Enabling Precision Medicine

Phenomics Australia
Enabling precision medicine

NCRIS
National Research Infrastructure for Australia
An Australian Government Initiative

High Throughput Transcriptomics

Enabling discovery and driving translational medicine with high throughput technologies

VCFG Victorian Centre For Functional Genomics

Peter Mac Peter MacCallum Cancer Centre Victoria Australia

High Throughput Transcriptomics

Overview

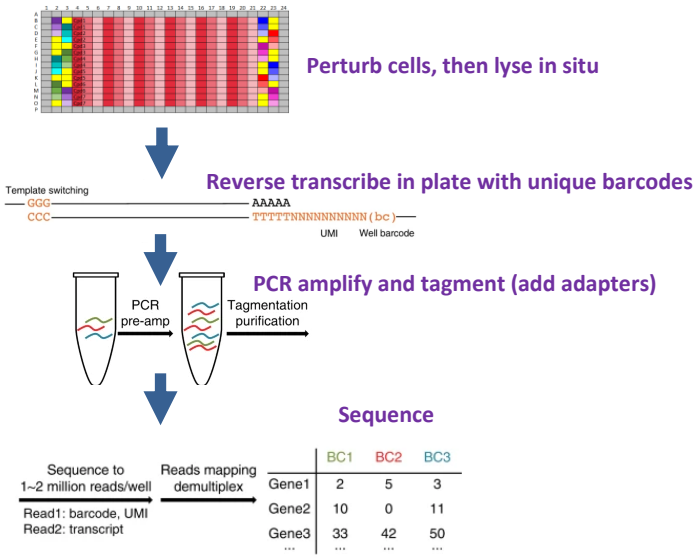
Multiplexed Analysis of cells (MAC-Seq) is a low-cost, versatile tool for high-throughput RNA-seq in a miniaturised plate format. MAC-seq allows you to take cells grown in 384 well format under virtually any setting, extract RNA and perform the sequencing reaction *in situ* using unique barcodes, then multiplex the samples in one sequencing run. Data analytics then deconvolute the barcodes to identify the transcript profiles of each well. This is a particularly powerful methodology when coupled with screening strategies using compounds or gene targeting in combination with high content imaging and cellular phenotyping to rapidly triage targets of similar mechanism of action. MAC-seq projects are run in collaboration with the Molecular Genomics Core.

MAC-seq applications

MAC-seq can be applied to cells cultured in suspension, in 2D and in 3D matrices.

- Drug and biomarker discovery
- Characterisation of patient derived materials (precious small samples, confirmation cells respond the same as origin, Precision medicine)
- Compound MOA (Mechanism of Action) validation
- Time point assays (tumour progression / drug resistance evolution)
- CRISPR on/off-target validation
- Your own imagination – a powerful method that can be used in so many ways

MAC-seq methodology



Working in 384 well format means your experiments will be the most robust when automated. If you have already run a screen in the VCFG, then MAC-seq may be the secondary or tertiary analysis step to identify hits. Your experimental design will already be well thought out. If your cell model has not started in the VCFG we highly recommend having a conversation to establish the project parameters.

1- See VCFG team for experimental design
2- Adapt assay to 384 well format
3- Coordinate timing with VCFG
4- VCFG will coordinate timing with MGC
5- VCFG will manage the lysis and automated addition of barcode primers
6- MGC will perform QC and sequencing
7- Data QC and output will be discussed

How long does it take?

MAC-seq does require some optimisation, whether it be in 2D or 3D. We will need to ensure your assay is robust, that the time points collected are relevant and with sufficient cell numbers, and that your treatments haven't resulted in totally dead cells. It is highly recommended to always run a parallel plate that couples another experimental readout in parallel, even if it is just measuring cell viability or taking a single image as a future reference. All this does take time and depending on the stage of your experimental design, it may take a while to optimise. Never-the-less, optimise first ensures success!
For the process itself, sequencing and data return, expect a turn around of ~4 weeks.

With Support from NCRIS through Phenomics Australia and The University of Melbourne

