Cell Culture

The SV40-immortalized skin fibroblast cell line XP6BE derived from a 19-year-old female patient with xeroderma pigmentosum (complementation group D), was obtained from the Coriell Institute (Camden, NJ, USA).
Cells are cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 with high glucose (DMEM-F12 ; Sigma-Aldrich, D6429) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Passaging Protocol

1. **Prepare Materials**
   - Warm culture medium and Trypsin-EDTA to 37°C**.**
2. **Remove Old Medium**
3. **PBS Wash**
   - Rinse cells once with D-PBS (no Ca^{2+}, Mg^{2+}) (2 mL per for T75 Flask)
4. **Cell Detachment**
   - Add 1 mL of Trypsin-EDTA per T25 flask (adjust for other dish sizes).
   - Incubate for 2–5 minutes at 37°C, checking for detachment.
5. **Stop Reaction**
   - Add equal volume of pre-warmed medium to stop the trypsin reaction.
   - Gently pipette up and down to break clumps.
   - Count cells.
6. **Re-seeding**

Thawing Protocol

1. **Preparation**
   - Prewarm complete DMEM/F-12 medium (containing 10% fetal bovine serum [FBS] and antibiotics) and additional FBS to 37°C in a water bath.

2. **Thawing**
   - Retrieve frozen XP6BE cell vials from −80°C and immediately proceed to thawing.
   - In a sterile biosafety cabinet, quickly add ~1 mL of the prewarmed medium directly into the vial to initiate thawing while gently pipetting up and down to dilute DMSO and dislodge the cell pellet.

- Transfer the cell suspension promptly into a 15 mL sterile conical tube to minimize exposure time to DMSO.

3. **Dilution and Washing**
   - Slowly add 9 mL of prewarmed complete medium to the tube to dilute the residual DMSO.
   - Centrifuge at 300 × g (approximately 1200 rpm) for 5 minutes at room temperature.
   - Carefully aspirate and discard the supernatant without disturbing the pellet.
   - Resuspend the cell pellet in 4.5 mL of complete DMEM (with 10% FBS).
   - Transfer the cell suspension to a sterile T25 culture flask.

4. **FBS Supplementation**

- Supplement the culture with 500 µL of prewarmed FBS to achieve a final concentration of 20% FBS.
- Incubate the flask at 37°C in a humidified 5% CO_2 incubator.

Freezing Protocol

One confluent T75 flask yields approximately 4 cryovials.

1. **Preparation**

   - Aspirate and discard the culture medium from a confluent T75 flask.
   - Rinse cells gently with 5 mL of sterile PBS to remove residual serum. Discard the PBS.

2. **Cell Detachment**

   - Add 1 mL of 0.05% trypsin-EDTA solution to the flask.
   - Incubate for 5 minutes at 37°C to allow cell detachment.

3. **Neutralization and Collection**

   - Add 9 mL of complete culture medium (DMEM/F-12 supplemented with 10% FBS and 1% penicillin-streptomycin) to neutralize the trypsin.
   - Disperse cell clumps thoroughly by pipetting up and down 5–6 times.
   - Transfer the entire cell suspension into a 15 mL sterile conical tube.

4. **Centrifugation and Washing**

   - Centrifuge at 300 × g (approximately 1200 rpm) for 5 minutes.
   - Discard the supernatant and gently resuspend the cell pellet in sterile FBS
   - Repeat centrifugation at 1200 rpm for 5 minutes to wash.

5. **Freezing Medium Preparation**

   - Discard the supernatant and resuspend the pellet in 4 mL of medium consisting of 90% FBS and 10% DMSO.
   - Gently pipette to achieve a uniform cell suspension.

6. **Aliquoting and Labeling**

   - Dispense 1 mL of the cell suspension into each sterile cryovial.
   - Label each vial with cell type, passage number, and date of freezing.

7. **Freezing and Storage**

   - Place the cryovials in a freezer container containing isopropanol and store at −80°C for 72 hours.
   - After 3 days, transfer vials to long-term at -80°C freezer.

Transient transfections

Transient transfections are performed in T75 flasks using linear polyethyleneimine (PEI, MW 25,000; Polysciences, Inc., 23966) at a DNA:PEI ratio of 1:5.
A total of 15 µg of DNA is used per flask, consisting of 2.25 µg of the probe construct and 12.75 µg of pcDNA3.1(+) empty vector. Following overnight incubation, cells are detached, resuspended in phenol red-free DMEM/F-12 (Thermo Fisher Scientific, 21063-029), and seed into white 96-well plates with clear bottoms (Greiner Bio-One, Courtaboeuf, France) at a density of 40,000 cells/well (200 µL/well of a 2×10^5 cells/mL suspension).