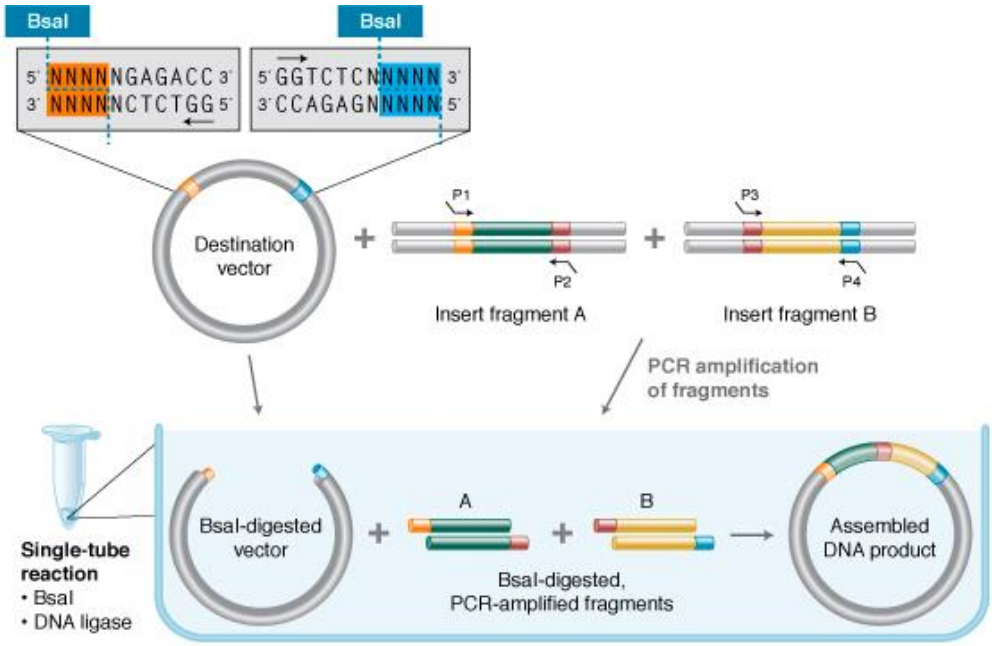
Golden Gate Assembly

Time : 2h

I. Principle

Golden Gate Assembly (GGA) is used for cloning one or more inserts into a vector by designing overlapping ends (**fusion sites** and **Type IIS restriction sites**) which can join the desired DNA together after cleavage by the non-palindromic restriction enzymes BsaI or BsmBI, followed by ligation to bind the DNA together.



https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/golden-gate-assembly

II. Material

- T4 DNA ligase
- Type IIS restriction enzyme
- 10X T4 DNA ligase buffer
- Vector
- Inserts
- MilliQ water
- PCR tube
- 10 and 20 µL pipettes

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III. Method

- In a PCR tube, annotated, introduce :
  - 0.5 µL of T4 DNA Ligase
  - 2 µL of 10X T4 DNA Ligase Buffer
  - 0.5 µL of Type IIS restriction enzyme
  - **100 ng of vector**
  - **Equimolar amounts of inserts**
  - Complete with water QS **20 µL.**

- Mix gently
- Place the tube on a thermocycler

Cycle :

Step		**Temperature**	**Time**
15 – 120 cycles	**Activation of the restriction enzyme**	37°C	5 min
	Activation of the Ligase	16°C	5 min
Inactivation of the enzyme		55°C	15 min
Inactivation of the ligase		85°C	20 min
Hold		4°C	∞