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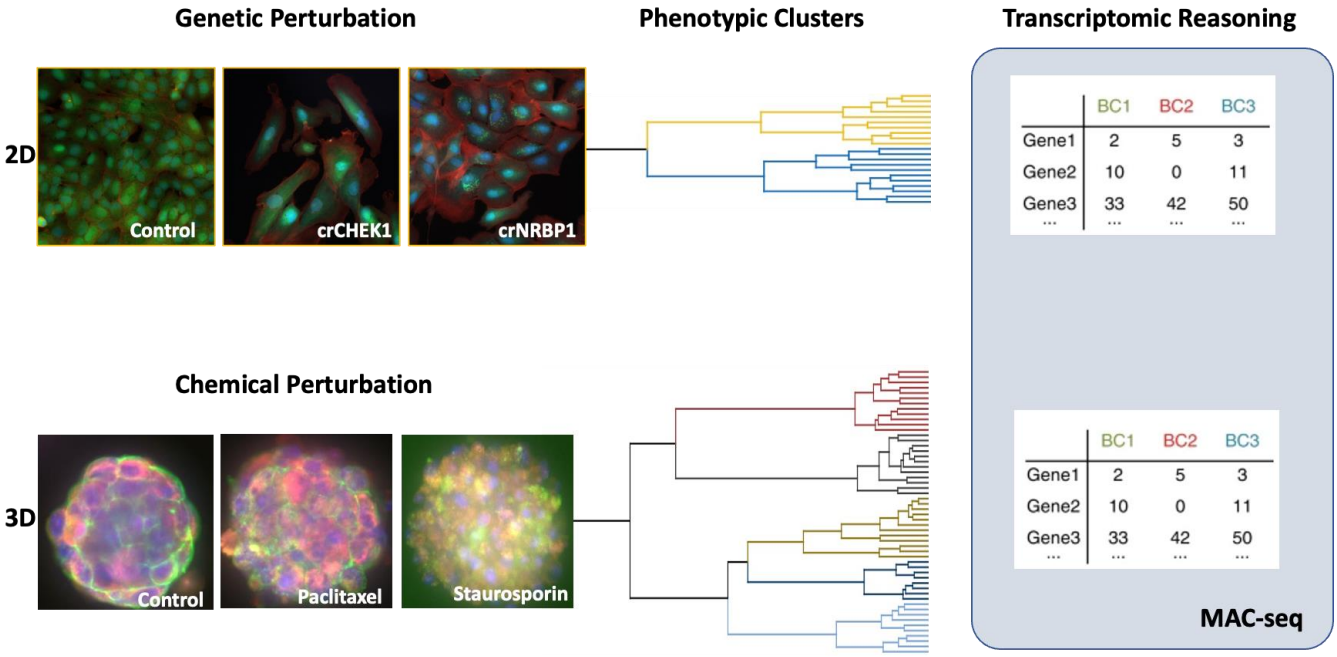
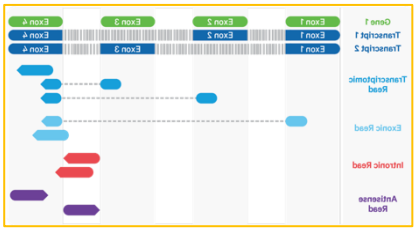
Coupling MAC-seq with high content imaging – 'Imaging Transcriptomics'

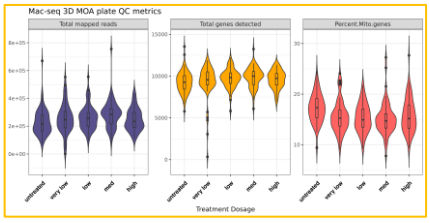


Use MAC-Seq to correlate high-content phenotypes with transcriptomes

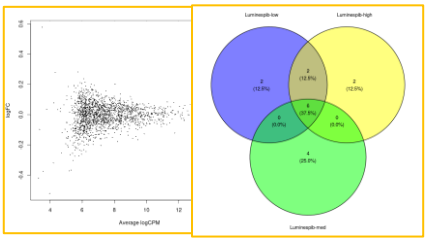
Image based high-content analysis can readily differentiate chemical or genetic perturbations in 2D or 3D cell models in the form of phenotypic clusters. However, imaging features that drive the cluster formation are difficult to interpret and the decision about which clusters to carry forward as hits is challenging. By integrating high-throughput transcriptomic data from the same sample, such high-content phenotypes can be explained by their RNA profiles. In addition, certain biological processes might not result in detectable phenotypic change. Having transcriptomic data will help researchers to capture and potentially identify the biological pathways regulating such processes.



Raw reads QC, read alignment



Read Count QC



Differential Gene expression analysis

Steps in the data analytics pipeline

Following sequencing the data is exported for raw read QC and read alignment. Once verified, read counts are extracted, QC'd and then analysis and data integration can begin.

Who will help me?

VCFG staff are with you every step of the way and will work with MGC to coordinate the delivery of your project. MGC will ensure all steps are QC'd and discussed with you prior to final sequencing.

The first pass data output will be a QC report and a read count per gene well.

Customised analysis will be discussed during the assay design phase and we will collaborate to help you build the best tools to answer your question. Image analytics will be supported by the VCFG. See Dr Mark Li, Dr Susanne Ramm (VCFG) and Stuart Craig, Dr Amanda Lee (MGC)

Li M, Yoannidis D, Ramm S, Luu J, Mir Arnau G, Semple T, Simpson KJ. Methods in Molecular Biology Cold Spring Harbor Press (B Jenkins Ed), 2023

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