

Platform for Advanced Cell Engineering

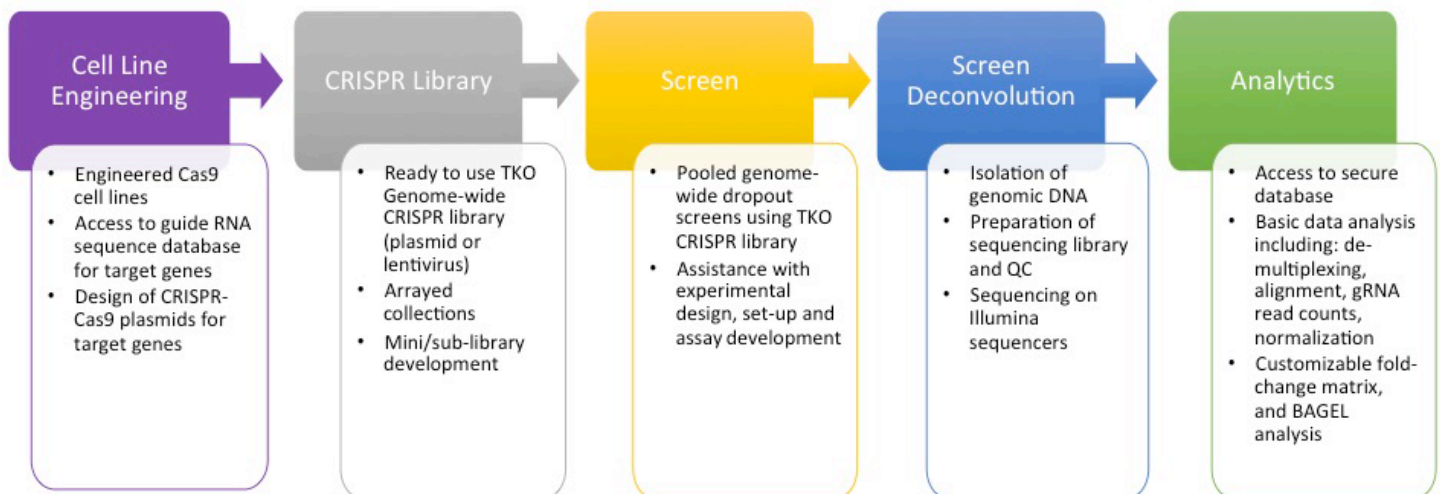
MISSION & OBJECTIVES

The underlying mission of the Platform for Advanced Cell Engineering (PACE) is to provide collaborative access to genetic reagents, technologies and data to the global scientific community in order to further our understanding of human genetics and ultimately the diseases that arise from genetic perturbations. As part of this mission, the Centre provides access to datasets from pooled genetic screens, allowing researchers from around the world to analyze the behavior of a given gene(s) of interest via short hairpin RNA (shRNA) dropout profiles. The datasets made available through the PACE website are searchable and freely downloadable. More recently, the Centre has advanced to using custom genome-scale CRISPR libraries for screens. The sequences for all guide CRISPR RNAs (gRNAs) in the 90,000-element primary library and 85,000-element supplemental library are also available for download, along with all of the screen datasets and related materials as published in Hart et al. *Cell*, 2015.

RESEARCH INTERESTS & EXPERTISE

As part of the RNAi consortium, Dr. Moffat helped to develop the first genome scale library of interfering shRNAs for use in human and mouse cells in 2006. With the wide adoption of genome-wide screening in the following years, the PACE (formerly called the Donnelly-Princess Margaret Screening Centre or DPSC, and before that the COLT platform) leveraged this technology to develop the first comprehensive platform for functional genetic screens and associated data analysis pipeline [Ketela et al., *BMC Genomics*, 2011; Koh et al., *NAR*, 2011].

CRISPR-Cas9 genome editing systems have gained popularity over the last 2 years and is now being utilized by many researchers around the world to undertake loss-of-function studies in mammalian cells. The Moffat lab has created the Toronto Knockout genome-scale CRISPR guide RNA library or TKO library for short. Using data from small, targeted CRISPR screens combined with bioinformatics approaches, the TKO was engineered for high specificity and minimal off-target effects. The TKO performs better in pooled genome-wide dropout screens (based on precision-recall metrics [Hart et al., *Cell*, 2015]) and will be invaluable for interrogating context-dependent fitness genes and synthetic lethal interactions. The platform is currently working on developing second-generation CRISPR systems, that have proven to be more efficient than first generation libraries and more cost-effective, as they are smaller in size.



