Virus-Free Gene Modification with Minicircle DNA for Advanced Cell Therapy

Dr. Dirk Winnemöller

PlasmidFactory GmbH

The CDMO and service provider for plasmid and Minicircle DNA

Agenda

Company Introduction

Minicircle (MC) Technology

Application Examples

- Generation of CAR-T Cells using the SB100X Transposon system
- Sleeping Beauty Transposon Engineering for HSC Therapy
- Non-Viral Manufacturing of Tumor-Specific TCR-T Cells
- CRISPR editing using a Minicircle donor DNA template

GMP production facility, PlasmidFactory GmbH, Bielefeld

Company Introduction

Our GMP capabilities at a glance

- 25 years of DNA manufacturing expertise
- Independent, flexible & trusted European CDMO
- Scalable production: Research → High Quality → GMP Grades
- Proprietary technologies: Minicircle, ITRPROTECT®, ITRRESCUE®, POLYARESCUE®, MIDGE®
- 3,500+ plasmid & Minicircle projects delivered
- 99.9% success rate

GMP Manufacturing You Can Trust

Your trusted CDMO partner

State-of-the-art GMP facility

- Dedicated building for GMP Grade plasmid & Minicircle DNA
- 450 m^2 cleanrooms suites (grades C & D)
- Facility designed to prevent cross-contamination

Flexible manufacturing with highest safety standards

- End-to-end single-use USP- and DSP
- Ph. Eur. / USP grade WFI supply
- EU GMP (Annex 1) / FDA compliant aseptic fill & finish
- QP batch release in-house

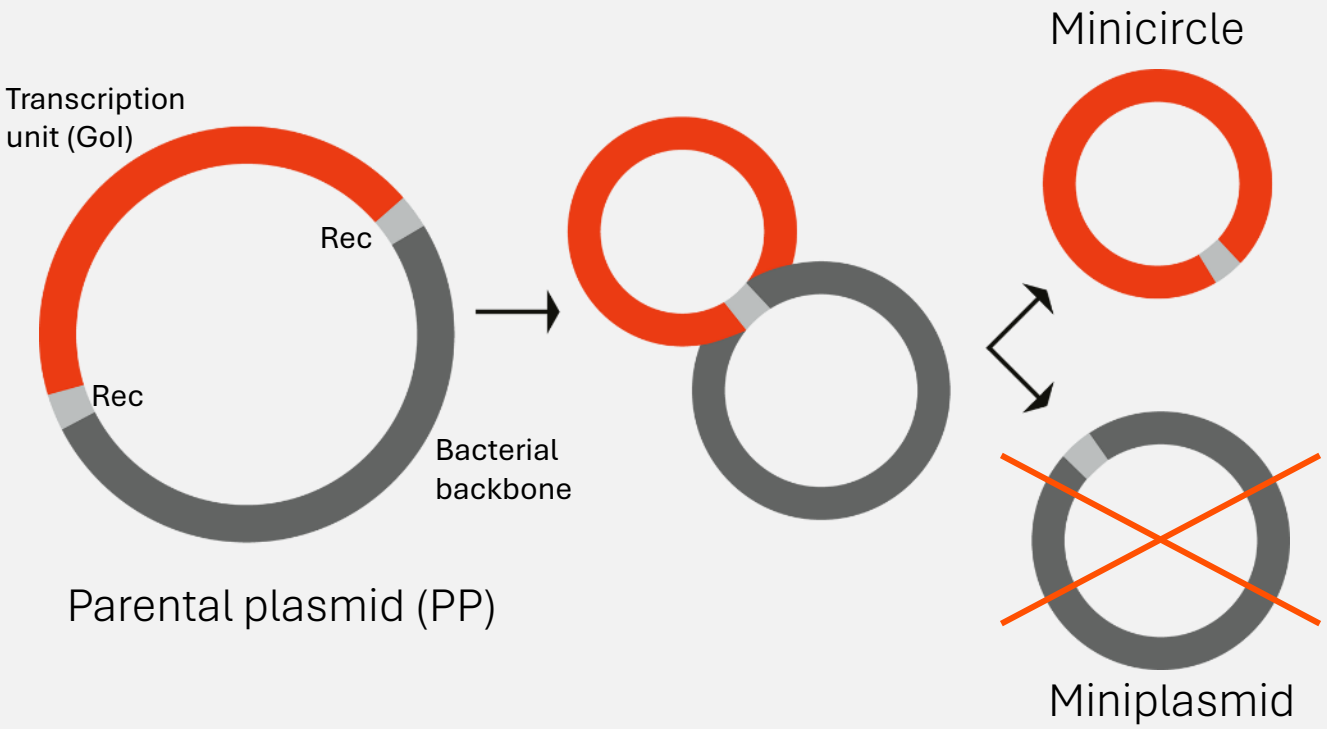
Full GMP compliance

- Data integrity measures and systems in place
- Annex 11 / 21 CFR Part 11
- EU GMP-Part 2 and AMWHV

Minicircle Technology: Proprietary Production Method

Minimalistic DNA vector reduced to the gene of interest (GoI)

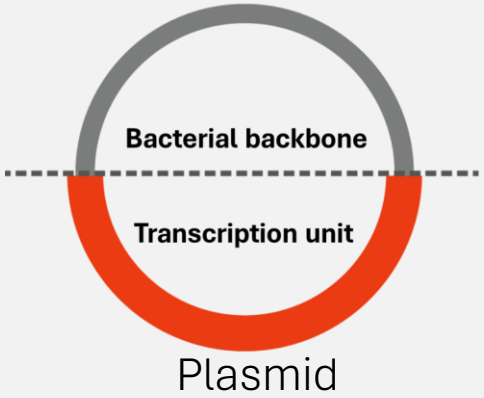
Minicircle production



Minicircle vs. plasmid features

- No bacterial backbone
- Small, supercoiled

- Bacterial sequences
- Larger size

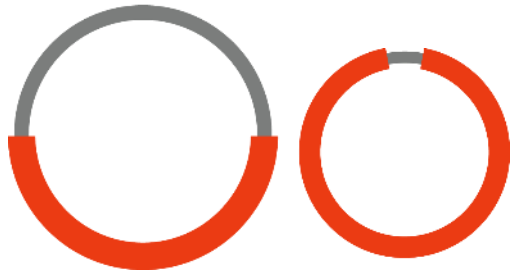


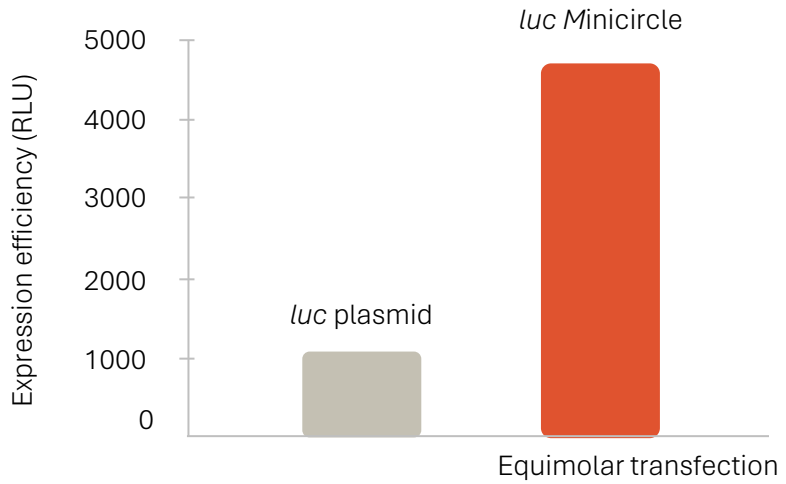
Minicircle: Benefits at a Glance

Advantages of bacterial backbone-free Minicircles in cell therapy

- Less immunogenicity
- Lower DNA toxicity
- Highter transfection efficiency
- Reduced transgene silencing
- Stronger, more stable gene expression
- Almost no cargo size restriction

