
Pooled screen delivery methodology
Druggable library covering 1980 genes , 10,000 guides (5 sgRNAs/gene)
Lentiviral sgRNA + Cas9 RNP electroporation
Activate T cells 24–48 h before electroporation (αCD3/αCD28)
Puromycin selection
>70–80% viability post-electroporation
Primary Human CD8+ T cells
Isolation
Transduction
Electroporation
Edited T cells
TCR stimulation
Cas9
sgRNA Library
Lentiviral Pool
SLICE
4–6 gRNAs/gene + ~5% non-targeting + safe-harbor control(e.g. AAVS1) + essential-gene sentinels


invitrogen
Attune CytPix
CytKick Max

Dear Gratiela Gradisteanu,

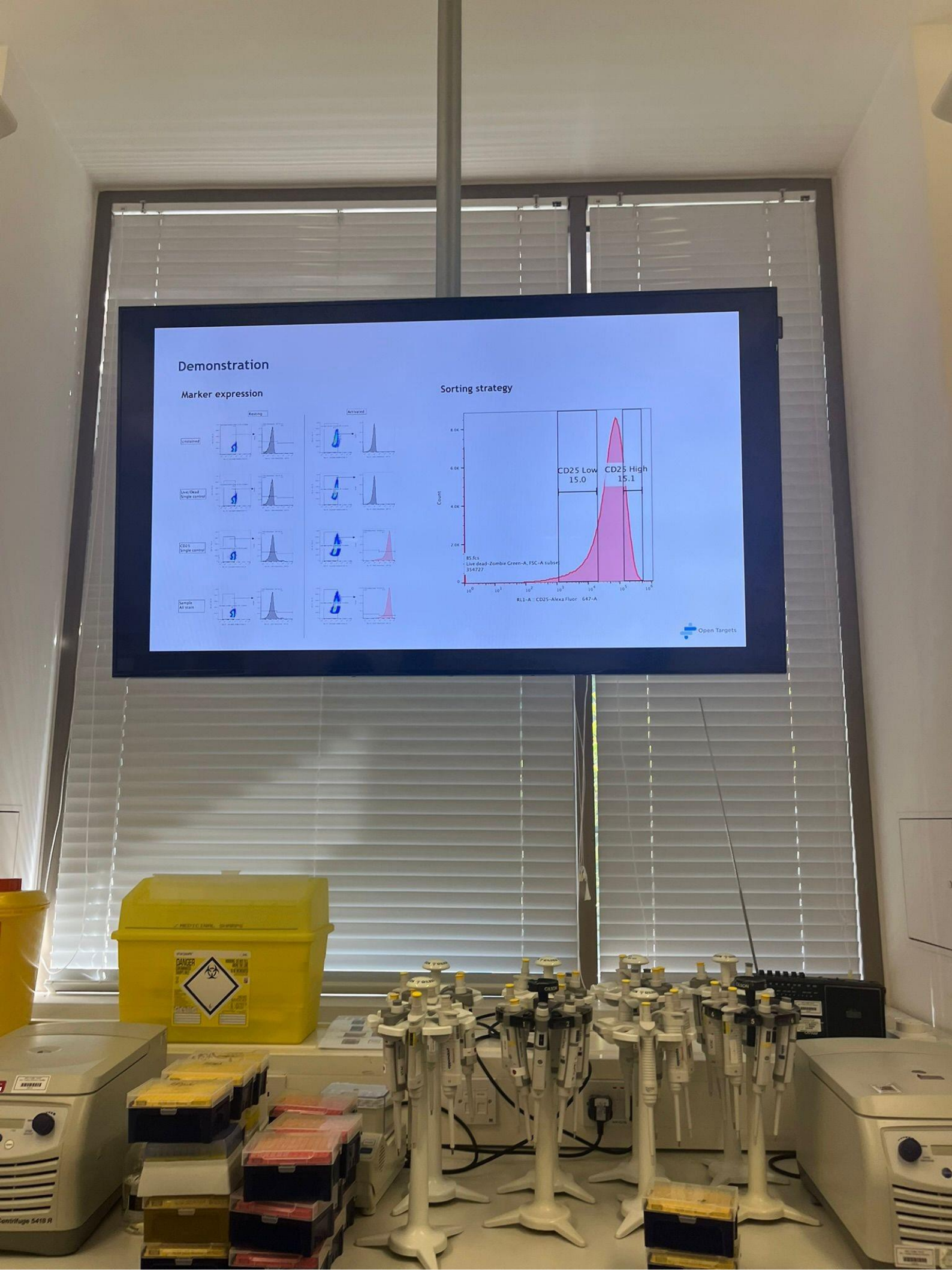
Wellcome Connecting Science: CRISPR Screening and Analysis

