Protocols

Usually we cook 5 L Media in a 5-L-Cup

LB Medium:
Per 5 L liquid medium

- 50 g peptone
- 25 g yeast extract
- 25 g NaCl

Mixed together in 4900 mL RO-water, set pH to 7,5 with NaOH and fill up to 5 L. Pour in 500mL shot flask and sterilize it by autoclaving. After cooling down to around 55°C antibiotics can be added.

LB Agar:
Per 5 L medium

- 50 g peptone
- 25 g yeast extract
- 25 g NaCl

Mixed together, add 4900 mL RO-water, set pH to 7,5 with NaOH and fill up to 5 L. Weigh in 10g Agar-Agar in each 500 mL-shot-flask and add the 500 mL mixture and sterilize by autoclaving. After cooling down to around 55°C antibiotics can be added. Pour the LB Agar into petri dishes under sterile conditions and let it cool down for 20 min.

Rehydration of plasmids from distribution kit:
Rehydration of plasmids from distribution kit:
Add 10 µL TRIS/HCl (pH=7,5) to well
-> 5-10 min wait
-> transfer to sterile Eppi
-> freeze at -20°C

Transformation:
Take 100µL of competent E. Coli and transfer 1µL of transfer DNA to the competent cells. Let chill for 30 minutes on ice. Freeze the rest of the DNA. After 30 minutes, heat shock the E. Coli to transform the Plasmid at 42°C for 90 seconds. Then let them recover on ice for 5 minutes. Add 1 mL LB medium (no antibiotic) to the incubation tube and incubate at 37°C for 30 min (Ampicillin) or 90 min (Chloramphenicol). Prepare a negative control (same steps but without plasmid)
After incubation take 4 agar plates (with antibiotic) for each transformation, add sterile glass balls to spread the cells and add 100µL LB to 2 of the plates. Add 1µL and 10µL of transformed cells into the plates with 100µL LB media. Add 100µL of cells into an agar plate, centrifuge the rest, discard the supernatant, so the Volume is reduced to about 100µL. Resuspend the pellet and transfer this to the 4^{th} agar plate. Incubate at 37°C overnight. Following day count the cells for quality measurements and store them at 4°C. In the afternoon prepare master plates (LB agar with antibiotic) and LB liquid media (with antibiotic). Choose 3 clones from each plasmid and transfer the colony form the petri dish to the master plate with a sterile toothpick and put the toothpick in 2 mL of liquid LB with antibiotic. Do this for each plasmid with 3 clones. Incubate overnight and do plasmid extraction with Mini Prep the next day and store the master plate at 4°C.

Cryo:
Add 1350µL bacteria in optimal growth phase to 150µL 100% glycerine. Vortex and freeze immediately at -80°C.

Gel electrophoresis:
1% Agarose (1,5g per Gel/150mL TAE Buffer)
2% Agarose Gel (3g Agarose/150mL TAE Buffer)
1 Gel (150mL)

- 1,5g/3g Agarose
- 150mL TAE (1x)
- 7,5µL peqGreen

Bring agarose with TAE to boil, cool down to around 55°C then add peqGreen. Pour into form and add well template. Put hard gel into electrophoresis aperture and add TAE until the Gel is covered in buffer.
Pipette 8µL of DNA Ladder into a well
Pipette up to 50µL of sample into the well after adding 6x DNA Loading Buffer (one fifth of the sample Volume)
Hook up the power supply and turn the power on. Turn power off when the dye has travelled ¾ of the gel length.

Restriction digest:
Aim for 750-1000µg plasmid
Total Volume adds up to 40µL

- Appropriate amount of plasmid solution to achieve 750-1000µg
- Add TRIS pH 7,5 to a volume of 34µL
- Add 4µL 10x Cutsmart buffer
- Add 1µL Enzyme 1
- Add 1µL Enzyme 2

Incubate at 37°C for ~1 hour
Deactivate Enzyme by incubation at 80°C for 20 min
Store at -20°C or proceed with gel electrophoresis or ligation.

DNA Ligation:
Aim for 500ng insert and plasmid DNA
Total Volume adds up to 30µL

- Add insert DNA to achieve around 500ng
- Add plasmid DNA to achieve around 500ng
- Add RO-water to a volume of 26µL
- Add 3µL 10x T4 Ligation Buffer
- Add 1µL T4 Ligase

Incubate at room temperature for 20-60 min
Deactivate T4 Ligase by incubation at 80°C for 20 min
Store at -20°C or proceed with transformation

Golden Gate Cloning:
DNA of backbone and inserts diluted, so that the concentration is 40nM.

- 1µL of each DNA sample
- 2µL 10x Cut Smart Buffer
- 2µL 20mM ATP
- 2µL BbsI (Backbone 1 or 3) /BsaI (Backbone 2) with 10 U/µL
- 2,5µL 1:10 diluted T4 Ligase with 400 U/µL
- filled up to 20µL with TRIS pH 7,5

PCR:
Protocol for 1 PCR adds up to 50 µL

- Tris (28,5µL)
- 5x Q5 buffer (10µL)
- 10 mM dNTPs (1µL)
- 10 µM forward Primer (1µL)
- 10 µM reverse Primer (1µL)
- 25 mM MgCl (3µL)
- Q5 Polymerase (0,5µL)
- DNA (5 µL)

Preparation of chemically competent E. coli cells
Materials and equipment
Solution TFB1: 30 mM potassium acetate, 10 mM CaCl2, 50 mM MnCl2, 100 mM RbCl, and 15% glycerol; adjust to pH 5.8 (with 1 M acetic acid), filter-sterilize, and store at 4°C (ready to use) or at room temperature (cool down before use).
Solution TFB2: 100 mM MOPS (or PIPES), 75 mM CaCl2,10 mM RbCl, and 15% glycerol; adjust to pH 6.5 (with 1 M KOH), filter-sterilize, and store at 4°C (ready to use) or at room temperature (cool down before use).

Preparation of chemically competent cells
• E. coli strain DH10B (or another appropriate strain) is inoculated from a glycerol stock onto an LB Agar plate; the inoculum is streaked on the plate using a loop to obtain individual colonies. The plate is incubated overnight at 37°C.
• Inoculate 10 mL of LB medium with a single colony and incubate the flask overnight in a shaker-incubator (37°C, shaking 180 rpm).
• The following day, transfer 2 mL of this culture to a flask containing 200 mL LB medium and incubate for about 2 h until OD600 reaches 0.6.
Cool down the cells on ice for 10 min. The cells are pelleted in a centrifuge for 5 min at 4,500 rpm (4,000 × g) at 4°C. The cells are resuspended in 0.4 volume of ice-cold TFB1.
• Repeat the centrifugation step. Resuspend the pellet in 1/25 volume of ice-cold TFB2.
• The cells are aliquoted 100 µL per tube and stored at −80°C.

Gel Extraction
Kits used:

- Wizard SV Gel and PCR Clean-up System (Promega)
- HiYield PCR Clean-up/Gel Extraction Kit (SLG)

Plasmid Extraction
Kits used:

- PureYield Plasmid Miniprep System (Promega)
- HiYield Plasmid Mini Kit (SLG)
- Monarch Plasmid Miniprep Kit (New England BioLabs)