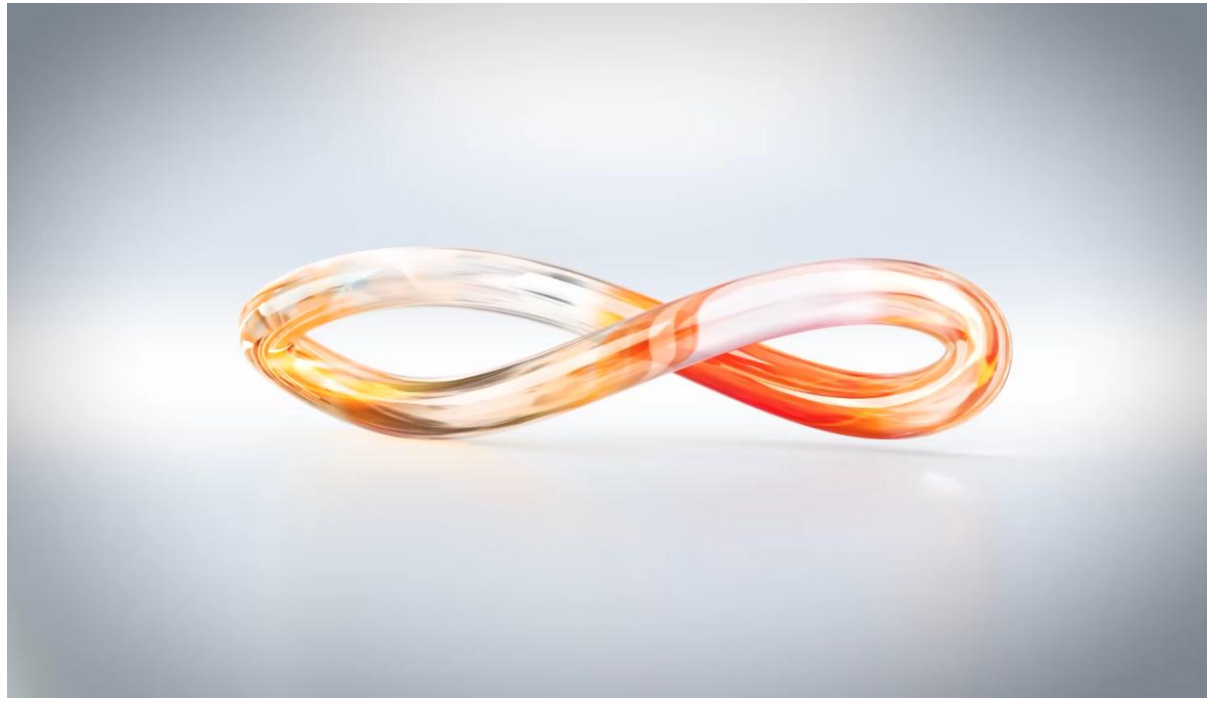
Successful in research & several clinical trials



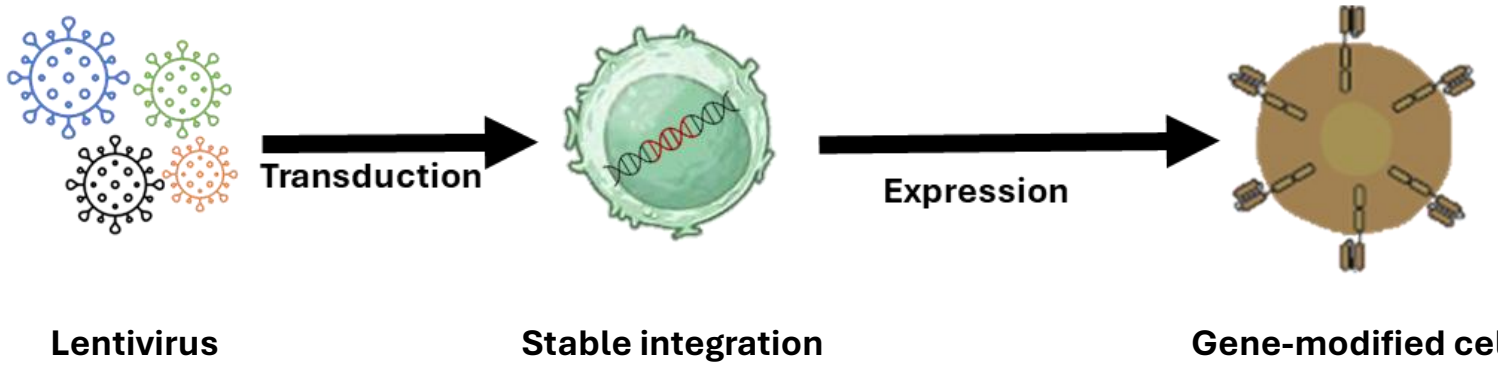


Application Examples

- **Generation of CAR-T Cells using the SB100X Transposon system**
- **Sleeping Beauty Transposon Engineering for HSC Therapy**
- **Non-Viral Manufacturing of Tumor-Specific TCR-T Cells**
- **CRISPR editing using a Minicircle donor DNA template**

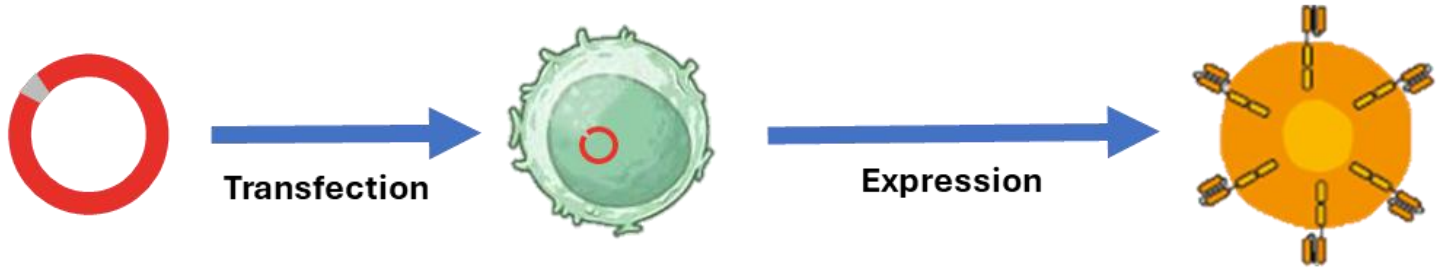


LVV vs. Non-Viral Gene Delivery Approaches



Lentivirus | Stable integration | Gene-modified cell

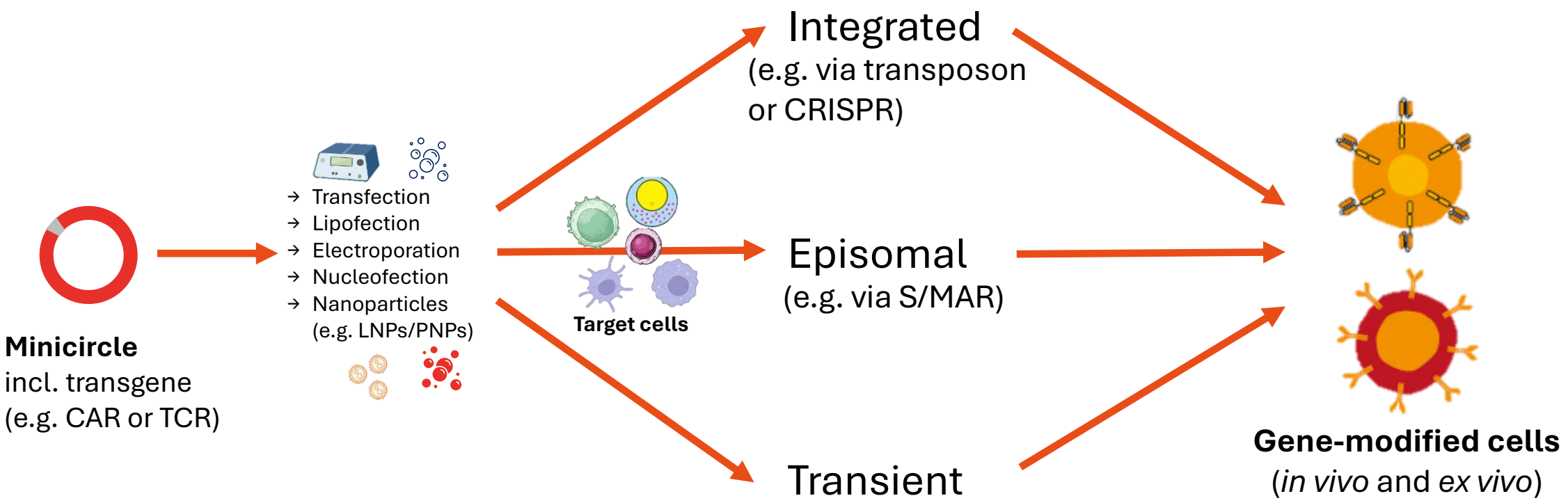
+ **Proven state-of the art technology**
+ **High delivery efficiency**
– **Complex manufacturing**
– **Time- & cost-intensive**
– **Cargo limits** (7–10 kb)



