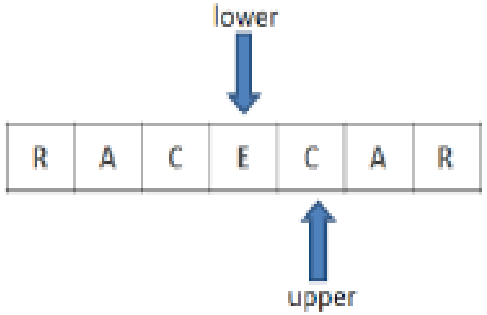
RESTRICTION DIGESTION, RESTRICTION MAPPING, DNA LIGATION

- Restriction enzymes (also called restriction endonucleases) are proteins made by many bacterial species, to defend against viral infections.
- Each restriction enzyme moves along a DNA molecule until it finds a specific recognition sequence in the DNA. The enzyme cuts the double-stranded DNA, resulting in DNA fragments.
- Over 3000 restriction enzymes that recognize short (4-8 bp) palindromic sequences have been discovered.

Restriction enzymes

- Isolated from bacteria as endogenous "restrictors" of bacterial pathogens. Evolved by bacteria to protect against viral DNA infection
- Recognize short specific sequences, often **palindromes** and cut DNA at highly specific points
- Recognize specific sequences
  - Four to seven bases
  - Each is unique
- Consistent results



Enzyme	Recognition Sequence
BamH I	GGATCC CCTAGG
Not I	GCGGCCGC CGCCGGCG
Sau3A I	GATC CTAG
Sac I	GAGCTC CTCGAG
Sst I	GAGCTC CTCGAG
Hinf I	GANTC CTNAG
Xho II	PuGATCPy PyCTAGPu

Digestion Procedure

- **Digestion**: the act of breaking down into pieces

Prepare Master Mix
Add buffer and enzyme in correct proportions.

⇩

Add restriction digest master mix to DNA
Mix thoroughly by flicking tube.

⇩

Incubate
Temperature and time depend on enzyme to be used.

⇩

View result by gel electrophoresis

Procedure:

1. The following components are added in a clean micro centrifuge and kept on ice. Restriction enzymes are added at the end.

a. Appropriate 10x buffer

b. DNA sample (0.5 ug)

c. 10 units of R.E (0.5 to 1 ul)

d. Deionized water to final volume of 10ul.

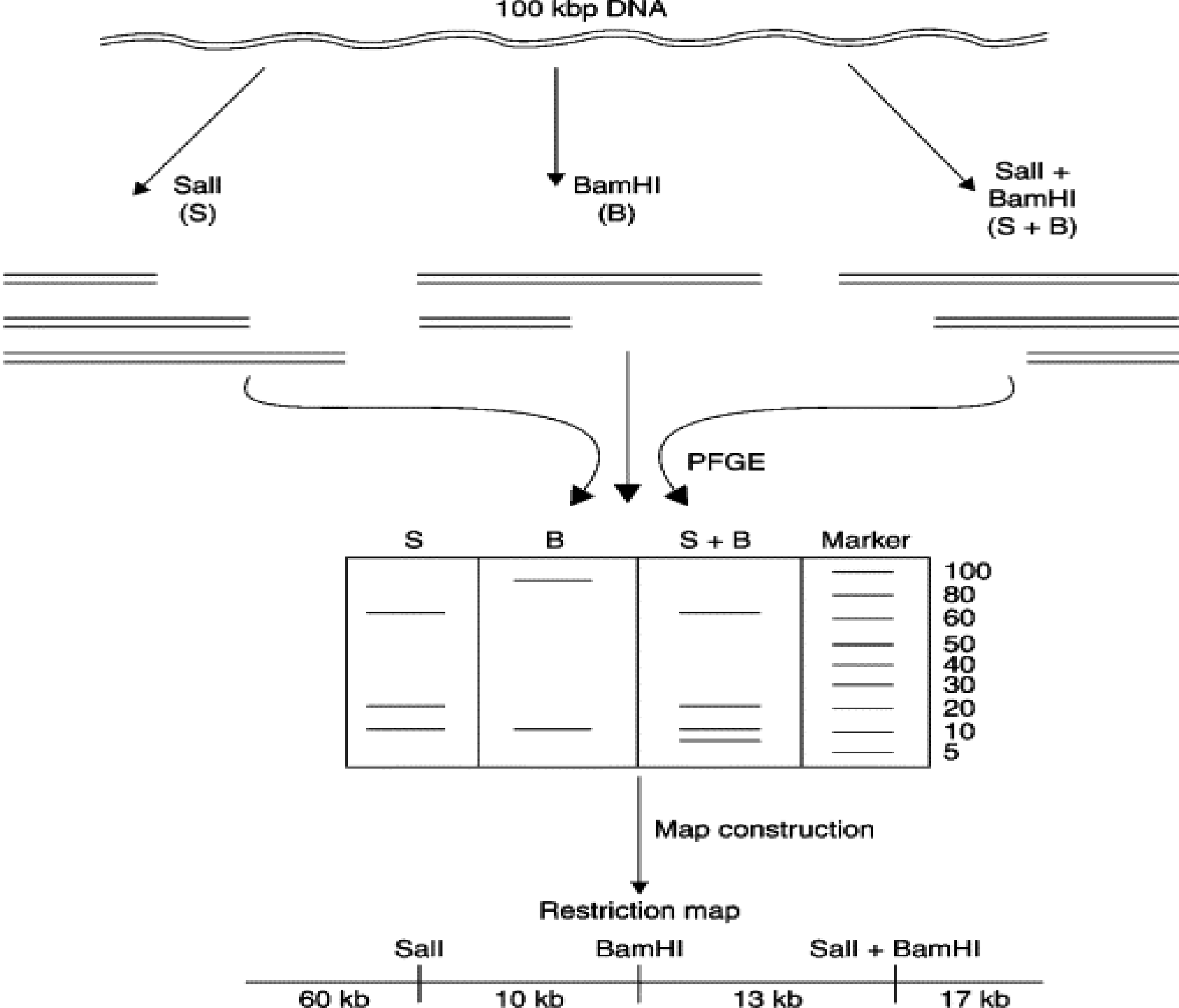
2. It was then mixed gently either by vortexing or by finger tapping.

