# First class: Basic skills in a molecular biology lab

a) Prepare digest with BamHI or BglII, for the following DNAs: FUGW, genomic DNA, FUCEdW.
b) Prepare 1% agarose gel
c) Precipitate genomic DNA
d) Run digest
e) Take pictures

# Basic Procedure: Liquid Handling

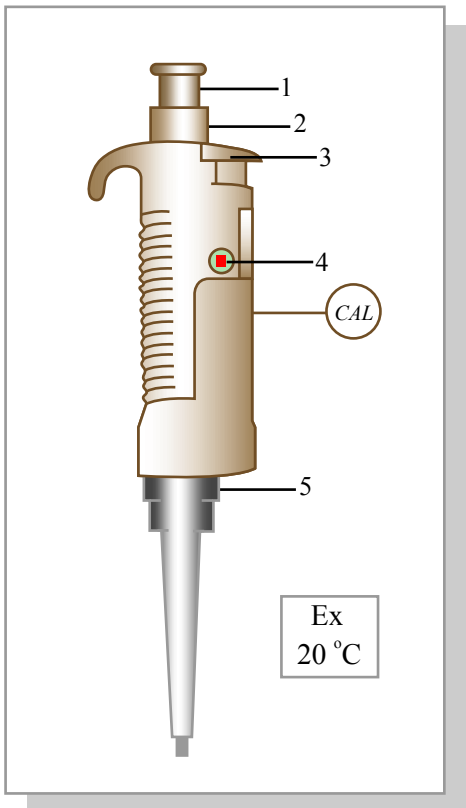


Figure by MIT OCW.

**1** Control button
First stop (measuring stroke); the aspirated volume is dispensed.
Second stop (blow-out); the liquid remaining in the tip is blown out.

**2** Setting ring
To set the volume
For fixed-volume pipettes, this ring is for adjustment purposes only.

**3** Ejection button
Tip ejection.

**4** Adjustment opening
with overlapping calibration seal for inserting the wrench during adjustment.

**5** Ejection sleeve
To extract liquids from long vessels, the ejection sleeve may be pulled off when the ejection button is held down.

# Basic Procedure: Liquid Handling 2

- 1000: 100-1000 µl. Use large tip.
- 200: 20-200 µl. Use small tip.
- 20: 2-20 µl. Use small tip.

# Basic Procedure: Centrifuge

- Have connector always outside
- Balance tubes.
- Complete stop before opening.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

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# Plasmid

- What is plasmid?
- What is the difference of plasmid and “usual” DNA?

# Plasmid

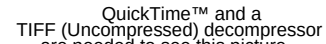
- What is plasmid?
  - Small circular DNA.
  - Replicated inside of host bacteria.
  - Can carry extra gene such as drug resistance gene.
  - Can carry foreign DNA.
- What is the difference of plasmid and “usual” or chromosomal DNA?
  - Size
  - Chromosomal DNA carries most (>%99.99) of necessary gene for bacteria
  - Bacteria can live without plasmid DNA but not without chromosomal DNA.

# Restriction Enzymes

- The critical component of DNA recombinant technology
- Why it is called “restriction” enzyme?
- How often do you see these “sites” in DNA?