Minicircle | **Flexible integration** (random,directed, stable, transient) | Gene-modified cell

+ **Faster development**
+ **Cheaper & better scalable**
+ **Easier handling** (e.g. no S2 needed)
+ **Bigger cargos** (up to 20 kb)
+ **Future-proof: enables virus-free *ex vivo* & *in-vivo* gene-delivery**

Overview of Non-Viral Gene Delivery Approaches



PlasmidFactory®

“We indeed have a very fruitful and productive scientific collaboration with PlasmidFactory for more than 10 years now.”

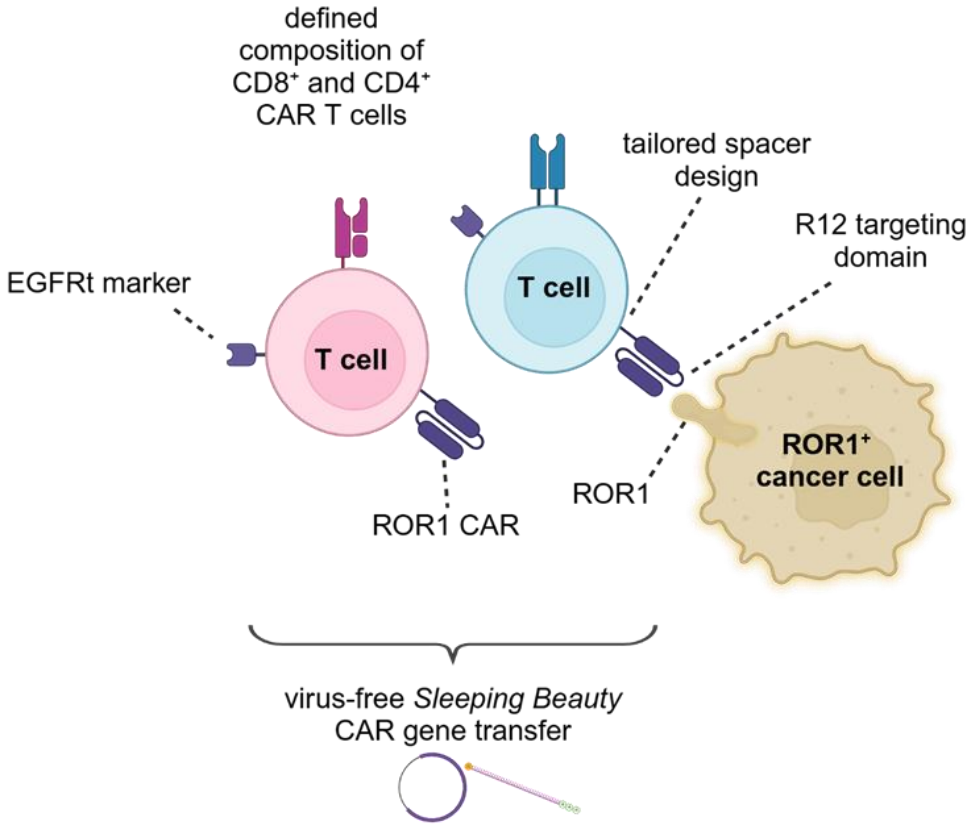
"A key inventive step for us was to use Minicircle DNA to encode the CAR which reduces the amount of DNA from a conventional plasmid which is 8-9 kbp to just 4 kbp.“

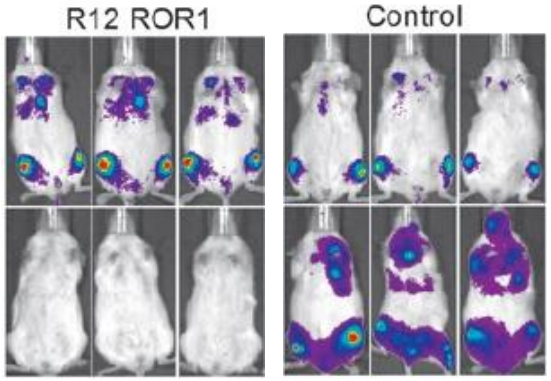
Prof. Dr. Michael Hudecek,
Universitätsklinikum Würzburg & Fraunhofer IZI

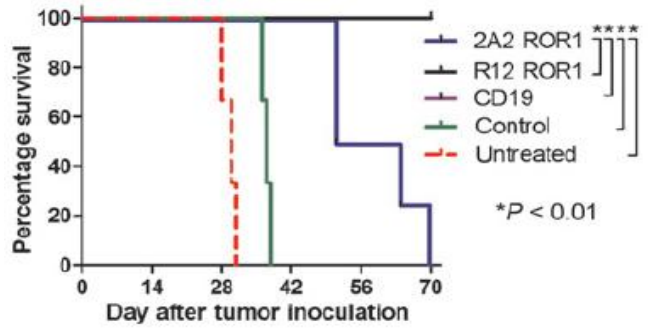
ROR1 CAR-T cells to treat hematologic and solid tumors

Slide courtesy of Prof. Dr. Michael Hudecek, Universitätsklinikum Würzburg & Fraunhofer IZI

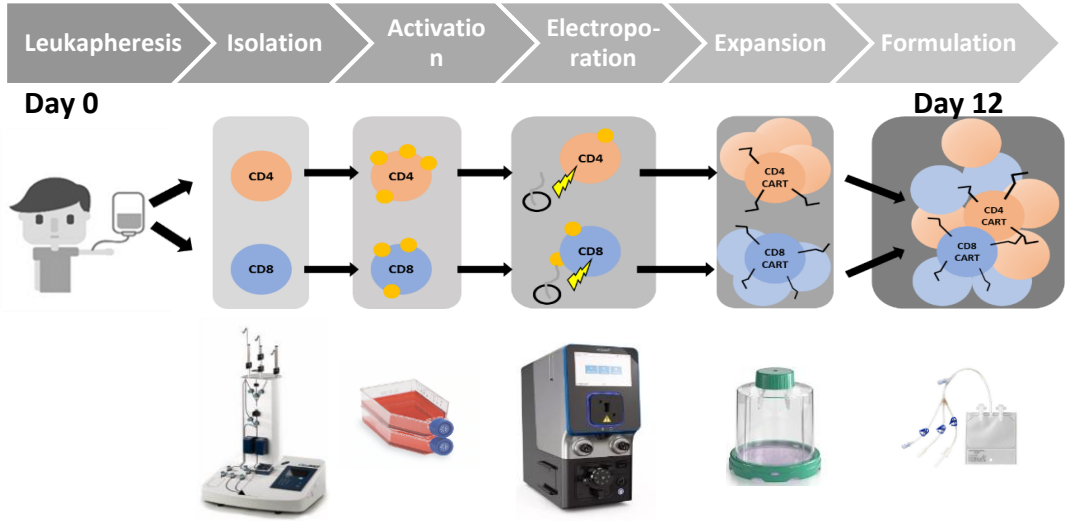
GMP CAR-T manufacturing with minicircle DNA SB gene-transfer







