Technical Note
CRISPR Detect

CRISPR Detect Compatibility Guidance

Introduction

Pooled single cell CRISPR screening pairs individual gene perturbations with rich whole transcriptome expression phenotypes. This approach has expanded understanding of gene function by quantifying changes in gene expression, regulatory networks, signaling pathways, and other complex signatures. CRISPR Detect brings the scalability of Evercode Whole Transcriptome technology to single cell CRISPR screening.

A typical single cell pooled CRISPR screening experiment begins by designing guide RNAs against target genes and choosing a screening methodology. Guide sequences are cloned into a plasmid vector containing the single guide RNA (sgRNA) scaffold. This plasmid pool is packaged into lentiviral vectors and transduced into the cell type of interest. Antibiotics or FACS are used to select cells expressing sgRNAs which are subsequently processed with Evercode Cell Fixation, Evercode Whole Transcriptome, and CRISPR Detect kits. After sequencing, analysis assigns sgRNAs to gene expression data at the single cell level (Figure 1). This note provides details on which methods and vectors are compatible with CRISPR Detect.

SINGLE CELL POOLED CRISPR SCREENS

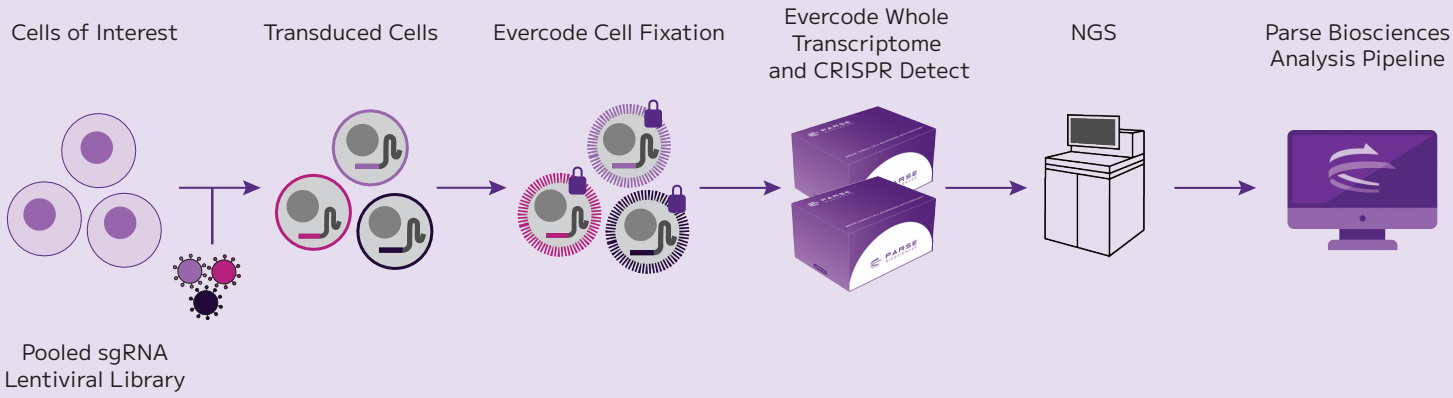

Figure 1. Single Cell CRISPR Screening Overview. Double stranded oligos with chosen sgRNA sequences are cloned into a vector, which is packaged into lentiviral particles. Target cells are transduced with the lentiviral sgRNA library, and cells expressing sgRNAs are selected with an antibiotic or fluorescent marker. sgRNA expressing cells are then fixed with Evercode Fixation and processed with Evercode Whole Transcriptome and CRISPR Detect for gene expression and sgRNA detection. After sequencing, the Parse analysis pipeline assigns sgRNAs and associated whole transcriptomes to individual cells.

