Spectinmyocin PCR Protocol

Introduction

Materials

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Procedure

Procedure + Materials

1 x 50µL reaction (the gel I gave you is for a 4 x 50µL reaction)

-added water first and polymerase last, the order of all other reagants is irrelevant
-thawed DNTP's and buffer on ice
-kept the phusion polymerase in the freezer until the reaction was fully prepared, and then kept it on ice until use
-mixed everything (except water) thoroughly by flicking tubes

31µL n.f. water
10µL Phusion high-fidelity (HF) buffer
2.5µL JTL035 (forward primer)
2.5µL JTL036 (reverse primer)
1.5µL DMSO (optional, but I use 3% v/v of DMSO in all of my PCRs because of my poor annealing regions in my project, can replace this with an extra 1.5µL of n.f. water)
1µL of P5trc (name of the plasmid, concentration is roughly 5ng/µL)
1µL 10mM DNTP's
0.5µL of Phusion polymerase

Total volume: 50µL

Thermo-cycler conditions

1. Initial denaturation: 98°C, 30s

(iterate the next step 35 times)
2a. Denaturation: 98°C, 5s
2b. Annealing: 62.9°C, 15s
2c. Elongation 72°C 30s

3. Final elongation: 72°C 10min

4. Hold: 4°C, forever (until you remove the samples, keep at 4°C)

Gel Electrophoresis

Used 1% agarose gel, poured 1x TAE over the gel to fully submerge it, and loaded 50µL into each well (if you aim to PCR purify, only load 5µL) along with a 1x 1kb ladder in the first well. Ran at 100V for 30 minutes (you might have to run it longer because I remember our machines were wonky). Image gel and if gel extracting, excise correctly sized bands. **Correctly sized band: ~1kb**

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