Hudecek et al. Cancer Immunol Res 2013; Wallstabe et al. JCI Insight 2019

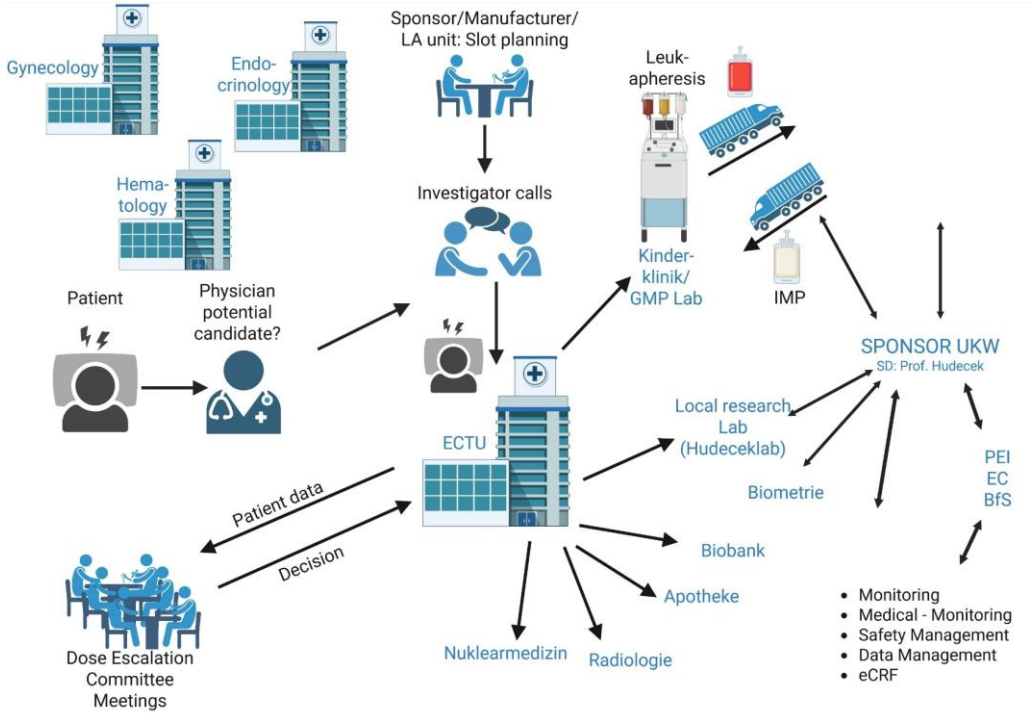


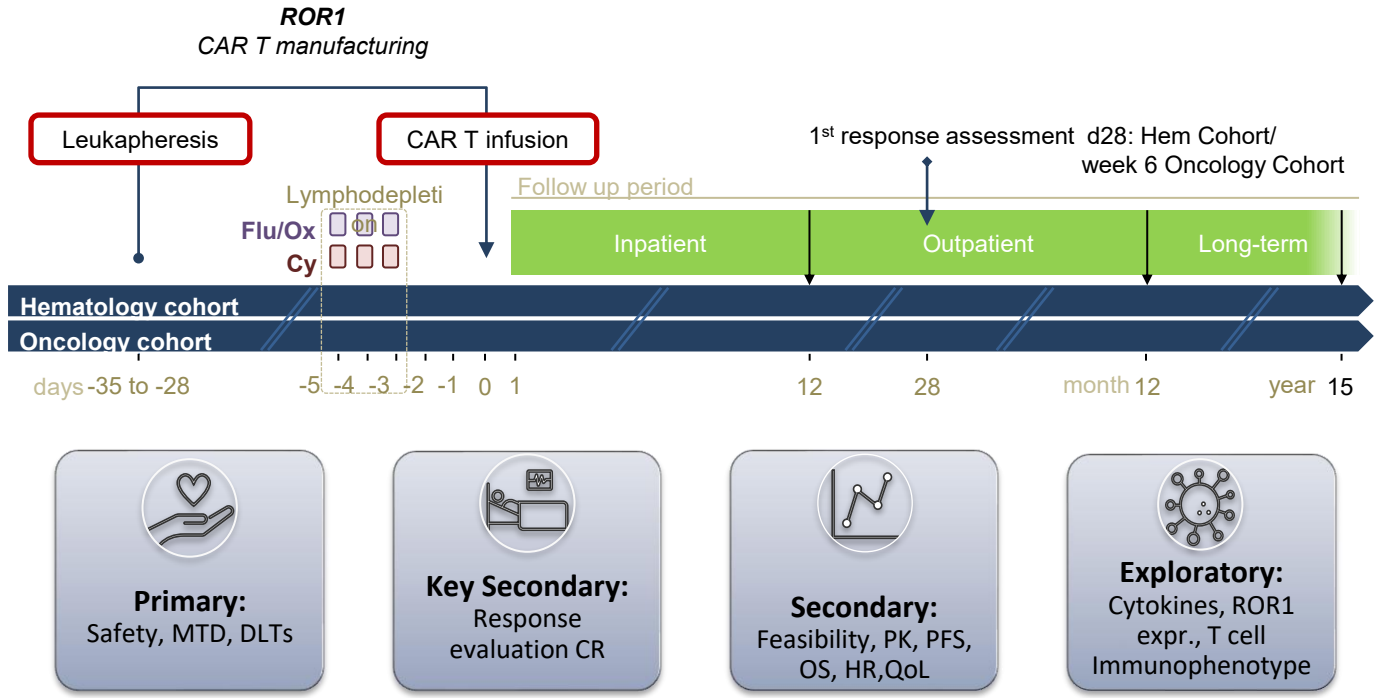
Fraunhofer IZI

GMP manufacturing established in Proof-of-Concept Initiative project (2018-2021). PI: Hudecek

ROR1 CAR-T cells to treat hematologic and solid tumors

Slide courtesy of Prof. Dr. Michael Hudecek, Universitätsklinikum Würzburg & Fraunhofer IZI

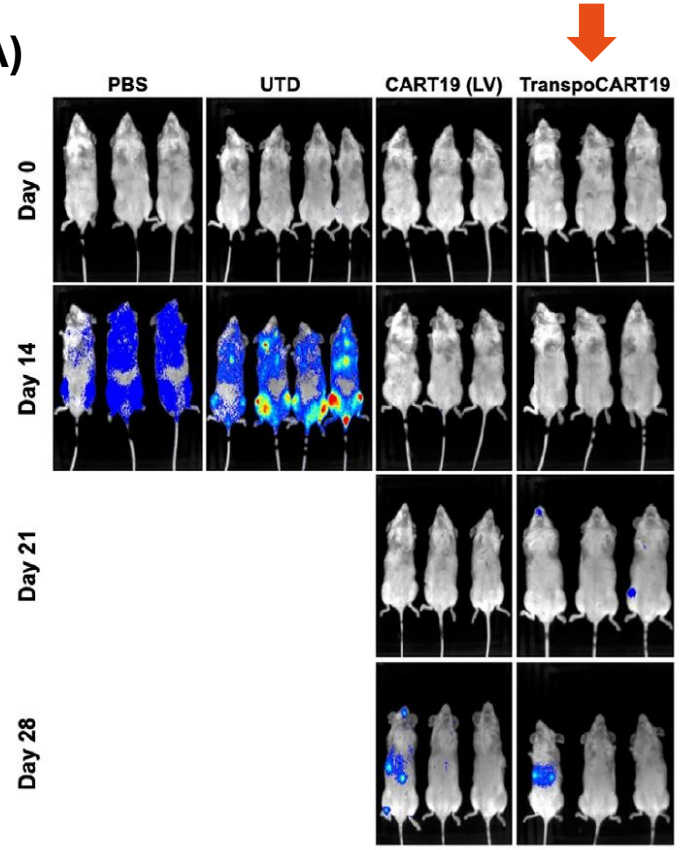




Four patients with adrenocortical cancer enrolled; three CAR-T products manufactured and administered

Clinical Scale Generation of functional CAR-T Cells using a Minicircle-based Sleeping Beauty Transposon System

A)



