ECLIPSE BIOINNOVATIONS

FLI-Seq

Fast Library Insert Sequencing Prep following CRISPR Screens

HIGHLIGHTS

Fewer PCR Reactions & Cycles

FLI-Seq enriches for sgRNA-containing sequences removing more than 99.8% of unwanted gDNA

Reproducible sgRNA results

High correlation between replicates with only 100-fold coverage

Less sequencing

Single end 50bp reads sufficient for analysis

Introduction

CRISPR screens are loss-of-function screens that are widely used to identify a small number of genes out of the whole genome responsible for a particular function or phenotype. After CRISPR screening, genomic DNA is harvested from the selected pool of cells and PCR is performed to isolate and identify the single guide RNA (sgRNA) sequences resulting from the screen. Currently, numerous PCR reactions with many cycles are necessary to do this and often researchers are met with failure due to the excess gDNA present.

Fast **L**ibrary **I**nsert Sequencing, or FLI-Seq, facilitates the efficient generation of sgRNA libraries from CRISPR screens by enriching for the relevant sgRNA-encoding gDNA regions. The FLI-Seq method generates libraries with far fewer PCR reactions and PCR cycles, thereby reducing PCR duplication and PCR artifacts. Optimized PCR amplification primers yield library fragments beginning at the sgRNA sequence thereby requiring very short read lengths and simplifying data analysis. FLI-Seq simply generates high quality sgRNA libraries with high technical reproducibility and requires less time, less starting material, and less reagents than other approaches for processing CRISPR screens.

FLI-Seq Workflow

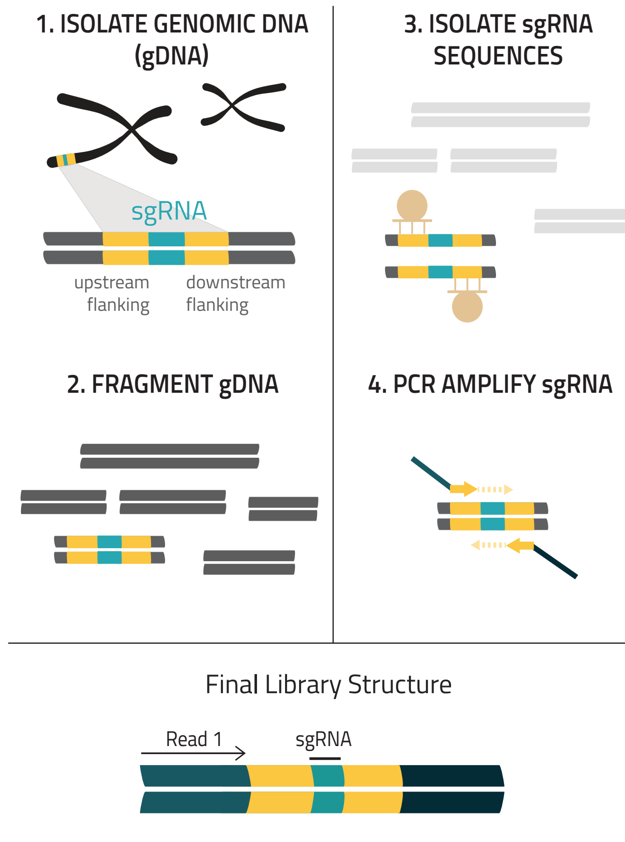

Figure 1. FLI-Seq library preparation is performed after collection of genomic DNA from the pool of cells selected during CRISPR screening. The gDNA is fragmented, the sgRNA sequences are enriched, and PCR is performed on the purified sequences. SE50 reads are sufficient to identify sgRNA sequences.

Specifications

Input Requirements	Up to 600 µg genomic DNA	
Sequencing Recommendations	Instrument	Illumina
	Sample Depth	3x the input cell count
	Run Parameters	SE50

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sgRNA libraries with fewer PCR reactions and PCR cycles

FLI-Seq precisely enriches for sgRNA-containing sequences within input gDNA thereby removing over 99.8% of unwanted gDNA going into PCR amplification. With sgRNA enrichment, fewer PCR cycles are needed compared to alternate methods, resulting in fewer PCR artifacts and PCR duplicates.

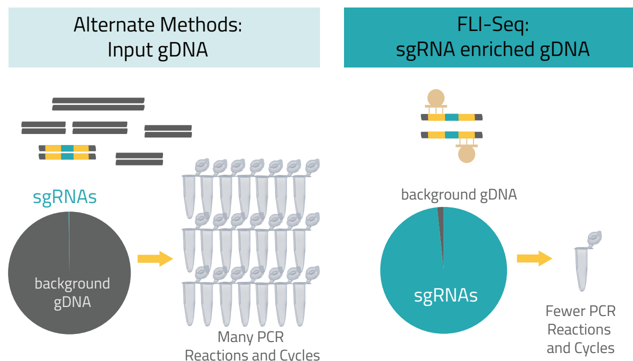

Figure 2. FLI-Seq removes 99.8% of unwanted background gDNA before PCR so you obtain your results with fewer PCR reactions and fewer cycles.

Kit Options

Kits can process up to 600 µg gDNA from screens using the following lentivirus delivery systems:

1. LentiGuide Puro
2. LentiCRISPR v2
3. LentiPool
4. Contact us for a custom kit for a different lentivirus delivery system

Reproducible sgRNA results at lower coverage

FLI-Seq libraries are high quality, showing highly reproducible results across replicate experiments at only 100-fold coverage (R=0.94 between technical replicates and R=0.87 between biological replicates). This high reproducibility in library generation allows one to perform library screens with less starting material (fewer cells, less media etc), and reduces the amount of sequence data and data analysis required to obtain results from a CRISPR screen.

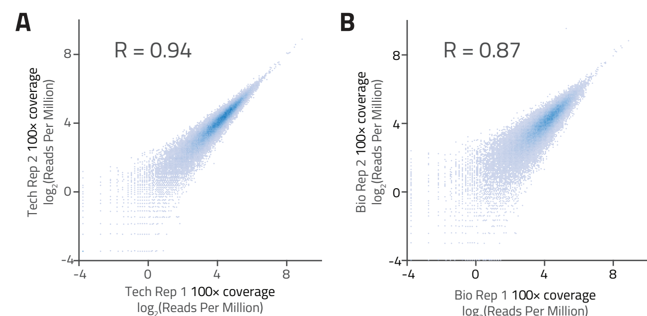

Figure 3. Correlation between libraries of both (**A**) technical and (**B**) biological replicates using an input material equivalent to 20 µg gDNA is incredibly high using the FLI-Seq kit.

References

EL Van Nostrand, SA Barnhill, AA Shishkin, DA Nelles, E Byeon, T Nguyen, YE Wong, NC Gianneschi, GW Yeo. *Unbiased identification of nanoparticle cell uptake mechanism via a genome-wide CRISPR/Cas9 knockout screen.* **bioRxiv** 2020.10.08.332510

Ordering information

More information about FLI-Seq library preparation kit online at www.eclipsebio.com or contact us at info@eclipsebio.com.

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