3. Reaction mixture was incubated at 370C for 2 hrs.

4. Then the digested DNA is separated in agarose gel electrophoresis.

Restriction Mapping

- Restriction mapping is a method used to map an unknown segment of DNA by breaking it into pieces and then identifying the locations of the breakpoints. This method relies upon the use of proteins called restriction enzymes, which can cut, or digest, DNA molecules at short, specific sequences called restriction sites.
- After a DNA segment has been digested using a restriction enzyme, the resulting fragments can be examined using a laboratory method called gel electrophoresis, which is used to separate pieces of DNA according to their size.


100 kbp DNA
SalI
(S)
BamHI
(B)
SalI +
BamHI
(S + B)
PFGE
S
B
S + B
Marker
100
80
60
50
40
30
20
10
5
Map construction
Restriction map
SalI
BamHI
SalI + BamHI
60 kb
10 kb
13 kb
17 kb

Restriction Digest Analysis

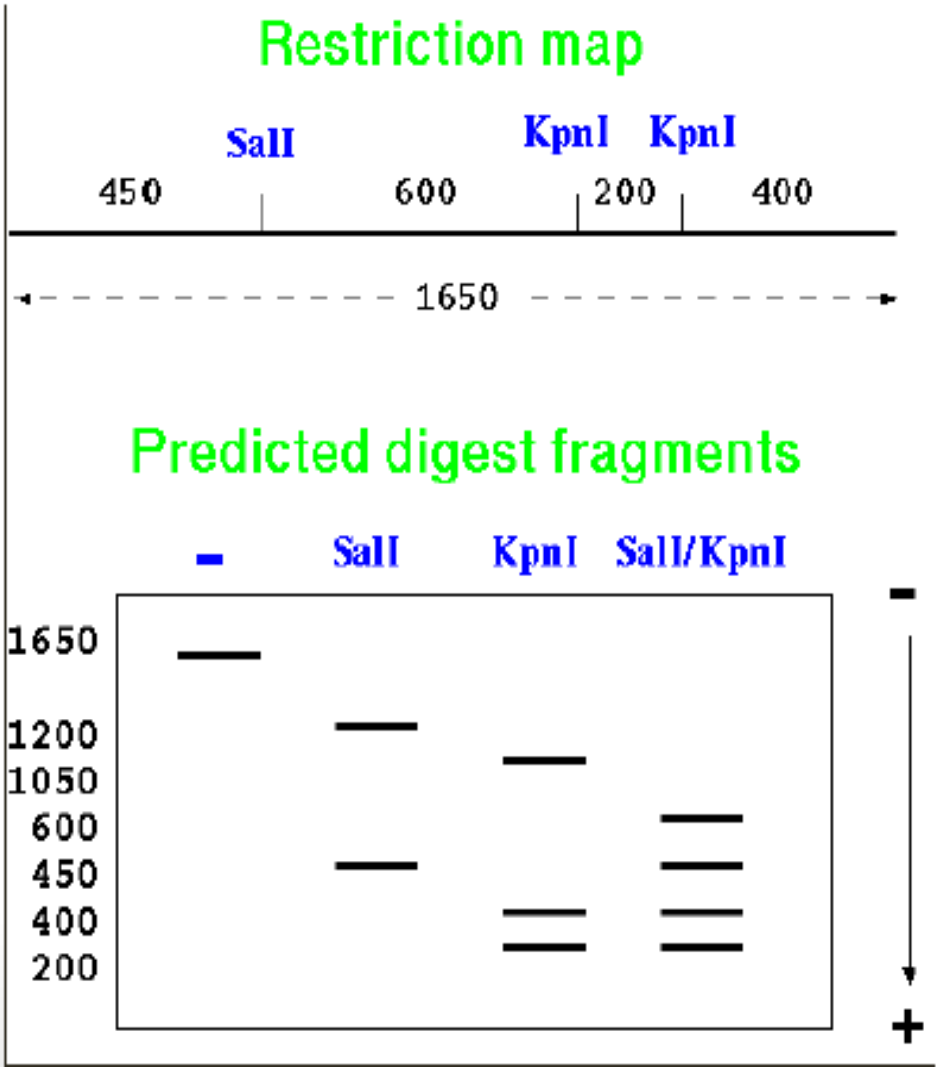
- ***Aim**: the digested fragments must be separated and identified.*

