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Amaxa Electroporation

5E6 cells per reaction

Make the media: 20% FBS, 1x antibiotic/antimycotic, 1x glutamine in monocyte media.

Spin the cells (each reaction has its own tube), 10 min @ 90g

During spin:
Put 2mL media in a 12-well plate into incubator for 5 minutes to get *CO2*, temperature levels right.

Mix tfxn soln. with supplement (mix entire amounts of each with each other)

100uL of soln/supplement + 2ug DNA for each reaction, flick to mix

Get plastic pipette @ RT

Set “Y001” on the Amaxa machine

Transfer 500uL media from the 12-well plate into an eppie for each reaction. Get electroporation chambers ready.

After spin, suck off all the media with aspirator

Use P1000 to put 100uL transfection solution onto cells, resuspend gently and put cells in electroporation chamber.

Put chamber into machine and press Start

Use plastic pipette to suck up 500uL recovery media from eppie, flush the chamber, suck up cells and put back into the eppie.

Incubate at 37C for 30 minutes.

For each reaction put 1.25mL media into each of two wells on a 12 well plate. Put plate in incubator.

After 30 minutes transfer 500 uL/well to each 1.25-1.5 mL media in the 12-well plate.

Figure 2

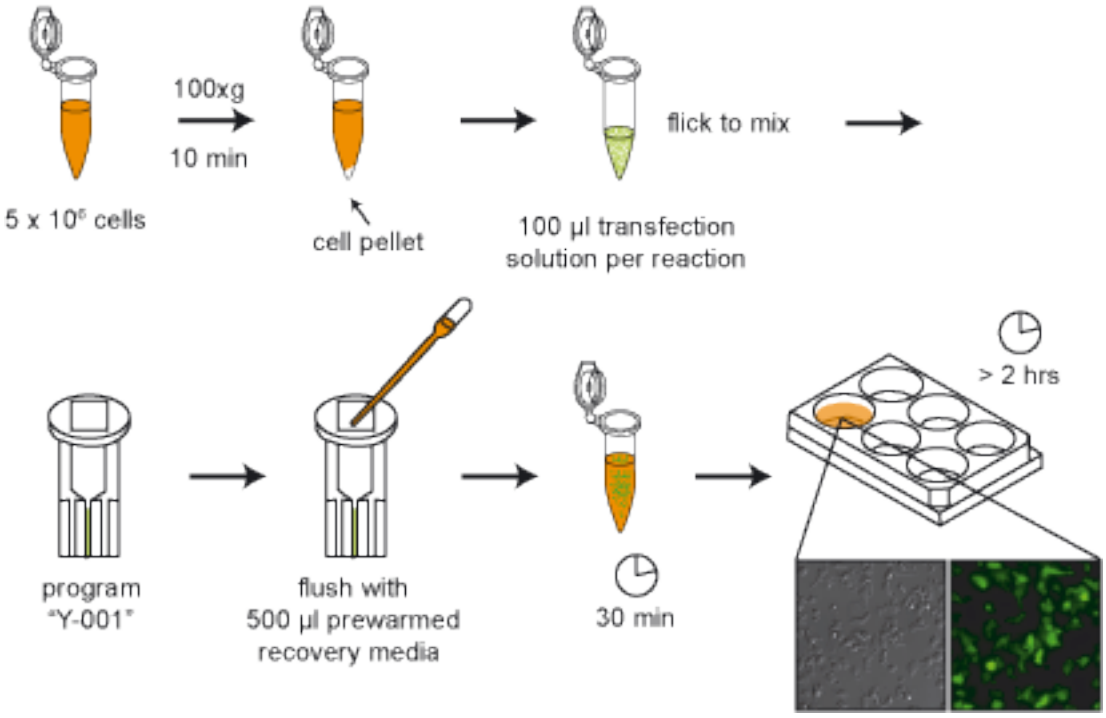

Fig. 2. Transient transfection of HL-60 cells with amaxa nucleofection. Spin ~5 million cells at 100xg. Aspirate supernatant and resuspend pellet in 100 µl transfection solution per reaction and nucleofect with amaxa program "Y-001". Flush with prewarmed recovery media and incubate in an eppendorf tube for 30 min. Transfer to a 6-well dish with 1.5 ml of recovery media; expression occurs after 2 hours. Shown is an example of HL-60 cells 5 hours after transfection with GFP visualized with DIC and fluorescence microscopy.

Note: I tend to see expression and good cell viability between 7-13 hours. AM 5/10/09