**Schwer Lab**

## Feeder cell depletion and genomic DNA preparation from ES cells

***PK cell lysis buffer for genomic DNA isolation from ES cells***
10 mM Tris-Cl (pH 8), 100 mM NaCl, 1 mM EDTA, 0.5% SDS, 0.2 mg/mL PK (stock is 20 mg/mL; use 1:100 to 1:200 dilution)
*Store buffer without PK at RT.*

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|---|---|
| 0.5 mL | 1 M Tris-Cl pH 8 |
| 1 mL | 5 M NaCl |
| 0.1 mL | 0.5 M EDTA pH 8 |
| 2.5 mL | 10% SDS |
| MilliQ water to 50 mL total volume | |

**PROCEDURE**

1. Remove ES medium. Wash 10-cm ESC dish once with 12 mL PBS (RT).

2. Cover cells with 2 mL 0.1% trypsin with chicken serum and incubate at 37°C **for 8 min**.

3. Pipette up and down 5× with P1000 tip. Stop the trypsin reaction by adding 4 ml of ES cell culture medium. Resuspend the cells by pipetting them gently up and down four times with 5-mL pipette.

4. Add the ES cell suspension to fresh 10-cm dish (no gelatin, no feeder cells). Incubate the dish at 37°C for 30 min.

5. Most of the MEF feeders will be loosely attached to the plastic surface, whereas the ES cells will be in suspension. Move the dish very carefully, taking care not to disturb these layers. Very carefully tilt the dish and collect 2 mL of supernatant and transfer to fresh gelatinized dish containing 10 mL of ES cell medium (~1:3 passaging).

6. Next day: remove medium completely and add 10 mL PK cell lysis buffer supplemented with 1:100 PK. Return dish to incubator. Incubate o/n (up to 36 h).

7. Transfer 10 mL PK cell extract to 50-mL conical tube. Let cool to RT. Add 2 volume (=20 mL) isopropanol (RT). Gently invert to mix. DNA will precipitate.

8. Fish out DNA and transfer to 15-mL conical containing 5 mL 70% EtOH (RT) to wash.

9. Remove DNA fibers and transfer to 1.5-mL microcentrifuge tube. Remove all EtOH and let air dry.

10. Resuspend DNA in TE for genomic DNA (reduced EDTA amount: 10 mM Tris-Cl pH 8, 0.1 mM EDTA).

11. Let resuspend o/n. This DNA is very viscous (not sheared). Heat to 55°C for 10 min before gently pipetting with cut P1000 tip to gently resuspend.