Adapted from Gelles Lab Protocol by Marc Ridilla and Linnea Lemma
Updated 2018-11-08 by Linnea Lemma

Dogic Lab Protocol for Transforming and Expressing Proteins in *E. Coli*

Day 1: Transformation

(1) Use Addgene (http://www.addgene.org/) to figure out the necessary antibiotic for the plasmid and how much to add

(2) Make media for plates

10g/L sodium chloride
5g/L yeast extract
10g/L tryptone
15g/L agar for plates to make gel
each plate needs ~30mL
Procdure:

- Use a beaker and stir bar
- add < (total volume) of water
- weigh and add media components
- add agar straight to bottle (with stir bar) for autoclaving
- add well stirred solution
- leave cap loose
- add indicator tape
- autoclave (liquid, 45min)

(3) Add antibiotics

make stock antibiotic (at 1000x concentration) if needed
allow media to cool (~30min) to touch
-- don't want agar to harden
-- needs to be cool enough to not damage the antibiotic (50C)
add antibiotic at required concentration
pour into plates
allow agar to set (~1hr—not time sensitive)

(4) Transform cells

get competent cells – put on ice (Rosetta 2 plysS)
get DNA – put on ice
in a sterile environment: add 1uL of DNA to each competent cell eppendorf (10uL)-- don't mix, pipette into the middle
leave on ice for >= 5min
heat shock at 42.5C for 45sec
put on ice for "recovery" 2 min
in a sterile environment: add 300uL of antibiotic-free media
put in incubator (37C) for >=1hr

(5) Spread cells on plates

label plates (date, DNA, cells used, Hi/Lo, initials)
in a sterile environment:
sterilize cell spreader using eth. and flame
add 50uL of starter onto Lo and the rest onto Hi for each DNA

allow to cool (antibiotics get damaged above ~50C)
spread cells well around plates
store upside down in incubator (37C) overnight

Day 2: Prepare Flasks, Begin starter growth

(1) Check plates for growth
negative control has no growth
Hi has more colonies than Lo
seal one plate from each DNA (either Hi or Lo—want several colonies but dilute enough to pick a single one) and place in cold room to halt growth
(2) Make media (2XYT)
16g/L tryptone
10g/L yeast
5g/L NaCl
For BCCP plasmids, add 24mg/L (100uM) biotin
Procedure:
- the largest beaker can hold 5L so concentrate as needed from there
- use big beaker and large stir bar
- make enough media for growth flasks and starter flasks
- add foam stoppers, cover with foil & indicator tape
- autoclave for 45min
(3) Begin starter growth
allow media to cool
add antibiotics (100ug/mL Amp, 25ug/mL Chlor)
label flasks
scrape a single colony from each plate with pipette tip and place in each of the small flasks
place an unused pipette tip in negative control flask
put all flasks in incubator (37C, 200RPM)
make glycerol stock tubes (0.9mL 20% glycerol—sterilized by filter)

Day 3: Grow

(1) Check that overnight cultures have growth (are cloudy)
(2) Make glycerol stocks (0.9mL of 20% glycerol, 0.9mL of overnight—freeze at -80C for future use)
(3) Inoculate broth with 1/200th volume of the overnight culture (2.5mL for 0.5L media)
(4) Grow in air shaker (250rpm) at 37C and follow OD600
(5) Induce expression at OD600=0.5-0.6.
- remove flasks and place in cool water
- add 1/1000th volume of 1M IPTG (final concentration is 1mM)
- reset shaker thermostat to 18C
- OPTIONAL: add 1/200th volume of 40mM Rifampicin 2hrs after induction (increases purity, decreases yield)
(6) Shake overnight at 20C (~12-20hrs)

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Day 4: Harvest

(1) Pour cells into centrifuge bottles and spin at 5000G for 30min
(2) Pour off most of the supernatant—save 50mL or whatever you want to resuspend in.
(3) Resuspend pellet in 50mL of media and transfer to falcon tubes
(4) Spin in swinging bucket centrifuge at 4C at maximum speed for 30 minutes.
(5) Pour of supernatant. Freeze cells at -80C until ready to purify.
(6) Rinse flasks with bleach. Rinse 3x volume with water. OPTIONAL: Autoclave dry for 60min.