## LNCaP cell line ATCC # CRL-1740

The LNCaP cell line is an epithelial cell line derived from a human prostate carcinoma. LNCaP cells grow as a loosely adherent layer of cells that are prone to growth in clusters. Do not plate LNCaP cells too sparsely or they will not grow well. Under the conditions described below they double every 48-60 hours.

## Cell culture

Cells are grown in RPMI 1640 media containing penicillin (100 units/ml), streptomycin (100  $\mu$ g/ml), and 10% FBS at 37°C in 5% CO<sub>2</sub> incubator.

- 1. Thaw the vial containing LNCaP cells (ATCC # CRL-1740) in a 37°C waterbath. Transfer cells to 10 mL RPMI 1640 media in a 15 mL conical tube.
- 2. Centrifuge 700 rpm for 5 minutes at room temperature.
- 3. Resuspend cell pellet in 10 mL RPMI 1640 media and transfer to a 10-cm dish.
- 4. Grow LNCaP cells until about 70-80% confluency (approximately 5 X 10^6 cells).
- 5. Wash with warm PBS and trypsinize to detach cells.
- 6. Collect cells with 10 mL RPMI media and plate the cells at a high density. To maintain the cells, plate about 1 X 10^6 cells per 10-cm dish (i.e., 2 mL of the resuspension plus 8 mL RPMI). To achieve a large number of cells (i.e., 1 X 10^8 cells), plate 5 mL of this resuspension plus 15 mL RPMI media onto 2 separate 15-cm dishes. When the cells have grown to 80% confluency, trypsinize and plate onto 2 500-cm dishes. Starting from the initial thaw, growing 10^8 LNCaP cells may take at least 2-3 weeks.