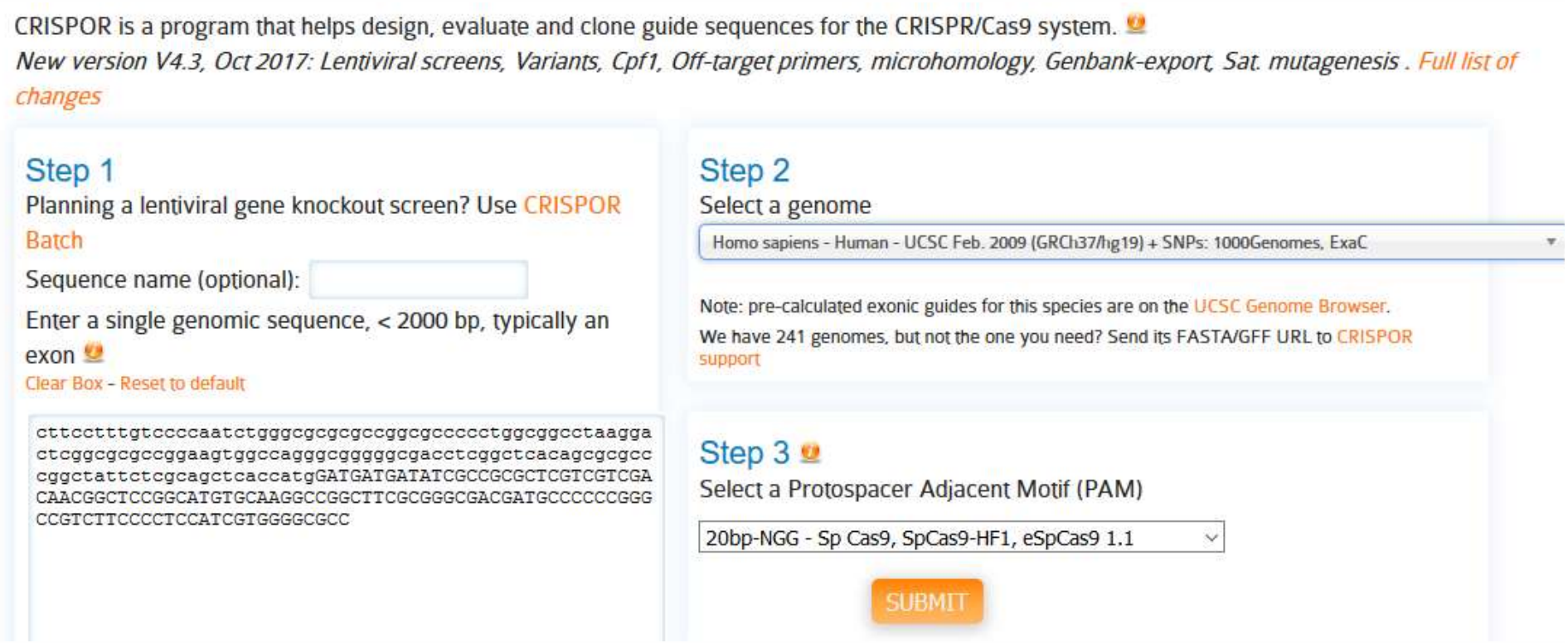
Selecting gRNA

4. Selection of target sequence/ gRNA design:

Plenty of webtools available:

