

Selecting gRNA

4. Selection of target sequence/ gRNA design:

Plenty of webtools available:

- Predicted- on-target activity <https://omictools.com/genome-editing-category>
- Predicted off-target activity
- ... much more

CRISPOR is a program that helps design, evaluate and clone guide sequences for the CRISPR/Cas9 system. 🤖

New version V4.3, Oct 2017: Lentiviral screens, Variants, Cpf1, Off-target primers, microhomology, Genbank-export, Sat. mutagenesis . [Full list of changes](#)

Step 1

Planning a lentiviral gene knockout screen? Use **CRISPOR**

Batch

Sequence name (optional):

Enter a single genomic sequence, < 2000 bp, typically an exon 🤖

[Clear Box](#) - [Reset to default](#)

```
cttcctttgtccccaatctgggcgcgccggcgccccctggcggcctaagga
ctcggcgcgccggaagtggccagggcgggcgacctcggctcacagcgcc
cggctattctcgcagctcaccatgGATGATGATATCGCCGCTCGTCGTCGA
CAACGGCTCCGGCATGTGCAAGGCCGGCTTCGCGGGCGACGATGCCCCCGGG
CCGTCTTCCCCTCCATCGTGGGGCGCC
```

Step 2

Select a genome

Homo sapiens - Human - UCSC Feb. 2009 (GRCh37/hg19) + SNPs: 1000Genomes, ExaC

Note: pre-calculated exonic guides for this species are on the [UCSC Genome Browser](#).
We have 241 genomes, but not the one you need? Send its FASTA/GFF URL to [CRISPOR support](#)

Step 3

Select a Protospacer Adjacent Motif (PAM)

20bp-NGG - Sp Cas9, SpCas9-HF1, eSpCas9 1.1

SUBMIT

<http://crispor.tefor.net/>

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Predicted guide sequences for PAMs

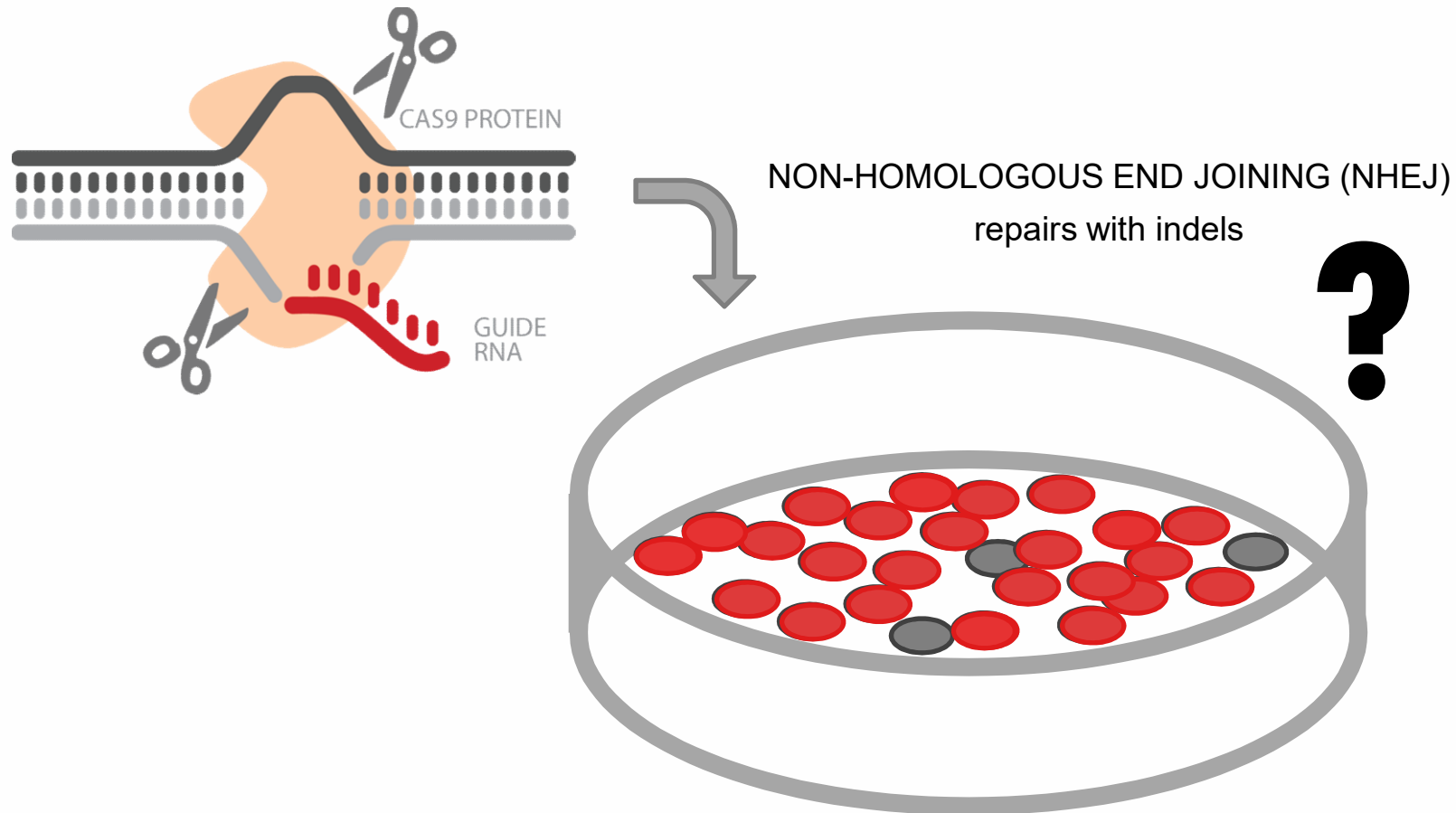
Ranked by default from highest to lowest specificity score (Hsu et al., *Nat Biot* 2013). Click on a column title to rank by a score.

