Protocol for colony PCR

We use TaKaRa EX Taq premix to amplify target fragment.

Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for 2 hours at 37°C with vigorous shaking (~ 220 rpm).

EX Taq premix: 5ul

Forward primer (10 uM): 0.4ul

Reverse primer (10 uM):0.4ul

dd H_2O: 3.8ul

DNA template (Culture medium containing the colony): 0.4 ul

The reaction condition settings are as follows:

95°C 2min (98 °C 10s 55 °C 30s 72 °C 1kb/min) 72 °C 10min

Cycles: 30

Protocol for PCR with PrimeStar HS DNA polymerase

PrimeSTAR® Premix: 25ul

Forward primer (10 uM): 0.8ul

Reverse primer (10 uM):0.8ul

DNA template: 0.4 ul

dd H2O: 8ul

The reaction condition settings are as follows:

98 °C 3min (98 °C 10s 55 °C 5s 72 °C 1kb/min) 72 °C 10min

Cycles: 30

Protocol for Agarose Gel Electrophoresis

Weigh moderate agarose powder and 1xTAE buffer;

Add agarose powder and 1xTAE buffer to a flask;

Heat up until the solution is homogeneous, avoiding boiling. If it boils, move away from the heat until it "calms down" and put it back on the heat until the agarose is completely dissolved.

While heating, prepare the bed in which the gel will polymerize. Make sure that it is well balanced and tight, and that the "comb" is well placed.

When homogeneous, add 2 μL of SYBR SAFE DNA Gel Stain to the solution and mix well.

Pour the solution into the bed and clear all its bubbles with a tip.

Carefully pull out the "comb";

Place the gel in the electrophoresis chamber;

Add enough TAE Buffer so that there is about 2-3 mm of buffer over the gel;

Mix the samples with loading dye in a 10:1 ratio. Put the samples into the wells, as well as marker into the first well.

Run the gel at 120V for about 30 minutes;

Note: For different size of gels, we have 25 ml, 50 ml and 100 ml agarose gel. And we often use 0.7%, 1% and 1.5% agarose gel for different samples.

Protocol for double digestion

We use Takara restriction enzymes to generate DNA fragments.

Pipette the following into a 0.2ml microfuge tube:

Enzyme A: 1 uL

Enzyme B: 1 uL

10x buffer: 2 uL

DNA: around 1ug

ddH2O: up to 20 uL

incubate at recommended temperature (37℃) for almost 4 hour;

Purify the digestion product through a 1.5% agarose gel electrophoresis.

Protocol for Gel Purification with OMEGA Gel Extraction Kit

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 mL.

4. Add 1 volume Binding Buffer (XP2).

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

6. Insert a HiBind® DNA Mini Column in a 2 mL Collection Tube.

7. Add no more than 700 µL DNA/agarose solution from Step 5 to the HiBind® DNA Mini Column.

8. Centrifuge at 10,000 x g for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Repeat Steps 7-9 until all of the sample has been transferred to the column.

11. Add 300 µL Binding Buffer (XP2).

12. Centrifuge at maximum speed (≥13,000 x g) for 1 minute at room temperature.

13. Discard the filtrate and reuse collection tube.

14. Add 700 µL SPW Wash Buffer.

Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see

Page 5 for instructions.

15. Centrifuge at maximum speed for 1 minute at room temperature.

16. Discard the filtrate and reuse collection tube.

Optional: Repeat Steps 14-16 for a second SPW Wash Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

17. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

Note: It is important to dry the HiBind® DNA Mini Column matrix before elution.

Residual ethanol may interfere with downstream applications.

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

19. Add 30-50 µL Elution Buffer or deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH.

If eluting DNA with deionized water, make sure that the pH is around 8.5.

20. Let sit at room temperature for 2 minutes.

21. Centrifuge at maximum speed for 1 minute.

Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

22. Store DNA at -20°C.

Protocol for ligation

We use Takara T4 DNA Ligase to clone restriction enzyme generated DNA fragments.

Test the concentration of the DNA sample(s);

Pipet the following into a 0.2ml microfuge tube:

Linearized vector DNA: around 100ng

Insert DNA (at 3:1 molar excess over vector): variable

10x ligation buffer: 1uL

T4 DNA Ligase: 1 uL

ddH2O: up to 10 uL

Vortex thoroughly and spin briefly to collect drops; Incubate the mixture at 16 degree for 60min; Use the ligation mixture for transformation;

Protocol for mini-prep with OMEGA E.Z.N.A.® Plasmid DNA Mini Kit I.

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for 12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.
2. Centrifuge at 10,000 x g for 1 minute at room temperature.
3. Decant or aspirate and discard the culture media.
4. Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 6.

5. Transfer suspension into a new 1.5 mL microcentrifuge tube.
6. Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

7. Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms.

Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

8. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1) Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2) Centrifuge at maximum speed for 30-60 seconds.
3) Discard the filtrate and reuse the collection tube.

10. Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.
11. Centrifuge at maximum speed for 1 minute.
12. Discard the filtrate and reuse the collection tube.
13. Add 500 µL HBC Buffer.

Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

E.Z.N.A.® Plasmid DNA Mini Kit I Spin Protocol.

14. Centrifuge at maximum speed for 1 minute.
15. Discard the filtrate and reuse collection tube.
16. Add 700 µL DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 6 for

instructions.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

19. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

20. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

21. Add 30-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

22. Let sit at room temperature for 1 minute.

23. Centrifuge at maximum speed for 1 minute.

Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

24. Store DNA at -20°C

Reference: OMEGA E.Z.N.A.® Plasmid DNA Mini Kit I

Protocol for transformation with ligation reaction system with TIANGEN DH5a competent cell

Get the competent cell from -70℃ and wait for its fusion;

Add 50ul competent cell into a 1.5ml centrifuge tube;

Add the entire ligation product (10ul) into the tube;

Mix and incubate on ice for 30 min;

Heat pulse for 90sec at 42℃;

Put back the tube on ice and incubate for 3min;

Add 450ul LB non-antibiotic agar plates and incubate at 37℃ for 45min;

Plate the culture on LB plate containing corresponding antibiotics;