- Predicted- on-target activity
- Predicted off-target activity
- … much more

https://omictools.com/genome-editing-category



http://crispor.tefor.net/

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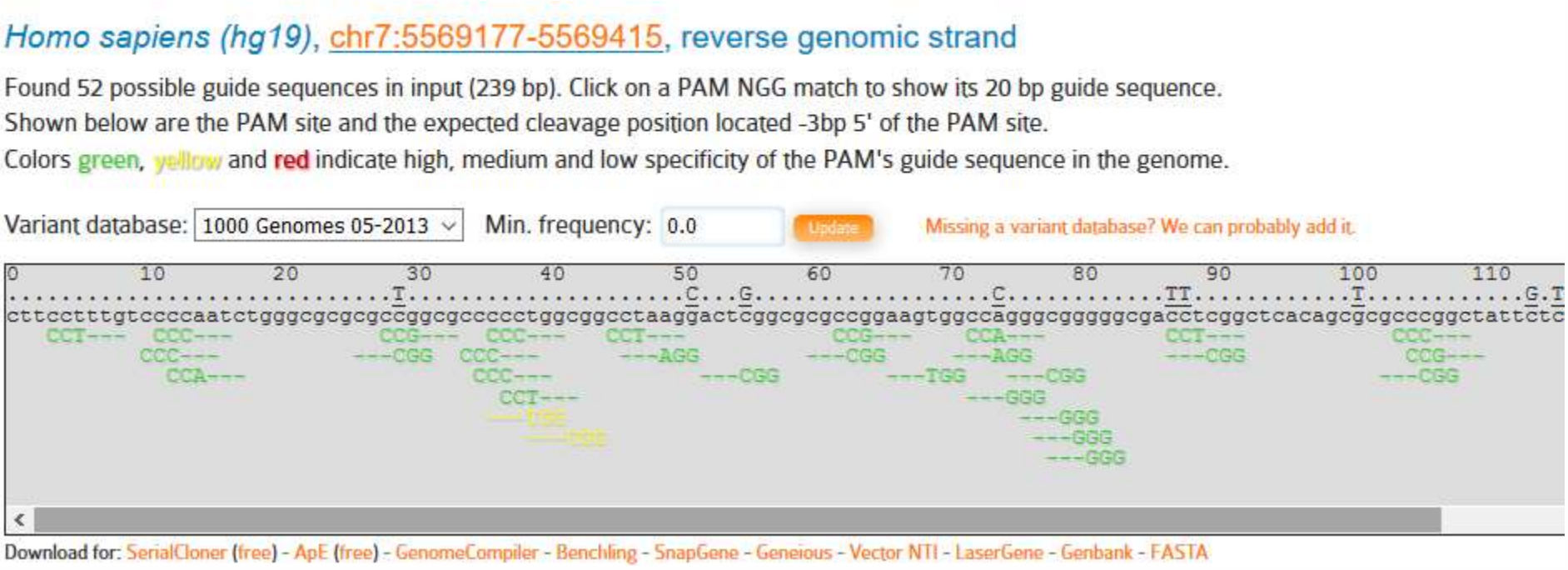

GENETHON
CURE THROUGH INNOVATION

Selecting gRNA

4. Selection of target sequence/ gRNA design:

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Predicted guide sequences for PAMs

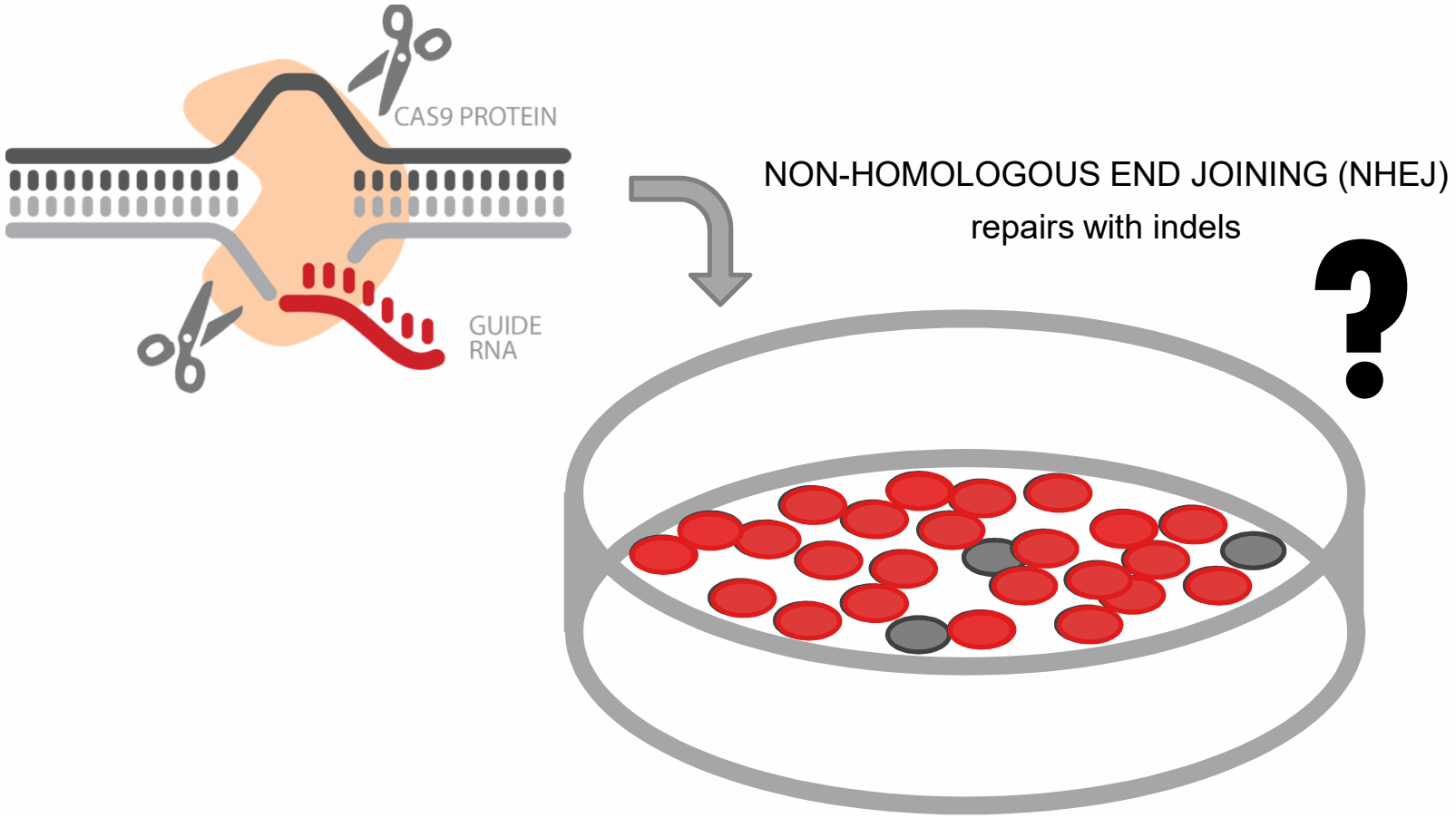
Ranked by default from highest to lowest specificity score (Hsu et al., Nat Biot 2013). Click on a column title to rank by a score.
If you use this website, please cite our CRISPOR paper in Gen Biol 2016.

