IDTDNA.com Directions for miniGENES

Plasmid Resuspension

1. Centrifuge tube before opening
2. Resuspend DNA in 80.0 µL of IDTE buffer
   - should reach a concentration of 50.0 ng/µL
   (Total of 4000 ng in tube (4 µg))
3. Incubate @ room temp for 30 minutes.
4. Centrifuge for 2 minute

- Dilution for transformation:
  - add 2.0 µL DNA to 999.0 µL diH_2O
    ↳ will provide 0.1 ng/µL concentration.
  - use 1-2 µL of dilution for each transformation.

- Dilution for PCR concentration: 10 ng/µL

  use 1.0 µL of 50 ng/µL DNA, mix w/ 4.0 µL diH_2O.

  = 5.0 µL 10 ng/µL

IDT Primer Dilutions:

"mamba EcoRI Fwd" 3.0 nmol
Resuspend w/ 300.0 µL → 10.0 µm Concentration.

"Mamba NotI Rev" 2.9 nmol
Resuspend w/ 290.0 µL → 10.0 µm Concentration

do Aliquots 20 µL of 10 µM conc
2 µL Stock + 18 µL diH_2O ✓
both ✓

NEB Q5 High Fidelity 2x master mix PCR

Purpose: Amplify mamba CDS sequence from IDT minigene vector while also adding EcoRI and NotI restriction sites.

Reaction mixture Total 50.0 µL

25.0 µL Q5 High Fidelity 2x master mix
2.5 µL mamba EcoRI Fwd 10 µM
2.5 µL mamba NotI Rev 10 µM
1.0 µL DNA template (10 ng/µL)
+ 19.0 µL Nuclease free dH2O
50.0 µL total volume

Negative control Rxn

25.0 µL Q5 High fidelity 2x mastermix
2.5 µL mamba EcoRI Fwd
2.5 µL mamba NotI Rev
+ 20.0 µL Nuclease Free dH2O
50.0 µL Total volume.

Amplification protocol

- Thermocycler settings.

98°C (30) seconds

25-35 cycles {
98°C 5-(10) seconds
58°C 10-(30) seconds
72°C 20-(30) seconds
}

72°C 5.0 minutes
4°C ∞

~~~ 45 mins
1 hr 21 mins
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