Generation of Plasmid cDNA

References

1. Park, S. Y., & Ferreira, A. (2005). The generation of a 17 kDa neurotoxic fragment: an alternative mechanism by which tau mediates beta-amyloid-induced neurodegeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(22), 5365–5375. https://doi.org/10.1523/JNEUROSCI.1125-05.2005
2. Kit Protocol: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/subcloningefficiencydh5alpha_man.pdf

Kit

1. Subcloning Efficiency™ DH5α Competent Cells, Thermo Fisher, Catalog# 18265017

Preparing Luria Agar Plates

1. Dissolve 10 g Luria Agar in 250 ml MilliQ Water
2. Autoclave on liquid setting for 20 minutes
3. After autoclaving, cool on hot plate with stir bar
4. When cool to touch, add appropriate antibiotic (AMP 100 µg/ ml; KAN 30 µg/ ml)
5. Pour ~25 ml per plate

Preparing Luria-Bertani (LB) Broth

1. Dissolve 2.5 g in 100 ml MilliQ Water
2. Autoclave on liquid cycle for 20 minutes

Protocol

1. Prepare DNA for transformation (make 0.1 µg/µL sample DNA in DNAse-free sterile water and thaw the provided pUC19 control DNA as a positive control to ensure the transformation worked).
2. Thaw 50 µL aliquots of DH5α cells on ice (takes ~5-10 minutes).
3. Add 5 µL of the 0.1 µg/µL sample DNA solution to aliquoted tube of DH5α cells and 2.5 µL pUC19 control DNA to another tube of DH5α cells (do not pipette mix!! Mix by gentle "flicking" of the tubes).
4. Incubate on ice for 30 minutes.
    a. While this incubates, set water bath to 42°C and warm sterile LB Broth (no antibiotic; 1 ml per transformation) to 37°C in dry incubator.
5. Heat shock cells for 20 seconds in a 42°C water bath.
6. Immediately place cells back on ice for 2 minutes.
7. Add 950 µL warmed sterile LB Broth to each tube.
8. Shake in the 37°C dry incubator for 1 hour at 225 rpm

    a. During shaking, pre-warm selective plates to 37°C.
9. Centrifuge tubes for 2 minutes at 8000 RPM in tabletop microcentrifuge.
10. Discard 500 µL supernatant and mix remaining solution by pipetting.
11. Spread 50-100 µL of DH5α-containing broth from each transformation on pre-warmed selective plates (may want to plate 2 different volumes to ensure well-spaced colonies).
    a. Store remaining transformation reaction broth at 4°C.
12. Incubate plates overnight at 37°C.
    a. After 1st 30 minutes, flip them upside-down.
13. Next day, inoculate an isolated colony in selective LB Broth using one of the miniprep/midiprep/maxiprep protocols below.
14. Innoculating miniprep culture.
    a. Add 1 ml LB Broth + appropriate antibiotic in 15 ml tube.
    b. Pick colony from freshly transformed plates using sterile pipette tip.
    c. Inoculate by dropping pipette tip into Broth and replace cap.
    d. Incubate at 37°C overnight shaking at ~250 to 300 RPM.
    e. Next day, begin DNA extraction using Qiagen Miniprep kit.
15. Innoculating midiprep culture.
    a. Add 25 ml LB Broth to 125 ml flask, cover with foil, and autoclave.
    b. Once cool, add antibiotic.
    c. Pick colony from freshly transformed plates using sterile pipette tip.
    d. Inoculate by dropping pipette tip into broth and cover.
    e. Incubate at 37°C overnight shaking ~250 to 300 RPM.
    f. Next day, begin DNA extraction using Qiagen Midiprep kit.
16. Innoculating maxiprep culture.
    a. Make a starter culture by adding 3 ml sterile selective LB Broth medium with appropriate antibiotic in a sterile 14 ml round bottom tube.
    b. Pick a single colony from a freshly streaked selective plate using a sterile pipet tip and drop tip into starter culture.
    c. Incubate at 37°C for 8 hours at 250 rpm.
    d. In a sterile 1 L Erlenmeyer flask, dilute the starter culture 1/1000 in selective LB Broth (250 µL starter culture + 250 ml selective LB Broth) and grow for 12-16 hours at 37°C at 250 RPM.
    e. Next day, begin DNA extraction using Qiagen Maxiprep kit.