⇒ Stable Cell Line Project

→ Goal: - > 95% cells express product of plasmid construct
- Expression will be verified biochemically & optically

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→ Subgoal: Use antibotic resistance as selection marker for cells that
* * stably express GFP plasmid
- Find optimal conc. of antibotic (Geneticin: G418, Life Technologies)
- Find optimal/appropriate plating density of cells

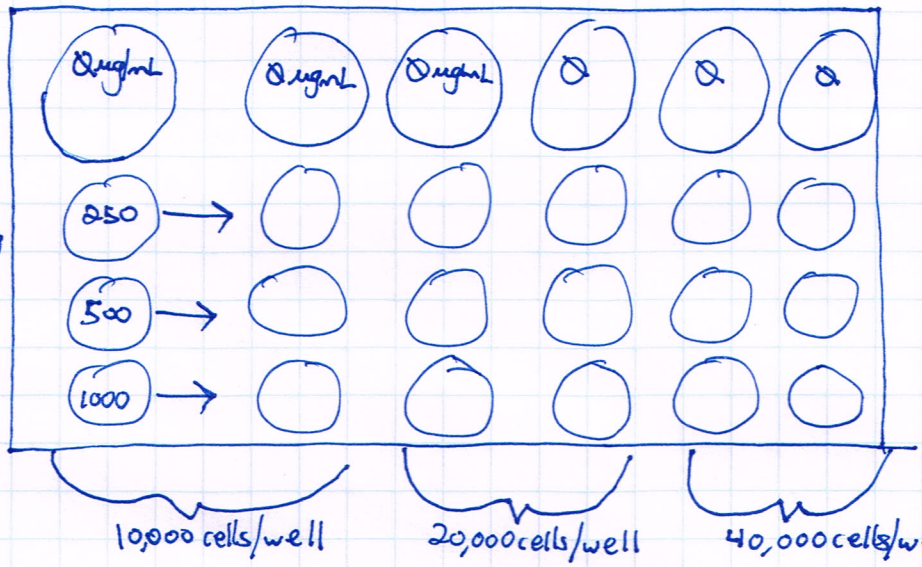
→ Procedure:
- Transfect normally in standard media
- 24 hrs. post-plating replace w/ Geneticin-containing media
conc. range from 0 ug/mL to 1000 ug/mL : 0, 250, 500, 1000 ug/mL
- Media change every day, qualitatively note % dead cells
- Run exp. at least 7 and potentially 14 days.

note HERG-HEK cell line is maintained @ 400 ug/mL

Set-up/layout:

→ 24-well plate w/ duplicates

→ variables:
cell plating density
geneticin conc.



Have 1 million cells/mL in media. Each well contains/holds 500 uL total volume.

~~(1,000,000 cells)(1 mL) =~~

$$\left(\frac{1{,}000{,}000\ \text{cells}}{\text{mL}}\right)(V_1) = (10{,}000\ \text{cells})(0.5\ \text{mL})$$

$\frac{1{,}000{,}000\ \text{cells}}{\text{mL}}$ & need 10,000 cells

∴ need 10 uL of stock cell suspension + 490 uL media for 500 uL total.

⇒ 10k: 10 uL cell stock + 490 uL media
20k: 20 uL cell stock + 480 uL media
40k: 40 uL cell stock + 460 uL media