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PCR Amplification of Desired Gene

iGEM Dusseldorf[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.8fuhtnw

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MATERIALS TEXT

water
Green HF buffer
dNTPS
F Primer
R Primer
Template DNA
Phusion DNA Polymerase
1 % agarose gel
10000x GelRed nucleic acid stain
1x TAE buffer
Load DNA ladder

1 Pipette PCR components in the following order. Always set up at least two replicates.

2

# Reactions	[µl]	Thermocycler Conditions	Rounds
Water	32.5	1. 98°C for 0:30 min	
Green HF Buffer	10.0	2. 98°C for 0:10 min	5x
dNTPs (10 mM)	1.0	3. X°C for 0:20 min	
F Primer (10 µM)	2.5	4. 72°C for X min	
R Primer (10 µM)	2.5	5. 98°C for 0:10 min	30x
Template DNA (10 ng/µL)	1.0	6. 72°C for X min	
Phusion DNA Polymerase	0.5	7. 72°C for 5:00 min	
Total	50.0	8. 4°C until use	

3 For step 3, use the annealing temperature of the gene-specific sequences of your primers.
For step 4 and 6, adjust the extension time based on the amplicon size (30 s / kb).

4 Prepare a 1 % agarose gel by dissolving agarose in 1x TAE buffer by microwaving (~ 3 min, swirling every min).
Add 2.5 µL of 10000x GelRed nucleic acid stain per 50 mL gel and mix well by swirling.
When the conical flask is almost cool enough to touch (3-5 min after microwaving), pour slowly in gel tray and then insert the combs. Use wide-wells for large PCR reaction volumes.
Gel will be solidified after 45-60 min. Remove combs and add enough 1x TAE buffer to cover the gel.

5 Load 2-3 µl DNA ladder and whole PCR samplesinto the gel pockets.
Connect lid (DNA will migrate to the + electrode). Turn on power supply and run at 80-110 V depending on the thickness and size of the gel.
Examine gel after 30-60 min of electrophoresis and run until the marker reached the desired height.
Extract bands of the expected height from the gel with and clean up the fragments with an extraction kit.

6 Thermocycler Step 3: 5 cycles with annealing temperature that is equal to the forward or reverse primer with lower Tm. Has to be adjusted for every gene.

7 Termocycler Step 4: Adjust the elongation time with 30 s for each kb.

8 Termocycler Step 6: 30 cylces with 72 °C for elongation and adjusted extention time. Change time with 30 s per kb.

9 If not enough DNA was produced run another PCR after DNA extraction with the purified amplicon as a template and skip the 5 initial rounds.

10 Other optimisation: Increase time to 40 sec/kb