UNIQUE CAPABILITIES

1) Toronto Knockout Lentiviral CRISPR Library

As part of an effort to generate next-generation genetic screening tools, the PACE has been integral in collaborating to create an ultracomplex genome-scale CRISPR guide RNA library to facilitate high-resolution functional genomics. Version 1.0 of the Toronto Knockout (TKO) CRISPR library consists of an ~90,000 element primary library and ~85,000 element supplemental library of CRISPR guide RNAs targeting all human coding genes and can be applied for systematic identification of essential genes in human cells [Hart et al. *Cell*, 2015; <http://tko.ccb.utoronto.ca/>]. Based on initial screening and analysis we created TKO v3.0, which has a smaller number of guides with higher specificity and editing efficiency and reduction in cost due to the library being smaller.

2) Cell Line Engineering and Screening Assay Development

The PACE has generated a collection of cancer cell lines stably expressing Cas9, as well as vectors for efficient cloning of gRNAs. We have also designed an open-access database of validated guide sequences for gene targeting as well as a secure database for screening analytics. We can provide assistance with screening design and assay development.

3) Lentiviral shRNA library

The PACE facility houses The RNAi Consortium collections and provides access to the library of ~240,000 unique lentiviral-based shRNAs targeting ~19,000 genes from each of the human and mouse genomes through collaboration. The PACE helped to develop the largest profile of essential genes across a set of three cancer types [Marcotte et al. *Cancer Discovery*, 2012] and the first negative genetic interaction network in isogenic cancer cell lines [Vizeacoumar et al, *Mol Sys Biol*, 2013]. In addition, the Moffat lab has developed novel analytical approaches and gold standards for genome-wide pooled lentiviral RNAi screening and analysis [Hart et al, *Mol Sys Biol*, 2014]. TRC collections are available in both pooled and arrayed formats.

4) Lentiviral Barcode Library

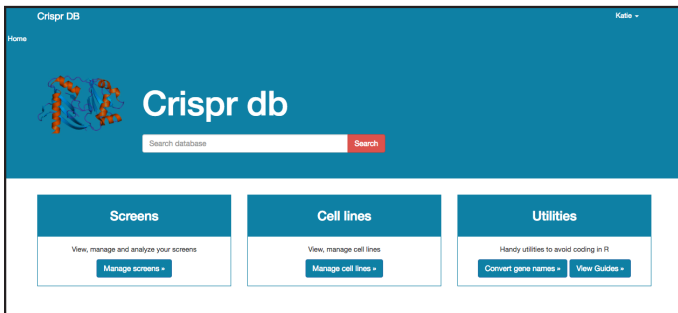
In order to enable the monitoring of clonal dynamics from tumors to treatments, the PACE has helped to construct a lentivirally-delivered barcode library that confers a unique molecular barcode to each successfully transduced cell. By marking cell populations with stable, genomically integrated barcodes that are heritable through cell divisions, one can quantify the complexity of cell populations expanded over time, infer the incidence of population bottlenecks, and determine the approximate number of cells that founded a population.

All of these molecular tools are made available by the Centre on a collaborative and cost-recovery basis along with standard protocols to employ the tools in various contexts.

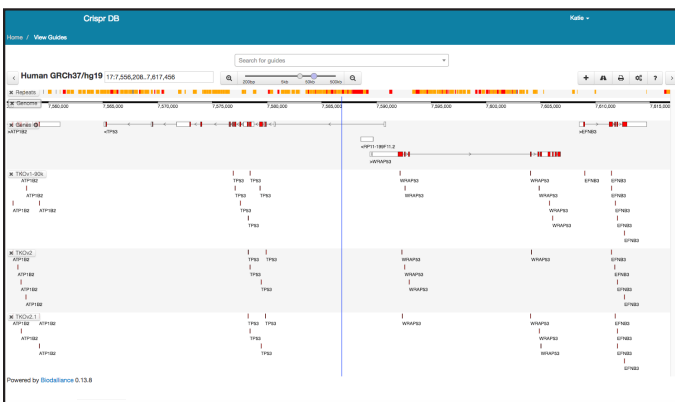
INFRASTRUCTURE & EQUIPMENT

Originally integrated within the Moffat lab on the 8th floor of the Donnelly Centre, the facility expanded to its own dedicated location on the Donnelly Centre's 3rd floor with funding from the Canada Foundation for Innovation (CFI) to enhance functional genetic screening capacity. In addition to developing and providing access to the molecular tools required for functional genetic screens, the PACE provides researchers with access to data analysis pipelines and expertise for screen deconvolution. Functional genomics, proteomics, and bioimaging projects generate huge amounts of biological data, which require substantial computing power for both data analysis and storage. The "bioinformatics core" at the Donnelly Centre supports the infrastructure required to manage the data from genome-scale screens, which can be accessed by collaborators through a web portal.

