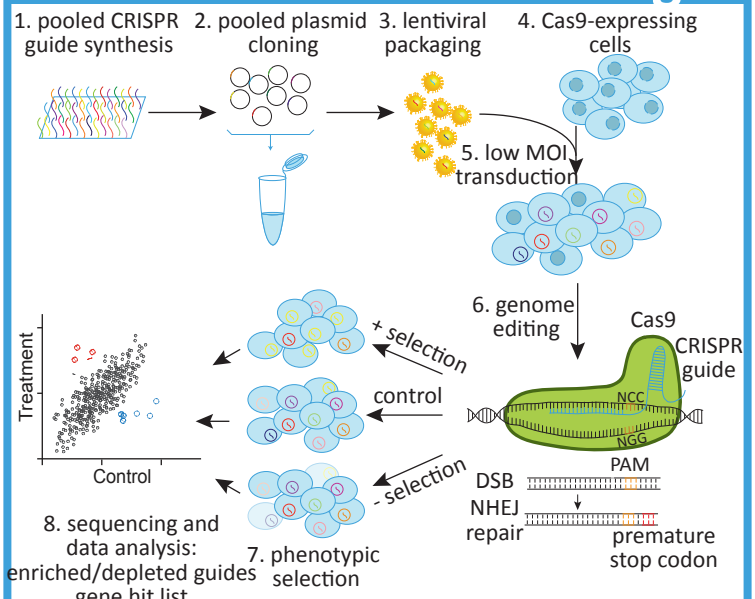
SciLifeLab

CRISPR-Cas9 screening at the CRISPR-based Functional Genomics facility

Abstract

The CRISPR-based Functional Genomics (CFG) facility is a SciLifeLab core facility offering pooled CRISPR/Cas9 screening. The CRISPR-Cas9 system allows targeted gene knockout, editing, or transcriptional modulation. One powerful application of this technology is in pooled screening, where thousands of genes can be targeted in parallel in a population of cells. This population is then enriched for a phenotype of interest, typically by growth advantage, drug selection, or cell sorting. Finally, enriched and depleted guide RNAs are determined by Next Generation Sequencing (NGS). All CRISPR guide libraries at CFG include a unique molecular identifier (UMI). This allows massively parallel lineage tracing and lineage dropout screening with improved reproducibility, precision and accuracy.
CFG supports pooled CRISPR-Cas9 screening from planning to data analysis. In addition to CRISPR loss of function knockout screening, CFG now offers CRISPR-i(nhibition) and a(ctivation), in which nuclease-dead dCas9 is fused to transcriptional effectors and targeted to elements of interest. CRISPR-i is particularly useful for targeting ncRNAs and for work in primary cells, while CRISPR-a allows gain-of- function screens. We also have a workflow where CRISPR-Cas9 screening is coupled to single cell transcriptomics (CRISPR-scRNASeq) and are developing in situ guide sequencing and imaging. Finally, we are setting up a CRISPR-X based method to direct saturating mutagenesis to elements of interest using CRISPR-Cas9 base-editing.

Pooled CRISPR screening



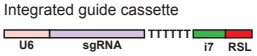
Unique Molecular Identifiers (UMIs)

Pooled Screen Confounders

Varying outcomes of editing
Experimental System
- p53 pathway status of cells
- Cell number
- Varying Cas9 expression
- Genetic heterogeneity

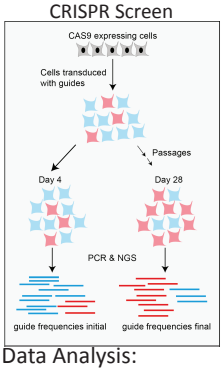
Couple guide to Random Sequence Label

Integrated guide cassette



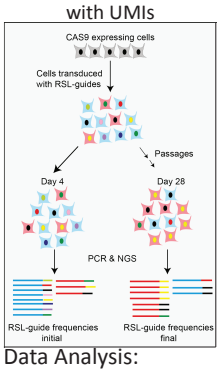
guide + RSL = UMI

Conventional Pooled CRISPR Screen



Data Analysis:
1. Total Count Analysis

Pooled CRISPR Screen with UMIs



Data Analysis:
1. Total Count Analysis
2. Lineage Dropout

UMIs enable massively parallel lineage tracing
UMIs increase precision and accuracy of screen, allowing use of fewer cells
UMIs support novel QC

Schmierer et al 2017

Genome editing in cell lines

From strategy design to delivery, validation and assistance in downstream selection of edited clones, Karolinska Genome Engineering Core Facility offers a range of applications: cellular model systems (knock-outs, knock-ins), reporter lines and more.

