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Biomedical Engineering

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Overhang PCR

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1 Reaction mixture

Component	Quantity/mass/final concentration	Volume (µL)
H_2O	Fill up to 50 µL	..
5x fusion buffer	1x	10 µL
Plasmid DNA	10 ng (stock … ng/µL)	..
Forward primer	0.5 µM (10 µM stock)	2.5 µL
Reverse primer	0.5 µM (10 M stock)	2.5 µL
dNTP mix	200 µM (10 mM stock)	1 µL
Fusion DNA Polymerase	1 U/50 µL (2 U/µL stock)	0.5 µL
Total		50 µL

2 Cycling conditions

Step	Temperature (°C)	Time (sec)	Cycles
Denaturation	98	30	1
Denaturation Annealing Extension	98 … (45-72) 72	10 20 .. (20 sec/kb)	30
Final Extension	72	10 minutes	1
Cooling	4	Hold	1

Important:

- Wear gloves to protect the DNA from DNAses!
- Add the components to reaction mixture in the order shown above
- Keep dNTP mix on ice before addition to reaction mixture
- Take the DNA polymerase out of the -30 °C freezer only after all components have been added to the reaction mixture and the PCR machine has been programmed. Do not touch the bottom of the polymerase tube.
- Mix reaction mixture well before start of PCR

3 DpnI digestion

- Add 1 µL FastDigest DpnI
- Program PCR machine:
  - 5 min at 37°C
  - 5 min at 80°C

4 PCR purification (Qiagen)

- Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.
- For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil)
- Place a QIAquick spin column in a provided 2 ml collection tube
- To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s
- Discard flow-through. Place the QIAquick column back into the same tube.
- Collection tubes are re-used to reduce plastic waste.
- To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s
- Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the column for an additional 1 min.
- Place QIAquick column in a clean 1.5 ml microcentrifuge tube
- To elute DNA, add 42 µl water to the center of the QIAquick membrane. Let the column stand for 1 min, and then centrifuge for 1 min.
- IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick
- membrane for complete elution of bound DNA.

5 DNA quantification

- Before measuring any samples, be sure to ‘blank’ the spectrophotometer using the solution the DNA is resuspended in, but with no DNA added. 'Blanking' measures the background inherent to the machine and your solvent.
- If using a NanoDrop to measure your samples, place 2 µL of PCR DNA onto the pedestal.
- Close the lid and click measure, be sure to record the concentration and purity.
- Note: Purity is measured under the 260/280 column (A good purity ranges from 1.80-2.00).
- Repeat for each sample.