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CROP-seq Single Cell CRISPR Screening

Background

An early method to pair sgRNA and gene expression at a single cell level relied on detection of a barcode on a polyadenylated transcript. This original Perturb-seq method requires complex cloning to match individual barcodes to sgRNAs. Researchers have found that the barcodes often become uncoupled from their sgRNA pair, complicating analysis. CROP-seq was developed to enable detection of the sgRNA sequence from a polyadenylated transcript rather than a barcode. Also developed to avoid barcodes, direct capture Perturb-seq captures the sgRNA using a reverse transcription (RT) primer annealing to its scaffold.

Mechanism

CROP-seq takes advantage of a feature of lentiviral integration to express both active sgRNAs and a longer polyadenylated transcript containing the sgRNA sequence. In CROP-seq, a human U6 (hU6) promoter and sgRNA are cloned in the 3′-long terminal repeat (LTR). During lentiviral integration, the 3′-LTR including the hU6-sgRNA sequence are duplicated in the 5′-LTR (Figure 2A and 2B). Consequently, three transcripts are expressed: two polymerase (Pol) III transcripts and a Pol II polyadenylated transcript (Figure 2C). The non-polyadenylated Pol III transcripts are processed into functional sgRNAs, and the polyadenylated Pol II transcript includes the hU6-sgRNA sequence. This transcript is captured in RT with an oligo-dT primer.

Advantages

CROP-seq avoids the limitations of the original Perturb-seq with a straightforward cloning strategy that analyzes the sgRNA sequence. Unlike direct capture Perturb-seq, it does not require modification of the sgRNA scaffold, which is useful in large CRISPR screens where it isn't feasible to optimize each sgRNA scaffold.

