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CRISPR platform for Target Identification and Validation at Evotec

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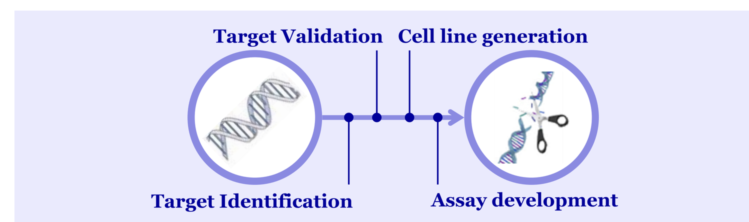
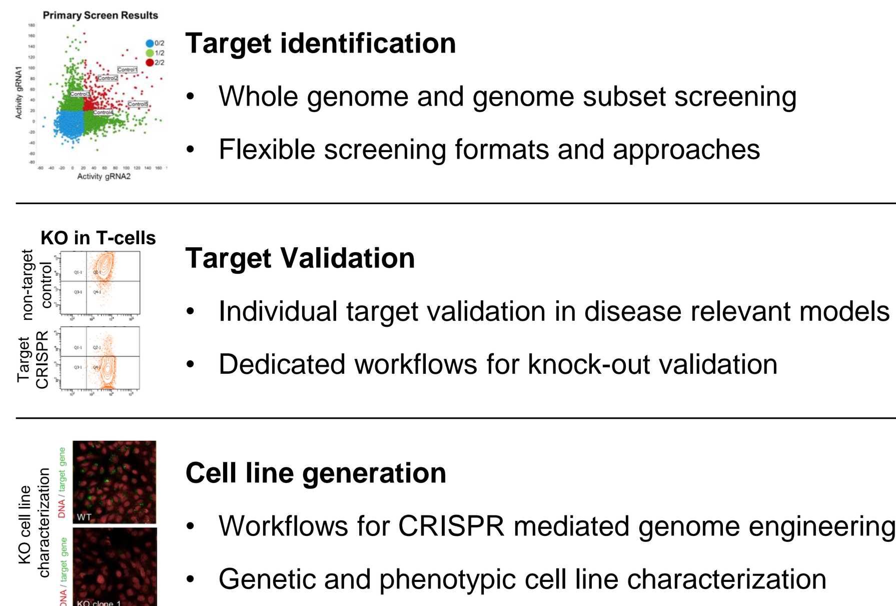
Abstract

The CRISPR/Cas9 system has revolutionized biomedical research and has created new opportunities for drug discovery. Evotec has fully integrated a CRISPR/Cas9 technology platform for target identification, validation, and genome engineering into its existing portfolio of state-of-the-art drug discovery services. Evotec offers comprehensive functional genetic screening for target identification, a powerful approach to identify novel disease-relevant targets in a genome-wide manner. We apply both pooled and arrayed screening using either lentivirus-based, lipofection-based or electroporation-based approaches depending on project requirements and biological context. In addition to experimental design and execution, Evotec provides sophisticated bioinformatic analysis of the screen results to aid with hit identification and annotation. Furthermore, we have developed approaches for genetic target validation, in advanced disease-relevant models such as primary cells and induced pluripotent stem cell (iPSC)-derived systems to follow up hits from genetic screens. Here we present two case studies: (1) a lentiviral pooled CRISPR dropout screen to identify novel druggable synthetic lethality partners for a genetic feature that causes a "mutator" phenotype in many different tumor types followed by extensive target validation; and, (2) an arrayed RNP-based CRISPR screen in primary human T-cells with using a multi-color FACS readout for novel target identification in immuno-oncology space at druggable genome level followed by target validation in multiple functional assays using purified different human primary T cell subsets. These two case studies exemplify integration of the CRISPR technology within Evotec´s comprehensive readout technologies for target identification followed by rapid transition from a target idea towards a validated target hypothesis feeding into target-based drug discovery. Evotec has also developed dedicated workflows to generate CRISPR/Cas9-mediated genome engineering and KI/KO cell line generation, which are applied in projects for target validation and target discovery.

