PCR

Introduction

PCR (the polymerase chain reaction) uses a polymerase and DNA oligonucleotides to amplify a length of double-stranded DNA. For general purpose PCR, I like to use the Q5 polymerase from NEB: the product documentation is here.

Materials

- 200 μl thin-wall PCR strip tubes, one per reaction
- Forward and reverse primers, 10 μM working stock
- Template DNA, 1 ng/ul
  - Make SURE that you have diluted out the template DNA appropriately! Miniprep-concentration template will screw up your PCR!
- Q5 master mix, thawed on ice
- Ice bucket with ice
- Empty 10 μl or 200 μl tip box
- Gel for gel electrophoresis
- Parafilm
- 6X NEB Purple Loading Dye
  -

Procedure

Set up your PCR program

1. Use tmcalculator.neb.com to compute the annealing temperature **Ta** of your reaction based on your primers' melting temperatures.

   Make sure to select **Q5 2X Master Mix** under "Polymerase/Kit"

2. Compute the extension time **Tex** based on your predicted product length: 30 seconds per kilobase. So if my amplicon is 1500 bp, my extension time is 45 seconds

3. Program the thermocycler with the following (there should be a "Q5" program you can easily edit):

Table1

	A
1	Heated lid --> 105°C
2	HOLD at 98°C
3	98° for 30"
4	Cycle 30 times:
5	-- 98° for 5 seconds
6	-- Ta for 15 seconds
7	-- 72° for time Tex
8	Close cycle
9	72° for 2'
10	Store at 8°C

4. **Start the program on the thermocycler.** It will heat the lid, heat the block to 98° then hold at that temperature.

Set up the PCR reactions

5. Use the following table to fill in your template and primers for each reaction you're running. For a 20 μl reaction, use 1 ul of forward primer (10 uM), 1 ul of reverse primer (10 uM), 1 ul of template (1 ng/ul), 7 ul nuclease-free water, and 10 ul 2X Q5 master mix.

Table2

	A	B	C	D
1		**Q1_PhlF_QX**		
2	**Fwd Primer**	1 ul LS Cas13 2.0 Fwd		
3	**Rev Primer**	1 ul LS Cas13 2.0 Rv		
4	**Template**	1 ul pENTR L1_PhlF_L2		
5	**Water**	7 ul		
6	**2X Q5 MM**	10 ul		

6. Take the grey platform off of the tip box. Fill the top box with ice, then replace the grey platform. Fill the box with water until the level is just below the grey platform.

7. Label the PCR tubes.

8. Set up the PCR reactions. Start with the water; end with the Q5 master mix.

9. Working quickly, re-cap the PCR reactions. Flick several times to mix, then pulse spin in the tube microfuge. Return the tubes to ice.

10. Carry the tubes to the pre-heated thermocycler. Put the tubes in the block, close the lid and then press the button to continue through the hold and run the rest of the program.

Analyze the PCR reaction with gel electrophoresis

11. Prepare or secure a gel.
12. Slice a small piece of parafilm off of the roll.
13. Spot 4 ul of water in a droplet on the parafilm. Add 1 ul of the PCR reaction and 1 ul of loading dye.
14. Pipette up and down with a 5 ul pipettor, then load in a well on the gel.
15. Run the gel.
16. If there is a single clean band, proceed to clean the reaction up with a QIAgen PCR cleanup column.
17. If there are multiple bands but you see the one you want, proceed to a gel extraction of the band you want.