Download as Excel tables: Guides / Off-targets / Saturating mutagenesis assistant

Position/ Strand	Guide Sequence + *PAM* + Restriction Enzymes + Variants ☐Only G- ☐Only GG- ☐Only A-	Specificity Score	Predicted Efficiency Show all scores		Out-of-Frame score Click on score to show micro-homology	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by CFD off-target score ☐exons only ☐chr7 only
			Doench '16	Mor.-Mateos			
35 / rev	CGAGTCCTTAGGCCGCCAGG GGG -G---C---------- --- Enzymes: *NlaIV, AcyI, BfoI, HinP1I, BseDI, BanI, BstNI, BslI, SspDI, StyD4I* **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...

GENETHON
CURE THROUGH INNOVATION

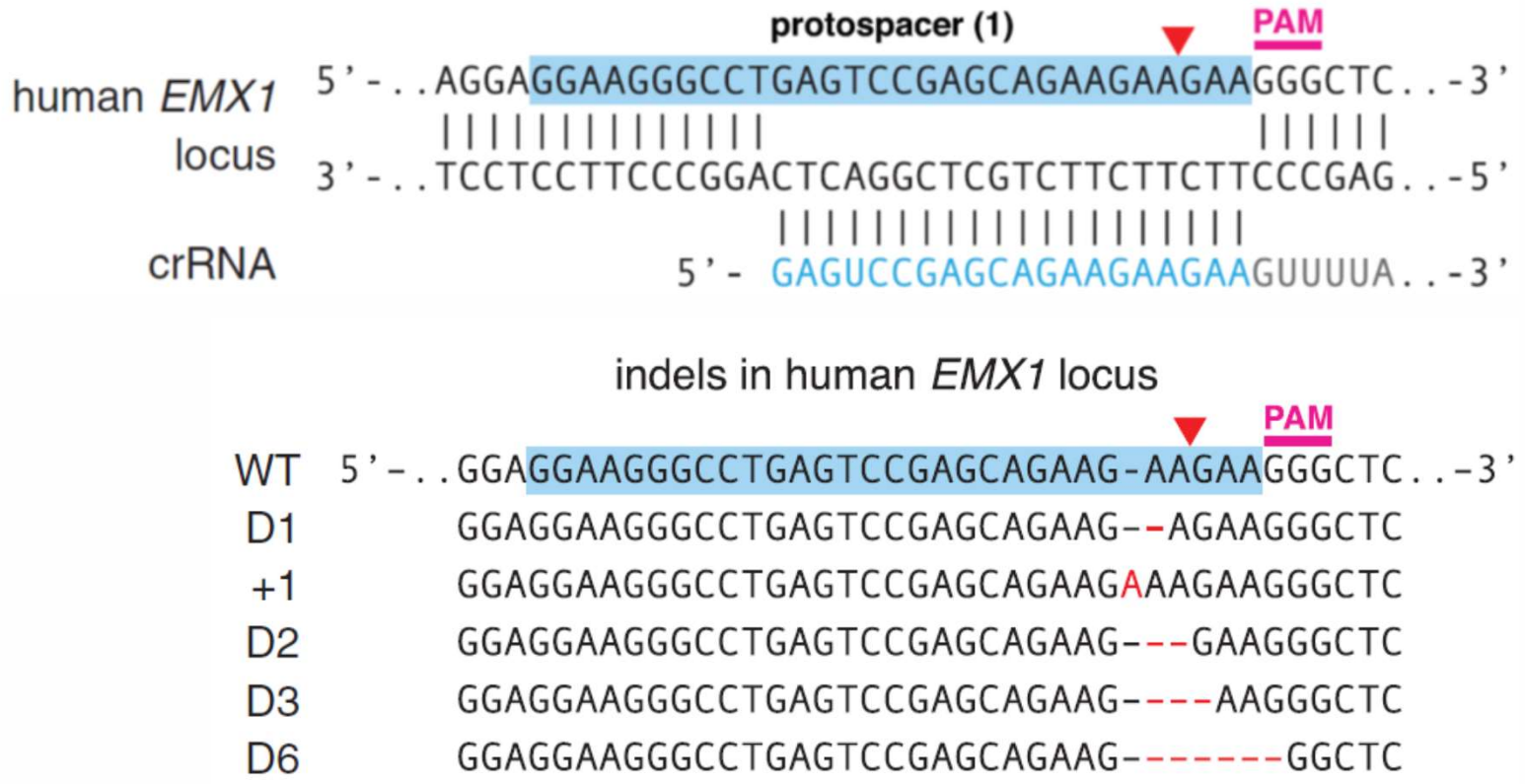
Measuring Cas9 Efficacy



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