CRISPR/Cas9-based target identification, validation and cell line generation approaches



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



Target identification

- Whole genome and genome subset screening
- Flexible screening formats and approaches

Target Validation

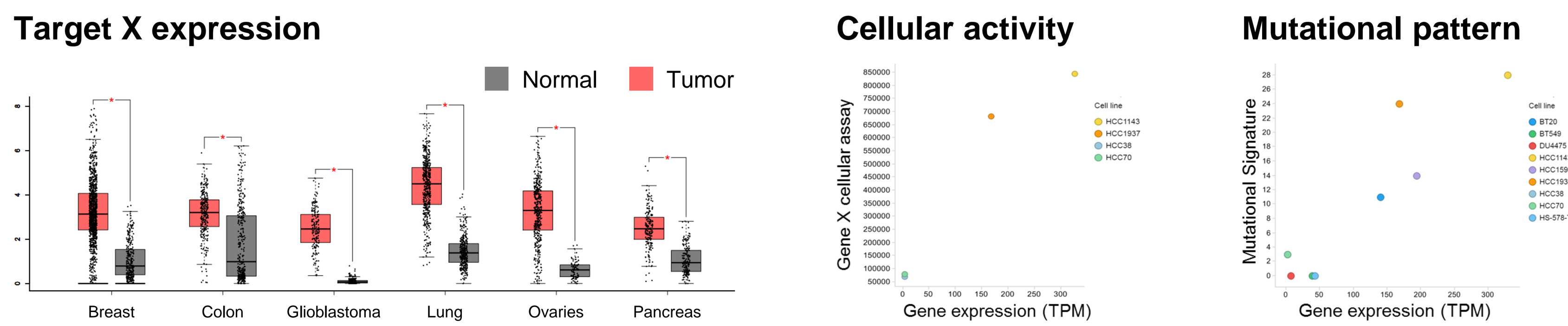
- Individual target validation in disease relevant models
- Dedicated workflows for knock-out validation

Cell line generation

- Workflows for CRISPR mediated genome engineering
- Genetic and phenotypic cell line characterization

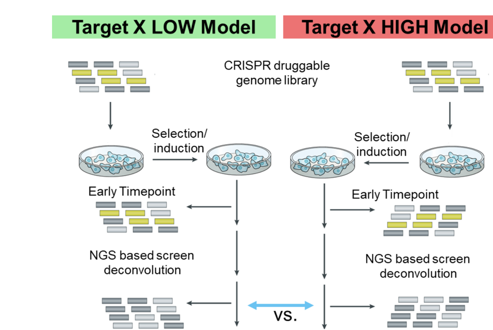
Case study 1 – Pooled CRISPR Screening

Identification of novel synthetic lethality interactions in oncology



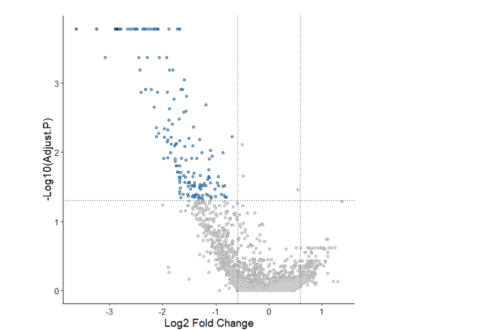
- Target X is found frequently upregulated across tumor types
- Upregulation is associated with an increase in mutation rate and induction of a specific mutational pattern
- *In vitro* models recapitulate disease biology: Target X expression correlates with activity and mutational pattern
- Identification of synthetic lethal partner warranted to deplete cells with high mutational burden and thereby restrict tumor plasticity

Synthetic lethality screening



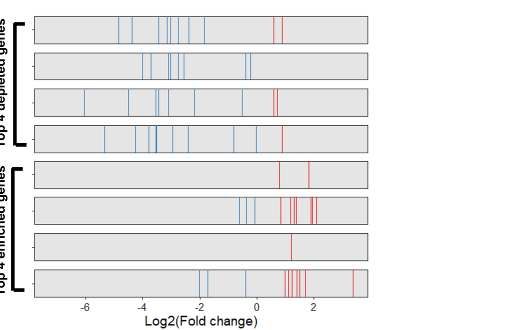
- Bioinformatic mapping of NGS reads and data analysis
- Comparison of genes dropping out between HIGH and LOW models
- Hit identification with high statistical power

Hits from the primary screen



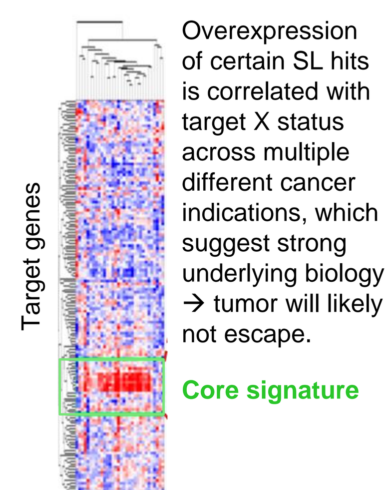
- Volcano plot displaying the log2 Fold Change and adj. P value for all genes identified in the screen

sgRNA performance analysis



- Bioinformatic analysis of the top targets
- Visualization of the rank of sgRNAs targeting top selected genes

