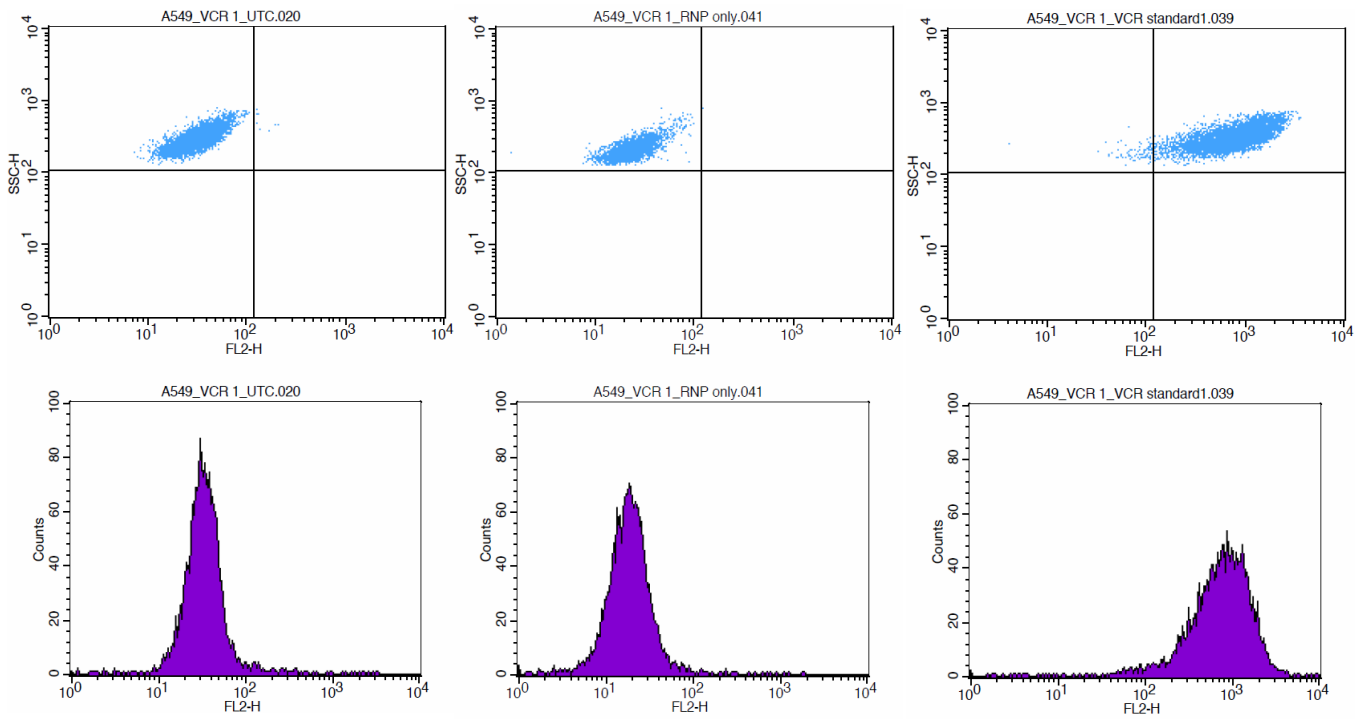
A549: transfection of Cas9/gRNA RNP



Untransfected Cells | RNP Only | Viromer CRISPR

VCR-01LB-00 /-01 /-03

Fig. 1: FACS monitoring for evaluating RNP transfection with Viromer® CRISPR

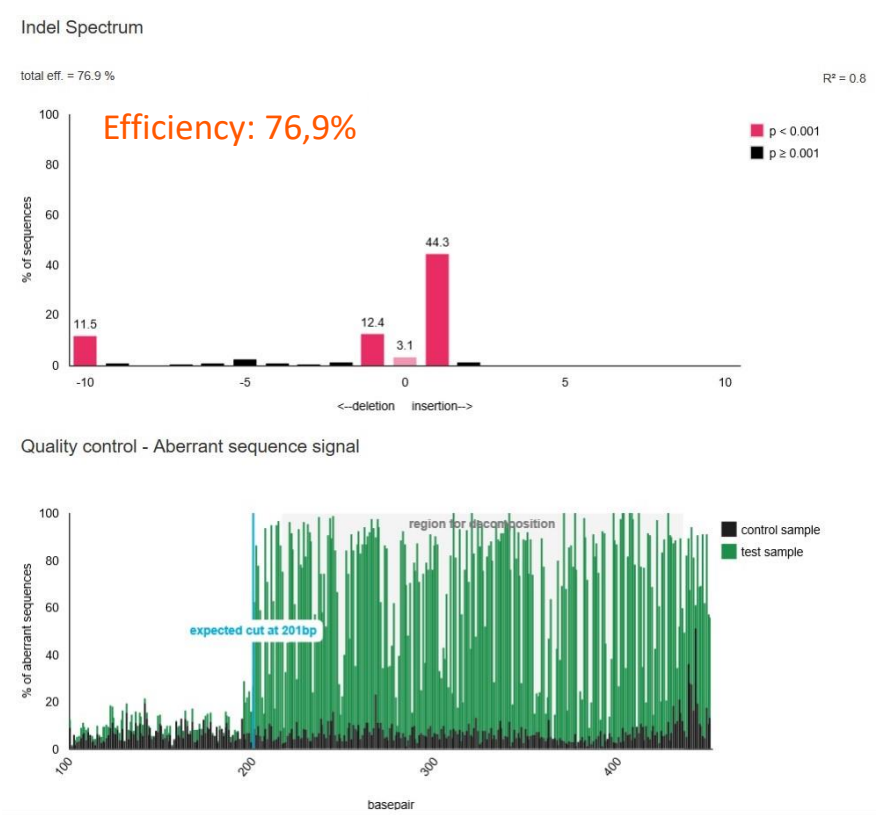
>90% of transfected cells
No sign of toxicity