- Fragments are separated by agarose gel electrophoresis. [Agarose is a large polysaccharide].

- Gel electrophoresis: your DNA will move through spaces in agarose against a current

- DNA has a negative charge and will migrate towards the cathode

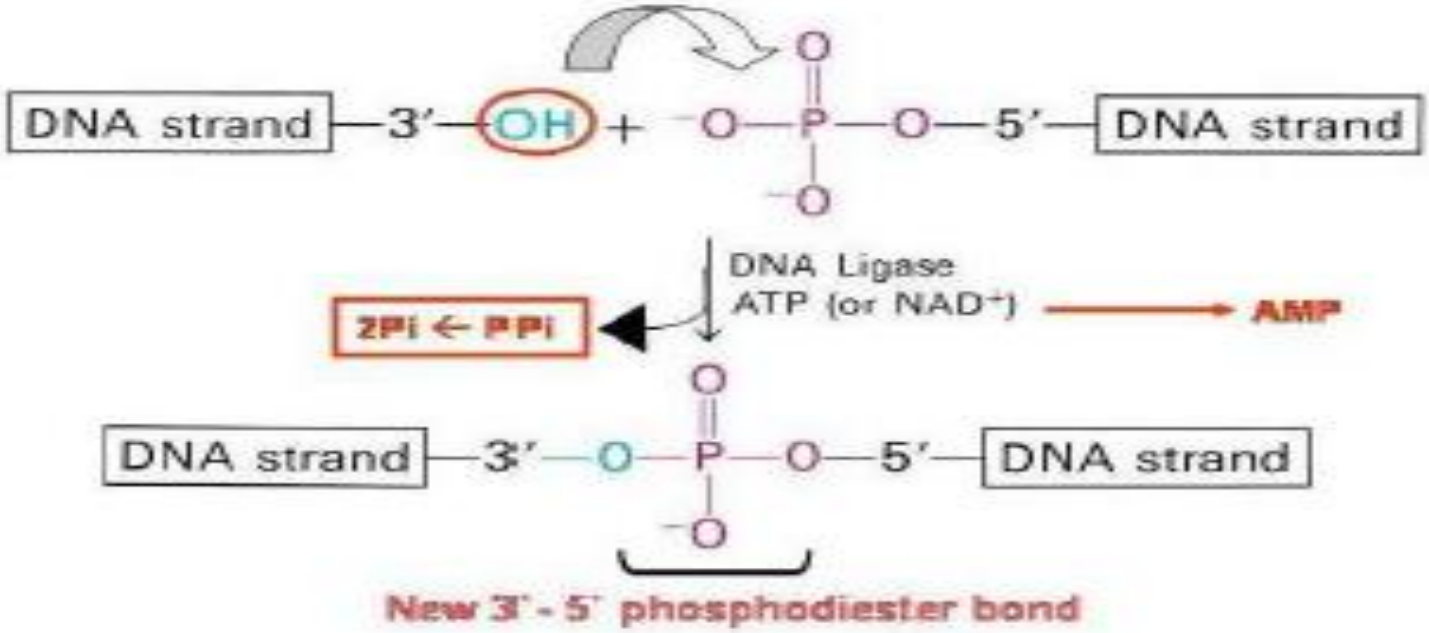
Restriction Digest Analysis



- Length=1650bp
- *SalI* yields two fragments (1200bp and 450bp)
- *KpnI* cuts at 2 sites giving 3 fragments
- *SalI* and *KpnI* cut 3X yielding 4 fragments

