

PCR Assembly

Aim:

- Assembling compatible parts into a single sequence. This protocol was used to assemble spider silk from the two parts we ordered.

Timeframe:

- Preparation: 15 minutes
- Wait-time: 2 hr
- Overall: 2 hr 15 min

Materials:

- Q5 DNA polymerase
- Q5 Reaction Buffer (5x)
- dNTP mix (10 mM each)
- Primers (10 μ M each)
- DNA template part 1 (1 ng/ μ L)
- DNA template part 2 (1 ng/ μ L)
- Milli Q water

Procedure:

1. Add the following to a 0.2 mL tube (1x 50 μ L reaction) and mix by pipetting up and down:

Component (concentration)	Volume (μ l)
Q5 reaction buffer (5x)	10
DNA template part 1 (1 ng/ μ L)	1
DNA template part 2 (1 ng/ μ L)	1
dNTP (10 mM each)	1
Milli Q water	Adjust so that final reaction volume is 48 μ l.

2. Reaction conditions

Step	Temperature ($^{\circ}$ C)	Time (s)
1	98	30
2	98	10
3	54.8	10
4	72	48

5	Repeat 2 to 4	5x
6	72	5'00"*
7	20	Hold

3. Add to the previous components and ensure all components are well mixed:

Component (concentration)	Volume (μl)
Forward primer (10 mM)	1
Reverse primer (10 mM)	1

4. Reaction conditions

Step	Temperature ($^{\circ}$C)	Time (s)
1	98	30
2	98	10
3	54.8	10
4	72	48
5	Repeat 2 to 4	31x
6	72	300
7	20	Hold

5. Follow this assembly by gel electrophoresis to check for the correct length.