

Tips and tricks of the trade

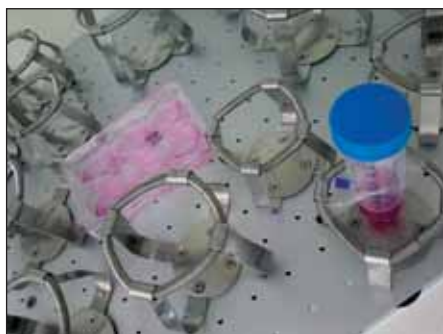
Rockin' Harder

Sometimes even slight changes in traditional protocols may have considerable influences on the outcome. One such example is given by Tobias Wimmer, who simply modified the mixing step during lipofection of mammalian cells to significantly enhance the transfection efficiency.

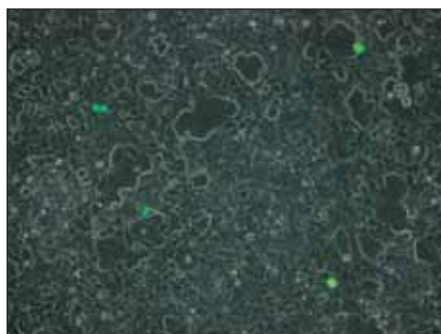
Lab Hint

Lipofection of mammalian cells using commercial transfection reagents or kits from different vendors is usually based on very similar protocols. The transfection reagent is first mixed with DNA and incubated for about half-an-hour to form DNA lipofection reagent complexes. In the next step, the latter are added to plates containing adherent cells and the cells are mixed with the transfection solution by gently rocking the plates back and forth.

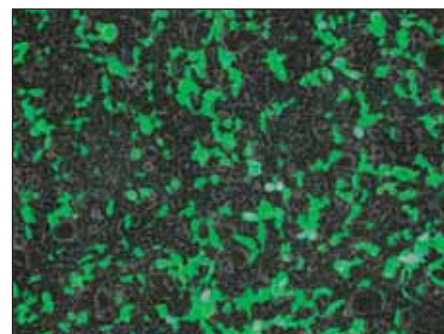
Subsequently, the cells are incubated at 37°C in a CO₂ incubator for a couple of days, in the hope that many cells will take up the DNA. But quite often, the transfection efficiency is pretty lousy. We usually get about one percent transfection efficiency if we apply the above-mentioned stand-



The efficiency of lipofection increases dramatically when the cells are resuspended and vigorously mixed on a lab shaker.



HEK 293 cells before (left) and after lipofection according to the modified protocol. Transfected cells show a green fluorescence.



ard protocol to transfect, e.g. HEK 293 cells. One may, however, increase the transfection efficiency dramatically with a very simple trick. Instead of dropping the DNA lipofection reagent complexes directly onto the adherent cells, we first resuspend the cells with trypsin/EDTA or accutase and transfer the cell suspension to a 50 millilitre falcon tube. We then add the DNA lipofection reagent complex and incubate the cell suspension for five to ten minutes. Afterwards, the cells are vigorously mixed on a lab shaker at 50 to 80 rpm. Some of the cells are killed during shaking, however, they may be easily removed by changing the culture media as soon as the cells reattach to the plates.

A slightly modified protocol may be applied to more robust cells, e.g. epithelial cells such as HeLa cells. In this case, the transfection complexes are dropped on the adherent cells as in the standard protocol,

however, the plates are then fixed on the lab shaker and agitated at 50 to 80 rpm for five to ten minutes (see figure showing lab shaker).

By simply applying this stronger agitation step, we have increased our transfection efficiencies from one percent for the "gentle mixing" standard protocol to more than 80 percent for our modified "hard rockin'" transfection protocol.

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