

UMMS RNAi Core Facility Protocol: Production of TRC or pGIPZ Viral Supernatant

We routinely use QIAGEN Effectene Transfection Reagent, which works very well for us. Detailed protocols are provided with the kit. The protocol below has been slightly modified from the QIAGEN kit protocol, in that it uses slightly more DNA.

Reagents

293T cells (ATCC)

293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted.

Cell culture medium

Effectene reagent (Qiagen)

EC buffer (comes with the QIAGEN Effectene kit)

Enhancer (comes with the QIAGEN Effectene kit)

TRC or pGIPZ plasmid DNA (purchased from the RNAi Core Facility)

psPAX2 (Addgene)

This is the packaging vector

pMD2.G (Addgene)

This is the envelope vector

0.45 µm filter (Millipore)

Method

Day 1: Plate 1.0×10^6 to 1.2×10^6 293T cells in a 6-well plate.

Day 2:

- a. In a sterile microfuge tube, combine 1 µg of TRC or pGIPZ plasmid DNA with 1 µg psPAX2 and 0.5 µg pMD2.G (2:2:1 ratio) in 100 µl EC buffer. Add 3.2 µl Enhancer. Mixed by brief vortexing and then spin down to collect the contents of the tube. Incubate at room temperature for 5 minutes. Add 10 µl Effectene reagent, mix by brief vortexing and incubate for another 20-30 minutes at room temperature.
- b. During the incubation, re-feed the 293T cells (that have been plated out the day before) with 1.6 ml of fresh medium
293T cells peel off easily, use extreme care to re-feed the cells.
- c. After the 20-30 minute incubation, add 0.6 ml medium to the DNA-Effectene mixture. Mix well and drop carefully onto the cells.

Day 3: Re-feed the transfected cells with 2.5 ml fresh medium.

293T cells peel off easily, use extreme care to re-feed the cells.

Day 4: 48 hours after infection, filter the supernatant through a 0.45 µ filter, aliquot and store at -80°C until ready for use.