If you use this website, please cite our [CRISPOR paper in Gen Biol 2016](#).

Download as Excel tables: [Guides](#) / [Off-targets](#) / [Saturating mutagenesis assistant](#)

Position/ Strand 🗨	Guide Sequence + PAM + Restriction Enzymes 🗨 + Variants 🗨 <input type="checkbox"/> Only G- <input type="checkbox"/> Only GG- <input type="checkbox"/> Only A- 🗨	Specificity Score 🗨	Predicted Efficiency 🗨 Show all scores		Out-of- Frame score 🗨	Off-targets for 0-1-2-3-4 mismatches + next to PAM 🗨	Genome Browser links to matches sorted by CFD off-target score 🗨 <input type="checkbox"/> exons only <input type="checkbox"/> chr7 only
			Doench '16	Mor.-Mateos	Click on score to show micro- homology		
35 / rev	CGAGTCCTTAGGCCGCCAGG GGG -G---C----- Enzymes: <i>NlaIV, AclI, BfoI, HinP1I, BseDI, BaniI, BstNI, BslI, SspDI, StyDI</i> Cloning / PCR primers	82	63	64	71	0-0-3-7-64 0-0-0-0-1 74 off-targets	4:intergenic:RP11-268I9.3-RP11-268I9.4 4:intron:STK32B 3:intergenic:RP11-438O11.1-RPS7P5 show all...