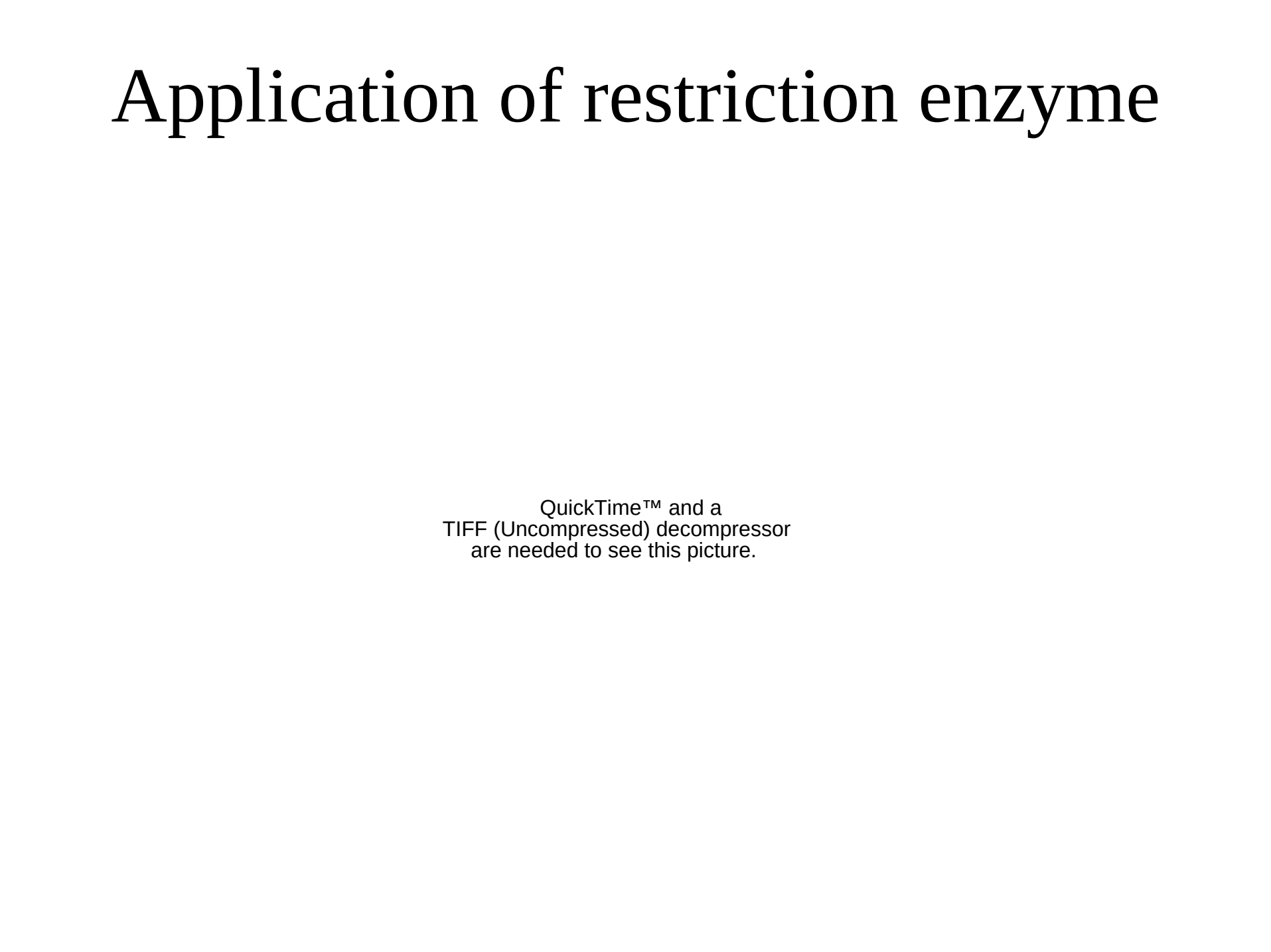
BamHI


BglII

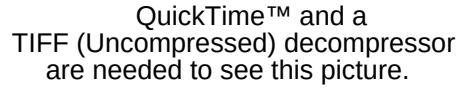



- Why restriction enzyme is useful?

# Application of restriction enzyme

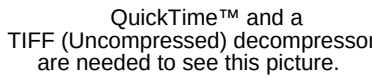


# Separation of DNA fragment

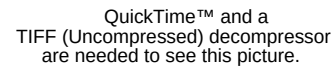



- What is the relationship of migration distance and molecular weight?
  - Include your analysis in the report.

# Visualization of DNA in agarose gel




- Ethidium bromide (EtBr): Possible carcinogen.



# Protocols

Preparation of the digest

Cut 3 µ of each type of DNA with BamHI or BglII.
For BamHI use buffer 2 (blue)
For BglII use buffer 3 (red tube)

Cut the following:

- FUGW with BamHI
- FUGW with BglI FUGW with no enzyme
- FUGW with both BamHI and BglII (in buffer 2)
- FUCed with BglII
- FUCed with no enzyme
- Genomic DNA with BglII

(per tube)
3 µl of DNA
2 µl of the appropriate buffer (red or blue)
14 µl of water
1 µl of BSA (bovine serum albumin)
0.5 µl of enzyme (BamHI or BglII)

Why do you think we have BSA?

Mix by gently tapping.
Spin for 3 seconds
Incubate in 37C waterbath for at least 40 minutes (while waiting, start preparing the gel )

# Protocols

## Preparation of the agarose gel

Measure 100 ml of TAE buffer in measuring syrinder
Pour 50 ml of TAE buffer in a 200 ml bottle
Measure 1 gram of agarose in a weigh boat
Add agarose to bottle and swirl thoroughly
Loosen cap and put in microwave for 2 minutes at low power (4)
Make sure that contents do not boil and spill over
Add the rest of TAE to the bottle and mix well by swirling
Add 5 µl of ethidium bromide to liquid in bottle
Swirl (without making bubbles)
Cast get with two 12 tooth combs (one on top row and one in bottom)
Add agarose into cast and let solidify
Wait 15 minutes (while you wait, precipitate the genomic DNA as explained below)
Once is solid, remove comb, turn the cast 90 degrees, and add approx. 400 ml of TAE buffer, until the liquid starts to cover the gel

# Protocols

Precipitation of DNA (this is separate experiment to be done while waiting for digestion and electrophoresis)

Into a 1.5 ml eppendorf tube, add:

40 µl of genomic DNA
5 µl of sodium acetate 3 M
100 µl of 100% ethanol

Mix well by shaking

Spin in centrifuge at maximun speed for 5 minutes

Discard supernatant in waste bucket

Add 1 ml of 70% ethanol

Discard supernatant in waste bucket (make sure that your remove all you can by inverting tube on a piece of paper and gently tap the tube to eliminate all remaining drops)

Air dry tube for 5 minutes

Add 10 µl of water to bottom of tube

Tap bottom of tube to ensure mixing of water and pellet

Spin down for 3 seconds

Do you see something on the bottom?

# Protocols

## Running of DNA digests and precipitation

Load 4 µl of 1Kb DNA ladder in first well

Mix 2 µl of loading buffer (LB tube) with each of the digests that you prepared before

Load 23 µl of each of the digest+loading buffer in each well

Add 3 µl of loading buffer to the precipitated genomic DNA, and load into a well

The loading order should be:

1kb ladder-FUGW (BaMHI)- FUGW (BglII)-FUGW (BamHI and BglII)-FUGW (no enzyme)- genomic DNA (BglII) -FUCedW (BglII)- FUCed (no enzyme)-precipitated genomic DNA (plus empty wells)

Once everything is loaded, set voltage to 150 volts and start running the gel
Run gel for 15 minutes
Stop gel and take image with UV light

# Report

- What is the relationship between migration distance and molecular weight?
- How can you calculate unknown DNA size?
- How many BamHI and BglII sites are there?
- How are they arranged?
  - Draw a “restriction enzyme map” of FUWG
- What is the smeary pattern?

(No introduction, method, etc needed for today. Just the result of you analysis and a map of the plasmid. Write about possibilities.)