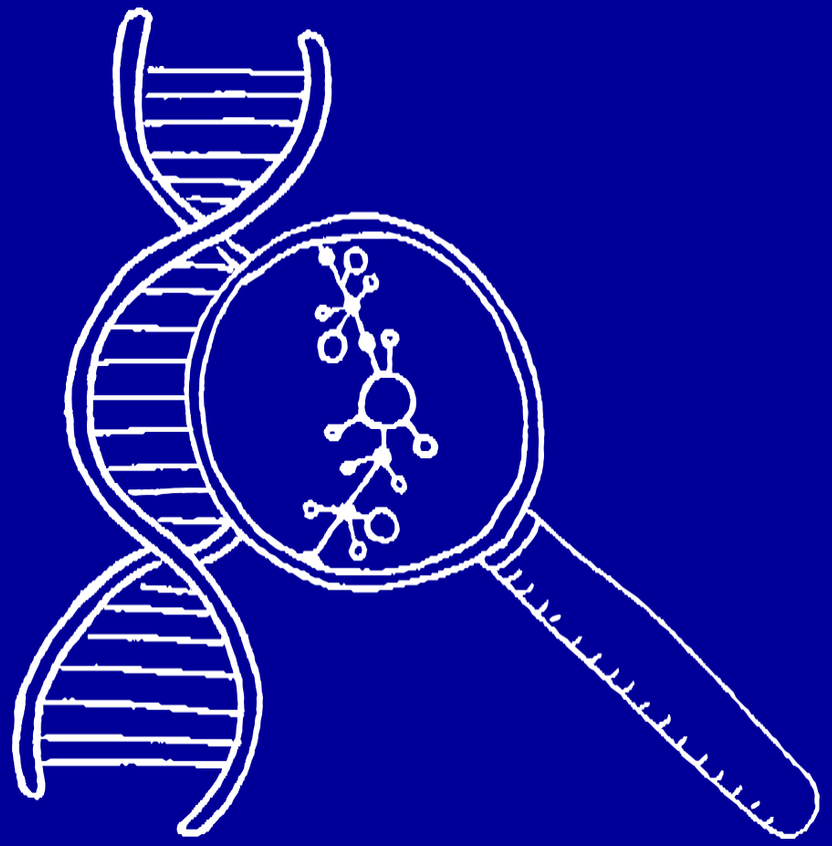
evotec

#RESEARCHNEVERSTOPS

Evotec Gene Therapy

Genome Editing Capabilities



Gene editing at Evotec GT

Overview of capabilities

Overview

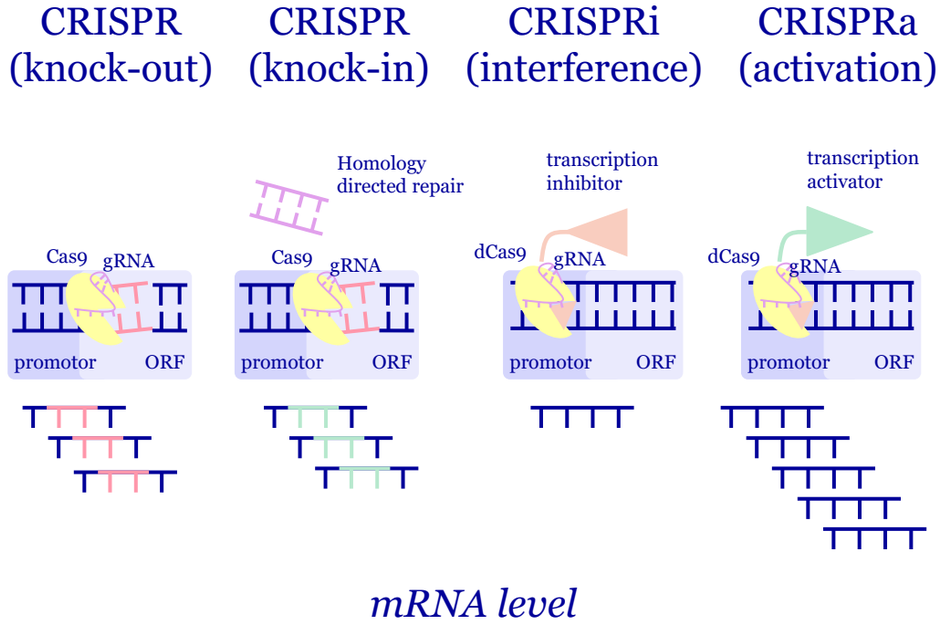
Expertise

- Application of genome editing and other genetic approaches at different stages of drug discovery process
- Dedicated team of scientists with strong background in genome editing
- Plug and play integration into existing Evotec platforms
- Close interaction with project teams ensures broad applicability and high success rates
- Broad experience in different disease areas

Output

- *In vitro* and *in vivo* validated genome editing products
- Optimized gene editing and delivery conditions for efficient editing with minimal off-target effects
- Assessment of gene editing efficacy in animal models of disease
- Optimized and streamlined protocols

CRISPR toolbox for genetic approaches



Within Evotec's scientific network we integrate a broad spectrum of technologies

CRISPR, clustered regularly interspaced short palindromic repeats.

Genome Editing Leadership @ Evotec GT

First-hand experience gained from the CRISPR/Cas pioneers at the Max Perutz labs Vienna

Vera Schoft

Head of Novel Technologies

- PhD in genetics at the University of Vienna, Austria
- Vienna Biocenter, Gene Editing Core Facility, 5 years
- Evotec GT since 03/2021

>20 years Academia and Contract Research

- Genome editing (8 years experience)
- Molecular Biology
- RNA Biology
- Epigenetics

Karolina Hilse-Koller

Sen. Res. Scientist Novel Technologies

- PhD in Biomedicine and Biotechnology at the University of Veterinary Medicine Vienna
- Vienna Biocenter, Gene Editing Core Facility, 3 years
- Evotec GT since 11/2021

>12 years Academia and Contract Research

- Genome editing (5 years experience)
- Molecular Biology
- Mitochondrial Research

*Support by the in vitro **technical** team as required*