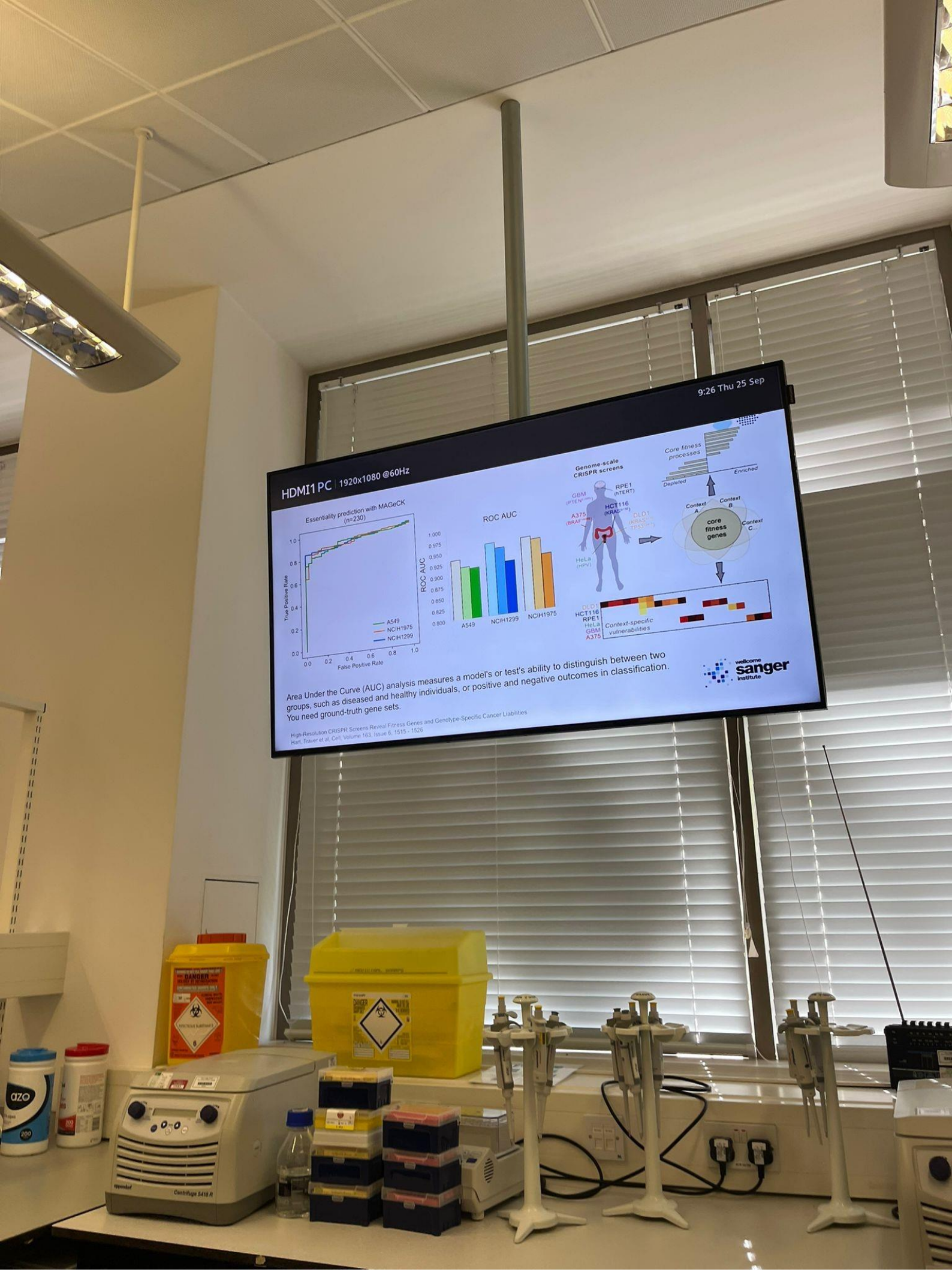
21/09/2025 – 26/09/2025

Congratulations! I am pleased to confirm that you have been selected to attend this event. The bioinformatics portion of the course will be held virtually on Monday 28 July - Friday 1 August 2025. Please be aware all times are British Summer Time, you should take this into account when planning your attendance if you are based in a different time zone. The laboratory course will be held onsite at the Wellcome Genome Campus from Sunday 21 - Friday 26 September 2025.

Acceptance

I am pleased to confirm you have been awarded a bursary towards your fees. This has been reflected in the invoice. Please note this is not transferable and we cannot offer any funding to cover travel expenses or other related costs.


Demonstration
Marker expression
Resting
Activated
Unstained
Live/Dead Single control
CD25 Single control
Sample All stain
Sorting strategy
CD25 Low 15.0
CD25 High 15.1
Count
B5.fcs
- Live dead-Zombie Green-A, FSC-A subset
154727
RL1-A :: CD25-Alexa Fluor 647-A
Open Targets


HDMI1 PC | 1920x1080 @60Hz
9:26 Thu 25 Sep
Essentiality prediction with MAGeCK (n=230)
True Positive Rate
False Positive Rate
A549
NCIH1975
NCIH1299
ROC AUC
ROC AUC
A549
NCIH1299
NCIH1975
Genome-scale CRISPR screens
RPE1 (hTERT)
HCT116
DLD1
HeLa (HPV)
A375
Core fitness processes
Depleted
Enriched
Context A
Context B
Context C
core fitness genes
HCT116
RPE1
HeLa
GBM
A375
Context-specific vulnerabilities
Area Under the Curve (AUC) analysis measures a model's or test's ability to distinguish between two groups, such as diseased and healthy individuals, or positive and negative outcomes in classification.
You need ground-truth gene sets.
High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities
Hart, Traver et al. Cell, Volume 163, Issue 6, 1515 - 1526
wellcome sanger institute


Quality control
1
Measure growth
Monitor OD600, growth rate, and biomass yield → avoid severe fitness penalty
2
Quantify SCFAs
GC-MS or HPLC to measure acetate, butyrate, propionate, lactate, ethanol. Expect butyrate ↑ 2–5×, ethanol ↓ 30–50%
3
Redox balance
If NADH accumulates (detected by redox dyes, NADH/NAD+ assays, or stunted growth), then:Reduce CRiSPRa strength on bcd/etfAB.R
4
Stability tests
Passage engine
strain over ≥1
generation
ensure tr
persiste