Next generation CRISPR screens in human cells

Myllia Biotechnology, previously known as Aelian Biotechnology, combines CRISPR screening with single-cell RNA sequencing, leveraging two transformative technologies to enable genetic screening for complex phenotypes.

A paradigm for next generation CRISPR screens

We utilize the CROP-Seq (for "CRISPR droplet sequencing") workflow, developed by Myllia´s co-founder Christoph Bock, to map the impact of thousands of genetic perturbations on the global transcriptome at single-cell resolution, thus effectively establishing a paradigm for next generation CRISPR screens. Our powerful approach has broad applications in identifying novel drug targets, elucidating unknown mechanisms of actions of drugs and understanding genetic variants linked to disease risk.

End-to-end screening platform

Myllia has built an end-to-end genetic screening platform that includes a proprietary guide RNA design algorithm, superior cloning of guide RNA libraries, reliable manufacturing of lentiviral guide RNA libraries, performance of the actual CRISPR screen, single-cell isolation and library preparation, next generation sequencing and the ensuing bioinformatic analysis. We routinely conduct screens of hundreds of thousands of single cells in one experiment and intend to scale the technology even further.

How we can help

RNA sequencing is widely used to profile drug responses and disease states. In many cases, such drug or disease signatures are not sufficient to decipher the underlying mechanisms and pathways. Truly understanding cellular responses requires a functional perturbation of the system. At Myllia, we utilize CRISPR screening as a discovery engine to dissect disease mechanisms and to deconvolve drug responses at high throughput.

Target identification
Target identification is the first step of a drug discovery campaign and begins with a screen identifying possible 'druggable' targets and their role in the respective disease. Myllia's unique CROP-Seq screening technology in combination with the best available cellular models supports identification of the critical genes and pathways driving certain disease states.

Mechanism of action of drugs
Understanding how drugs act in the complex environment of a cell remains one of the critical aspects of drug discovery and development. CROP-Seq delivers transcriptional profiles associated with drug action and indicates which genes impact the drug profile, thus providing unique insights into its mechanism of action. It also uncovers genes that modify drug responses, thus paving the way for combination therapy.

Variant-to-gene mapping
Genome-wide association studies have identified thousands of genetic variants that are linked to disease. Unfortunately, many of these loci lie in non-coding regions of the genome. Pinpointing the gene(s) whose expression is regulated by these regions would elucidate novel drug targets that are causally linked to disease. Myllia has built a CRISPR interference platform that can map disease-associated variants to genes in an unbiased fashion.

For additional information, please get in touch.

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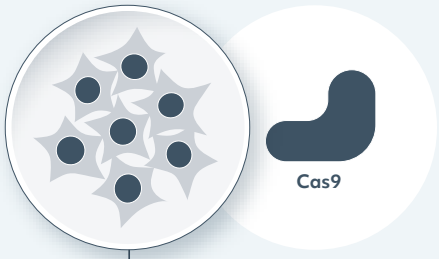
CRISPR screening

CRISPR screening has revolutionized the unbiased annotation of gene function, but most screens done so far have been confined to rather simplistic read-outs (usually life/death of the target cells). Combining CRISPR perturbation with single-cell sequencing allows researchers to assess much more complex phenotypes, thus effectively broadening the scope of these screens. At Myllia, we use the CROP-Seq technology to perturb cells with CRISPR and profile transcriptional outcomes by RNA sequencing at single-cell resolution. Importantly, our technology is applicable across a wide range of cell types including primary cells. The latter include primary human T cells which are of great interest for the discovery of novel targets in immuno-oncology.

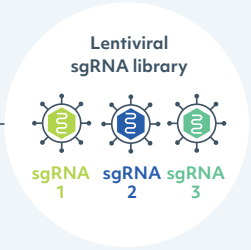
CAS9-EXPRESSING CELL LINE

Every CRISPR screen starts in a cell line that harbors Cas9.



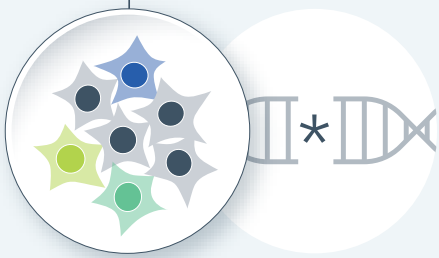
LENTIVIRAL GUIDE RNA LIBRARY

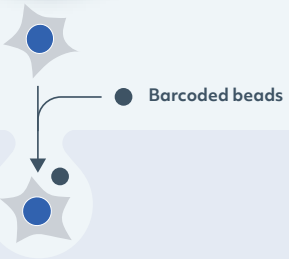
Cells are infected with a pooled lentiviral single-guide RNA (sgRNA) library.



CRISPR PERTURBATIONS

Following perturbation with Cas9 and a suitable guide RNA, every single cell in the pool will carry a knockout for a different gene.





Single-cell RNA sequencing

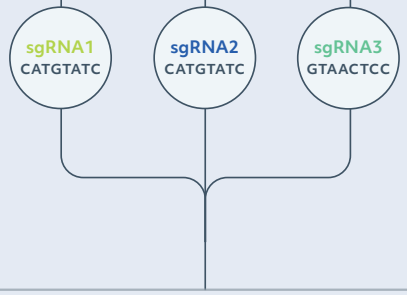
The CROP-Seq ("CRISPR droplet sequencing") technology measures transcriptome responses to CRISPR perturbation. It offers the flexibility of arrayed CRISPR screens at the scale of pooled CRISPR screens, thus providing a synergy of the two widely popular screening paradigms.

SINGLE CELL LIBRARY PREPARATION

Each single cell is then encapsulated in a lipid droplet together with a barcoded bead. Reverse transcription occurs on the surface of the bead, thus creating a unique transcriptomic fingerprint for each cell.

GUIDE RNA MAPPING

Mapping of the guide RNAs will connect each single-cell transcriptome to the guide RNA perturbation that caused the transcriptomic phenotype.



NGS AND BIOINFORMATIC ANALYSES

Single-cell sequencing datasets are typically large and complex. We are routinely analyzing these and are providing analyses that are customized to the needs of our clients.

Datlinger et al. (2017) in *Nature Methods*, Pooled CRISPR screening with single-cell transcriptome readout

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