Genotyping Protocol

2021.05.11 김명준

For genotyping, genomic DNA (gDNA) must first be obtained:

1. In case of genotying mice, obtain tissue sample via ear-punch, toe-clipping, or tail-cut and place sample in a 1.5ml tube
2. Add 350ul SNET Lysis Buffer (0.2M Tris, 0.5M NaCl, 0.01M EDTA, 1% SDS in H_2O) and 5ul Proteinase K Solution (20mg/ml)
    a. In case of genotyping cells on culture plates, first aspirate media and wash twice with PBS. Afterwards, add 350ul SNET Buffer and 5ul Proteinase K Solution to each well, pipette up and down to detach cells, and transfer to 1.5ml tubes
3. Mix well by vortexing for ~10 sec and then spin-down the tubes
4. Incubate at 56°C
    a. For tissue samples, overnight incubation is recommended; for culture plate samples, 4 hours is recommended
5. After incubation, add 200ul Phenol/Chloroform/Isoamyl alcohol (PCI) solution
6. Vortex for 30 sec; the solution should turn cloudy white color
7. Centrifuge at 13,000 rpm at 4°C for 15 min
8. Carefully remove tubes from the centrifuge and transfer only the top clear layer to new 1.5ml tubes
9. Add 200ul Isopropanol and 40ul 0.1 Sodium Acetate to each tube, mix by inversion, and incubate at 4°C for 60-90 min
10. Centrifuge at 13,000 rpm at 4°C for 15 min, and carefully aspirate supernatant
11. gDNA pellet will likely be visible at the bottom of the tube
12. Wash the pellet twice with 70% Ethanol
    a. add 700ul 70% Ethanol, mix by inversion, centrifuge, and aspirate supernatant
13. After the last wash, aspirate supernatant and dry the pellets for ~20 min
    a. Aspirate as much supernatant as you can without disrupting the pellet, and place the tube upside down on a paper towel to dry
14. Resuspend pellet in 20ul DEPC-treated Ultra-Pure Water
15. Store at 4°C

For Genotyping, you must have the correct primers available. Sequences for primers specific for the gene of interest can be found on Jackson Laboratory website, or may be listed in journals

1. First, obtain PCR premix tubes
    a. These tubes contain DNA polymerase, dNTPs, reaction buffer, and loading dye that are needed for PCR and Gel Electrophoresis
2. Prepare a working concentration for each primer to be used by diluting to 20 pmol
3. Add 1 ul of each primer to be used and DEPC-treated water up to total of 19 ul
    a. Number of primers may vary; some genotyping require 3 primers (usually a reverse primer common for both Wild and Mutant gene, and a forward primer for each of Wild Type and Mutant genes); some recommend 4 primers (a set for mutant gene, and another set for internal control)
4. Add 1 ul of gDNA
    a. Measuring the concentration of each gDNA sample is not required, but because the concentrations will be different, the strength of the bands after gel electrophoresis may also vary
    b. If you feel that the concentration of gDNA may be too low, or want a very strong band, add increased amount of gDNA and decrease the amount of water added on Step 3 (the reaction volume should be set to 20 ul total for each sample)
5. Mix by flicking the PCR tubes gently and spin down the tubes
6. Place on PCR machine and run the cycle
    a. The PCR cycle may differ for different genes of interest; For genotyping mice bought from Jackson Laboratory, their website has specific PCR cycle for each genotype; Others can be run on standard PCR cycles
7. After PCR cycles are complete, prepare 1.2% or 1.5% agarose gel in TAE buffer (0.5x or 1x)
8. Place the gel in gel electrophoresis chamber and fill the chamber with TAE buffer until the gel is completely covered
9. Add 9ul DNA ladder on the first well and 9ul PCR products in each of the following wells
10. Run gel electrophoresis and visualize gel on Gel Imaging Systems
11. Using the DNA ladder, you can approximate the weight of each band(s) on each sample and identify which contain the Wild Type gene or the Mutant gene