

Protocols

Preparation of TE Buffer

Materials

- Tris-HCl: 2ml
- EDTA: 0.5ml
- dH₂O: 197.5ml

Protocol

1. Mix the above materials.
2. Place the mixture in a medium bottle and cover with aluminum foil
3. Autoclave. Following autoclave, store it at room temperature.

Preparation of LB medium

Materials

- Tryptone: 10g
- Yeast Extract: 5g
- NaCl: 5g
- dH₂O: 1000ml

Protocol

1. Put the above materials into a beaker, cover with aluminum foil, and mix with a stirrer.
2. Autoclave. Following autoclave, store it in a medium bottle at 4 °C.

Preparation of Ampicillin

Materials

- Ampicillin sodium salt: 0.5g
- Sterile water: 5ml

Protocol

1. Mix the above materials and vortex.
2. Filter the mixture through a syringe filter.
3. Dispense the amount to be used into a tube and store at -20 °C. (If you use 50% ethanol instead of sterile water, you do not need to dispense.)

Preparation of LB Agar Plates

Materials

- Tryptone: 4g
- Yeast Extract: 2g
- NaCl: 2g
- Agar (not agarose): 6g
- Antibiotic (ampicillin): 400µl
- dH₂O: 400ml

Protocol

1. Put Tryptone, Yeast Extract, NaCl, and dH₂O into a beaker, cover with aluminum foil, and mix with a stirrer.
2. Add agar and autoclave.
3. Mix with a stirrer.
4. When the solution cools to about 60 °C, add ampicillin and mix.
5. Transfer the mixture to a Petri dish on a clean bench. When the agar has set, stack the Petri dishes, cover them with plastic wrap, and store them at 4 °C.

Resuspending gBlocks® Gene Fragments (IDT)

Materials

- DNA
- TE Buffer

Protocol

1. Prior to opening, centrifuge the tube at a minimum of 3000×g to ensure that the material is at the bottom of the tube
2. Add TE to reach a final concentration of 10ng/μℓ.
3. Vortex briefly
4. Incubate at 50 °C for 20 minutes.
5. Briefly vortex and centrifuge.

Gibson Assembly (NEB)

Materials

- DNA
- Gibson Assembly® Master Mix (E2611)
- Sterile water

Protocol

1. Determine the amount of each material according to the table below.

	2-3 Fragment Assembly	4-6 Fragment Assembly	Positive Control
Total Amount of Fragments	0.02-0.5 pmols (X μℓ)	0.2-1 pmols	10μℓ
Gibson Assembly Master Mix (2×)	10μℓ	10μℓ	10μℓ
dH ₂ O	10-Xμℓ	10-Xμℓ	0
Total Volume	20μℓ	20μℓ	20μℓ

2. Add sterile water, DNA, and Gibson Assembly Master Mix to the microtube in that order.
3. Incubate samples in a thermocycler at 50 °C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled. Following incubation, store samples at -20 °C.

DNA purification

Materials

- DNA
- Wizard SV® Gel and PCR Clean-up System (Promega)

Protocol

1. Add an equal volume of Membrane Binding Solution to DNA.
2. Insert SV Minicolumn into Collection Tube.
3. Transfer DNA to the Minicolumn assembly. Incubate at room temperature for 1 minute.
4. Centrifuge at 16,000 × g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
5. Add 700μℓ Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
6. Repeat Step 5 with 500μℓ Membrane Wash Solution. Centrifuge at 16,000 × g for 5 minutes.
7. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
8. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
9. Add 50μℓ of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × g for 1 minute.
10. Discard Minicolumn and store DNA at 4°C or -20°C.

Dilution of TAE Buffer (10×)

Materials

- TAE Buffer(10×): 100ml
- MiliQ: 900ml

Protocol

1. Mix the above materials and store them in a medium bottle at room temperature.

Preparation of agarose gel

Materials

- TAE Buffer: 20ml
- Agarose: 0.2μℓ
- Midori Green: 1μℓ

Protocol

1. Put TAE Buffer and Agarose in a beaker and cover with plastic wrap.
2. Melt the Agarose in a microwave oven.
3. When the solution is cool enough not to be hot to the touch, add the Midori Green.
4. Pour the gel into the casting stand and set the comb.
5. When the gel hardens, cover with plastic wrap and store at 4 °C.

Agarose Gel Electrophoresis

Materials

- DNA: 1μℓ of each
- DNA Ladder: 5μℓ
- Loading Dye (6×): 1μℓ×(n+1)
- Sterile water: 4μℓ×n
- TAE Buffer

(n: Number of samples)

Protocol

1. Add sterile water 4μℓ, Loading Dye (6×) 1μℓ, and DNA 1μℓ to the PCR tube.
2. Add DNA Ladder 5μℓ and Loading Dye (6×) 1μℓ to the PCR tube.
3. Place the agarose gel into the gel box.
4. Fill the gel box with TAE Buffer until the gel is covered.
5. Loading the samples.
6. Run the gel at 100V for 15-25 minutes.
7. Check the bands by UV irradiation.

