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RNAi SCREENING IN DROSOPHILA CELLS

Equipment/material needed:

1. Multidrop (ThermoElectron Corporation, #5840300)
2. Mithras Platereader LB940 (Berthold)
3. 384well LIA white plates (Greiner, #781073)
4. Alufoil seals (Costar, #6569)
5. DDAB (Sigma, D2779)
6. Schneider's Drosophila Medium (Invitrogen, #11720-034)
7. Celltiter Glow (Promega, #G7571)
8. VP186L (24-channel wand) (VP Scientific, #VP186L)

Assay: Viability assay
Plate type: LIA plate white (Greiner, #), 384 well
Cell type: Drosophila S2
dsRNA/well: 5µl of 50ng/µl (total per well: 250ng)

Preparing the plates

- Thaw the assay plates for approximately 1 hour at room temperature
- Spin down the plates for 1min at 1000rpm
- Wipe each plate with 70% ethanol (to avoid contaminations during stacking)
- Remove the lids of the plates
- Pipette the controls in the corresponding wells (5µl of 50ng/µl dsRNA)
- Cover the plate on top of each stack with a lid (again to avoid contaminations)

Day 1:

Prepare the multidrop

- Rinse the tube with:
  - 70% Ethanol
  - ddH20
  - serum free Schneider's Drosophila Medium (Invitrogen)

Preparation of the cell suspension

- Scrape the cells of each flask, pool and count them
- To set up the desired cell count (for example 2 mio cells per ml) spin down the required volume of cell suspension. *Note: to account for the dead volume of the multidrop an additional of 8ml is required.*

- Remove the supernatant and resupend the cell pellet in the calculated volume of serum-free medium (SFM)
- Now your cells are ready for screening! Immediately process with the next steps because your cells start starving in SFM…

Performing the assay

- Seed 15µl of your prepared cell suspension (in this example: 30.000 cells/well) with the Multidrop. *Note: adjust cell number depending on cell type and duration of assay*
- Starve the cells for 45-60min at room temperature
- Add 20µl serum containing Schneider's Drosophila and DDAB (stock: 0.4mg/ml, use 0.2µl per well, depending on the cell line this might differ) medium on top of the cells. *Note: DDAB is a cationic liposome reagent to improve the transfection of dsRNA into the cell.*
- Seal the plates with alufoil and spin them 1min at 1000rpm
- Incubate the plates at 25°C

Day 2-4:

- Incubation at 25°C

Day 5:

Readout

- Spin the plates 1min at 1000rpm
- Remove the seals
- Remove the medium from wells using a 24 channel wand
- Add 20µl Celltiter Glow per well (1:3 diluted in PBS)
- Incubate 15min at RT (protect plates from light!)
- Start readout using the Mithras plate reader (no filter)