Product description

RPE1 FRT/TR Cell Line can be used to generate cell lines with tetracycline inducible expression of genes of interest. The cell line has a randomly integrated FRT locus and Tet repressor for Flp-In expression. For example, the originator of the cell line used this cell line to generate tetracycline-inducible cell lines expressing APC15-IRES2-mRuby, APC15-3xflag and Cyclin B1-L45A-HA using the FLIP-in system and a modified pCDNA5/FRT/TO vector. This cell line is resistant to Zeocin (from FRT) and Blasticidin (from TR). It also carries resistance to hygromycin and puromycin, inherited from the parental RPE1 cell line. After Flp-In integration, selection is performed using Geneticin (a neomycin analogue), because the hygromycin resistance cassette in the original pcDNA5/FRT/TO plasmid was replaced with a neomycin resistance marker. The FRT and TR cassettes are stably integrated, so additional co-selection with Blasticidin or Zeocin is not required.

Name: RPE1 FRT/TR Cell Line

Research fields: Genetics

Parental cell: RPE1

Organism: Human

Tissue: Eye

Growth properties: Adherent cell line

Conditional: Yes

Conditional description: Tetracycline-inducible expression of genes of interest.

Production details: This tetracycline-inducible RPE1 cell line was created by random integration of an FRT site and a Tet repressor gene into retinal pigment epithelial 1 (RPE1) cells. The inventors used electroporation for transfection, specifically with Invitrogen's Neon transfection system. Cells were allowed to recover for 2–3 days post-transfection, then split into media containing Geneticin at a final concentration of 0.5 mg/mL (ThermoFisher Scientific, 910131027). This selection medium was refreshed every 2–3 days for approximately 10–14 days until cell death ceases. Standard media included high-quality, tetracycline-free FBS. For plasmid ratios during transfection, a 4:1 ratio of pOG44 (Flp-recombinase expression plasmid) to the gene-of-interest plasmid (pcDNA5/FRT/TO/Neo + gene of interest) was used.

Biosafety level: 1

Cellosaurus id: CVCL_VP32

Contributor(s)

Inventor: Jonathon Pines
Institute: University of Cambridge

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapour, until ready for use.

Recommended medium: 500 mL Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (Sigma: D6241), 17.3 mL of 7.5% sodium bicarbonate, 10% FBS, 1% Pen/Strep, 1% 200 mM Glutamax and 1 mL Amphotericin B.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Handling instructions

1. Please ensure that vials are frozen when received, and store at <-130 °C long term. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a viable cell count using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension to a 50 mL conical tube using a pipette. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
5. Wash by adding 15 - 20 mL of medium **dropwise**, while gently swirling the tube.
6. Centrifuge the cell suspension at **250 x g for 5 minutes** at room temperature.
7. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
8. Gently add required volume of culture medium and transfer to a T25 flask or 10 cm dish and incubate. Examine the cultures after 24 hours and subculture as required.

References

- Mansfield et al. 2011. Nat Cell Biol. 13:1234-43. PMID: 21926987

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: RPE1 FRT/TR Cell Line, was invented by Jonathon Pines (CancerTools.org #153242).