In Plate Transfection of hEK293 Cells by Lipofectamine 2000 (384-well format) for 3 replicate plates of arthropod and human receptors

Supplies:

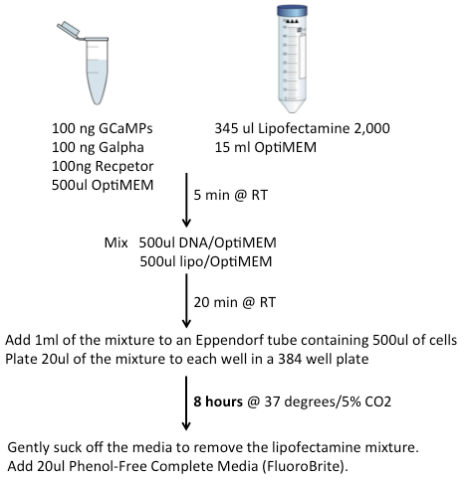
Lipofectamine 2000 – Invitrogen #11668-019
Opti-MEM – Invitrogen #51985-034
FluoroBrite DMEM Media – Invitrogen #A1896701
Greiner black 384 well plate - #781091
Greiner transparent 384 well plate - #784201
pGP_CMV_GCaMP6s_(Kan) - Addgene #277314.1040753
pME18s_Gα15_(Amp)
pME18s_NPYLR7_(Amp)

Day 1: Seeding cells

Seed cells for transfection in 75cm^2 flask. Aim for cells to be ≥80% confluent on day 2.

Day 2: Transfection

The 384 well plate will have single transfection with GCaMP6s, Gα15 in every well and receptors in relevant wells. Ensure that cells are ≥80% confluent in flask before transfection.



You can check on transfection efficiency by looking for GFP+ cells in the dish after several hours.

Keep transfected cells in the TC incubator overnight in phenol-free media so that they adhere well to the bottom of the 384 well plate. Want to end up plating ~5,000 cells/well (1x10^6 cells/ml has worked well although we can go up to 2x10^6 cells/ml comfortably).

Day 2: Reading

Check the cells in the transmitted light microscope @ HTSRC to ensure that they are healthy and adherent.

Prepare the compound plate (Greiner #784201) we need to fill column 23 with vehicle (Reading Buffer) and column 24 with our positive control (FMRFa3).
Need 15ul/well, 3x concentrated (the Hamamatsu will dispense 10ul of the compound/well which will contain 20ul of media).

Plate set up

TRANSFECTION PLATE 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G	str_217		str_217		str_217		No receptor		No receptor		No receptor		str-217		NO RECEPTOR	NO RECEPTOR	NO RECEPTOR	NO RECEPTOR		NPYLR7	NPYLR7	NPYLR7	NPYLR7	
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

LIGAND PLATE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H													Blank		Blank	agonist	DEET	DEET + agonist		Blank	agonist	DEET	DEET + agonist	
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								