Cells: A549 human lung adenocarcinoma cells
Cas9: Cas9 3NLS, gift from IDT
gRNA: HPRT1 crRNA + ATTO550 tracrRNA, gift from IDT

Protocol:
- RNP ratio 1:1
- Standard conditions (25nM final RNP)

Read-out: FACS after 24h

A549: transfection of Cas9/gRNA RNP



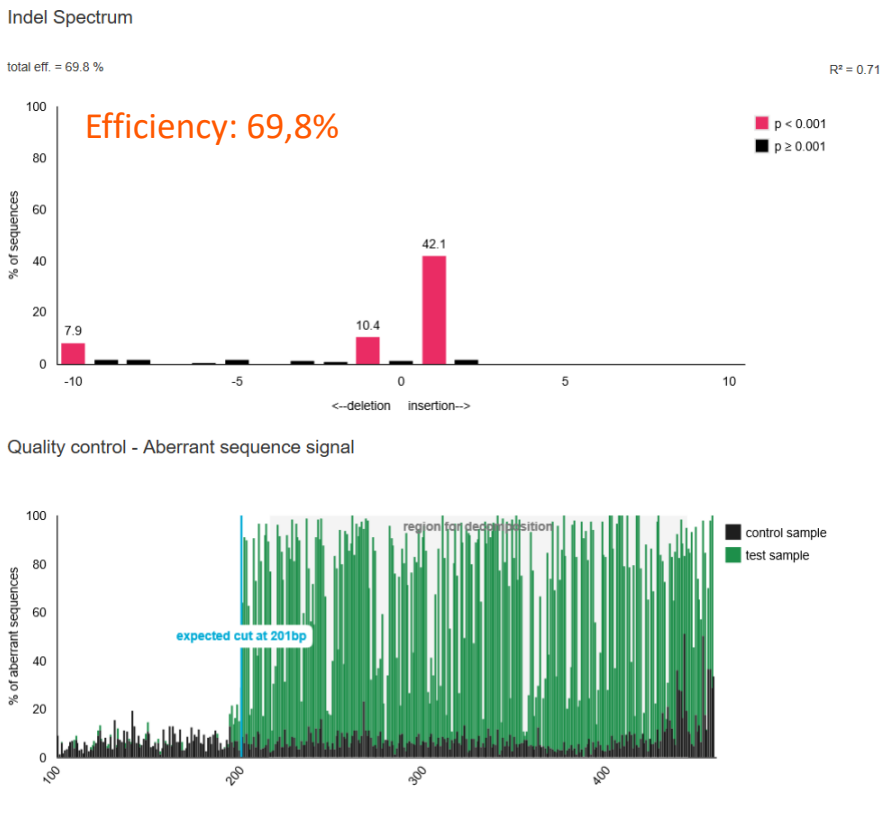
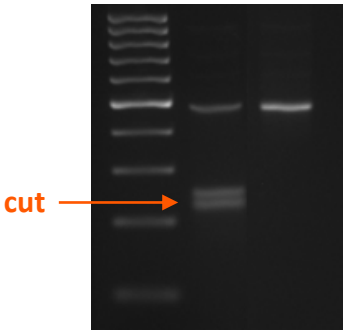


