BME

MOLECULAR BIOLOGY EXPERIMENT

VECTOR ENDONUCLEASE RX

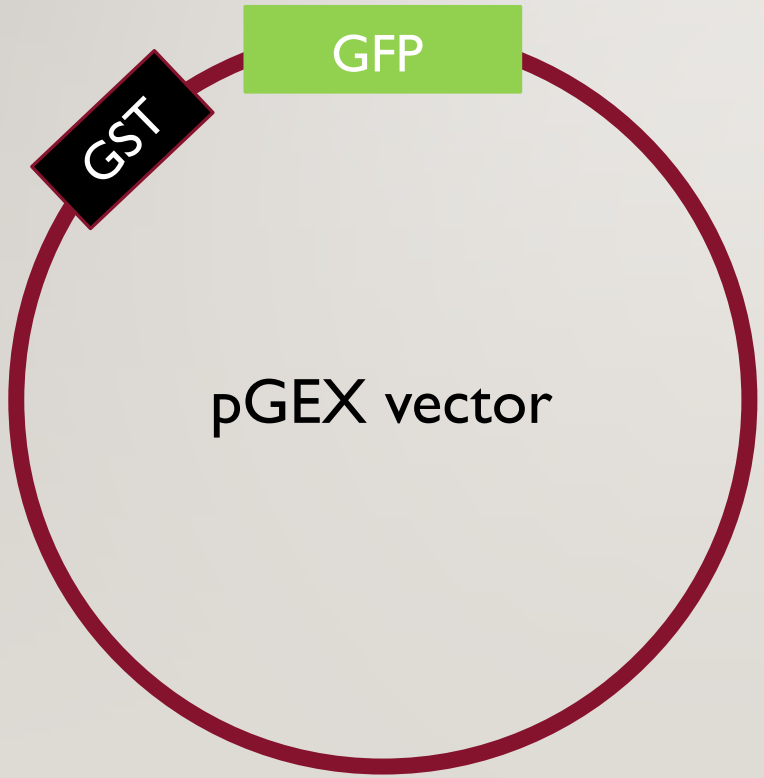
SKKU BME

3RD GRADE, 2ND SEMESTER

TODAY

- Vector endonuclease reaction
- DNA electrophoresis

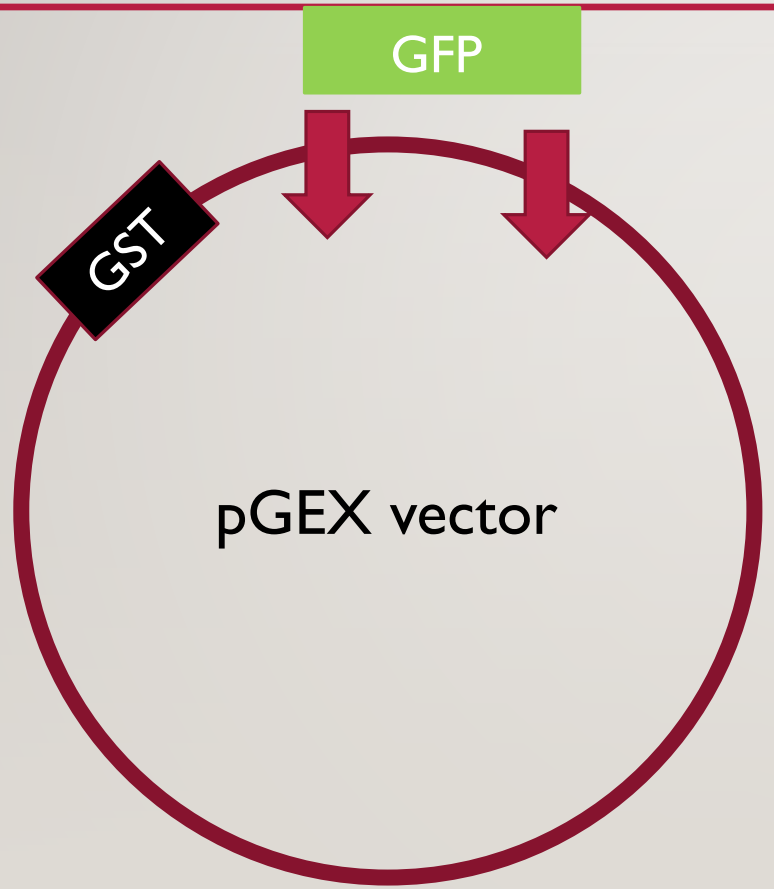
LET'S SEE THE BIG PICTURE AGAIN!



