HBL 100 Metafectene Transfection Method

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Materials:
Plasmid pEGFP-N-fusion protein vector (Clontech)
Sterile 35mm-well tissue culture dishes
Sterile Eppendorf tubes
Glass coverslips
Opti-MEM 1 (GIBCO)
HBL100: human transformed breast cell line

Optimization of the transfection for HBL 100 cells:
HBL100 cells were grown in a 35mm cell culture dishes with glass coverslips in DMEM with 10% calf serum to near 50% to 90% confluency. Then ready for transfection with METAFECTENE.

Optimization of the transfection with METAFECTENE was carried out as follows:
1. Tube A: 250µl Opti-MEM (serum-free cell culture medium) medium with plasmid DNA 1 to 4µg.
2. Tube B: 250µl Opti-MEM (serum-free cell culture medium) medium with the indicated amount of METAFECTENE transfection reagent (use 3:1 up to 6:1 METAFECTENE to DNA).
3. Combining tube A and B, mixed by gentle tapping of tube.
The tubes were allowed to stand at room temperature for 20 to 30min min for lipid-DNA complexes to form.
4. During this time, the cells were wished with fresh Opti-MEM medium.Then add 1ml Opti-MEM medium to the dishes. At the end of the incubation time, the lipid-DNA complex mixtures were pipetted onto the cells.
5. Incubated at 37°C incubator under 5% CO2 more than 4hr, then change to regular medium for culture another 24 or 48h.
6. 24 or 48h post transfection starting time, the cells were either observed under fluorescent microscope or fixed with cold 80% methanol/20% acetone at–4°C for 15 min. the cells were mounted.
The transfection rate was determined by counting the per cent of fluorescent cells expressing the EGFP-tagged protein under any fluorescent microscope.

Conclusions:
1. HBL100 cells is one of tough cell line for us to transfection. Using the Metafectene transfection conditions for HBL100 cells have relatively good transfection efficiencies of more than 40% . In comparison, other the lipid-based transfection had low efficiencies.
2. The DNA amount to METAFECTENE can be used lower and if using DNA up to 2 µg and 12 µl METAFECTENE reagent, you can get ideal efficiencies.
3. Although HBL100 cell is not active proliferative cell line, whatever you use 50% or 90% confluency of cell, it will not affect the transfection efficiencies.

Figure Legend: HBL100 cells were transfected with a plasmid encoding for a dynamin-associated EGFP fusion protein using the METAFECTENE reagent according to the conditions described in this study. A) Phase contrast image of the cells B) Fluorescent image of the transfected cells shows that about 40% cells express the GFP fusion protein. C) Overlay of A and B.