Calcium Phosphate Transfection of Adherent Cells

This protocol was designed for 293 cells which are a human renal epithelial cell line transformed by adenovirus E1A gene product: 293T cells are a derivative which also express SV40 large T antigen, allowing episomal replication of plasmids containing the SV40 origin and early promoter region. They have the unusual property of being highly transfectable by the following CaPO₄ transfection protocol, up to 50-80+% efficiency. The Phenix retroviral packaging line is derived from a particularly transfectable clone of 293T.

The quality of DNA is very important. Double CsCl-banded and dialyzed preps work best. Qiagen-purified DNA is also supposed to work, although not as well. Source of calcium chloride is also important, we have found JT Baker to be the best, cat# 1332-01.

The cells transfect best at higher density. They are small cells, and optimal density is around 2.5-3.5 x 10⁶ cells per 6 cm dish, plated the night before. This achieves approximately 60-70% cell confluence the day of the transfection. If you believe your gene may cause some toxicity, especially for growth, use the higher density plating. For immunofluorescence, can probably be plated at lower density with some decrease in efficiency. The cells are poorly adherent to glass and plastic; pipette solutions with care, from the side, using wide mouth pipettes. Washing cells in PBS with Ca and Mg may help keep them attached.

Immediately prior to transfection, change medium to 5 mls fresh medium. Failure to do this will decrease transfection efficiency. Some people add 25 μM chloroquine (from 1000X stock in PBS-stored at –20°C), but we have not found that this appreciably improves transfection efficiency.

Add DNA (usually 10 μg) to ddH₂O to a volume of 438 μl total, then add 62 μl 2M CaCl₂. Then add 500 μl 2X HBS pH 7.05 dropwise while vortexing, or bubbling. Add this solution directly to the cells, dropwise through the medium, scattering the drops uniformly and GENTLY over the surface of the plate. It is important to add the precipitate to the cells within 1 minute of adding the HBS.

Return cells to the incubator for 7-10 hours (we have let this go overnight in some cases with reasonable results). There should be a very fine sand-like precipitate visible. Change the medium to 3 mls fresh medium. (If chloroquine was used 8-9 hours is probably best and significant toxicity occurs after 11 hours).

The next morning I change medium again to 3 mls fresh medium if I am going to later harvest retroviral supernatant or do starve or stimulation type experiments. Otherwise, cells may be harvested 36-48 hours after transfection for protein or processed for immunofluorescence analysis. I have obtained nice levels of protein expression from SV40-containing plasmids as early as 24 hours post transfection. Typically, transfection with pJ3omega β-gal DNA and staining shows 20-50% blue cells, and MFG β-gal has give us 50-80%.
2X HBS recipe (500 ml):

8.0 g NaCl.
0.37 g KCl
106.5 mg Na$_2$HPO$_4$ (anhydrous, 201.1 mg if 7X H2O)
1.0 g dextrose
5.0 g Hapes

Add 450 ml ddH2O, adjust pH to 7.05 with NaOH and bring final volume to 500 ml.
Sterile filter through 0.45 µM filter, discarding first few mls through and store at RT, cap on tight.