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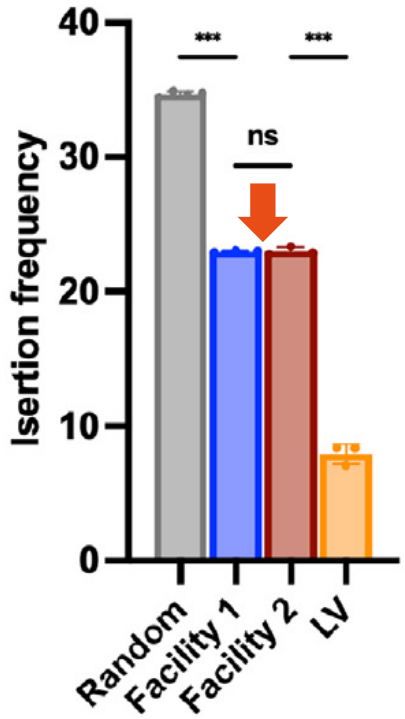


Díez *et al.*, 2025, Mol Ther Methods Clin Dev

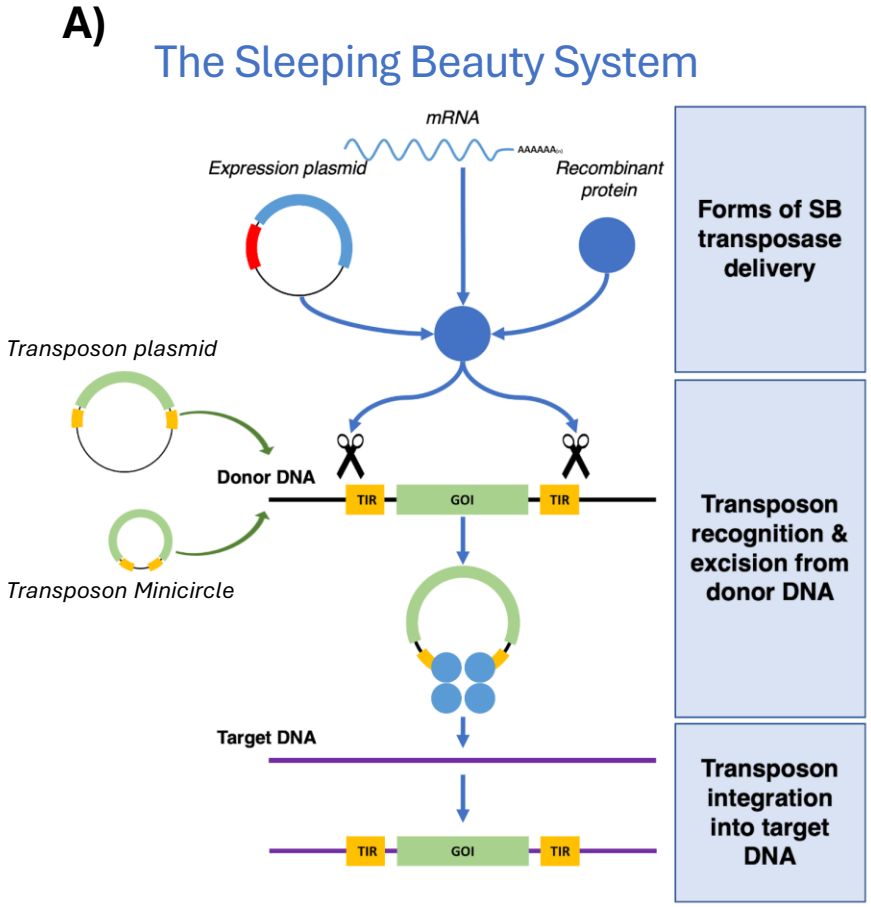
- MC approach results in equal tumor eradication to LVV (A)
- MC shows improved genomic safety vs. LVV (B)
- MC approach results in prolonged survival equal to LVV (data not shown)
- MC un-detectable in final CAR T product (data not shown)

Article title: Generation and GMP scale-up of human CAR-T cells using non-viral Sleeping Beauty transposons for B cell malignances

Efficient Non-Viral Gene Delivery into HSC using a Minicircle-based Sleeping Beauty Transposon System

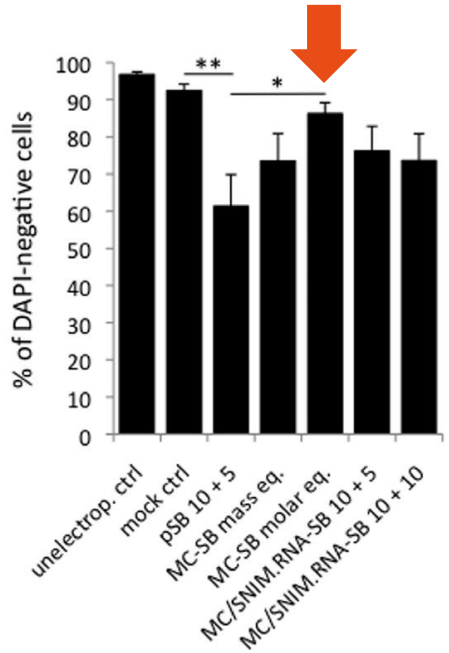
PlasmidFactory®

A)

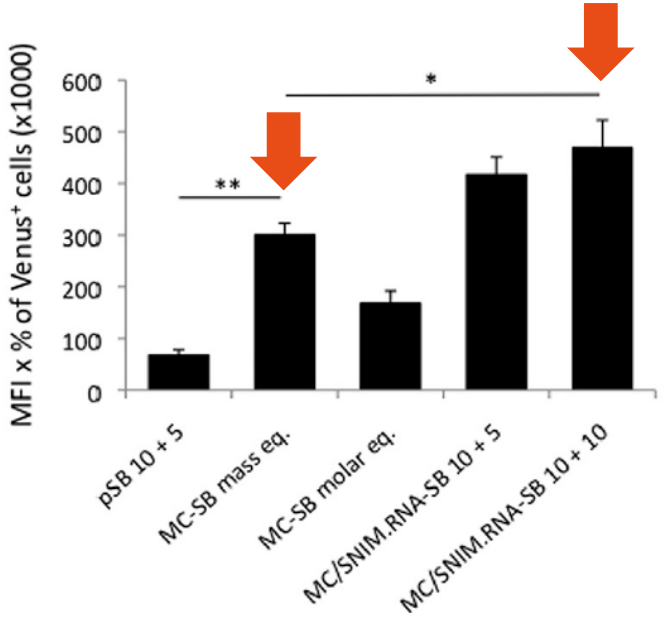


Adapted from Ivics et al. (1997) Cell

B)



C)



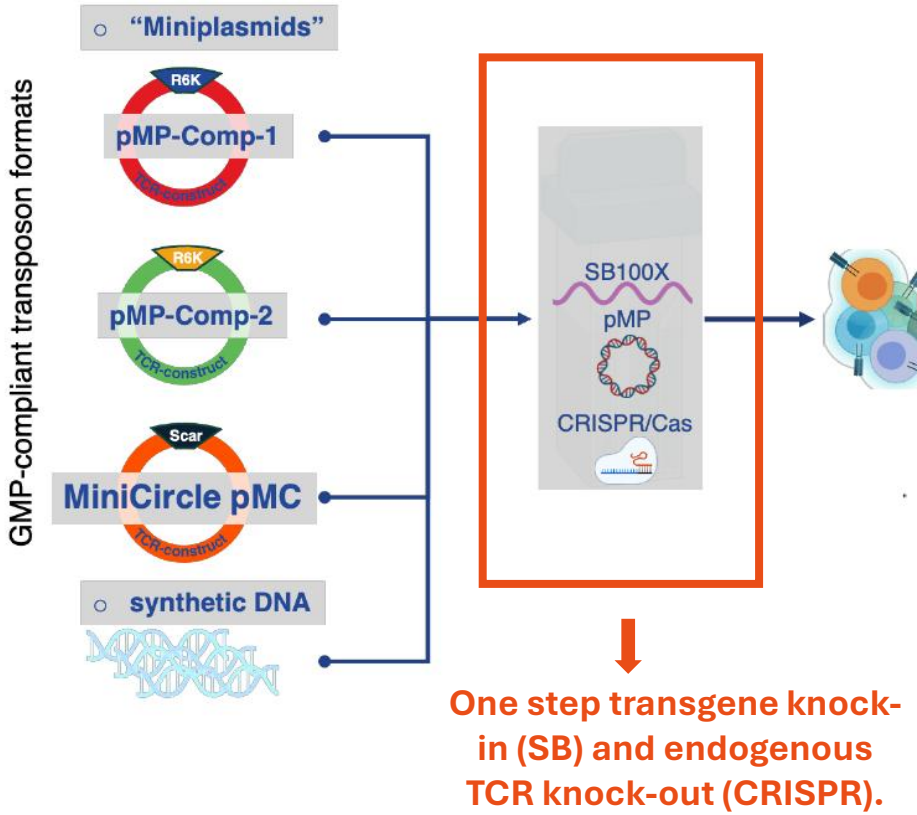
Holstein *et al.*, 2018, Mol Ther Nucleic Acids

