

How CRISPRnano Works

Any sequencing data

ONT

Illumina

PacBio

Upload FASTQ files

Input Data and Results
Single or multiple clones
Multiple genotypes
Off-Target

Step 1: Input Data

GENERAL ANALYZER

PRIME EDITING ANALYZER

RESULTS

BULK/SINGLE CLONE VIEWER

OFF-TARGET ANALYSIS

TEST DATA

Enter sequencing data

FASTQ Files (*):

Choose files 6 files

Data type:

- Illumina
- Illumina**
- Nanopore
- PacBio or Nanopore Duplex

Maximum expected genotype (ML mode only):

10

BOOSTING mode

Use machine learning classification model, faster but less accurate

Upload FASTQ files

97_S97_L001_R1_001 05/02/202... FASTQ Sequence T...
98_S98_L001_R1_001 05/02/202... FASTQ Sequence T...

Select data type

Choose BOOSTING mode for fast analysis

Provide genotype details

Gene Name:

AHR

gRNA (*): (? Click here!)

TTTGAAGACATCAGACACATGCAG

NOTE: this is pegRNA for Prime Editing Mode

Targeting mutagenesis oligonucleotide (Knock-in), OR Base Editing:

Knockin-only mode (exclude INDEL)

Non-homology end joining KI

Enter Gene Name

Opportunity for custom-made reference!

Enter Target or Knock-in site sequence

Custom-made reference or self-designed!

Step 2: Multiple Clone View Results

GENERAL ANALYZER

PRIME EDITING ANALYZER

RESULTS

BULK/SINGLE CLONE VIEWER

OFF-TARGET ANALYSIS

TEST DATA

GENOTYPE RIMA



A1 (HET) A2 (HET) A3 A4 A5 (KO) A6

B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12

D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12

E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12

F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12



- in-frame indels
- out-of-frame indels
- no indel
- targeted mutagene

GENOTYPE RIMA



A1 A2 A3 A4 A5 A6

B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12

C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12

D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12

E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12

F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12

Click on RIMA for viewing repairing pathway

SUMMARY: Gene: AHR | File A1: 97_S97_L001_R1_001.fastq

Total aligned reads: 1281 | Outframe indel: 764 READS, (59.64%) | Inframe indel: 517 READS, (40.36%)

targetingOligo reads: 0 READS, (0.00%) | No indel: 0 READS, (0.00%) | Read ambiguous: 0

MH-del: 24.04 | NHEJ: 0.00 | NHEJ-KI: 0.00 | HDR-KI: 0.00 | Other EJ: 75.96

ANNOTATION

- DUPLICATED INSERTION
- MICRO HOMOMOLOGY
- NORMAL SNP/INSERTION

TTTGAAGACATCAGACACATGCAG X

REFERENCE 5'>3' TGA AAAA CCTAGGCATTGATTTTGAAGACATCAGACATGCAGAAATGAAAAATTTTTCA

GROUP 1 TGA AAAA CCTAGGCATTGATTTTGAAGACATCAG-----AAATTTTTCA TYPE: Inframe Indel, (-15 bp) | 517 Reads, 40.36%
RIMA classified as: other EJ (MH-del 1bp)

GROUP 2 TGA AAAA CCTAGGCATTGATTTTGAAGACATCAG-----ATTTTTCA TYPE: Outframe indel, (-17 bp) | 456 Reads, 35.60%
RIMA classified as: other EJ (MH-del 1bp)

GROUP 3 TGA AAAA CCTAGGCATTGATTTTGAAGACAT-----AATGAAAAATTTTTCA TYPE: Outframe indel, (-10 bp) | 308 Reads, 24.04%
RIMA classified as: MH-del

Scroll down and view summary of generated genotypes in respective sample

Reference sequence on top and genotypes below! Click and scroll left/right to view total sequence!

Step 3: Single Clone View Results

SAVE HTML Indel Excel Indel CSV Alignment (.svg) Print PIE

GENOTYPE

RIMA

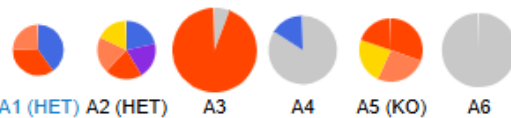
SECONDARY ANALYSIS

Save data for publication or downstream analysis!

