Kerstin Spirohn Boutros Lab
August 2010

DUAL CHANNEL RNAi SCREENS 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. Effectene Kit (Qiagen, #301427)
6. Schneider's Drosophila Medium (Invitrogen, #11720-034)
7. VP186L (24-channel wand) (VP Scientific, #VP186L)
8. Dual-luciferase kits: various, consult specific protocols

Assay: Dual Channel Reporter Assay
Plate type: LIA plate white (Greiner), 384 well
Cell type: Drosophila SL2
dsRNA/well: 5µl of 50ng/µl (total per well: 250ng)

Day 1:

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 while stacking them later)
- Remove the lids of the plates
- Add controls to the corresponding wells (5µl of 50ng/µl dsRNA)
- Cover the plate on top of each stack with a lid (again to avoid contaminations)

Prepare the multidrop

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

Preperation of the cells

- Scrape the cells of each flask, pool and count them
- To set up the desired cell count (for example 2mio/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! Seed them immediately, because the cells start starving …

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*
- Starve the cells for 45-60min at room temperature
- Add 20µl serum containing Schneider's Drosophila medium (SCM) on top of the cells (use the same order of plates as used for pipetting of cells)
- Seal the plates with alufoil and spin them 1min at 1000rpm
- Store the plates at 25°C

Day 2:

- remove the alufoil from the plates
- rinse the multidrop tube

  - 70% Ethanol
  - ddH2O
  - serum containing medium

Preparation of the transfection mix:

- Plasmid transfection in 20µl SCM using Qiagen Effectene Kit
  → firefly expression plasmid, *Renilla* expression plasmid, pathway inducer (depending on the specific assay)

  Each assay requires the optimization of the transfection-procedure, here are some general guidelines:

  Add 100µL EC buffer per 1µg DNA
  Add Enhancer in 1:8 ratio (8µL per 1µg DNA), incubate 10min at RT
  Add Effectene in 1:10 ratio (10µL per 1µg DNA), incubate 15min at RT
  Add SCM to obtain a final volume of 20µl transfection mix per well

  (add extra volume for Multidrop dead volume).

- Add 20µL to each well, seal plate with aluminium foil
- Spin plates 1min at 1000rpm
- Incubate at 25°C

Day 3-X:

- Incubation at 25°C

Day X+1:

Dual-luciferase Readout

- Spin the plates 1min at 1000rpm
- Remove the seals
- Remove the medium from wells using a 24 channel wand
- Lyse cells in lysis buffer
- Add firefly and *Renilla* substrates according to the manufacturer's description