Information | Time: prep: 15-20 min. PCR run: ~1h 40 mins

Highlights:
-- When using PCR with phage DNA, one has the choice of either working directly with phage lysate or with purified phage DNA (additional centrifugation + filtered sterile). For us, lysate worked once but we had to switch to purified phage DNA for better results.
-- Please study the PCR machine setup and try out different things when troubleshooting. For us, lowering the annealing temperature seemed to work better for phage DNA

Before PCR: decide what you want to amplify, desing primers (as described in "Primer Construction")**.** Have purified phage DNA stock (we found it didn't work with just the lysate...)

After PCR: run a gel to see if the PCR worked as described in "Gel", also do PCR cleanup (Protocol: according to cleanup manual in box), digest the inserts and the vector with the same enzymes, run a gel and cut out, cleanup.
then: insert the amplified fragment into a vector via Ligation

What we need

- PCR-tubes + caps (small tubes of ~100µl, 10 together)
- ddH20
- buffer (Phusion)
- primer forward + reverse | -20°C
- Phage DNA (5-15ng stock) | -20°C
- dNTP's
- phusion enzyme (keep at -20°C)

Procedure

PCR tubes ALWAYS FOLLOW THIS ORDER PRECISELY. DO NOT CONTAMINATE THE ENZYME. DO NOT TAKE OUT THE ENZYME/POLYMERASE WITHOUT -20 RACK (KEEP ON RACK AS MUCH AS POSSIBLE)

- Note: Do on ice if assembly takes you more than 10mins!
- Note: wear gloves! (duh)
- Thaw the buffer, primers, Phage DNA (~10ng/µl stock), keep on ice

Into each tube, add (for a 20µl reaction):
- primer forward 1 uL (10umol stock -20°C)
- primer reverse 1 uL (10umol stock -20°C)
- Phage DNA 1 uL (13ng/uL -> Dilute stock before)

Mastermix (prepare for samples+1)
- ddH20 (fill up to 20uL)
- phusion buffer 4 uL (if 10X)
- dNTP's 1 uL, keep on ice
- put dNTP's back in -20
- take out phusion enzyme/polymerase in -20 rack, add 1 uL to mix
- put enzyme back in -20

- Add 17µl mastermix to each tube (on ice if you have many samples). Tip: quickly spin down mastermix before.

Calculations examples for 1X:

Component	20µl RXN	50µl RXN
ddH2O	add to 20µl	add to 50µl

5X Phusion Buffer	4µl	10µl
Forward Primer	1µl	2.5µl
Reverse Primer	1µl	2.5µl
Template DNA	1µl (~10ng)	1µl (~10ng)
10mM dNTPs	0.4µl	1µl
Phusion DNA Polymerase	0.2µl	0.5µl

- Note: with Phage Lysate we used 3µl, but only worked the very first time... since then we used purified phage DNA

- close tubes with caps, cut off excess tubes, flick softly to mix, spin down

- put in PCR machine

PCR machine setup:

Heated lid: 111°C
Temperature: 98°C for 30 sec
35 PCR steps
Starting temp: 98°C for 15 secs
Annealing temperature: 58.5°C for 30 secs (theoretically: lowest Tm of both primers + 2°C) (we realized phage DNA seems to need lower temperatures to work, according to the primers 62°C should have been fine!)
elongation temp. 72°C (determined by enzyme)
elongation time: 45sec. (theoretically: 15-30 sec. per 1kb (our fragment are up to 2.5kb))
10 mins at 72°C
4°C at end for infinite time

enter: volume of the PCR: for us 20uL / 50µl

- Edit program: adjust elongation step according to the longest construct (here: 2.5kb)

- Give in volume, press OK

- Runs for ca 1h 40mins

- Cools down to 4°C when finished, take out and keep at 4°C or at -20°C

Storage

Store the PCR product at -20°C. You can run a gel directly or after storing it. Also do PCR cleanup afterwards according to manual.

For the 1%gel run use 1 uL PCR product with 1µl 6x purple loading dye, 3µl ladder.