SWITCH TO SINGLE CLONE VIEW

Click here to go to single clone view

Select sample of interest for detailed single clone information



You receive detailed information on detected genotypes and quality control

GENOTYPE RATIO

RIMA RATIO



SUMMARY: Gene: AHR | File: A1-97_597_L001_R1_001.fastq

Total aligned reads in bulk: 1281
in-frame indels: 517 READS, (40.36 %)
out-of-frame indels: 764 READS, (59.64 %)
no indel: 0 READS, (0.00 %)
targeted mutagenesis (KO): 0 READS, (0.00 %)

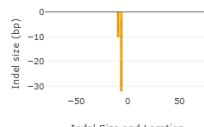
MH-del: 24.04 %
NHEJ: 0.00 %
NHEJ KI: 0.00 %
HDR KI: 0.00 %
Other EJ: 75.96 %

ANNOTATION
DUPLICATED INSERTION
MICRO HOMOLOGY
NORMAL SNP/INSERTION

REFERENCE 5'-->3' TGA AAAACCTAGGCATTGATTTTGAAGACATCAGACACATGCAGAAATGAAAAATTTTTCA
GROUP 1 TGA AAAACCTAGGCATTGATTTTGAAGACATCAG... AAAATTTTCA
GROUP 2 TGA AAAACCTAGGCATTGATTTTGAAGACATCAG... AAAATTTTCA
GROUP 3 TGA AAAACCTAGGCATTGATTTTGAAGACAT... AAAATTTTCA

TYPE: Inframe indel, (-15 bp) | 517 Reads, 40.36 %
RIMA classified as: other EJ (MH-del 1bp)
TYPE: Outframe indel, (-17 bp) | 456 Reads, 35.60 %
RIMA classified as: other EJ (MH-del 1bp)
TYPE: Outframe indel, (-10 bp) | 308 Reads, 24.04 %
RIMA classified as: MH-del

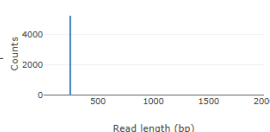
Indel Size and Location



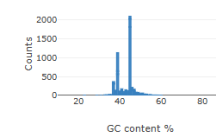
Quality Score Histogram



Read length (bp)



GC content



Step 4: Off-Target

GENERAL ANALYZER

PRIME EDITING ANALYZER

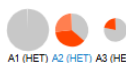
RESULTS

BULK/SINGLE CLONE VIEWER

OFF-TARGET ANALYSIS

TEST DATA

Gene Name: ACTB
gRNA (*): (? Click here!)
GGAAATCGTGCGTGACATTAAGG
NOTE: this is pegRNA for Prime Editing Mode



Select clone

TOTAL ALIGNED READS	ACTB	ACTA2	ACTA1	ACTG1	ACTG2
in-frame indels:	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)
out-of-frame indels:	117 Reads, (100.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)
no indel:	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)	0 Reads, (0.00%)



OFF TARGET Analysis (optional)

List off-target Gene names (separate by ,): (? Click here!)

ACTA2, ACTA1, ACTG1, ACTG2

Off-target analysis will check up to 4 other genes in a single run

List of offset sgRNA: Enter off target gRNA sequences

If off-target sgRNA empty, system will take the previous sgRNA to find region of interest
sgRNA gene 1

TGAGATTGTCGGGACATCAAGG

sgRNA gene 2

CGAGATCGTGCGGACATCAAGG

sgRNA gene 3

GGAAATCGTGCGGACATCAAGG

sgRNA gene 4

AGAAATTGTGCGAGACATCAAGG

Use the correct gene order used in the list above!

START OFFTARGET ANALYZER

Click here for off-target analysis

OFF-TARGET ALIGNMENT SUMMARY: Gene: ACTG2 | File: 32190ac0838998df6f4a79ee2f80496905fea757_EXP-NBD196_barcode02.fastq

AGATATTAGAGATTTTAA

REFERENCE--> AAGCAGGTCCCTCAGTCGACATATTAGAGATTTTAA



No off-target effect



Off-target effect

General considerations for off-target analysis

Off-target identification tools:

- https://ccsm.uth.edu/CRISPROffT/
- NCBI Blast
- gRNA design pages like Chopchop

For sample preparation it is recommended to prepare gene amplicons separately and include a wildtype control!