

### **Lipid siRNA transfection**

For some cell lines, either electroporation or cationic lipids may be used. Basically any tfx protocol will require you to work out conditions for every cell line. Sometimes between 70-90% or more cells can be transfected with cationic lipids. With a bit of tweaking, and depending on the cell line, you can drive siRNAs into almost 100% of the cells with the following protocol:

Seed cells high, so that they are 85%+ on the day of transfection:

For one 10 cm dish:

40 ul Lipofectamine 2000

20 ug DNA

100-300 pmoles siRNA

#### tube 1

add the siRNA and/or DNA to 2 ml SF-DMEM (no drugs added)

#### tube 2

add the lipid to 2 ml SF-DMEM (no drugs added)

mix these two tubes immediately, for a volume of 4 ml, and then add to cells which have been washed 2X with SF-DMEM. Incubate 3-6 hours, then add normal media. Check for expression or knockdown 36+ hours post-transfection. Knockdown by siRNAs usually last 3-5 cell doublings.

*When oligofectamine is used, the identical protocol is used, but:*

Seed cells at 15% confluency, perform tfx with 30 ul oligofectamine and siRNAs, then repeat tfx 36 hours later. Analysis is performed when cells are 85-90% confluent. I have not calculated the transfection efficiency with oligofectamine.