LHSR-AR culture conditions +/- androgen treatment

From: Duke/UNC/UT/EBI ENCODE group
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1. Source: Genetically engineered in the lab of William Hahn at the Dana Farber Cancer Institute, Boston, MA.

2. Lineage: Human prostate epithelial cells (PrEC) were obtained from Lonza (catalog CC-2555) and propagated in appropriate media as described below. These cells were infected with amphotropic retroviruses encoding the SV40 large T antigen (L), the telomerase catalytic subunit *hTERT* (H), the SV40 small T antigen (S) and an oncogenic allele of H-*ras* (R) to create LHSR cells. These cells were then infected with a pWZL retrovirus encoding a blasticidin selection cassette that contained a wild-type androgen receptor (AR) cDNA. The final cell line product was then termed LHSR-AR.

3. Donor Information: Multiple human donors, all of whom are HIV-1, Hepatitis B and Hepatitis C negative.

4. Karyotype: Diploid

5. Medium: We maintain LHSR-AR cells in PrEBM prostate epithelium basal medium (Lonza, cat # CC-3165) + PrEGM SingleQuots (Lonza, cat # CC-4177) prepared by adding the SingleQuots to the medium in a sterile fashion, followed by passing the growth medium through a 0.22 µm filter. The PrEGM SingleQuots contain the following growth factors and supplements: BPE, hydrocortisone, hEGF, epinephrine, insulin, triiodothyronine, transferring, gentamicin/amphotericin-B and retinoic acid. We do not use any different medium prior to androgen exposure. For androgen induction, we treat cells with R1881 (methyltrienolone, Perkin Elmer, cat #NLP005005MG) from a 2.5 mM stock in 70% ethanol to a final concentration of 1 nM in the appropriate medium. See below for induction instructions.

6. Growth conditions: Grow at 37.0°C, atmosphere of 95% air, 5% CO2

7. Protocol of cell growth: We typically grow LHSR-AR cells in 150 or 100 mm polystyrene tissue culture dishes depending on number of cells required. We use 20 mL of media for 150 mm dishes, and 10 mL of media for the 100 mm dishes. We change media

the day after splitting or seeding, and every other day thereafter. We split these cells soon after they reach 80% confluence.

To split: Obtain the Reagent Pack (Lonza, cat # CC-5034) that contains Trypsin/EDTA solution, Trypsin neutralizing solution and HEPES buffered Saline Solution. We use 1 mL of Trypsin/EDTA for a 100 mm dish, and 2 mL for a 150 mm dish. Remove media thoroughly, and then add an amount of HEPES buffered Saline Solution that is twice the volume of Trypsin/EDTA to be used. Allow the entire plate to be covered, and then aspirate off the solution. Cover the cells with the appropriate amount of Trypsin/EDTA and place in a 37.0°C incubator. Allows incubation for 2-5 minutes, rap the plate against the palm of your hand, and examine how many cells have detached under the microscope. If less than 90% of cells have detached, place dish back into the incubator for 30-second increments until enough cells have detached. Then, neutralize the Trypsin/EDTA by adding twice the volume of trypsin added of Trypsin neutralizing solution to a total volume 3x the amount of Typsin/EDTA. Transfer the detached cells to a 15 mL centrifuge tube and spin at 800 rpm x 5 minutes to pellet cells. Aspirate the supernatant, leaving a slight amount in order to leave the pellet undisturbed. Resuspend the pellet in an amount of media appropriate for splitting 1:2 or 1:3 as needed.

To prepare R1881 for induction, first dissolve the R1881 powder in 70% ethanol to a concentration of 2.5 mM. Serial dilutions of 1:100 and 1:25 in appropriate growth media create a solution of 1 uM. This 1 uM solution is diluted 1:1000 (final concentration 1nM) by adding the appropriate volume directly to the plate with media already in place. Cells are exposed to R1881 for 12 hours.

8. Cell Passage: After thawing, cells are harvested approximately before they reach passage 10. In order to begin a new culture from a frozen stock, we thaw the vial at 37.0°C for two minutes and then place the contents of the vial into a 100 mm polystyrene tissue culture dish containing 10 mL of growth media. After allowing the cells to attach for one day at 37.0°C in the incubator, we change media and then propagate the cells as described above. We do not spin down the contents of the cryotube to remove the DMSO, as this is more damaging than the effects of the DMSO residue once diluted by fresh media.