(Protocol donated by Mona Abed)

RNAi in Schneider cells:

1. **If cells intend to be transfected then it is preferred if the RNAi is performed in the late afternoon and the transfection the following morning.**
2. Resuspend a 10cm dish of S_2R cells and spin down at 1300rpm for 3min.
3. Remove supernatant and resuspend with 10ml Schneider media (S++-, Schneider media that has been supplemented with glutamine and P/S (**no serum**)).
4. Spin cells down as mentioned in 1.
5. Remove supernatant and repeat wash twice more.
6. Resuspend in 10ml S++- and count cells (using a cell counter or any other means available).
7. Add $2*10^6$ cells to an eppendorf tube along with 5ug of designated RNAi and bring to a final volume of 1ml with S++- media (you may have to calibrate the amount of dsRNA for every gene. I've found that 5ug usually works but for example it may be lethal for some genes and for others it may not affect at all).
8. Incubate at room temperature for 10min.
9. Transfer the 1ml of cells with RNAi to a well of a 6-well plate (move the media around in the well so that it covers the well evenly) and incubate at 25ºC for 1h.
10. Add 3ml of S+++ media (Schneider media supplemented with glutamine, P/S **and SERUM**)

Transfection of S2R cells using Fugene HD:

Required volumes of S++- and FugeneHD:

S++- (without serum at 37°C as mentioned above in the RNAi protocol) = 150µl x no. of reactions.
Fugene HD = 1µl per 1µg of DNA (1:1 ratio), 200-500ng DNA is required per plasmid.

1. Pipette Fugene HD into an eppindorf tube (you can prepare a mix if you have multiple samples with identical DNA concentrations).
2. Drip required volume of S++- (150ul per sample) onto required volume of Fugene HD and pipette up and down gently a few times.
3. Incubate at RT for 10 min
4. Drip the S++- and FugeneHD mix onto DNA in this order (pipette up and down a few times) and incubate for a further 23 min.
5. Drip S++-/Fugene-DNA mix onto cells (80% density).

Cells can be harvested 3 days post RNAi or 2 days post-transfection (this is usually a good enough range to cover the half life of most proteins).