1> Vector
- E.coli transformation & culture
- Miniprep – confirmation by enzyme cutting

2> Insert
- Primer making
- PCR amplification
- Cleaning
- Enzyme cutting for ligation

ENDONUCLEASE REACTION



1> Vector

E.coli transformation & culture

Miniprep – confirmation by enzyme cutting

Endonuclease reaction

2> Insert

Primer making

PCR amplification

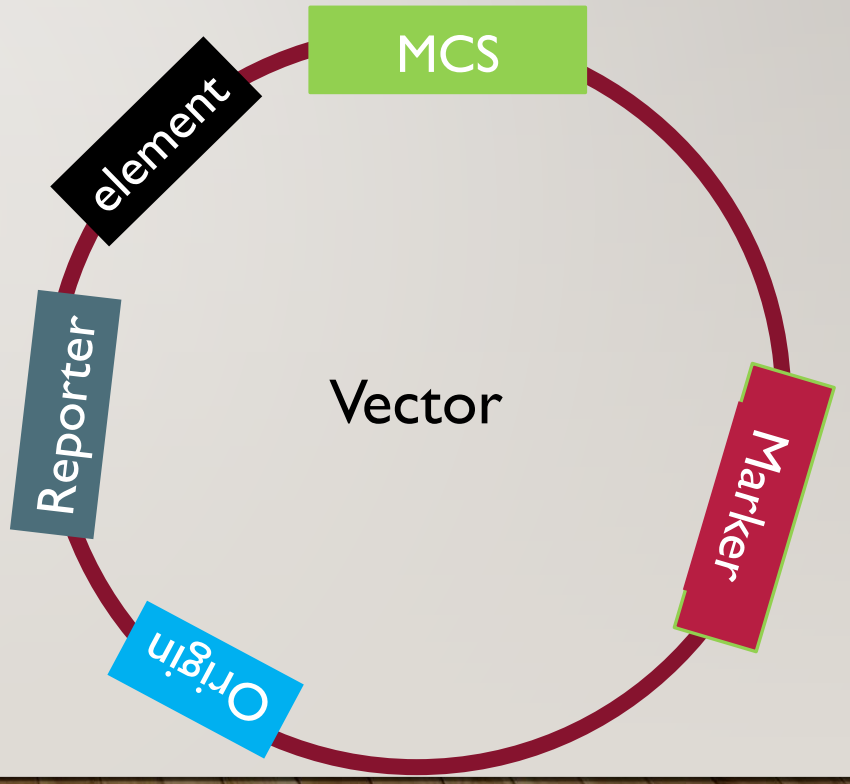
Cleaning

Enzyme cutting for ligation

WHAT ARE THE CRITICAL COMPONENTS IN VECTOR?

- Cloning sites
- Selectable marker
- Reporter gene
- Elements for expression
- Origin

Can you explain what are these?



https://www.youtube.com/watch?v=N3NVJ5nJqKw

5 TYPES OF VECTORS

- Plasmid
- Bacteriophage
- Cosmid
- Bacterial artificial chromosome
- Yeast artificial chromosome
- Human artificial chromosome

Home work!