- MC shows less cytotoxicity than plasmids (B)
- MC has improved long-term gene expression vs. plasmids (C)
- MC exhibits safer integration than viral vectors (γRV & LV; data not shown)

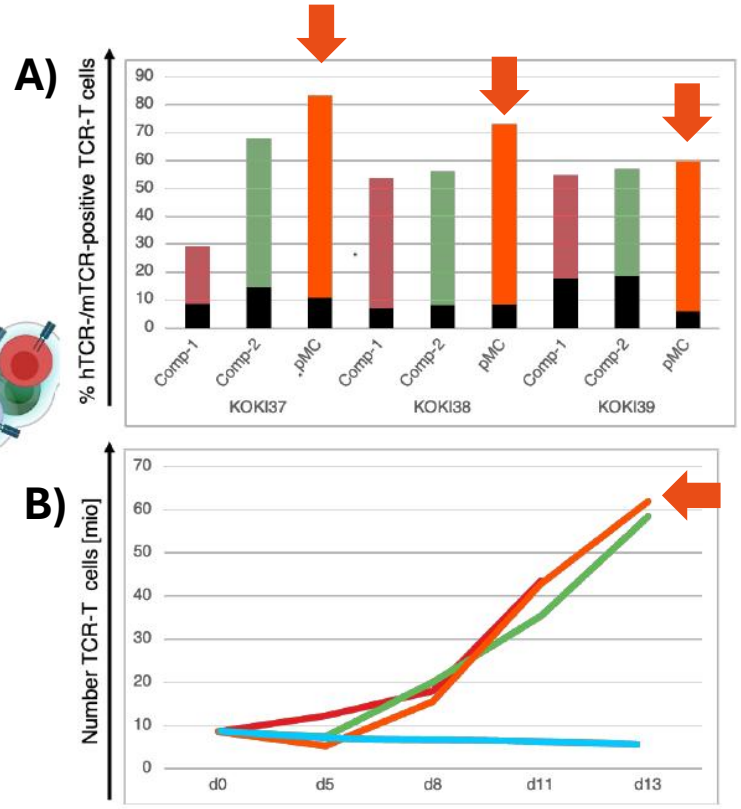
Article title: Efficient Non-viral Gene Delivery into Human Hematopoietic Stem Cells by Minicircle Sleeping Beauty Transposon Vectors

Non-Viral Manufacturing of Tumor-Specific TCR-T Cells for Immunotherapy of Solid Cancers using Minicircle

PlasmidFactory®



One step transgene knock-in (SB) and endogenous TCR knock-out (CRISPR).

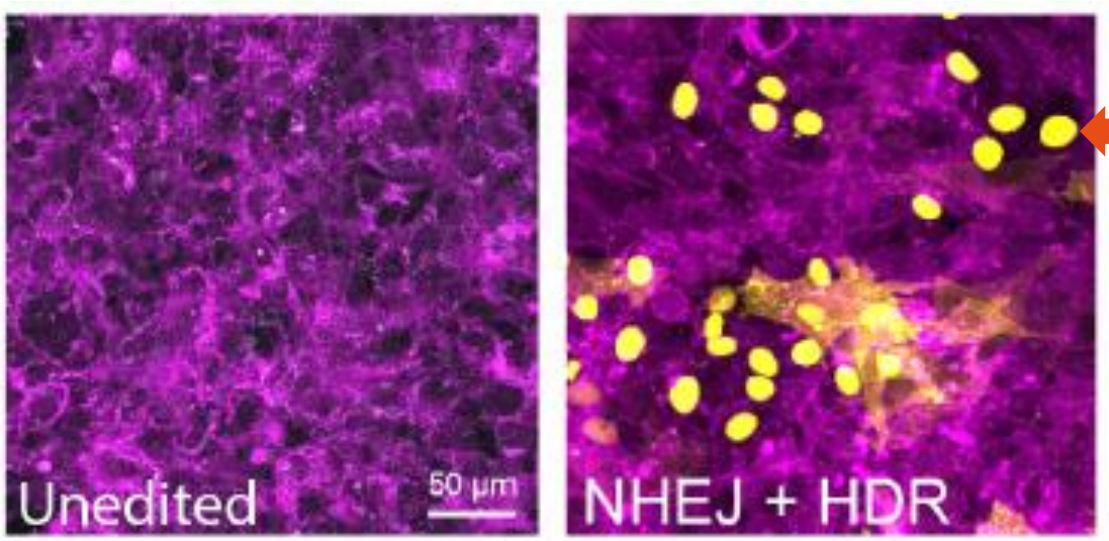


Lennerz *et al.*, 2025 ESGCT Annual Meeting

- MC approach achieves highest TCR knock-in combined with efficient endogenous TCR k.o. (A)
- Drastically reduced TCR-T cell numbers using synthetic DNA (B)
- MC is most effective in multi-vector setups (e.g. MC + mRNA; data not shown; see also Calviño et al., 2023, Frontiers)

HSDiagnomics TheryCell

CRISPR Editing using a Minicircle Donor DNA Template Carrying a Fluorescent Insertion Cassette



MC-mediated knock-in of nuclear EGFP

Mouse Embryonic Fibroblasts

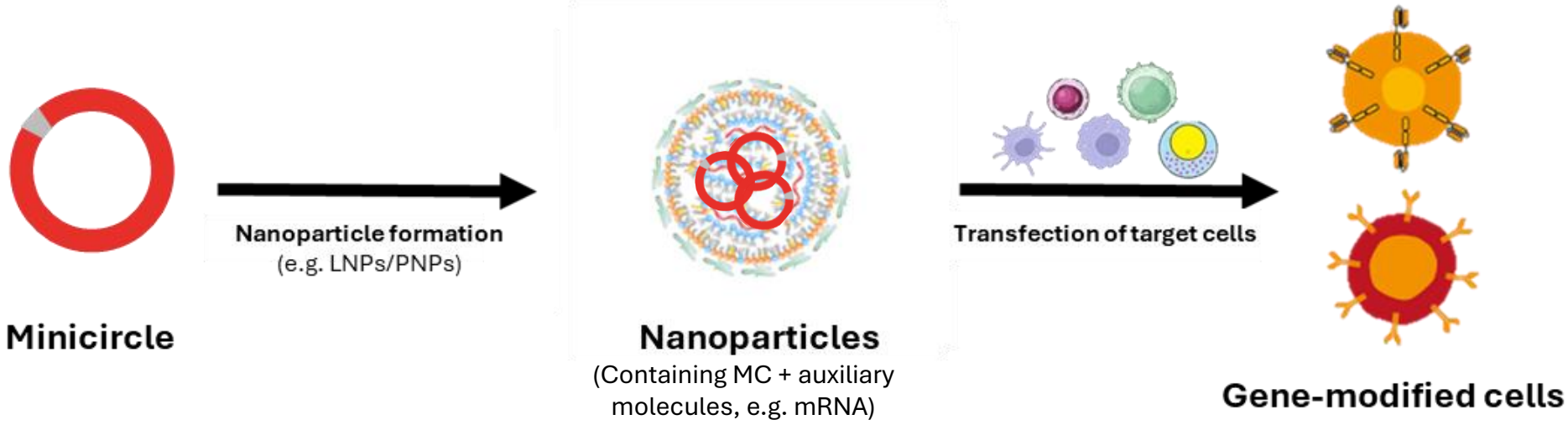
NHEJ – Non-Homologous End Joining
HDR – Homology-Directed Repair
HITI – Homology-Independent Targeted Integration