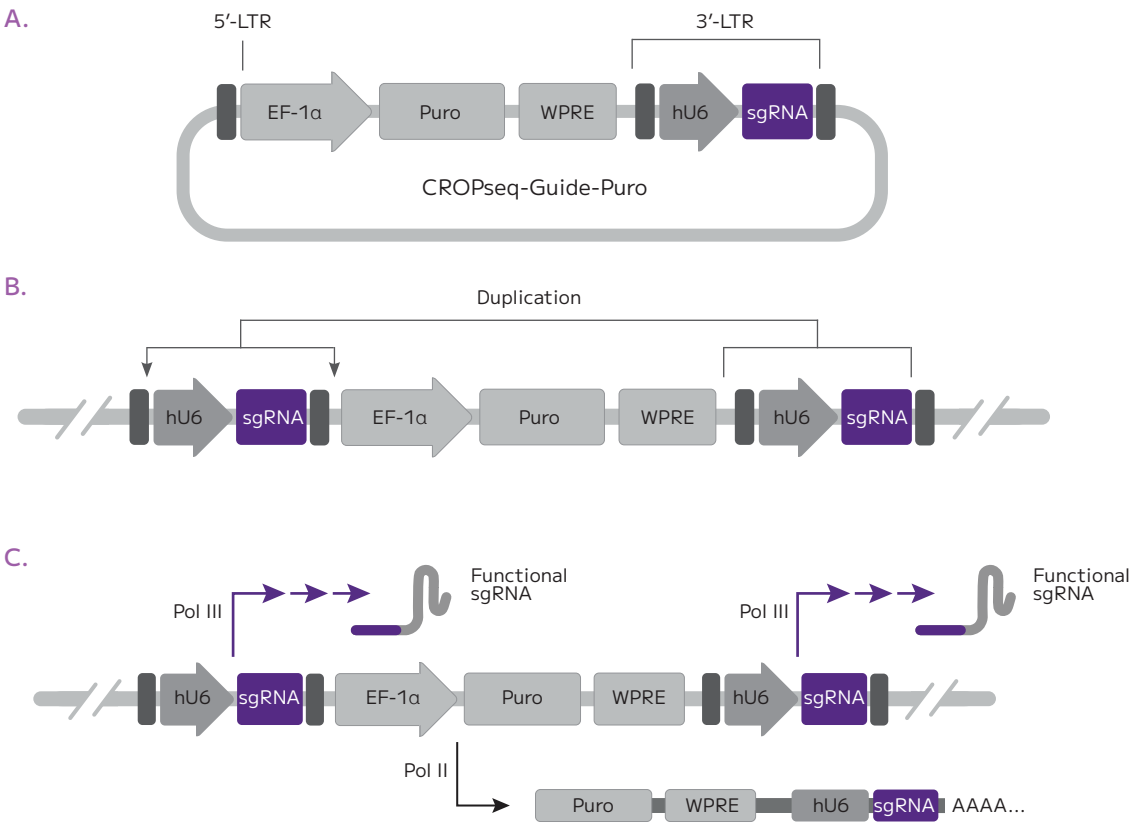

Figure 2. CROP-seq Method. There are a variety of vectors that use a similar method, but CROPseq-Guide-Puro is used as an example. (A) A simplified plasmid map highlights the position of the hU6-sgRNA cassette within the 3′-UTR. (B) After integration, the hU6-sgRNA cassette is duplicated. (C) The integrated vector expresses 3 different transcripts, including a Pol II polyadenylated transcript from EF-1α promoter that contains the sgRNA sequence.

CRISPR Detect

CRISPR Detect is compatible with CROP-seq and similar methods. Polyadenylated mRNA containing the sgRNA sequence is captured via oligo-dT priming during the first round of barcoding in Evercode Whole Transcriptome kits (Figure 3). The hU6-sgRNA sequence containing mRNA is enriched during cDNA amplification with a primer that anneals to the human U6 promoter. Amplified cDNA is then split between whole transcriptome library prep and CRISPR library prep. CRISPR PCR further enriches the sgRNA-containing mRNA with a semi-nested PCR using a hU6 specific primer. A sequencing ready CRISPR library is generated after the addition of adapters and indexes via PCR. After sequencing, transcripts and sgRNAs are assigned to cells using the combination of their cell barcodes.

Compatible Vectors

Key features of vectors compatible with CRISPR Detect are outlined in Table 1. Briefly, vectors must generate a polyadenylated version of the sgRNA expressed from a hU6 promoter.

Details of several vectors compatible with CRISPR Detect are outlined in Table 2. These include vectors with different methods of selection, including different antibiotic resistance markers and fluorescent proteins. The table includes vectors for applications beyond CRISPR knockout screening, such as CRISPR inactivation, CRISPR activation, and lineage tracing. It also includes a vector designed to enable CROP-seq screens with two sgRNAs per vector.

Length of the hU6-sgRNA cassette is constrained by decreased transduction efficiency, which makes Cas9 dual sgRNA screens incompatible with CROP-seq. Alternative Cas proteins with shorter sgRNA scaffolds can enable CROP-seq compatible dual guide screening, including the pRG212 vector that relies on Cas12a.

