**Plasmid screening for Golgi phenotype exp1 rev. 28.11.2022**

**DAY1 morning**:

Seed 1.2X104 Mz cell/well in two 96 image-plate

**DAY1 afternoon:**

**Control plasmid** PLASM\_00000247. pcDNA3.1 eGFP - CERT G243E

DNA transfection with MIRUS:

Plate 1:

|  |  |  |
| --- | --- | --- |
|  | **Id** | **Name** |
| 1 | PLASM\_00000137 | CERT WT-GFP |
| 2 | PLASM\_00000247 | pcDNA3.1 eGFP - CERT G243E |
| 3 | PLASM\_00000139 | CERT S135C-GFP |
| 4 | PLASM\_00000140 | CERT S132L-GFP |
| 5 | PLASM\_00000141 | CERT T251A-GFP |
| 6 | PLASM\_00000146 | CERT G243R-GFP |
| 7 | PLASM\_00000147 | CERT 166A-GFP |
| 8 | PLASM\_00000148 | CERT S138C-GFP |
| 9 | PLASM\_00000255 | pcDNA3.1 eGFP - CERT L330V |
| 10 | PLASM\_00000256 | pcDNA3.1 eGFP - CERT R366T |
| 11 | PLASM\_00000257 | pcDNA3.1 eGFP - CERT I381V |
| 12 | PLASM\_00000258 | pcDNA3.1 eGFP - CERT E424G |
|  |  |  |
|  | PLASM\_00000264 | pcDNA6.1 3HA - CSNK1G2 |
|  | PLASM\_00000265 | pcDNA6.1 3HA - PPM1L |

Plate 2:

|  |  |  |
| --- | --- | --- |
|  | **Id** | **Name** |
| 1 | PLASM\_00000137 | CERT WT-GFP |
| 2 | PLASM\_00000247 | pcDNA3.1 eGFP - CERT G243E |
| 13 | PLASM\_00000259 | pcDNA3.1 eGFP - CERT A449V |
| 14 | PLASM\_00000260 | pcDNA3.1 eGFP - CERT P500L |
| 15 | PLASM\_00000261 | pcDNA3.1 eGFP - CERT 497RVWPA501\_497PPPPP501 |
| 16 | PLASM\_00000262 | pcDNA3.1 eGFP - CERT P603A |
| 17 | PLASM\_00000263 | pcDNA3.1 eGFP - CERT A501C-A589C |
|  |  |  |
|  | PLASM\_00000264 | pcDNA6.1 3HA - CSNK1G2 |
|  | PLASM\_00000265 | pcDNA6.1 3HA - PPM1L |

Co-transfection CERT-wt /muts and PP or CSNK:

10:1 = Cert 0.025 g/well: PP/CSN 0.0025 g/well

0.0275 g/well of DNA

0.15 l/well of MIRUS

Up to 15l with Optimem

For each CERT plasmid I prepared the quantity for 20 samples in Eppendorf then I add 18.75ul in 3 well of working plate.



GFP-CERTwt or CERT mutants (100ng/ul) in 75ul



For empty - CN or PP plasmids I prepared the quantity for 120 samples in Eppendorf (447ul Optimem +3ul of plasmid 0.1ug/ul).

Prepare MIRUS diluted in Optimem for all the sample: 45ul MIRUS in 2205ul-tot 2250 ul (37.5ul/well)

Prepare a V bottom working plate for the transfection mix following the layout:





NB: CERT mutants name is the name of the plasmid.

During the incubation time change the plate medium adding 100l of medium/well.

After 20 min of incubation and added the Fluoro-Brite medium (193.75 up 250ul in each dilution) and dispense 50l/well following the layout

Plate Layout



**DAY2:**

Fix the plate after overnight transfection with 3%PFA (70l/well)

Wash ones with BioteK plate washer

Add 70l/well of GM130 (1:500) and anti HA (1:200) in BSA/SAP PBS

Incubate for 1h and half gently shaking

Wash ones with Biotek plate washer

Add 70l/well of Cy5anti-Mouse (1:200) Cy3 anti Rat (for anti-HA) and Hoechst (1:2000) in PBS

Incubate half an hour gently shaking

Wash twice with Biotek plate washer

Image with 40Xplan-Apo widefield

49 frame/well

Plate ID:

Plate1 65420

Plate2 65422