Transfection of DNA into MDCK cells to produce stable cell lines

The method employed is essentially the same as for Rat 2 cells but with a few subtle differences due mostly to the fact that MDCK cells are very resilient and not affected too much by the toxicity of the calcium phosphate precipitate.

Day1:

1. Split the cells from confluent dishes at a 1 dish into 4 ratio.
2. Ethanol precipitate the DNA samples.

Day2:

1. Check that the cells are OK.

   (p.m.)
2. Spin down the DNA as before.
3. Dissolve DNA and mix to precipitate as before. The calcium phosphate precipitate can be left on the cells for 16 to 18 hours ie overnight, so mixing the DNA and forming the precipitate should be done in the late afternoon.

Day3:

Remove the medium first thing in the morning, wash with PBS and PBS + a.m. 1mM EGTA as before. Add back non-selective medium for 4 to 6 hours.

Day4:

The cells should be fairly confluent with very little cell death. Trypsinize each p.m. dish and plate all the cells into three dishes with complete medium + selection antibiotic. For neomycin resistance use 500ug/ml G418, for hygromycin resistance use 175ug/ml hygromycin (Boehringer - Mannheim).

As with the Rat 2 cells the medium is changed every 3 to 4 days until large colonies are visible.