Tenant *et al.*, Mol. Ther., 2020

- Successful knock-in of fluorescence transgene cassettes (EGFP; yellow) using a MC as HDR- and HITI donor (only HDR shown)
- High compatibility with nucleofection, LNP, hydrodynamic tail vein injection and microinjection gene delivery approaches (data not shown)

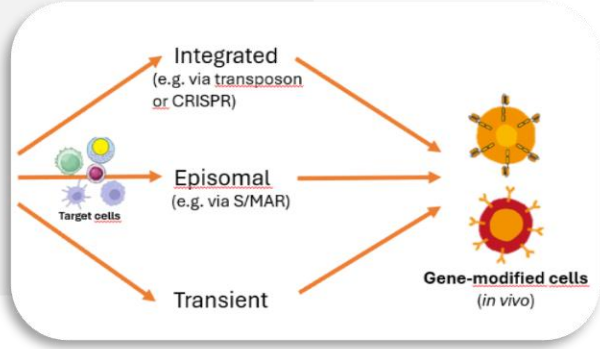
Article title: Fluorescent in vivo editing reporter (FIVER): A novel multispectral reporter of in vivo genome editing

Future Outlook: Minicircles as Ideal Vectors for Nanoparticles-Based *ex vivo* & *in vivo* Cell Therapies

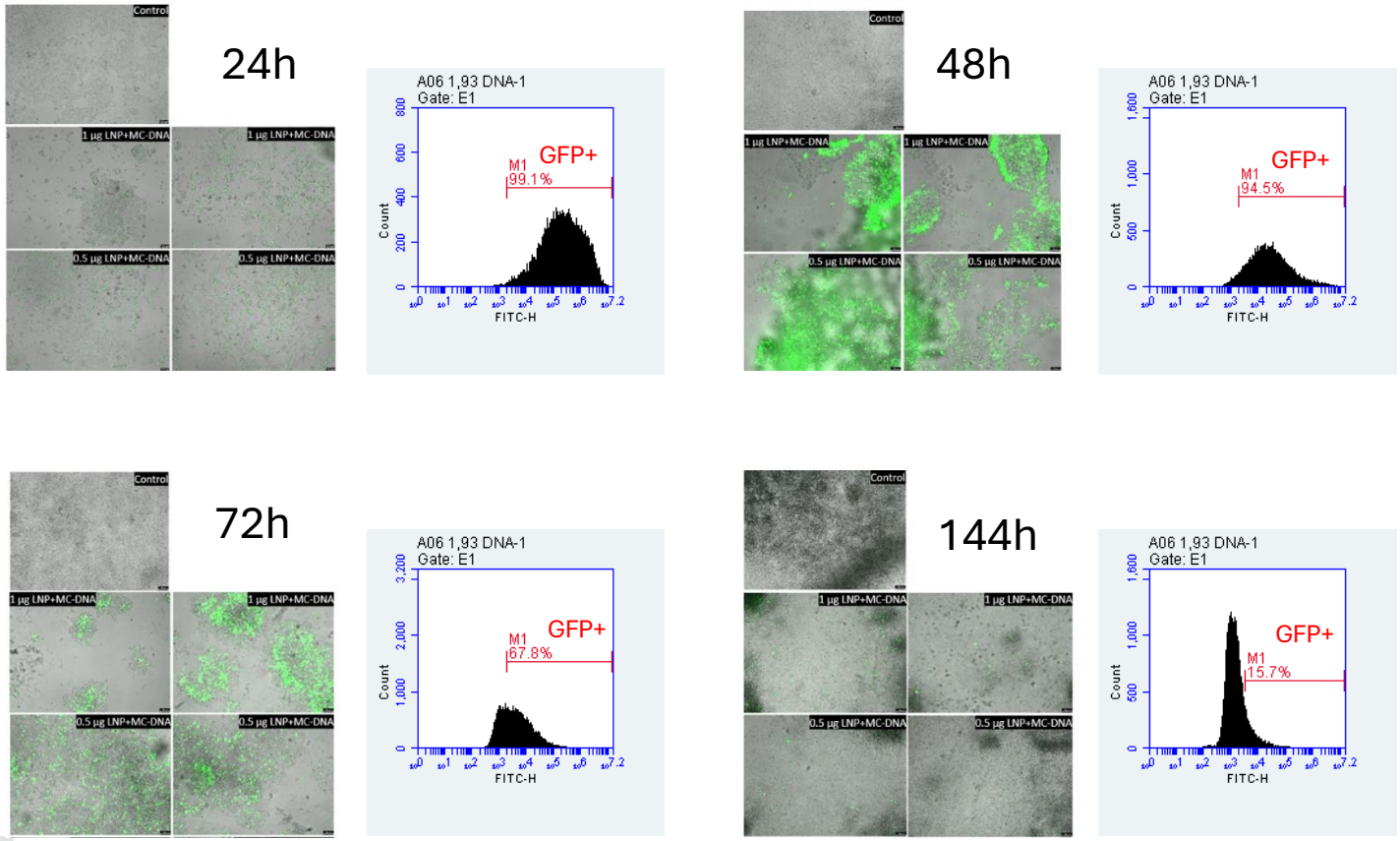


Minicircles: the ideal DNA vector for *in vivo* nanoparticle delivery:

- Non-viral, direct *in vivo* engineering of immune cells
- Versatile payload
- Broad use: CAR-T, TCR-T, TILs incl. solid tumors
- Safer, more scalable than viral vectors



Proof of principle: LNP-Transfection with Minicircle DNA



MC-GFP in HEK293

Karoline Czapla, 2025, Bielefeld University

- MC: well compatible with LNP transfection
- Efficient & successful gene delivery
- Strongest GFP expression at 48 h
- Minicircle-driven signal persists 24 h - 144 h
- mRNA: more short-lived expression, almost no detection from 48 h onwards
  (for comparison; data not shown)

Minicircle as Ideal Vector for Virus-Free Gene Delivery

Summary

- **Superior performance vs. plasmids**
  Bacterial backbone–free, small, supercoiled Minicircle DNA enables higher transfection and expression efficiency with lower DNA toxicity, reduced immune activation, and less gene silencing.
- **Enables versatile non-viral delivery**
  Compatible with a broad range of *in vivo* and *ex vivo* cell-engineering approaches, including LNP/PNP-based delivery and electroporation of transposon systems or CRISPR into T cells, NK cells, and HSCs.
- **Proven in several clinical trials**
  Backed by extensive research and clinical use (e.g. TranspoCART19, LION, CARAMBA) and peer-reviewed data, supporting virus-free, GMP-compliant manufacturing.
- **Reliable EU-based CDMO partner**
  PlasmidFactory offers >25 years of experience, ensuring consistent DNA supply from Research to GMP Grade.
- **Cost-efficient & faster alternative to viral methods**
  Reduced overall process costs & faster development times compared to viral vectors.

PlasmidFactory®

Thank you for your interest!