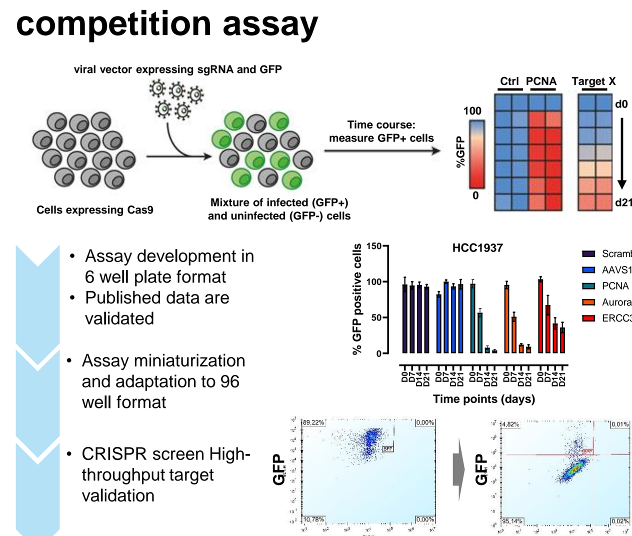
Hit Identification (TCGA analysis)



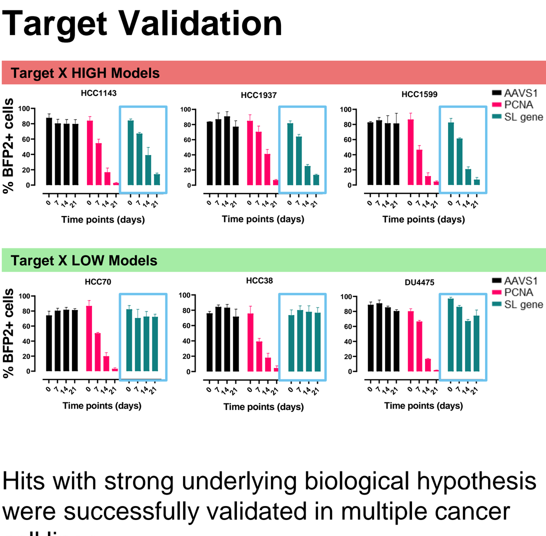
Overexpression of certain SL hits is correlated with target X status across multiple different cancer indications, which suggest strong underlying biology → tumor will likely not escape.

Core signature

Cell based CRISPR competition assay



Target Validation



Hits with strong underlying biological hypothesis were successfully validated in multiple cancer cell lines

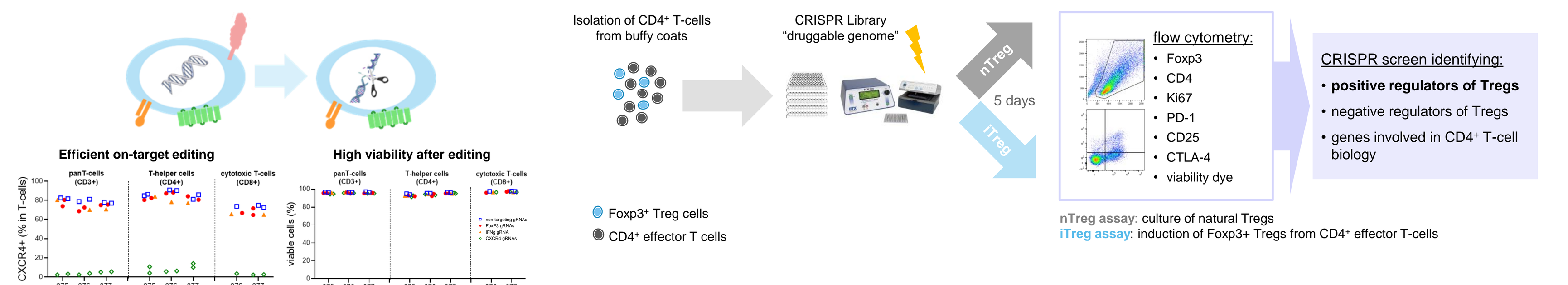
- High degree of validation of synthetic lethality partner across relevant models
- Implementing causality early in Target ID and validation workflow facilitates target selection

Case study 2 – Arrayed CRISPR Screening

Identification of novel targets that impact on T-regulatory cell lineage for I/O and I&I

