CRISPR screens with single cell resolution Q&A with our 10x-perts

With Chromium Single Cell CRISPR Screening, researchers can massively scale functional genomics workflows to profile perturbations across hundreds of different genes and detect single-guide RNA (sgRNA) with directly linked gene expression phenotypes. We've fielded a number of queries from scientists getting started with single cell analysis of CRISPR screens.

Understanding the System

Why are there two capture sequences, and which integration site do you recommend?
Two capture sequences are provided on Chromium Next GEM Single Cell 3' v3.1 Gel Beads in order to provide the end user experimental flexibility. Both capture sequences have been shown to perform well, and in controlled experimental conditions, provide very similar results.

Using gene expression analysis, I have identified a list of genes that are differentially expressed in cells at various developmental stages. How can I use Single Cell CRISPR Screening to validate these genes?
Starting with your list of differentially expressed genes, identify sgRNAs that target each gene of interest. The number of genes and guides for your experiment can be titrated based on parameters such as the number of cells per guide and targeted number of cells to recover. Next, obtain either a commercial lentiviral packaged library, or purchase the vectors and sgRNAs to perform cloning yourself and produce packaged lentivirus for transducing your cells. There are several ways to design a developmental stage–specific experiment. One option is to produce multiple distinct sgRNA pools that target genes active at different developmental periods, and transduce cells at different developmental stages with the corresponding guide pool. Another option is to create a single sgRNA pool that includes all genes of interest across developmental stages, and transduce cells at an early stage, observing how this affects development. Alternatively, a single sgRNA pool could be used to transduce cells at different developmental time points, testing to see if only the genes shown to be required during a specific stage have an effect on the cell transcriptome, or if novel gene functions can be identified. The broad array of experimental design possibilities makes Chromium Single Cell CRISPR Screening a powerful tool for dissecting developmental pathways in a high-throughput manner.

Is it possible to use one of the two capture sequences for CRISPR and the other for surface protein detection on the same cells?
10x Genomics does not currently support combining the CRISPR and Cell Surface Protein workflows. However, there is no technical limitation that would necessarily prevent this from working.

If I have only a few genes (e.g. 2–3), is it worth using a single cell CRISPR screen?
Even if you're looking at just a few genes, Chromium Single Cell CRISPR Screening can provide highly valuable data. For a screen of two to three genes, a pool of approximately 5 to 15 guides would be required, depending on how many guides per gene and non-targeting guides are selected. This type of experiment would lend itself well to running a single channel on a 10x Genomics microfluidic chip. For example, if you are looking at a homogeneous cell type, such as a cell line, you could target approximately 2,000 cells and obtain the 10x Genomics–recommended number of cells for each targeting and non-targeting guide. Perturbation effects across the two to three genes, as well as the impact of each individual guide, can be measured across the entire transcriptome at the single cell level.

Setting up the Experiment

What controls are ideal beyond the negative controls?
Beyond the non-targeting control guides, having multiple guides per target gene is recommended. To control for the effects and perturbation efficiencies of a given protospacer sequence, we recommend performing pilot experiments prior to starting large-scale screens, which would include using a smaller number of genes/guides and determining the best capture sequence/location for your specific experiment.

Do I need to do pre-validation work with the 10x Genomics guide RNA before running a full experiment on the Chromium Controller?
In general, no. 10x Genomics compatible guides have been shown to work in a variety of CRISPR systems, including CRISPRi, CRISPRa, and CRISPR cutting. If you would like to pre-validate the effectiveness of a 10x Genomics compatible guide, we suggest performing a pilot transduction using the guides of interest (including appropriate non-targeting control guides). A qPCR-based method could then be used as an orthogonal method to validate the perturbation (either upregulation or downregulation) of interest.

How can I test for gene knockdown/activation before performing Single Cell CRISPR Screening?
Analysis of Gene Expression Interference/Activation via qPCR: Total RNA can be extracted from transduced cells and used for the RT-qPCR experiments to quantify the level of gene expression interference/activation for the target gene of interest. Analysis of Gene Expression Interference via FACS: post transduction, a purified population of cells can be isolated using antibiotic selection and a relevant fluorescent marker, if one was expressed in the system, using flow cytometry.

How many cells do I need to recover, per targeting guide and per control guide(s)?
10x Genomics recommends recovering 100–200 cells per targeting guide to ensure enough statistical power for determining the significance of the perturbation. For non-targeting guide-containing cells, 10x Genomics recommends 500–1000 cells. These cells are critical for providing a baseline from which perturbations are calculated.

How many perturbations can I measure using a single 10x Genomics channel?
In general, a single microfluidic channel can test dozens of guides. For example, if a user wishes to load the maximum number of cells in a single channel (10,000), we recommend that 500–1,000 (i.e. 5–10%) of these cells be made up of non-targeting guide-containing cells and each targeting guide be represented by 100–200 cells (i.e. 1–2%). Using this setup, a single channel would allow for testing ~45–90 guides.

Is gene expression library read depth important, or does it play a factor in Single Cell CRISPR Screening?
Just as appropriate read depth is important for gene expression libraries, so too is read depth important for Single Cell CRISPR Screening. When determining the effect of a given perturbation, the dynamic range of the target gene's expression level is a key factor. Since the UMI count for a given gene is dependent on the overall read depth of the gene expression library, the deeper that library is sequenced, the higher the UMI count will be (up to the point where the gene expression library is saturated and there is a low likelihood of seeing a new UMI with each new sequencing read). The higher the UMI count for a given gene, the higher the likelihood that a change in its expression will be detectable and significant.

What is the sensitivity level of the assay in its ability to capture gRNAs expressed/present at low levels?
The 10x Genomics integrated Single Cell CRISPR Screening workflow is the first commercially available method for direct measurement of sgRNA expression. As such, it provides many users with their first opportunity to interrogate sgRNA expression at the single cell level. During product development, we found that differing levels of sgRNA transcripts can be identified, depending on the context of the sgRNA, capture sequence, capture sequence location, CRISPR systems, etc. If we normalize read depth for reads coming from a 10x Genomics CRISPR library to 1,000 usable GUIDE reads per cell, we have seen anywhere from 20 to 600 UMIs per cell (representing a ~30-fold range in expression). We can also see minor variations in the expression levels of sgRNAs across the different protospacer sequences used. This variation can be due to multiple factors, including how the protospacer sequence affects expression of that sgRNA, as well as its capture efficiency when using Chromium Single Cell CRISPR Screening. Our internal testing, as well as data from collaborators, has shown that Single Cell CRISPR Screening is able to assign guides to cells and measure significant perturbations across the range of sgRNA expression mentioned above. We consider anything below 100 UMIs/cell "low" expression.

Contact us

For more information please visit 10xgenomics.com/products/single-cell-crispr-screening
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