PlasmidFactory.com

PlasmidFactory 25 YEARS

PlasmidFactory GmbH
Meisenstraße 96 | 33607 Bielefeld | Germany

Available Quality Grades

Scientific Quality (SQ) Grade

Optimized DNA quality for basic research, pre-clinical and toxicology studies

- 4 parallel production lines
- Scalable process starting from 0.5 mg*
- 2 options available:
  - *Research Grade* for basic requirements
  - *CCC Grade* with ≥ 95% supercoiled DNA

Key features
- Fermentation-based

2 x 30L
2 x 20 L

High Quality (HQ) Grade

Starting material for GMP productions in early clinical phase

- 2 HQ facilities
- According to EMA guidelines
- Production scale 10 mg – 10 g**

Key feature
- Complete traceability

2 x 30L
2 x 200L

GMP Grade

Late clinical phase and market supply with direct human application

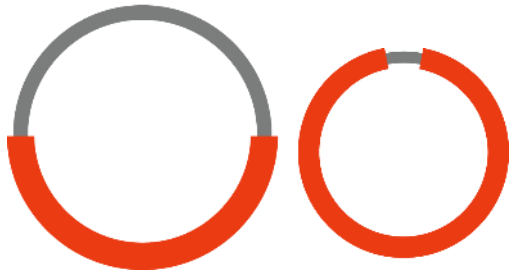
- State-of-the art GMP facility
- GMP-compliant production
- Scalable production process
- Tailormade for MC production

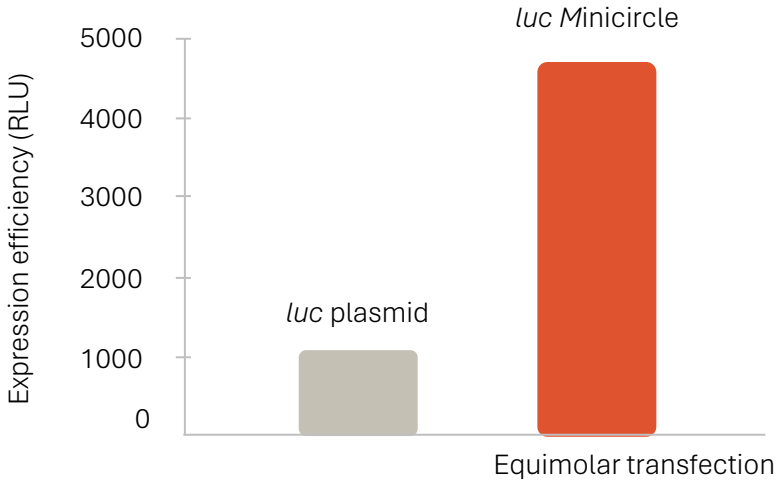
Key feature
- Single use equipment

2 x 40L

*0.5 mg for MC; 5 mg for plasmid DNA
**1 g for MC; 10 g for plasmid DNA; more on request

The McBox®: Test the Minicircle Yourself





Minicircle and plasmid DNA in one box

Compare Minicircle and the corresponding plasmid in your own experimental setting!

McBox® GFP

- MC.CMV-GFP, 0.1 mg
- pCMV-GFP, 0.1 mg
- WFI

McBox® lacZ

- MC.CMV-lacZ, 0.1 mg
- pCMV-lacZ, 0.1 mg
- WFI

McBox® luc

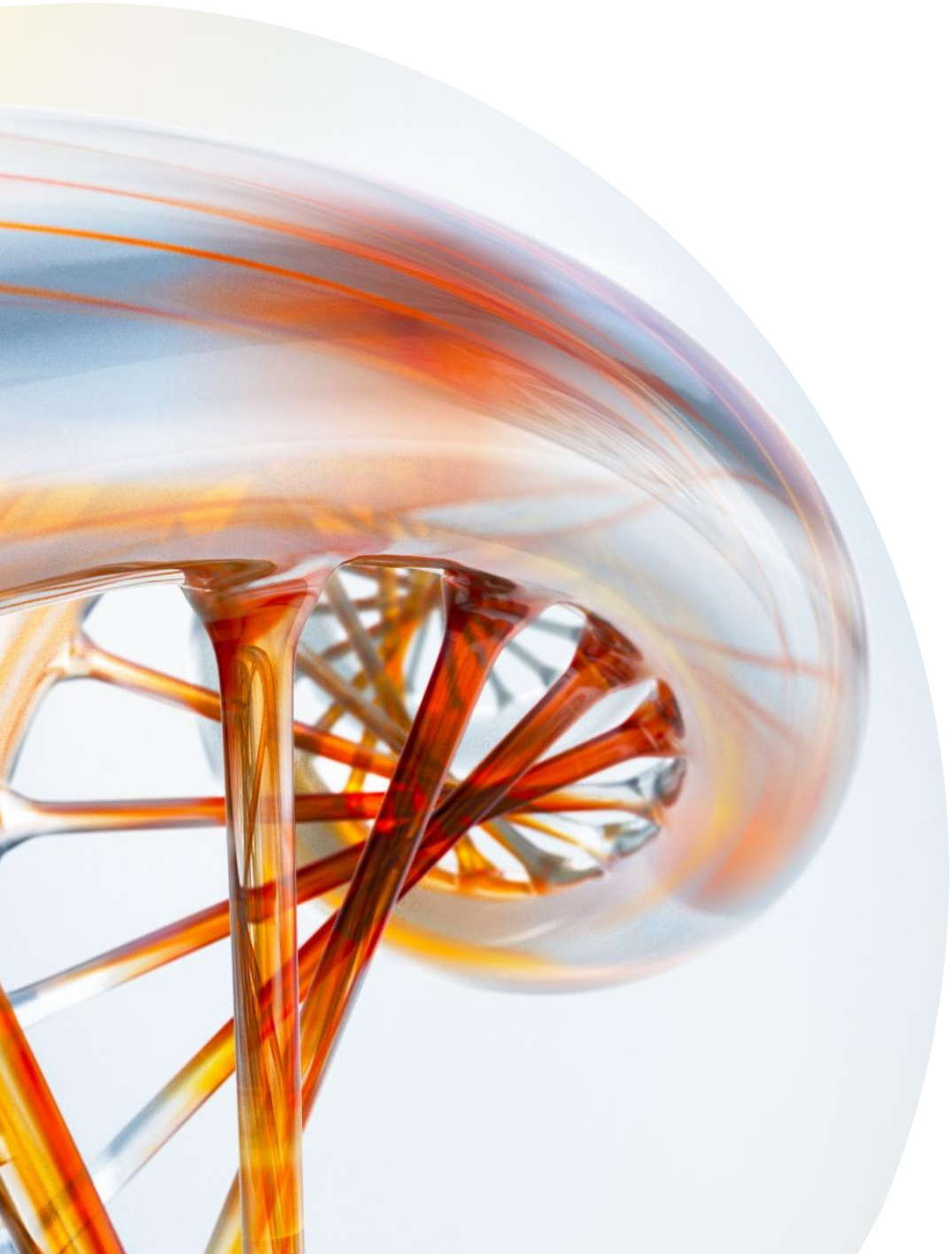
- MC.CMV-luc, 0.1 mg
- pCMV-luc, 0.1 mg
- WFI

Quality Grades Overview

Characteristics	Scientific Quality (SQ) Grade		High Quality (HQ)* Grade	GMP Grade
	Research Grade Plasmid	CCC Grade Plasmid | Minicircle	Plasmid | Minicircle	Plasmid | Minicircle
Produced by fermentation	✓	✓	✓	✓
Guaranteed amount of DNA	✓	✓	✓	✓
Adjustment of DNA concentration incl.	✓	✓	✓	✓
Customized filling included	✓	✓	✓	✓
Certified quality report incl.	✓	✓	✓	✓
Storage of glycerol stocks and retain samples for repeat orders	✓	✓	✓	✓
Antibiotic-free fermentation	✓	✓	✓	✓
Verified removal of bacterial endotoxins (LPS assay)	✓	✓	✓	✓
Removal of RNA and proteins	✓	✓	✓	✓
Removal of bacterial chromosomal DNA and oc-forms		✓	✓	✓
CGE analysis (ccc-supercoiled vs. oc-plasmid topologies)		✓	✓	✓
Extended specification		✓	✓	✓
Enzyme- and animal-free workflow		✓ | ✗	✓ | ✗	✓ | ✗
BSE/TSE-free certificate		✓ | ✗	✓	✓
Characterized and documented cell bank and pilot cultivation			✓	✓
Documentation according to GMP/GMP principles			✓	✓
Dedicated lab			✓	✓
QM system applied			✓	✓
Spatially separated upstream and downstream processes			✓	✓
Single use equipment for the entire process				✓
Compliant with applicable GMP-guidelines				✓

* HQ: High Quality Grade is produced in accordance with EMA guideline CHMP/BWP/2458/03 as the highest non-GMP quality standard

ccc: covalently closed circle
CGE: Capillary Gel Electrophoresis
LPS: Lipopolysaccharide
oc: open circular



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