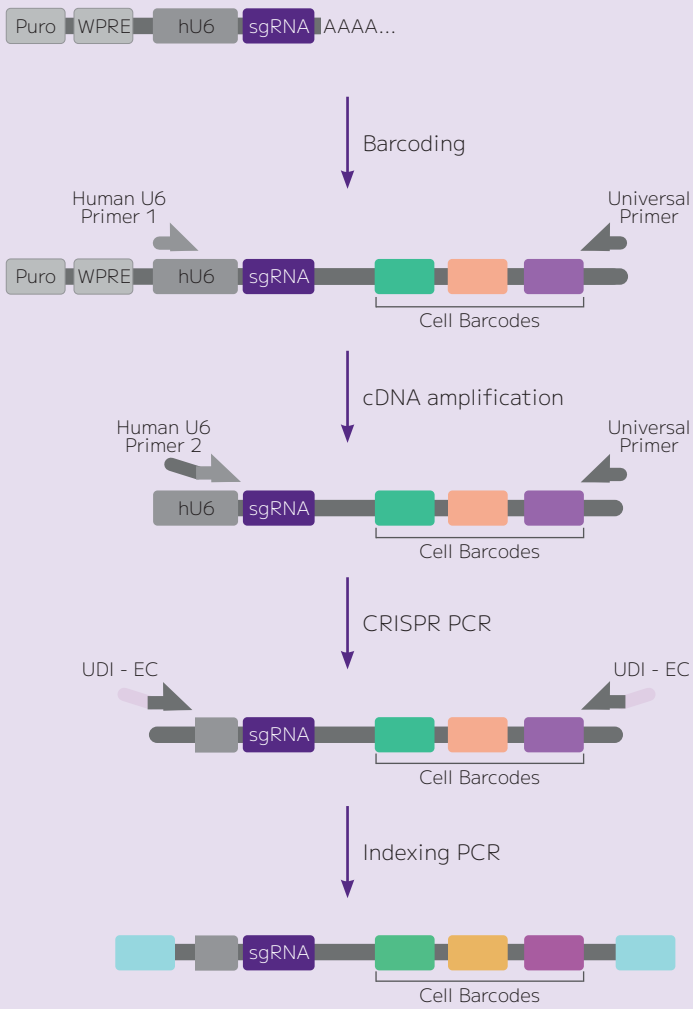

Figure 3. CRISPR Detect Mechanism. Outline of how CRISPR Detect captures sgRNA containing mRNA from CROP-seq vectors. Whole transcriptome library preparation is not shown.

Feature	Compatible
Vector type	CROP-seq
Reverse transcription primer	Oligo dT
sgRNA promoter	Human U6
Distance from Human U6 promoter to the polyA tail	~200-300 bp

Table 1. Features of Vectors Compatible with CRISPR Detect. Although most CROP-seq vectors are compatible with CRISPR Detect, these features provide more detail when assessing a particular vector.

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Vector	Selection	Other features	Addgene Number	Pubmed ID
CROPseq-Guide-Puro	Puromycin		86708	28099430
CROPseq-puro-v2	Puromycin	Modified sgRNA scaffold for increased Cas9 efficiency	127458	31626775
CROPseq-Guide-Zeo	Zeocin		127173	31626775
CROP-seq-opti	Puromycin	CRISPRi optimized backbone	106280	29457792
CROP-sgRNA-MS2	Puromycin or mCherry	sgRNA scaffold sequence contains two MS2	153457	32634384
CROPseq-Guide-EFS-SpCas9-P2A-EGFP	EGFP	EF-1α replaced with EFS promoter	99248	
EFS-EGFP-P2A-Neo-WPRE-U6-AsCas12aDR-AsCas12aDR (pRG212)	Neomycin or EGFP	Cas12a scaffold, enabling dual guide screeening	149722	32661245
Crop-Seq-BFP-WPRE-TS-hU6-BsmbI	BFP	Lineage tracing vector system	137993	35094325

Table 2. Compatible Vectors. A nonexhaustive list of CRISPR Detect compatible vectors with link to the original publication (if applicable) and the associated Addgene® Plasmid Number.

Incompatible Vectors

Because it relies on human U6 specific enrichment primers, CRISPR Detect is not compatible with vectors that express sgRNAs from a mouse U6. CRISPR Detect is not compatible with Perturb-seq vectors with barcodes or for direct capture.
If unsure of compatibility, our single cell experts can assess individual experiments.

Summary

CRISPR Detect and Evercode Whole Transcriptome kits enable CRISPR knockout, activation, and interference screens that can easily scale to millions of cells and hundreds of samples.

PRODUCT ORDERING INFORMATION

Product	Part Number
CRISPR Detect Guide RNA enrichment sufficient for 16 sublibraries	**CRS1010**
Evercode WT Mega v3 Up to 1,000,000 cells or nuclei and 96 samples	**ECWT3500**
Evercode WT v3 Up to 100,000 cells or nuclei and 48 samples	**ECWT3300**
Evercode Cell Fixation v3 Up to 12 samples	**ECFC3300**

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