Subcloning: digestion & ligation reaction protocol

Introduction

Subcloning is the procedure of moving a DNA fragment in a certain plasmid to another. This process requires digestion of a "parent" plasmid to obtain the DNA fragment and/or insertion plasmid with complementary sticky ends to the sequence it will be ligated with in the final construct. For that, a ligation reaction is also required. This protocol describes a single digestion and ligation reaction of a DNA fragment and plasmid with PstI and EcoRI restriction sites.

This protocol is adapted from iGEM's 3A Asembly protocol:

Materials

- DNA plasmids
- ddH2O
- Digestion cutSMART 10x buffer
- PstI restriction endonuclease
- EcoRI restriction endonuclease
- T4 DNA ligase
- Ligase 10x buffer

Procedure

The following procedure is for one digestion and ligation reaction, respectively.

Digestion

1. Mix 0.5 µl Digestion cutSMART 10x buffer with 4.3 µl ddH2O and add 0.1 uL of each enzyme. This is your master mix.
2. Transfer 4 µl of the master mix into a PCR tube.
3. Add 100 ng of DNA plasmid to the same PCR tube by adding 4 µl of DNA with concentration 25 ng/ µl.
4. Tap on the tube to mix or centrifuge for a few seconds to spin down the liquid.
5. Put the PCR tube into a thermocycler with the following settings for incubation and heat-inactivation of the enzymes.

Step	Temperature (°C)	Time (min)
1	37	30
2	80	20

3	4	Infinite

The sample may be stored in the -20 freezer.

Ligation (10 µl reaction mixture, 2 DNA parts)

1. Mix 1 µl 10X T4 DNA ligase buffer with 4.5 µl ddH2O in a PCR tube.
2. Add 2 µl of each part you want to ligate from the above digestion.
3. Add 0.5 µl of T4 DNA ligase.
4. Put the PCR tube into a thermocycler with the following settings for incubation and heat-inactivation of the enzyme.

Step	Temperature (°C)	Time (min)
1	16	30
2	80	20
3	4	Infinite

The sample may be stored in the -20°C freezer.

References

Parts.igem.org. 2020. *3A Assembly*. [online] Available at: <http://parts.igem.org/Help:Protocols/3A_Assembly> [Accessed 5 December 2020].