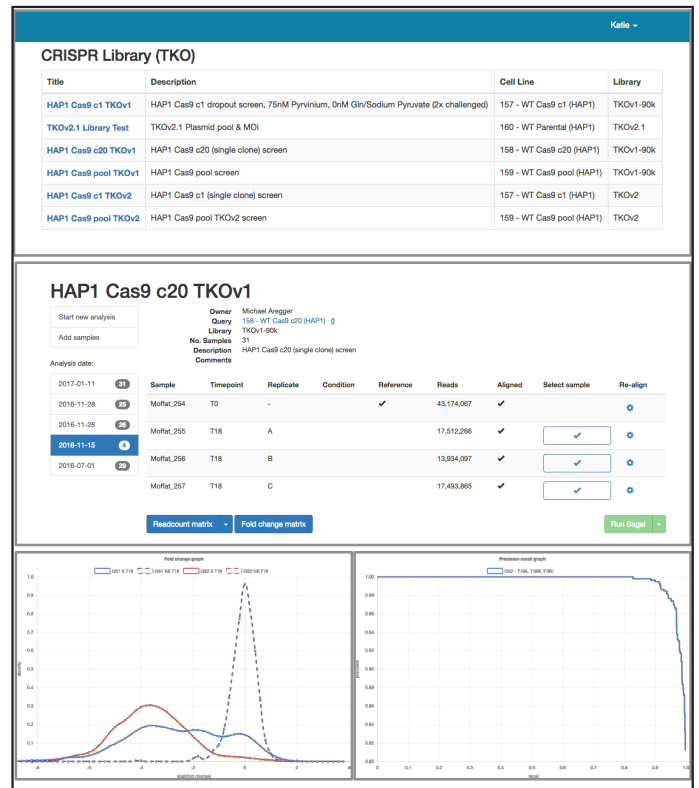
Secure Database Access



Visualized guide-RNA portal



Analytics



Retrieve raw or processed data
 Customize your BAGEL analysis

Assess screen quality using PR curves
 Downloadable Bayes Factor scores

SERVICES

With the current core team, the PACE carries out genetic screens utilizing the latest CRISPR TKO libraries and targeted gRNAs. Running at full capacity, the PACE performs five pooled CRISPR screens per month. This includes cell line infection, outgrowth and gDNA preparation. The PACE is focused on developing and implementing cell screening tools and methodologies for drug target and biomarker discovery. This includes off-the-shelf pooled RNA interference and CRISPR libraries, custom library construction, generation of “query cell lines”, next generation sequencing of samples from high-throughput forward genetic screens, data access portal, and sophisticated analytical tools for forward genetic screens.

Service	Description
Existing CRISPR Reagents	
Guide-RNA portal/database	Access to gRNA sequences based on target gene; access to database of screening results
Cas9 cell lines	Collection of cell lines engineered to stably express Cas9 nuclease (published in Hart et al., 2015)
CRISPR-related plasmids	Cas9 nuclease expression plasmids; control constructs
CRISPR libraries	TKOv1 180K gRNA library (virus pool format) TKOv2 70K gRNA library TKOv2.1 71K gRNA library TKO_mouse 71K gRNA library
Lentivirus production	Production of validated lentivirus for specific gRNAs and related constructs (purified, titred virus)
New CRISPR Reagents	
CRISPR library design & construction	Genome scale and mini-library [up to 10000 guides]
CRISPR clones	Individual and arrayed clones for targeted guides
Lentivirus production	Pooled library virus and individual guide virus production and validation
Cell line generation	Knockout and Cas9-expressing cell line generation
RNAi Reagents	
TRC Collection	Arrayed and pooled lentiviral shRNA constructs (provided as glycerol stock, plasmid or virus)
ORF Reagents	
ORFeome collection	Human ORFeome 8.1 (entry clone collection and lentiviral expression collection; provided as glycerol stock)
TCAC collection	Origene TrueClone expression constructs (mouse and human ORFs; provided as glycerol stock)

CONTACT INFORMATION

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