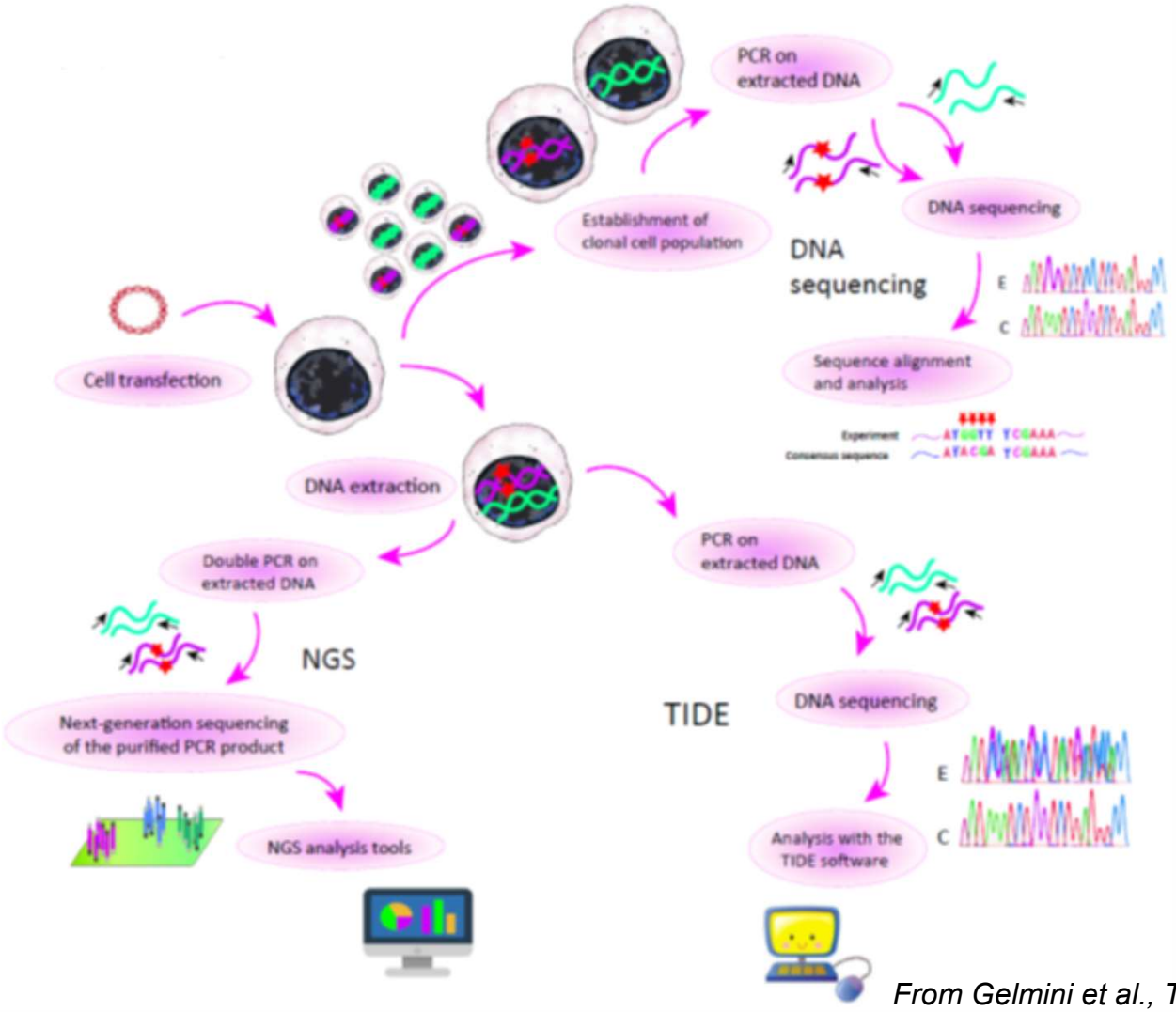
Measuring Cas9 Efficacy

1. Sequencing based techniques
2. Denaturation based techniques
3. Alternative techniques



Measuring Cas9 Efficacy

1. Sequencing based techniques

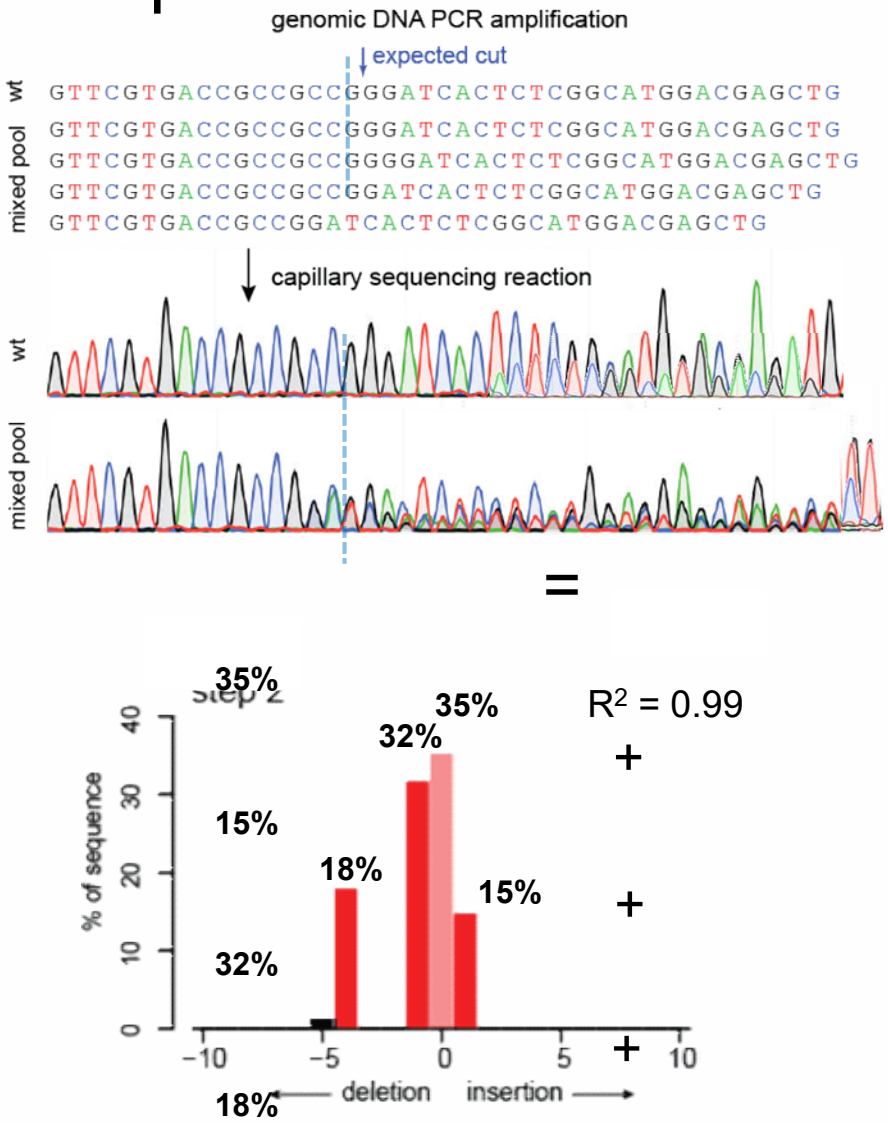


From Gelmini et al., Trends Biotechnol 2017

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