

Using genetic barcodes to explore functional heterogeneity in paediatric Burkitt lymphoma

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1. Barcoding cells

- Make a 4×10^5 / ml solution (2ml for each cell line)
- Add 0.5ml of the cell mixture to 4 wells
- Add relevant virus to the correct wells as follows
- Make the mixture as follows in eff

Well	Virus	medi	apoly - D-lysine	1000ul / HEPES	25ul
	volume		stock	stock	
A 1	250ul	250ul	5ul		25ul
A 2	150ul	350ul	5ul		25ul
A 3	50ul	450ul	5ul		25ul
A 4	0	500ul	5ul		25ul

- Centrifuge the cells at 1250 x g for 1.5 mins
- Use p1000 to gently resuspend in 2ml media and transfer to feeder plate
 - important to use microscope to check all / most cells are attached
- 4 hours later transfer cells to a 12 plate and add advanced media to each well
- Sort cells 48 hours later

IMPORTANT

- On the same day of transduction, set up a control well ready for expansion.

2. Sorting

- 30 minutes before sort, spin cells down & resuspend in 1ml media
- We want to sort 5 well each of 500, 1000 (1000 well plate wells)

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