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

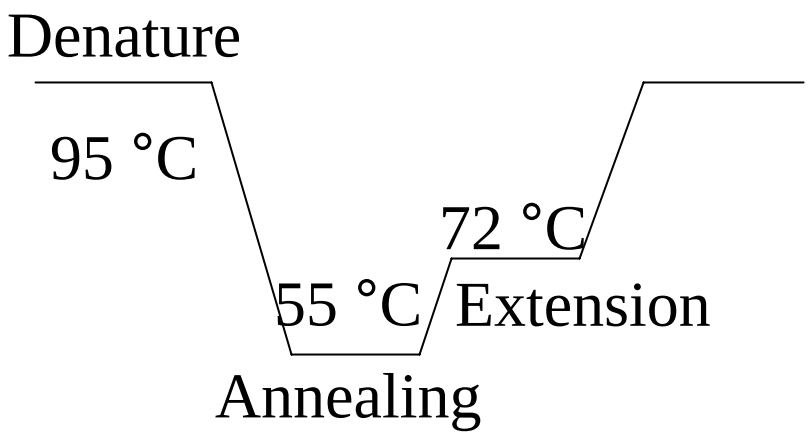
- Amplify DNA from single copy of DNA
  - Molecular Biology
  - Genetic
  - Paleontology
  - Forensic Science
  - Ethology
  - Etc.