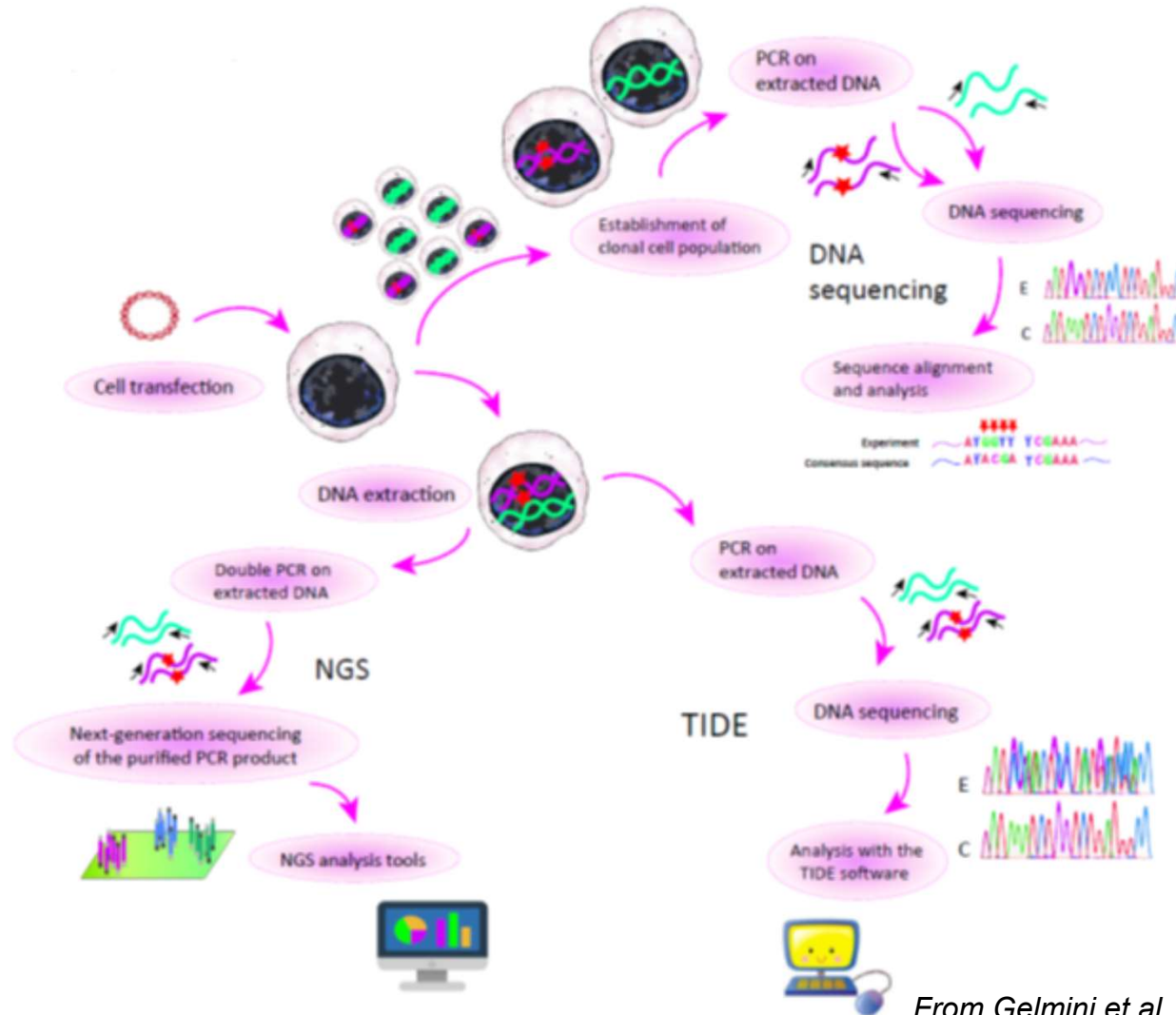
Measuring Cas9 Efficacy



How to measure the DSB efficacy of a CRISPR sgRNA and the nature of the mutations?

Measuring Cas9 Efficacy

1. Sequencing based techniques

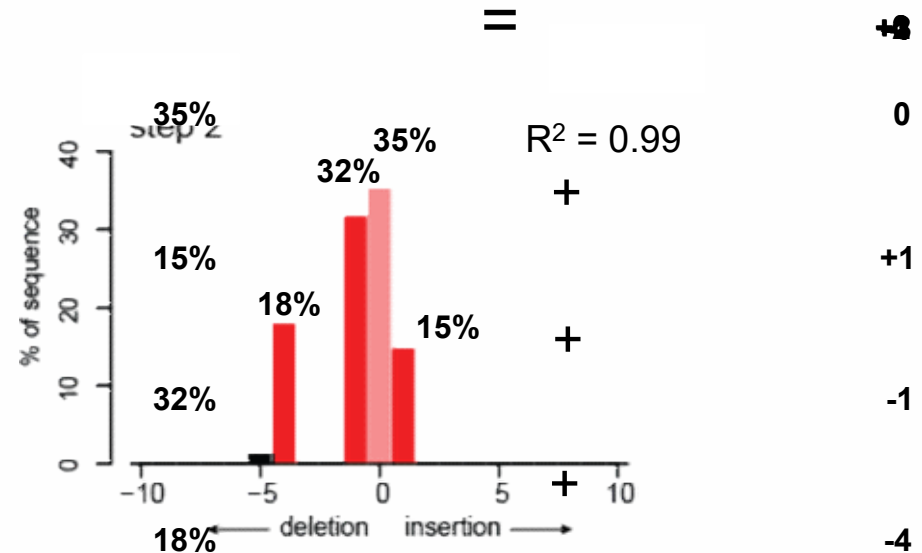
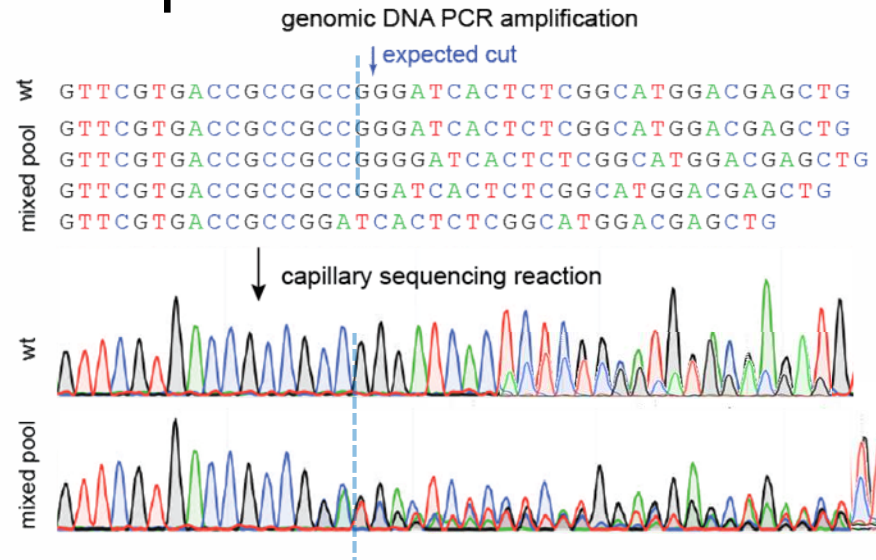


Measuring Cas9 Efficacy

1. Sequencing based techniques: TIDE



Tracking of Indels by DEcomposition



Eva K. Brinkman, Tao Chen,
Mario Amendola, Bas van Steensel
<http://tide.nki.nl>