

## Urothelium Cell Line (UROtsa) Growth conditions

From: Duke/UNC/UTA/EBI ENCODE group

Date: 8/24/10

1. Source of cells: University College London. A primary culture of urothelial cells was derived from the left ureter of a 12 year-old girl and immortalized by transfection with a temperature-sensitive SV-40 large T antigen gene as described by Petzoldt (1995). The Seed lab (Duke University) obtained the immortalized urothelium from the lab of Dr. D Sens (University of N. Dakota), who developed the cell culture model for the UROtsa cells (see Rossi et al. 2001).
2. Lineage of cells: normal human ureter cells.
3. Donor Information: 12 year-old female
4. Karyotype: normal in quantity and appearance
5. Media for cell lines:
  - a. Undifferentiated: DMEM-LG (low glucose, Sigma) supplemented with an additional 1mg/ml glucose and 5% serum (Gibco or Sigma, certified FBS).
  - b. Serum-free Differentiation: 1:1 mixture of DMEM and Ham's F-12 supplemented with selenium (5 ng/ml), insulin (5 ug/ml), transferrin (5 ug/ml), hydrocortisone (36 ng/ml), triiodothyronine (4 pg/ml), and epidermal growth factor (10 ng/ml).
6. Culture conditions: Cells are adherent and should be incubate at 37 ° in the presence of 5% CO<sub>2</sub>. Even though a temperature sensitive SV40 T antigen was used to immortalize the cells, we always maintain the cells at 37 C +/- 0.5 C and have no other data (or a phenotype) to suggest issues with variation outside of this range. At this stable temperature, the cells readily maintain immortalization. We have found that upon reanimation from liquid nitrogen, the cells grow better with 7% CO<sub>2</sub> for several passages, at which time the cells may be switched down to the more standard 5% CO<sub>2</sub>. UROtsa cells can be propagated in untreated plastic tissue-culture dishes or flasks with little difficulty.
7. Cell line maintenance: Cells should be fed at least every 3 days with new media. Cells are detached using a trypsin/EDTA mixture for 10 minutes at 37C and sub-cultured 1:3 or 1:4 into 20mL of new culture media. We do not include drug to maintain the T antigen. The line has been used extensively and this has not been routinely performed. In our experience, we do not have problems with repeated passage, suggesting that immortalization is readily maintained in the absence of drug.

For freezing, take a full T-75 flask that was fed the day prior (~90% confluent), trypsinize, centrifuge on slow speed, and bring up in 3 ml of growth media containing 10% DMSO. Aliquot into 3 cryovials. Allow the cells to freeze gradually by placing inside a Styrofoam container and into a -70C freezer overnight. The following day, cells can be transferred to liquid nitrogen. Upon reactivation from liquid nitrogen, cells should be cultured for several passages using the standard (serum-containing) culture medium and NOT the differentiation media.

8. Cell passage #: 15
9. UROtsa infection with uropathogenic *E. coli*:
  - a. *E. coli* cystitis strain UTI89 was grown overnight (18h) at 37C with aeration in LB media and sub-cultured 1:100 in fresh LB media the following day. The culture was then grown for an additional 24 h at 37C, this time without aeration, to induce the expression of type 1 pili (necessary for adherence and invasion into cultured bladder and urothelial cells). *E. coli* cells were enumerated by serial dilution and plating onto agar to count CFU.

- b. 24 hours prior to infection, the serum-containing (normal) growth media was removed from the UROtsa cells and replaced with approximately 20mL of serum-free media to induce uptake of bacteria. At the time of infection, old media was removed and replaced with 5 mL (low volume to ensure adequate contact between cells and bacteria) of a mixture of serum-free media and *E. coli* cells. UROtsa cells were infected with *E. coli* at an MOI (multiplicity of infection, meaning number of bacterial cells to number of eukaryotic cells) of 10:1.
- c. The cells were incubated with bacteria for 1 hour at 37C with 5% CO<sub>2</sub> to allow bacteria time to adhere and invade into cultured cells. After 1 hour, the media and non-adherent bacteria were removed. The cells were washed 3 times with sterile PBS. 15 mL of normal DMEM media supplemented with 100 mg/mL gentamicin was added back to the cells to kill extracellular bacteria for 1 hour. The media was then removed and replaced with media containing 50 mg/ml gentamicin to control extracellular bacteria for an additional 22 hours at 37C incubation with 5% CO<sub>2</sub>.
- d. UROtsa cells were harvested at 24 hours post-infection.

#### References:

- Rossi, MR, Masters, JRW, Park, S, et al. The immortalized UROtsa cell line as a potential cell culture model of human urothelium. *Environ health perspect.* 2001. 109(8):801-8.
- Petzoldt, JL, Leigh, IM, Duffy, PG, Sexton, C, and Masters, JRW. Immortalisation of human urothelial cells. *Urol Res.* 1995. 23:377-380.