Third class : PCR amplification of DNA

a) Set up PCR reaction of GFP and potassium channel
b) Check the size of expected PCR product by computer
c) Check PCR product on gel and take picture
d) Ethanol precipitate DNA
e) Set up digestion of PCR product and vector.

• Because PCR takes some time, we will start the reaction first. As you come in, please set up PCR reaction.
Usage of PCR

• Amplify DNA from single copy of DNA
  – Molecular Biology
  – Genetic
  – Paleontology
  – Forensic Science
  – Ethology
  – Etc.
Three components of PCR

- DNA template
- Two primers that anneals to DNA you want to amplify
- Heat-stable polymerase (Taq polymerase, Pfu polymerase etc)
Three steps of PCR

- Denature
- Annealing
- Extension

Denature 95 °C
Annealing 55 °C
Extension 72 °C
PCR

Four components of PCR
Template
Primer
Heat stable polymerase
dATP, dCTP, dGTP, dTTP
After 25 cycles of PCR, how many copies of DNA do you have?
$2^{25} = 33,554,432$ copies
PCR (polymerase chain reaction)

CaMKIIα cDNA

- Amplify coding region
- Add restriction enzyme site

XhoI - EcoRI
Introduction of Restriction Enzyme Site using PCR

Enzyme sites
Introduction of Restriction Enzyme Site using PCR
Introduction of Restriction Enzyme Site using PCR

Enzyme sites are incorporated into the PCR product
Enzyme digestion

ligation

Vector DNA (plasmid)

pTCAGA

T

--T

--CTCAG

Shrimp Alkaline phosphatase

--T

--CTCAGp
1. PCR reaction
2. Make 0.8% gel
3. Run PCR product on agarose gel
4. Ethanol precipitate PCR product
5. Set up the digestion of vector and insert