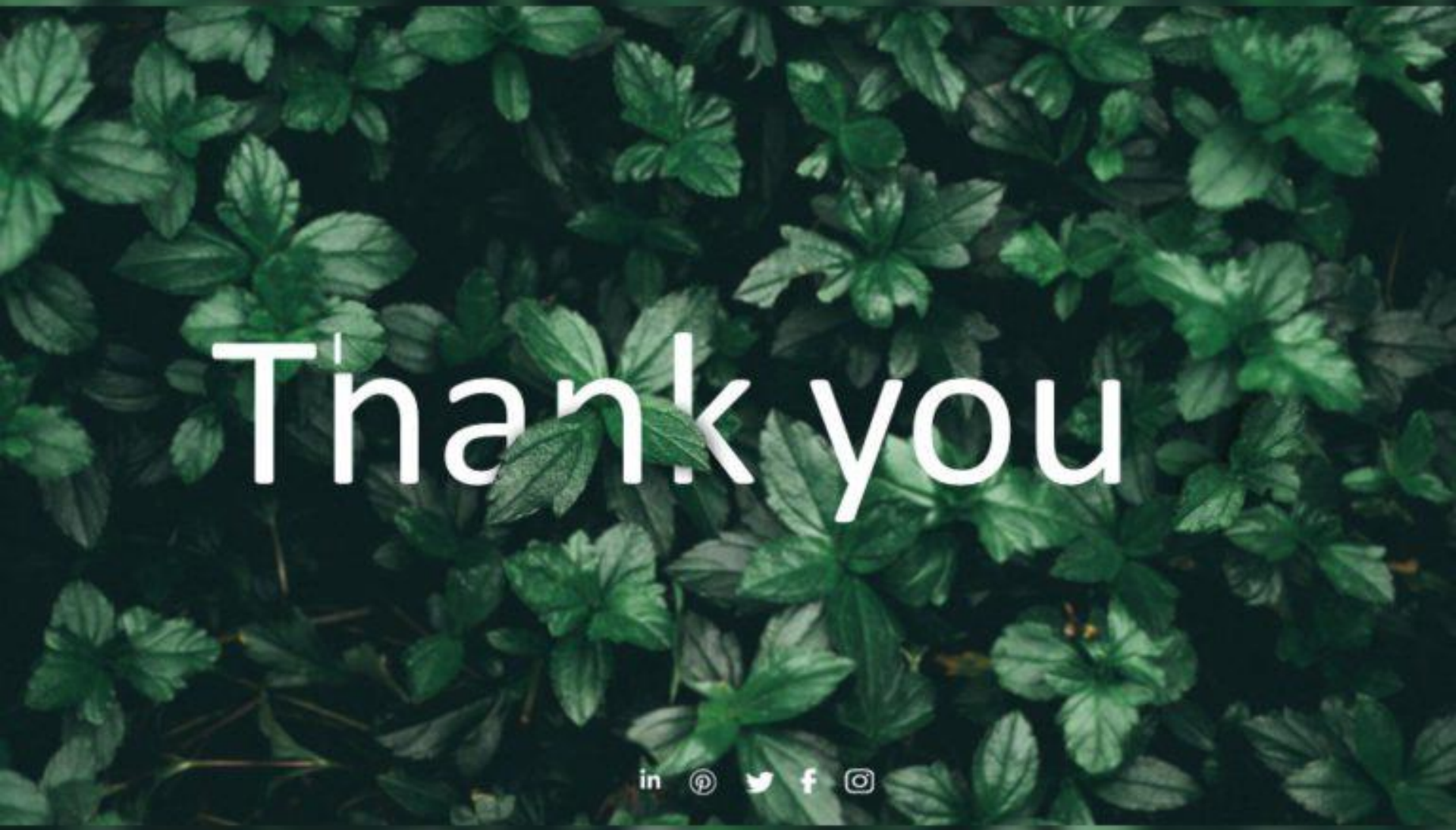
DNA Ligation

- Ligation is the joining of two nucleic acid fragments through the action of an enzyme .
- The ends of the fragments are joined together by the formation of phosphor-di-ester bonds between the 3'-hydroxyl of one DNA terminus with the 5'-phosphoryl of another .
- A co-factor is generally involved in the reaction, and this usually ATP or NAD+



DNA ligation protocol

- Thaw all reagents on ice.
- Assemble reaction mix into 10 µL volume in a microfuge tube. Reaction may be scaled up to 20 µL if DNA concentrations are low.
- Add reagents in following order: water, buffer, insert, vector, T4 ligase.
- Gently mix by stirring gently with pipette tip.
- Typical Incubation time and temperature is 15°C for at least 4 hours. Incubation at 4°C for overnight is commonly used.
- Prepare a control that excludes the insert. This allows for detection of re-annealing due to incomplete vector digestion (If vector is not completely digested the colonies generated by the vector reaction will greatly outnumber that of the vector + insert reaction).
- Ligation reaction can be inactivate d by incubation at 65°C for 20 minutes. This step is optional.
- Proceed directly to transformation reaction.


Thank you