Fig. 2: Sequencing data after RNP transfection with Viromer® CRISPR (results of T7 surveyor assay on 2 replicates)

Cells: A549 human lung adenocarcinoma cells
Cas9: spCas9 from OriGene
gRNA: HPRT1 A35524 gRNA from Thermo Fisher

Protocol:
- RNP ratio 1:1
- Standard conditions (25nM final RNP)

Read-out: T7 assay after 24h, sequencing using Tides



A549: transfection of Cas9/gRNA RNP

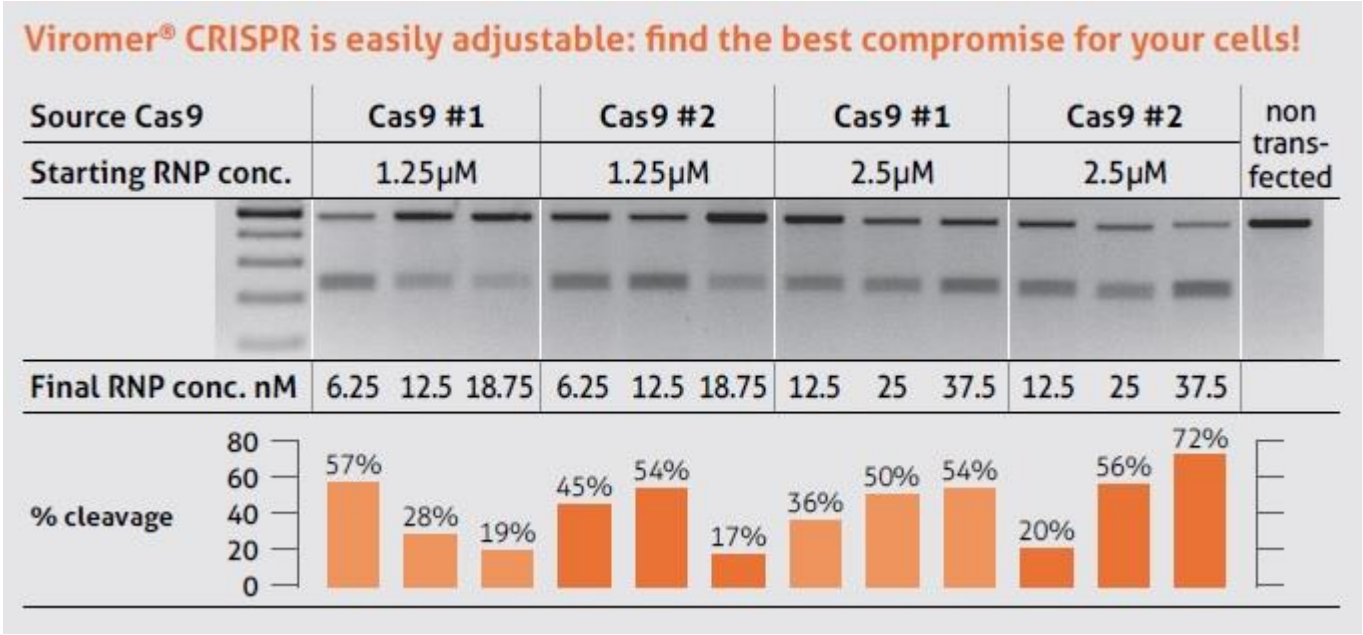


Fig. 4: Results of T7E1 assay 48h after RNP transfection with Viromer® CRISPR with various conditions and use of 2 different Cas9

17-72% of cleavage efficiency depending on starting conditions - Not always dose-dependent!

Cells: A549 human lung adenocarcinoma cells
Cas9: #1 from OriGene, #2 from LifeTech
gRNA: HPRT1 A35524 gRNA from Thermo Fisher

Protocol:
- RNP ratio 1:1
- Test of different conditons (1.25 and 2.525uM stock RNPs, with 3 different transfer volumes: 5, 10 and 15ul in 96-well, resulting in 6.25 to 50nM final RNP concentrations)

Read-out: T7 assay after 48h

All data from in-house experiments