Primer Ligation with T4

Introduction

In order to create the template DNA for our EXPAR-like protocol, we ordered 3 primers (from) for each microRNA, as shown in the picture below:
1. Template DNA -up,
2. Template DNA -down and
3. Template DNA -main

For more information check our Engineering Page.

The protocol is based on Neb's Ligation Protocol with T4 DNA Ligase (M0202).

Materials

› Materials

- › EXPAR template parts (up, down & main parts)
- › 10x T4 DNA Ligase Buffer
- › T4 DNA Ligase
- › Nuclease free Water

› Equipment

- › Pipettes & tips
- › Eppendorfs
- › Incubator

Procedure

1. Prepare Mix A (for each microRNA):

Mix A

	A	B
1	**Material**	**Volume**
2	PCR H2O	up to 20 ul final volume
3	Template DNA - up (10 uM)	1 ul
4	Template DNA - down (10 uM)	1 ul

2. Incubate mix A to 51^{o}C for 10 minutes (hybridisation of Template DNA - up and down)

3. Prepare Mix B (for each microRNA):

Mix B

	A	B
1	**Material**	**Volume**
2	10X T4 DNA Ligase Reaction Buffer	2 ul
3	T4 DNA Ligase	1 ul
4	Template DNA - main (10 uM)	1 ul

4. Add mix B to mix A while on ice

5. Incubate the final samples to 37^{o}C for 50 minutes (ligation of dsdna -up and down- with ssdna -main-)

6. Heat inactivate at 65°C for 10 minutes