WildSeq - Characterising cell pools to Create a Whitelist before in vivo studies

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0.1. Materials

- TransIT
- Plasmids (two packaging, one entry)
- Optimem
- GlutaMax
- HEK cells
- Heat inactivated FBS

0.2. Protocol

Do not use antibiotics in media.. And add g
(unless it has L-alanyl-L-glutamine dipepti

0.2.1. Day 1

- Seed HEK cells @ 4.5x10^{6} / 10cm dish late afternoon

0.2.2. Day 2

- Check density, if reasonable (between 80 and 95
  low wait till afternoon)
- Replace media on feeders with fresh medi
- Mix Opti-MEM + Transit 293, vortex and leave for 10 min @ RT es
  - 43ul Transit + 1.3ul Opti-Mem per T75
- Add plasmids (in table below) to the com
  and leave for 15 - 30 mins
- Add mixture dropwise and gently rock the
  place in an incubator
- Harvest virus 36-48 hours after and move

Lentivirus	10cm dish
Opi-mem	1ml
Transit293	33ul

Lentivirus	10cm dish
Pax.2	8.3ug
GagV MTR	2.8ug
lentiviral WildSeq library	11ug

1. Concentrating virus

Will concentrate with Lenti-X Concentrator

- Spin briefly @ 500g, then filter virus s flasks
- Mix 1 volume of Lenti-X concentrator to gentle inversion
  - Measure viral supernatant / 3, then ad
- Incubate mixture @ 4 °C for 30 minutes to overnight
- Centrifuge sample @ 1,500 x g for 45 min at 4 °C. After centrifuging pellet will be visible.
- Carefully remove supernatent, taking car supernatent can be removed with either a
- Gentley resuspend the pellet in 1/10th t complete DMEM, PBS, or TNE. The pellet c into suspension quickly
- Immediately titrate sample or store @ -70 °C in single-use aliquo

1.1. Results

This was successful. I replenished media on that with lenti-X concentrator for 3 hours

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Author: Chris Steel
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