Break-down of Core Genome Editing Activities

Services covering a broad spectrum of technologies

Proof of concept studies

- Validate client technologies against benchmark
- GE tools provided by client
- Assay set-up defined by client or by Evotec

Design of *in vitro* gene experiments[1]

- Selection of suitable editing tools, designs, and delivery systems tailored to project needs
- Plan and test indication specific concepts *in vitro*
- Applying specialized technology as needed (e.g. analytical assays, NGS, RNAseq)

Design of *in vivo* gene editing-based therapies[1]

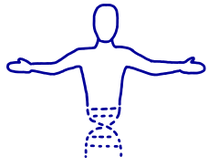
- Selection of suitable editing tools, designs, and delivery systems tailored to project needs
- Plan and test indication specific concepts *in vitro*
- *In vivo* proof of concept studies
- Assessment of gene editing efficacy in animal models of disease

Protocol establishment

- Adapting/setting up of gene editing assays:
  - Variable based on biological question
  - Bridging to specialized teams (e.g. analytics)
- SOP preparation

Product development up to clinical phase[1]

- Design and execute CTA/IND-enabling packages



Evotec GT capabilities

- Proven track record covering a variety of gene editing technologies
- Experience in state-of-the-art delivery modes (material produced in house or sourced externally)
- Full bandwidth of genetic and phenotypic analysis capabilities

1 Technologies may require licenses; MS: mass spectrometry; NGS: next generation sequencing

Hands-on experience with gene editing projects

Ample experience with multiple nucleases and delivery systems

Nucleases

- CRISPR/Cas9:
  - Wild type
  - Cas9 orthologs
  - dCas9
  - Nickase
  - Fusion proteins (transcriptional and epigenetic modulators, base editors, reverse transcriptase)
- CRISPR/Cas12 like nucleases
- Zinc Finger Nucleases (ZFN)
- TALENs
- megaTAL