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



PCR

Four components of PCR

- Template
- Primer
- Heat stable polymerase
- dATP, dCTP, dGTP, dTTP

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

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

PCR

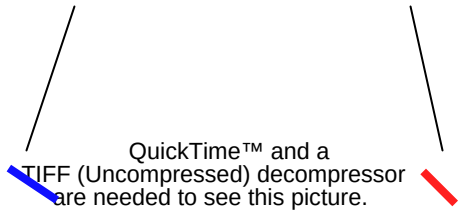
Amplify coding region

Add restriction enzyme site

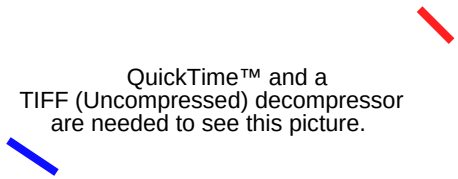
XhoI EcoRI

Introduction of Restriction Enzyme Site using PCR

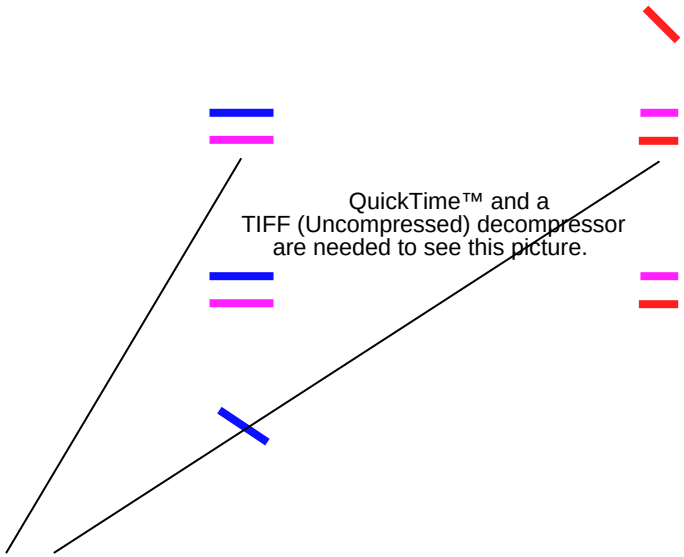
Enzyme sites



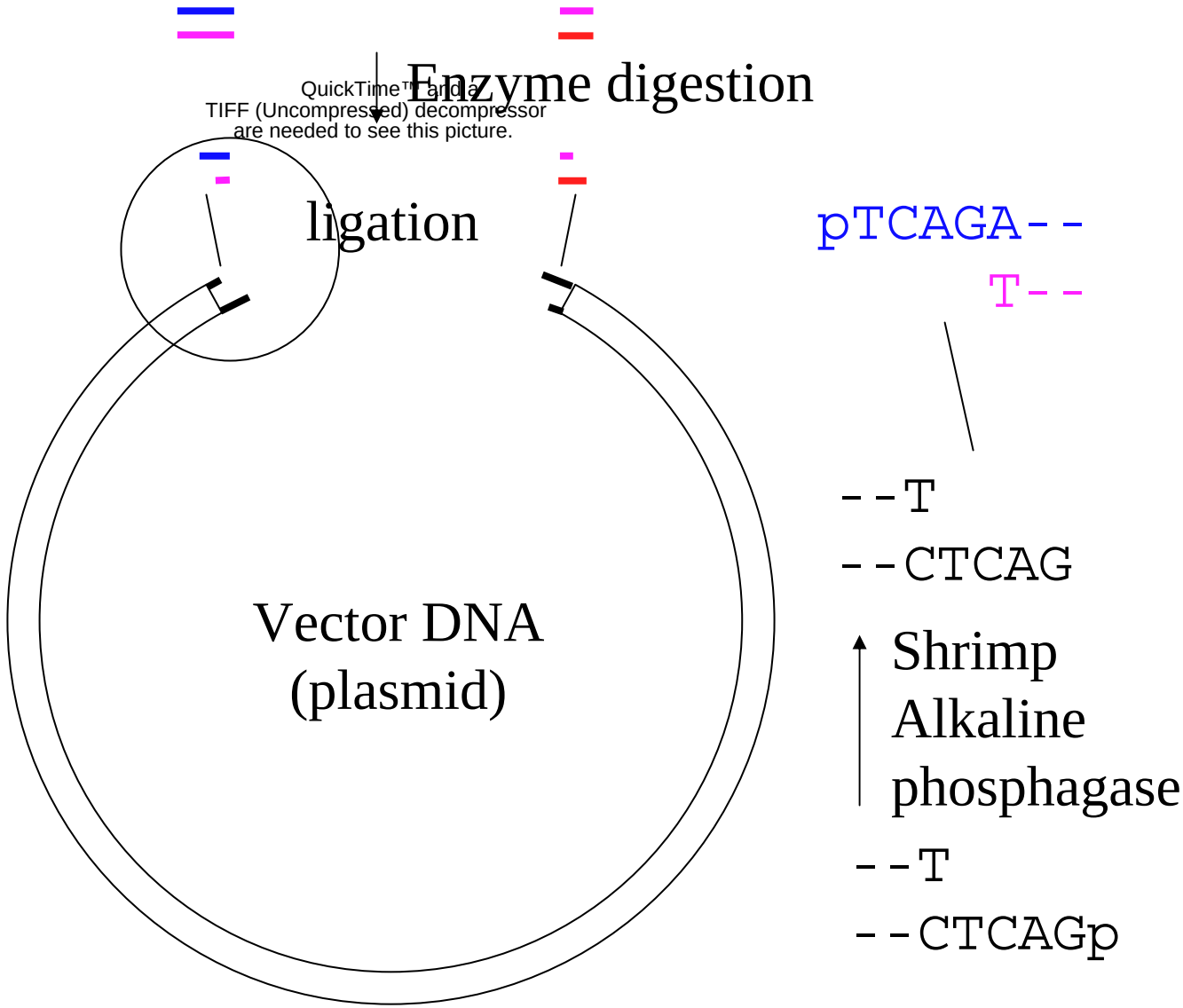
Introduction of Restriction Enzyme Site using PCR



Introduction of Restriction Enzyme Site using PCR



Enzyme sites are incorporated into the PCR product


QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.
Enzyme digestion
ligation
Vector DNA
(plasmid)
pTCAGA--
T--
--T
--CTCAG
Shrimp
Alkaline
phosphagase
--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