Transient transfection into 293T cells

(by Jun Takagi, Jun 15,2000)

Purpose

Transient transfection into 293T cells is a convenient way to overexpress and obtain both cellular and extracellular (secreted or membrane) proteins. 293 is a human renal epithelial cell line which is transformed by adenovirus E1A gene product. 293T is a derivative which also express SV40 large T antigen, allowing episomal replication of plasmids containing the SV40 origin and early promoter region. They (both) have the unusual property of being highly transfectable by the following $Ca_3(PO_4)_2$transfection protocol. Up to 50% efficiency is attainable.

Materials

• Complete medium: DMEM(high glucose) supplemented w/ 10% FCS (either w/ or w/o heat inactivation at 55°C/30min), NEAA, Na-pyruvate, and Gln (Pen/Streptmycin: optional)
• Transfection reagents:
i) 2M $CaCl_2$: 2M in H_2O, filter sterile, store at 4°C
ii) x2HBS: 8.0g NaCl, 0.37g KCl, 201mg (yes! mg) $Na_2HPO_4•7H_2O$, 1.0g glucose, 5.0g HEPES/500ml (adjust pH to 7.05 with NaOH and filter sterile, store at 4°C)
• DNA: QIAGEN miniprep grade OK. Solution in TE is compatible. Basically, no need to linearize DNA.

Procedure

Culture conditions

Growth will be very fast. Usually doubling time of <1 day is observed. Split cells every 3~4 days with 1:10 to 1:20 dilution and culture under 5% CO_2. Cells loosely attach, so you can detach cells with EDTA alone but brief trypsinization gives single suspension. Do not overtreat with trypsin.

Transfection of 293 cells

The following protocol is for 10 cm dish (medium: 10ml). If you use 6-well or 12-well plates, total volume of the medium should be 3ml and 1.5ml, respectively, and decrease the amount of each reagent accordingly.

1. Plate cells the night before to give 60-70% confluence at the day of transfection. The efficiency will decrease if reached 100% confluence. Less than 50% confluence may be OK but the amount of protein expressed will be low because of the small numbers of cells.

2. One hour prior to the transfection, change to medium containing 25μM chloroquine (from x1000 stock in PBS, stored at -20°C). Volume should be 10 ml per dish. (Chloroquine can be omitted, but increases efficiency about x2)

3. Add 10μg DNA to ddH_2O (1095 μl total) in 15-ml sterile tube, then add 155 μl 2M $CaCl_2$. When you are ready, add 1250 μl of 2xHBS dropwise while gently mixing. Add this mixture directly to the cells dropwise

through the medium. Do this within 1-2 min after adding 2xHBS. You will notice that the medium turns to orange. Make sure you evenly sprinkle the droplet over the entire area.

4. Incubate for 7-11 h. Very fine, dust-like precipitate visible. After incubation, rinse once and change to medium without chloroquine, again 10ml/dish.

5. Harvest cells, or culture supernatant, 48-72 h after transfection. For secreted protein, you can change media once (at day 3 or 4, save the collected sup) and give it another 3-4 days of culture. As long as the cells are alive, they produce proteins. However, the time when the secretion level reaches maximum varies among proteins.

References

1. Dubridge et al (1987)MCB,7,379
2. Heinzel et al. 1988, J.Virol. 62,3738
3. Pear et al.1993,PNAS,90,8392.