CRISPR/Cas9 Plasmid ES Cell Electroporation with Transient Puro Selection

Materials:

One 10cm gelatin coated dish of JM8.A3 ES cells in exponential growth.

Renew in the morning if you will do the electroporation in the afternoon.

Electroporation medium. KO-BL6 ES Cell Culture medium
Puromycin Selection Medium Trypsin + chicken serum (T+CS)
D-PBS 2 gelatin-coated 10cm dishes
Electroporation cuvette, 0.4 cm gap (BioRad # 165-2088)

Plasmids in TE:

1. PGKpuro
2. pX330 (Cas9) with desired sgRNA
   construct name:_________conc:_____μg/μl, μl for 15 μg____
   
   Or

3. X461(Cas9n) + desired sgRNA#1
   construct name:_________conc:_____μg/μl, μl for 10 μg____
   pX461(Cas9n) + desired sgRNA#2
   construct name:_________conc:_____μg/μl, μl for 10 μg____

Method:

1. Harvest JM8.A3 ES cells from plate by trypsinization with T+CS. Resuspend at a concentration of 10⁷ cells/ml in electroporation medium.
2. Add DNA and cells to electroporation cuvette.
   Add 15 μg X330 plasmid (Cas9) with desired sgRNA in TE, or 10 μg each of X461 plasmids
(Cas9n) with desired sgRNAs. Add 5 μg PGK-puro. Add 0.8 ml of cells at 10^7 cells/ml. Pipet gently up and down without introducing air bubbles into the sample.

3. Electroporate with BioRad electroporator at room temperature. 250 μF and 0.3 Kev. Time constant = ________

4. Dilute cells in 20 mls KO-BL6 media + penstrep.

5. Dispense cells to 2 x gelatin-coated P100s. Gently slide dishes forth and back, then side to side, to distribute the cells evenly across the surface.

6. Incubate overnight at 37°C in 5% CO₂ humidified incubator.

7. Renew next day with KO-BL6 media + penstrep.

8. For next two days, renew daily with KO-BL6 media + penstrep + 2 μg/ml puromycin.

9. For next four days, renew daily with KO-BL6 media + penstrep.

10. If the colonies are very small, or you don’t see any, let the dishes remain in the incubator for 2-4 more days without changing the media.

11. Harvest cells from P100s by trypsinization, pooling both duplicate P100s. Wash cell pellet 2x in D-PBS. Resuspend final cell pellet in 0.2 ml PBS and transfer to microcentrifuge tube for DNA extraction by Qiagen DNeasy kit.

**Trypsin + Chicken Serum**

Add 5 ml chicken serum, 5 ml 2.5% trypsin solution, and 186 mg EDTA to 475 ml PBS. Sterile filter, aliquot and store aliquots at -20°C.

**Electroporation Medium**

High Glucose DMEM (ThermoFisher catalog no. 11965092)
4mM Glutamine (ThermoFisher catalog no. 25030081)
0.1 mM 2-mercaptoethanol (Sigma-Aldrich catalog no. M-7522)
Penicillin/Streptomycin 50 units/ml (ThermoFisher catalog no. 15070063)
15% ES cell qualified fetal bovine serum
1000 U/ml LIF (EMD Millipore catalog no. ESG1107)

**KO-BL6 ES Cell Culture Medium**

KO-DMEM (Invitrogen 10829)
4mM Glutamine (ThermoFisher catalog no. 25030081)
0.1 mM 2-mercaptoethanol (Sigma-Aldrich catalog no. M-7522)
Penicillin/Streptomycin 50 units/ml (ThermoFisher catalog no. 15070063)
15% ES cell qualified fetal bovine serum
1000 U/ml LIF (EMD Millipore catalog no. ESG1107)
1% Non-essential amino acids (ThermoFisher catalog # 11140050)
Selection Medium.

Add Puromycin to KO-BL6 ES Cell Culture Medium
2 μg/ml puromycin (ThermoFisher catalog no. A1113803)