Remember - this is nothing more than logic.

Quality Control Grading - Notebook

The cutting produced on the gel.

The final map.