RESTRICTION ENZYME REACTION (AGAIN)

- **1U/ul**
- **Unit Definition**. **One unit** of **restriction endonuclease** activity is **defined** as the amount of **enzyme** required to produce a complete digest of **1** µg of substrate DNA (or fragments) in a total reaction volume of 50 µl in 60 minutes under optimal assay conditions as stated for each **restriction endonuclease**.

TODAY – TEMPLATE CUTTING

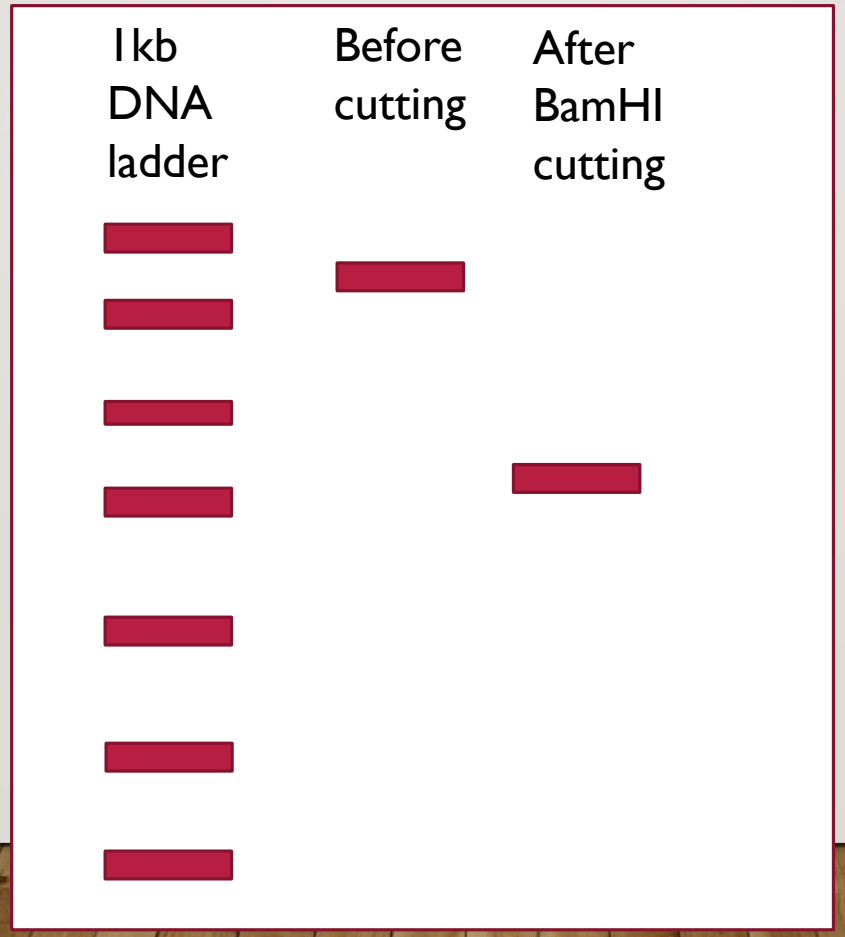
- Now you can ***make your own procedure***…
- What do you need?
- Template
- Enzyme
- Buffer
- DW

Let's make your own protocol!

WHAT WE WILL DO TODAY…

- Measure the *concentration of template*
- Calculate *how much amount of enzyme* is necessary for the Rx
- *Enzyme reaction* (BamHI first) – Confirm the full cutting
- Making *agarose gel* (with cyber safe)
- Confirm the enzyme reaction by *gel electrophoresis*
- *Enzyme reaction* (XhoI) overnight

TODAY'S FINAL FIGURE



HOMEWORK

- Find restriction enzyme buffers in NEB site and discuss about a problem when you want to use the EcoRV and FatI for template cutting.

(NEB site: https://www.neb.com/~/media/NebUs/Files/nebuffer-performance-chart-with-restriction-enzymes.pdf)

- What is the T4 DNA ligase?
- Find 5 types of vectors and explain their advantages and disadvantages.

NEXT WEEK

- DNA clearing
- Template and insert ligation