Applications

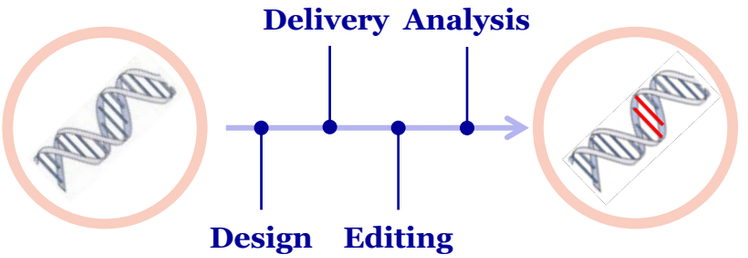
- Gene knock-out
- Gene knock-in
- Screening for the best ZFN pair
- Protocol development
- Proof-of-concept studies
- *In vitro*
- *In vivo*
- Organellar editing

Delivery

- Transfection (plasmids)
- Transduction (AAV)
- Electroporation (RNPs)
- Nucleofection
- AAV
- LNPs
- LNP plus AAV

Proven track record covering a variety of technologies:

- Richter et al., Sci Rep., 2018 Aug 15;8(1):12182
- Schoft et al., Nucleic Acids Res., 2007;35(11):3723-32
- Hilse et al., Biochim Biophys Acta, 2016 Jan.; 1857(1):72-78

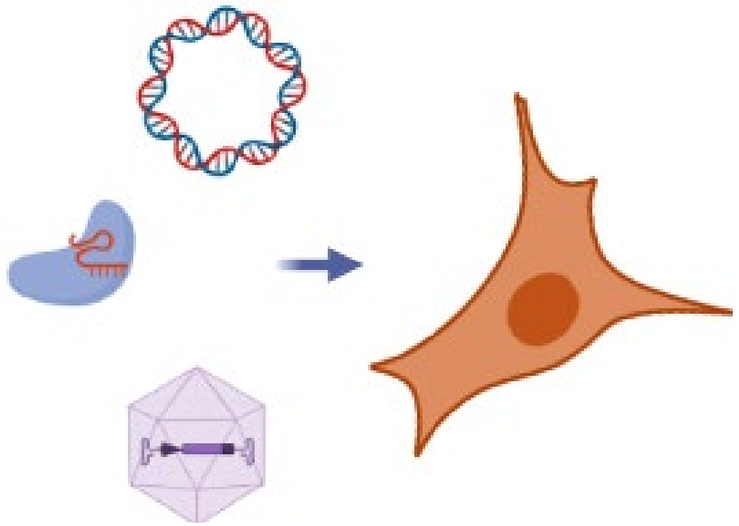


RNP: ribonucleoprotein; ZFN, Zinc finger nuclease; TALEN, Transcription activator-like effector nuclease; LNP, lipid nanoparticle.

Genome Editing Activities – Workflow

Customized solutions for clients

Transfer of gene editing components to cells



Choosing best suited methods for analysis

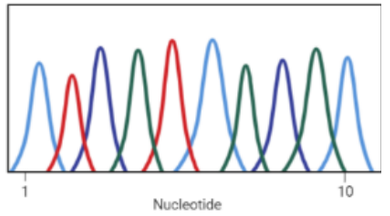
Genetic evaluation

- Sanger based INDEL analysis
- INDEL analysis by ddPCR
- Amplicon deep sequencing (short and long read)
- qRT-PCR