Build your screen

1. Assay

Survival
- \+ selection e.g. drug resistance
- \- selection e.g. drop-out screening

Cell Sorting (FACS)
- Reporter gene
- Antibody staining, e.g. differentiation state
- Cellular component staining

in vivo cell migration/activation/differentiation
- fluorescence-marked guide libraries allow tracking

Inducible Cas9 for temporal control

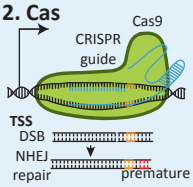
Cellular system

Species
Human ✔ Mouse ✔ Other- discuss!

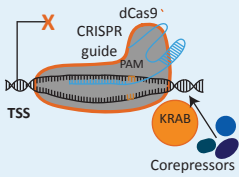
Cell type
- Cancer cell line- first choice = WT Cas9
- hTERT, many primary cells- DSB sensitivity, consider dCas9-effector

Longevity
- Immortal- two vectors
- Passages Limiting- one vector

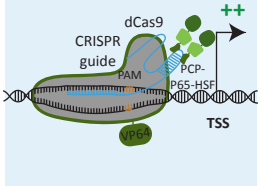
2. Cas



CRISPR knockout
WT Cas9
Loss of Function
Coding genes



CRISPRi(nterference)
dCas9-KRAB -> corepressors
Loss of Function
Coding or Noncoding elements



CRISPRa(ctivation)
dCas9-VP64
P65-HSF activator -> TRACR
Gain of Function
Coding or Noncoding elements

3. Guide Library

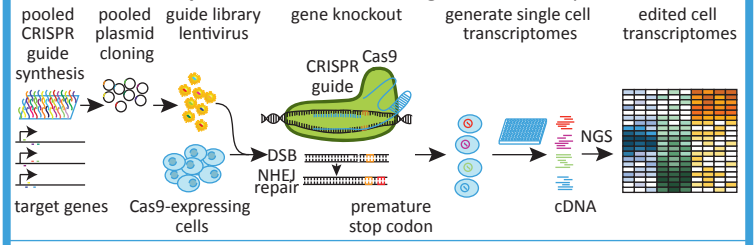
CRISPR knockout
Genome-wide: human/mouse
Chromatin/Epigenetic factors
Custom

CRISPR-i(nterference)
Genome-wide: human
Custom

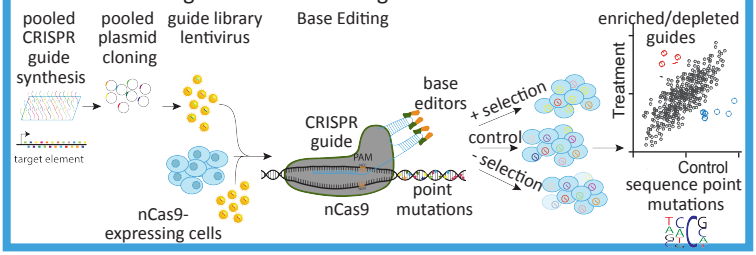
CRISPR-a(ctivation)
Genome-wide: human
Custom

All guide libraries include Unique Molecular Identifiers (UMIs)

CRISPR-scRNASeq: CRISPR screen with single cell transcriptome readout

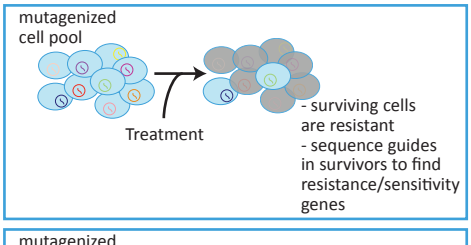


CRISPR-DIVA: sequence Diversification and Interrogation of VAriants targeted random mutagenesis



Workflow and Selection Examples

Cas9-expressing cell line generation
Pooled lentiviral CRISPR guide library
↓
low MOI transduction
↓
select for CRISPR guide integration
↓
culture of transduced cell pool
↓
assay-specific selection
↓
cell separation, *e.g.* -survival -cell sorting
↓
genomic DNA preparation, guide amplification, sequencing library preparation
↓
Next Generation Sequencing
↓
Data analysis -enriched/depleted guides -hit list



Performed by customer
Performed by CFG

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