Digestion

Materials

- Restriction enzyme
(EcoR I -HF, Hind III)
- NEB Buffer r2.1 (10×)
- DNA
- Sterile water

Protocol

1. Determine the amount of each material according to the table below.

	Insert	Vector
① Restriction enzyme	0.4μℓ of each (fixed)	0.4μℓ of each (fixed)
② Buffer (10×)	1(~2μℓ)	1(~2μℓ)
③ DNA	<1μg	<1μg
④ Sterile water	Total-(①+②+③)	Total-(①+②+③)
Total Volume	10(~20)μℓ	10(~20)μℓ

2. Add sterile water, Buffer, restriction enzyme, and DNA to the microtube in that order.

3. Incubate samples in a thermocycler at 37 °C for 1 hour. Following incubation, store samples at -20 °C.

Ligation

Materials

- Ligation Mix: 3 $\mu\ell$
- Sterile water: 18 $\mu\ell$
- DNA

Protocol

1. Prepare Mighty Mix by mixing Ligation Mix and sterile water.
2. Determine the amount of each material according to the table below.

				Negative Control
Vector	50ng (fixed)	A $\mu\ell$	X mol	A $\mu\ell$
Insert		B $\mu\ell$	5X~10X mol	B $\mu\ell$ (TE Buffer)
Mighty Mix		(A+B) $\mu\ell$		(A+B) $\mu\ell$

3. Add vector, insert, and Mighty Mix to the microtube. (For negative controls, add TE Buffer instead of Insert.)

4. Incubate samples in a thermocycler at 16 °C for 30 minutes. Following incubation, store samples at -20 °C.

Transformation

Materials

- Plasmid: 6 $\mu\ell$ of each (Recombinant plasmid and Negative Control)
 - Competent cells: 50 $\mu\ell$ \times n
 - SOC medium (or LB medium) 1ml \times n
 - LB Agar Plates: 2 Plates \times n
- (n: Number of samples)

Protocol

1. Add plasmid and competent cells to the microtubes.
2. Place the microtubes on ice for 30 minutes.
3. Heat shock at 42 °C for 45 seconds
4. Add SOC medium to the microtubes.
5. Place the microtubes at 37 °C for 30 minutes.
6. Spread 100 $\mu\ell$ onto the plate.
7. Centrifuge the rest of the mixture at 27°C, 3000 rpm for 1 minute.
8. Discard about 700 $\mu\ell$ of the supernatant.
9. Dissolve the precipitate by pipetting slowly and spread 200 $\mu\ell$ onto the plate.
10. Incubate at 37°C for 14~18 hours. Following incubation, cover samples in plastic wrap and store them at 4 °C.

Inoculation

Materials

- LB medium: 3ml \times n
 - Antibiotic (ampicillin): 3 $\mu\ell$ \times n
 - LB Agar Plates with colony
- (n: Number of samples)

Protocol

1. Mix LB medium and ampicillin.
2. Dispense 3ml into each culture tube.
3. Pick the colonies from LB Agar Plates using a yellow tip and eject them into a culture tube.

4. Incubate at 37 °C for 12~16 hours in a shaking incubator.

Miniprep (Promega)

Materials

- Bacterial culture 2.4ml×n
 - PureYield™ Plasmid Miniprep System
- (n: Number of samples)

Protocol

1. Centrifuge 1.2ml of bacterial culture for 30 seconds at maximum speed in a microcentrifuge.
2. Discard the supernatant.
3. Add an additional 1.2ml of bacterial culture to the same tube. Repeat Steps 1 and 2.
4. Add 600µl of TE buffer or water to the cell pellet, and resuspend completely.
5. Add 100µl of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.
6. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting.
7. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
8. Transfer the supernatant (~900µl) to a PureYield™ Minicolumn without disturbing the cell debris pellet.
9. Place the minicolumn into a Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 15 seconds.
10. Discard the flowthrough, and place the minicolumn into the same Collection Tube.
11. Add 200µl of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 15 seconds.
12. Add 400µl of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.
13. Transfer the minicolumn to a clean 1.5ml microcentrifuge tube, then add 30µl of Elution Buffer or nuclease-free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.
14. Centrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20 °C.

Measurement of DNA concentration (NanoDrop)

Materials

- DNA 1.5µl
- Buffer 1.5µl

Protocol

1. Start nanodrop2000
2. Perform calibration and blank measurement by entering one drop of 1.5µl buffer.
3. Clean the surface of the NanoDrop with KimWipes.
4. place 1.5µl per sample on the NanoDrop and measure the concentration.