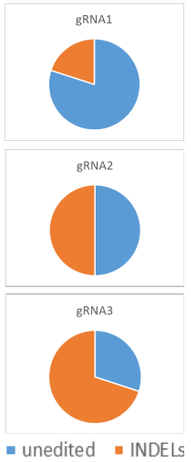
Phenotypic assessment

- Imaging
- Flow cytometry
- qRT-PCR
- Western Blotting
- Enzymatic activity

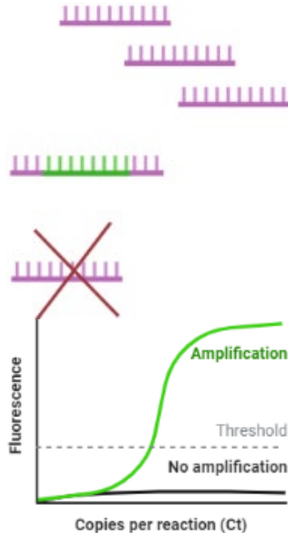
DNA sequencing



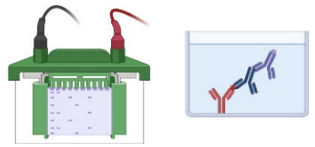
Analysis of cell pools



RNA analysis



Protein analysis



INDEL: insertion / deletion; ddPCR, digital droplet PCR

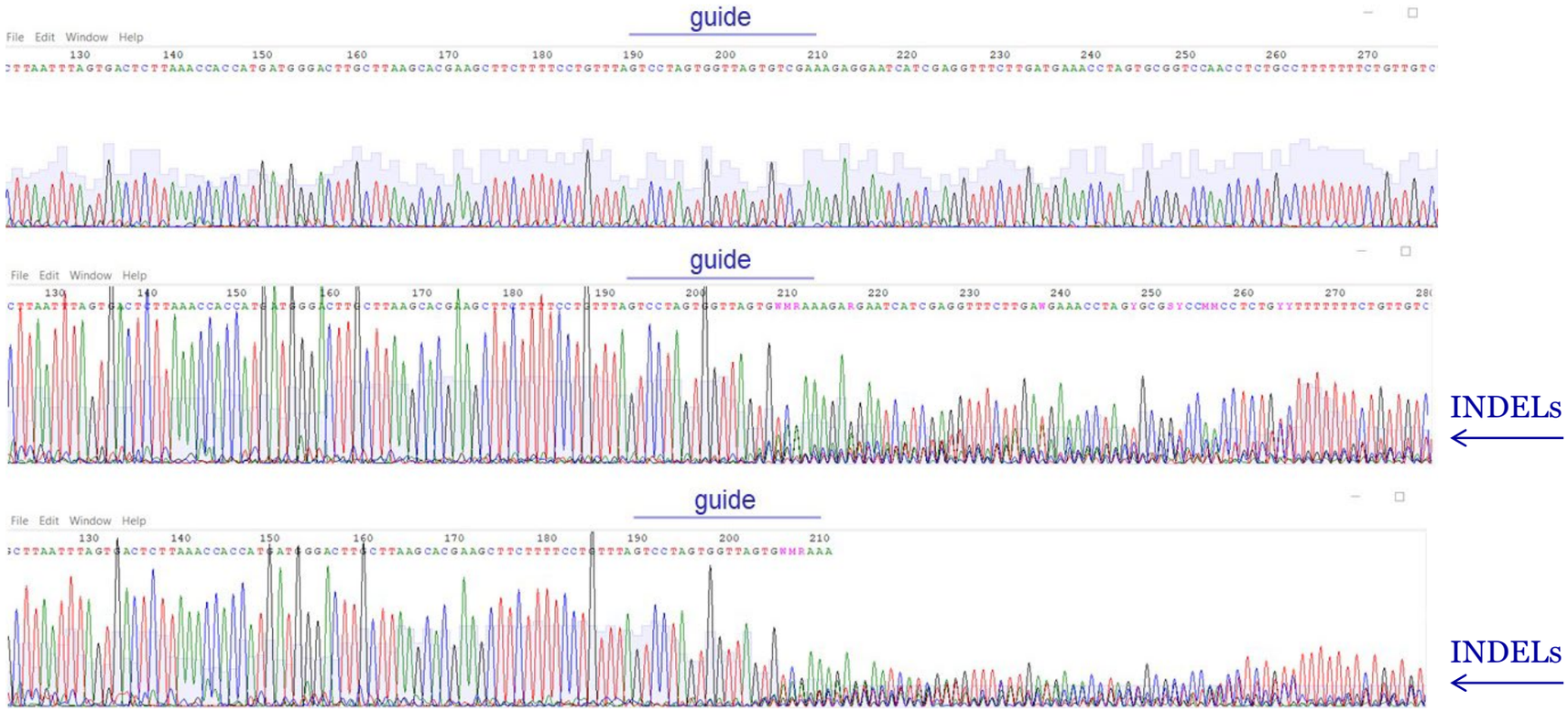
Example: Cas12-like nuclease-based gene editing