- Foxp3-expressing Treg cells are a sub-population of CD4+ T cells which suppress other components of the immune system via multiple mechanisms, e.g. inhibition of effector T-cells
- Importantly, Treg cells contribute to immune escape by tumors by suppressing anti-tumor immune responses
- Treg cells are recruited to tumors and CD4+ effector T cells are converted into Treg cells within tumors
- Affecting the generation and maintenance of the Foxp3+ Treg lineage can enhance anti-tumor immunity for I/O therapy

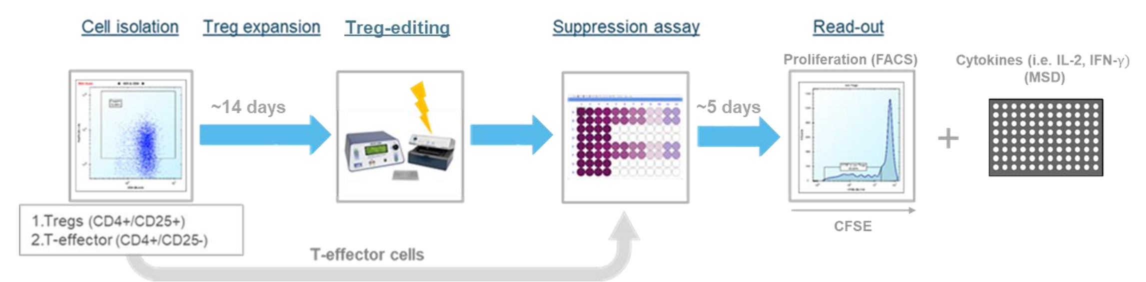
An arrayed CRISPR screen in primary human CD4+ T-cells for target ID



Efficient editing across human T-cell populations   An unbiased screening platform for novel Treg targets

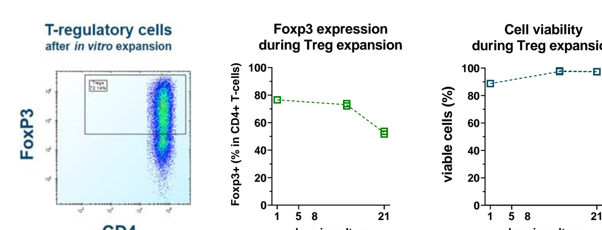
- Efficient manipulation of primary human T-cells using CRISPR established (T-cell subsets: CD4⁺, CD8⁺, γδ-T-cells, Tregs, etc.)
- "Druggable genome" CRISPR library (~8,000 genes) with a pool of 4 guides/gene was screened in arrayed format
- Effect of target-knockout on the frequency of FoxP3⁺ cells was monitored under two different assay conditions:
  1) Maintenance of Tregs (non-inducing medium condition; nTreg assay)
  2) Induction of Tregs (FoxP3 inducing medium; iTreg assay)
- Seven-color FACS readout: main parameter for hit ID was FoxP3. In total 220 assay plates were screened with a plate success rate of 93.6% (Z' > 0.4).
- Two hit lists were generated for targets of which knockout led to either:
  1) decrease in FoxP3⁺ cells; 321 hits (≥3-fold MAD1) (relevant for I/O)
  2) increase in FoxP3⁺ cells 453 hits (≥3-fold MAD1) (relevant for I&I)

T-cell suppression: A relevant *in vitro* assay to capture Treg biology

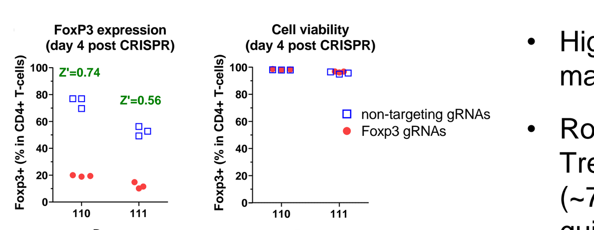


- CD4⁺/CD25⁺Tregs purified from healthy donors and expanded for 2 weeks before target KO by CRISPR/Cas9.

