LNCaP culture conditions +/- androgen treatment

1. Source: ATCC # CRL-1740.

2. Lineage: Isolated in 1977 by J. Horoszewicz from needle aspiration biopsy of the left supraclavicular lymph node of a patient with confirmed metastatic prostate carcinoma, androgen receptor and estrogen receptor positive, contain a T877A mutation in their androgen receptor

3. Donor Information: 50 year old Caucasian male, B+ blood type

4. Karyotype: Markedly aneuploid, modal chromosome number 84

5. Medium: We maintain LNCaP cells in RPMI 1640 (VWR, cat # 4500-396) + 10% FBS (MediaTech, cat # 35-010-CV) + 0.5 mg Plasmocin/500 mL of media (InvivoGen Cat # ANT-MPT). Prior to androgen exposure, we grow the cells in RPMI 1640 + 10% Charcoal Dextran Stripped (CDS) FBS (Gemini BioProducts, cat #100-119) + 0.5 mg Plasmocin/500 mL of media. 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. 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 LNCaP cells in 150 or 100 mm polystyrene tissue culture dishes depending on number of cells required. Media is changed at minimum twice per week, or before media becomes orange. Cells are split soon after reach 80% confluence as they rarely form a uniform monolayer, but rather begin to clump together.

To split: Remove media, rinse 1x with sterile PBS to completely remove elements of serum that may inhibit trypsin, then add 1-2 mL of .25% Trypsin-EDTA (depending on culture dish volume) and place in incubator for 2-3 min. After this, tap dish gently to dislodge cells and examine under microscope to ensure that cells have been dispersed from dish. Place back in incubator as needed until most of the cells have been dislodged. Then, add 6-8 mL of complete growth media to neutralize trypsin, and pipet vigorously to break apart any clumps that may form during tapping.

If preparing for androgen treatment, split the resuspended cells into dishes containing charcoal dextran stripped serum medium, and change media to RPMI 1640 + 10% CDS-FBS after 24 hours. We add R1881 the evening following this media change. 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 1 nM) by adding the appropriate volume directly to the plate with media already in place. Cells are exposed to R1881 for 12 hours.

If splitting the cells for maintenance, split the resuspended cells into dishes containing complete media. We typically split these cells 1:2-1:6 as needed.

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 five 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. Despite the 5% DMSO in the frozen stock, LNCaP cells tend to attach and grow quite rapidly. If desired, one can take the thawed stock, spin

down the cells, remove the supernatant containing DMSO, resuspend the cells in 1 mL of fresh media and then place in the tissue culture dish.