Sequencing data for an edited gene of interest in a cell pool

Untreated cells

Transfected cells (plasmid)

Electroporated cells (RNP)

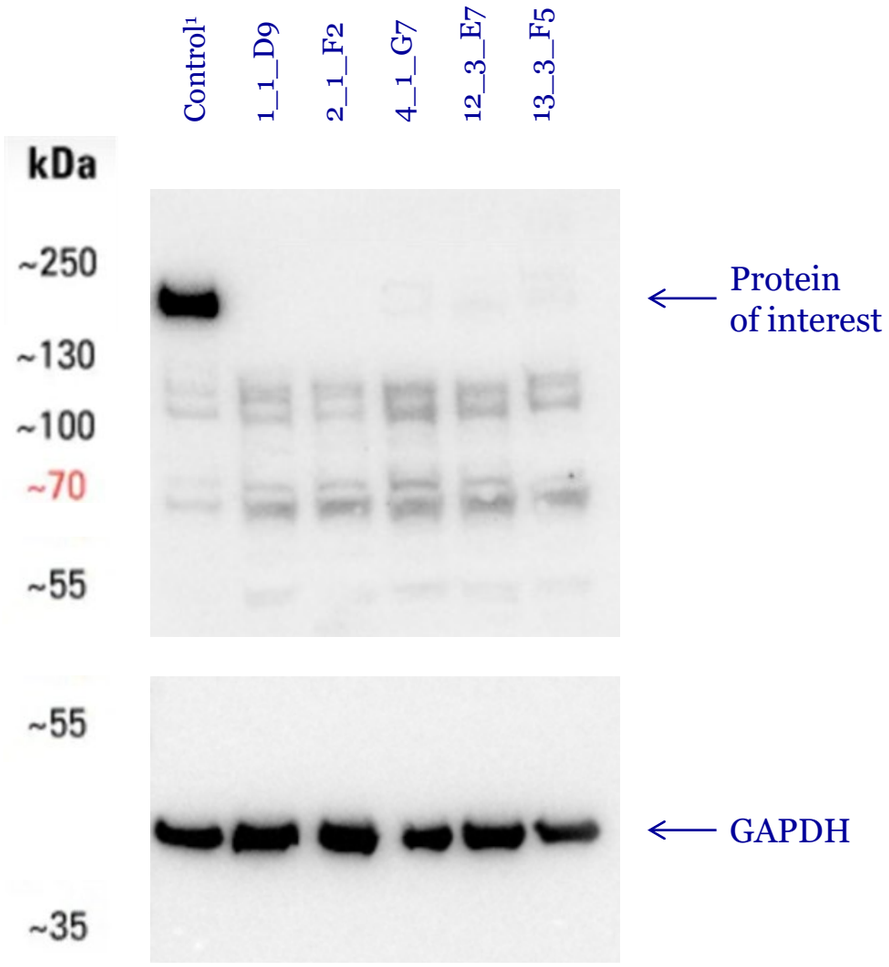


Sanger sequencing reveals the frequency and spectrum of small insertions and deletions (INDELs) generated in a pool of cells treated with CRISPR/Cas12

RNP: ribonucleoprotein; INDEL: insertion / deletion

Generation of a single clone ko cell line for a gene of interest

Western blot analysis of Huh-7 single clone knock-out candidates



Example for a knock-out project

For the generation of knock-out lines Huh7 cells were treated with CRISPR/Cas9 and separated into single cells. Clones were grown and gene knock-out was confirmed by Sanger Sequencing (not shown). Western Blot analysis confirmed the lack of the protein of interest in the cell clones.

1 Untreated cells

evotec

#RESEARCHNEVERSTOPS

Business Development
114 Innovation Drive,
Milton Park, Abingdon
Oxfordshire OX14 4RZ, UK

T: +44.(0)1235.86 15 61
F: +44.(0)1235.86 31 39
info@evotec.com