In vitro expansion of purified Tregs

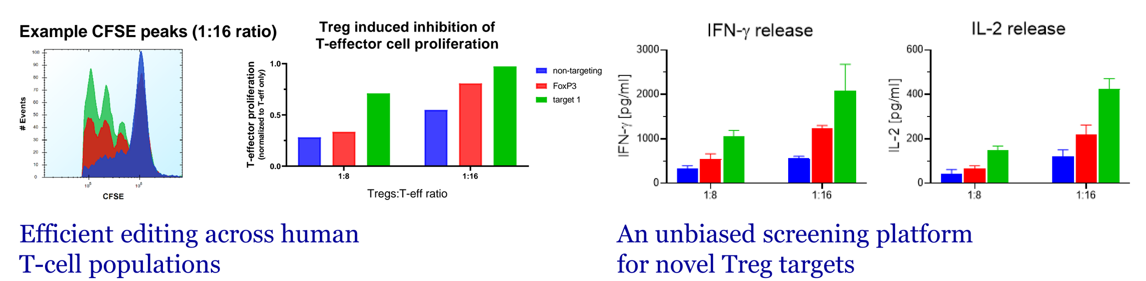


CRISPR-editing of expanded Tregs



- High viability and FoxP3 expression maintained during Treg expansion
- Robust CRISPR-editing of expanded Tregs with a good assay window (~70-80% FoxP3 reduction with control guides) and high viability
- Effect of target KO on FoxP3 expression, cell viability and cytokine release can be assessed

Target validation in T-cell suppression assay



- Effect of target KO on Treg immune-suppressive function assessed by monitoring effector T-cell proliferation and cytokine release (i.e. IL-2, IFN-γ)

Efficient editing across human T-cell populations   An unbiased screening platform for novel Treg targets

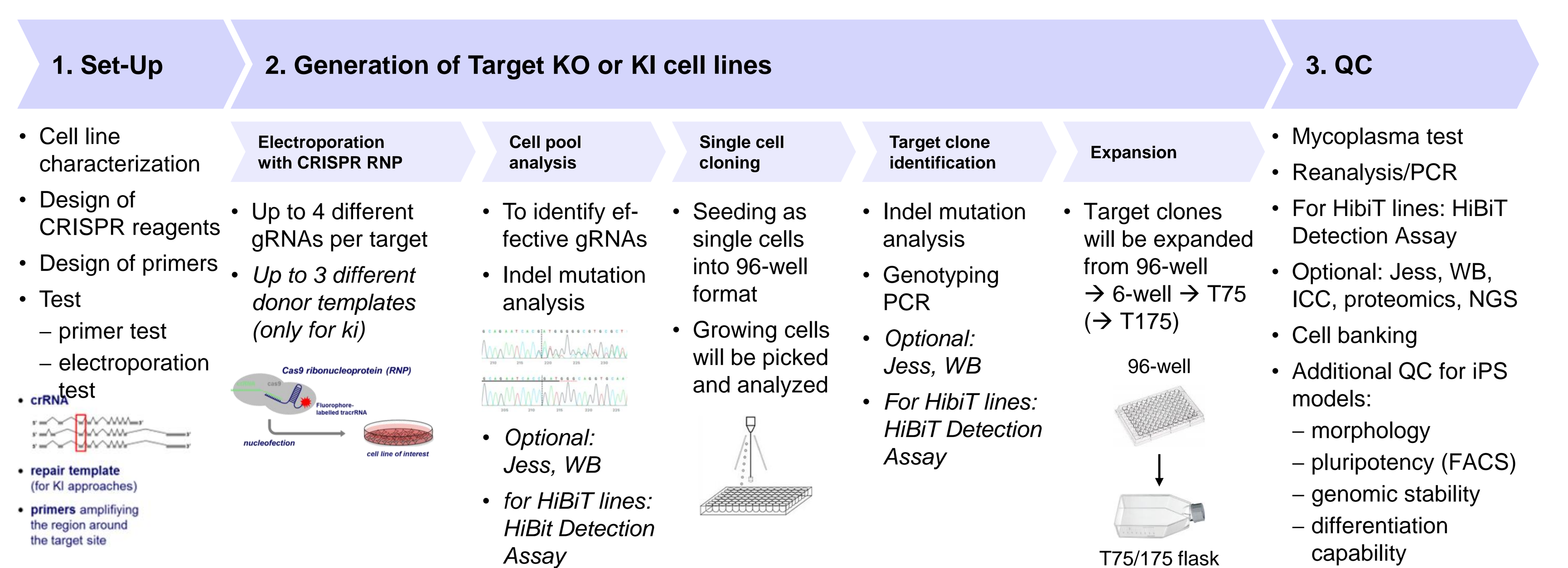
Knock-out and knock-in cell line generation using CRISPR/Cas9

Lenti CRISPR knock-out (KO) cell pools

- Fast generation time
- Applicable for many cell lines and primary cells
- 100% KO is usually not achieved
- Validation by next-generation sequencing

Clonal KO or knock-in (KI) cell lines

- Generation time: 10 – 14 weeks
- not applicable for cells with limited passaging number
- 100% KO or KI achieved
- Validation by Sanger sequencing



Genome editing workflow: Generation of CRISPR HiBit KI, KI or KO cell lines