PlasmidFactory®
The Minicircle Company

Why DNA Quality Matters – From Research to GMP

Dr. Martin Schleef - Founder of PlasmidFactory GmbH

Agenda

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1. DNA as the Foundation of Cell & Gene Therapy
Role of DNA vectors in modern CGT development

2. Plasmid Design Challenges
How sequence and vector architecture influence performance

3. Manufacturing of High-Quality DNA
Key steps from fermentation to purified plasmid DNA

4. Quality Control and Product Robustness
Ensuring consistency from research to GMP

5. Advanced DNA Vector Technologies
Minicircle DNA and next-generation vector formats

6. PlasmidFactory’s Expertise
Supporting CGT programs from early research to clinical development

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DNA as the Foundation of CGT

Role of DNA vectors in modern CGT development

High Quality DNA by PlasmidFactory

PlasmidFactory supports researchers and therapy developers

- Robust plasmid production
- Advanced vector formats
- Seamless transition from research to GMP

25+ years experience | 3500+ plasmid projects | CGT focus

Company info

The CGT Toolbox

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Cell and gene therapy development relies on several key components

Cells
- Patient-derived cells
- Production cell lines

Gene transfer technologies
- Electroporation
- Lipid nanoparticles, PEI, ...
- Viral delivery

Vectors
- AAV
- Lentivirus
- Plasmid / Minicircle DNA
- RNA

All vector systems ultimately depend on high-quality DNA.

DNA Vector Formats

Common non-viral DNA formats

Plasmid DNA
- Most widely used backbone

Linear DNA fragments
- Proprietary MIDGE® vectors

Minicircle DNA
- Backbone-free expression vectors

Vector design strongly influences:
- Transfection efficiency
- Safety
- Manufacturing feasibility
- Regulatory acceptance

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DNA Design Challenges

How sequence and vector architecture influence performance

Plasmids Are Not Equal

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Plasmids widely differ in:

- Size
- Sequence complexity
- Topology
- Regulatory elements

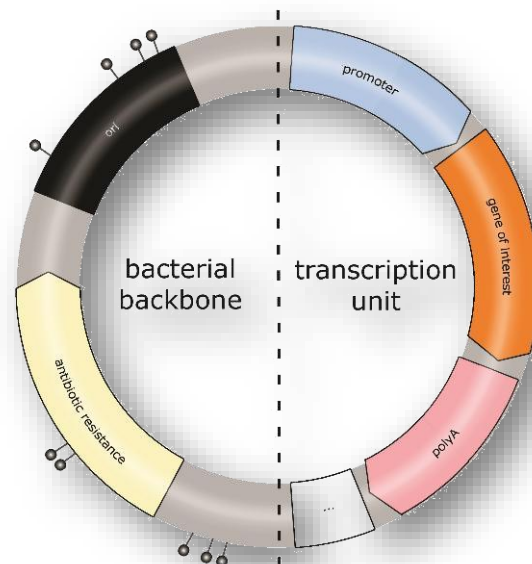
These differences strongly affect:

- Scalability
- Stability
- Manufacturability
- Biological performance

Structure in Textbooks

Ori = origin of replication
AB^R = selection marker

GOI = gene of interest



Topology of DNA Vectors

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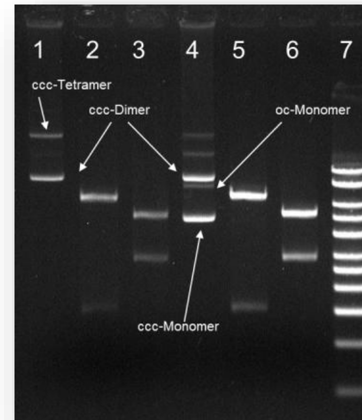
UNIVERSITÄT BIELEFELD

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

Plasmids widely differ in:

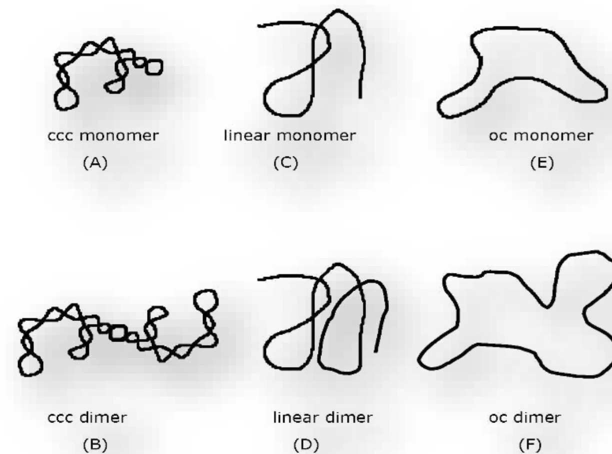
- Size
- Sequence complexity
- Topology
- Regulatory elements



Lane 1: Dimer form of the Plasmids
Lane 2: Dimer form *Bam*HI digested
Lane 3: Dimer form *Hind*III digested
Lane 4: Mono-/Dimer form of the Plasmids
Lane 5: Monomer form *Bam*HI digested
Lane 6: Monomer form *Hind*III digested
Lane 7: 1 kb ladder (DNA Size Marker)
(PlasmidFactory; Art. No. MSM-850-50)

Transformation of a 8393 bp Plasmid

Control digest
*Hind*III: 5417 bp + 2976 bp
*Bam*HI: 1525 bp + 6868 bp



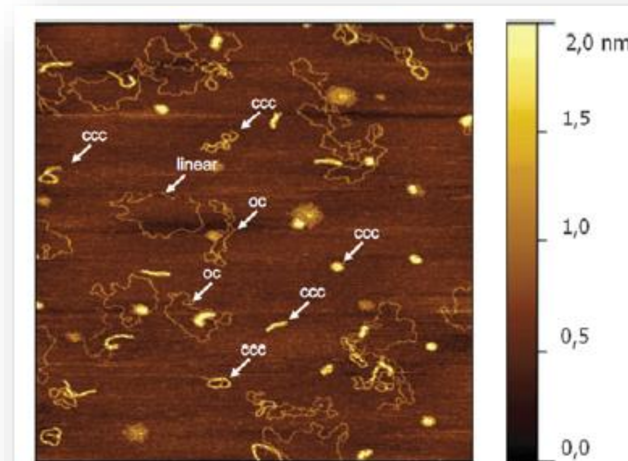


FIG. 2. AFM image of the 7-year-stored plasmid DNA. The AFM image shows ccc plasmid DNA of different degrees of condensation and oc forms (without linear forms, chromosomal DNA aggregates, or other contaminants). The figure shows an AFM image with an edge size of 3 μm. The altitude

Walther et. al, 2013

Impact of Plasmid Size

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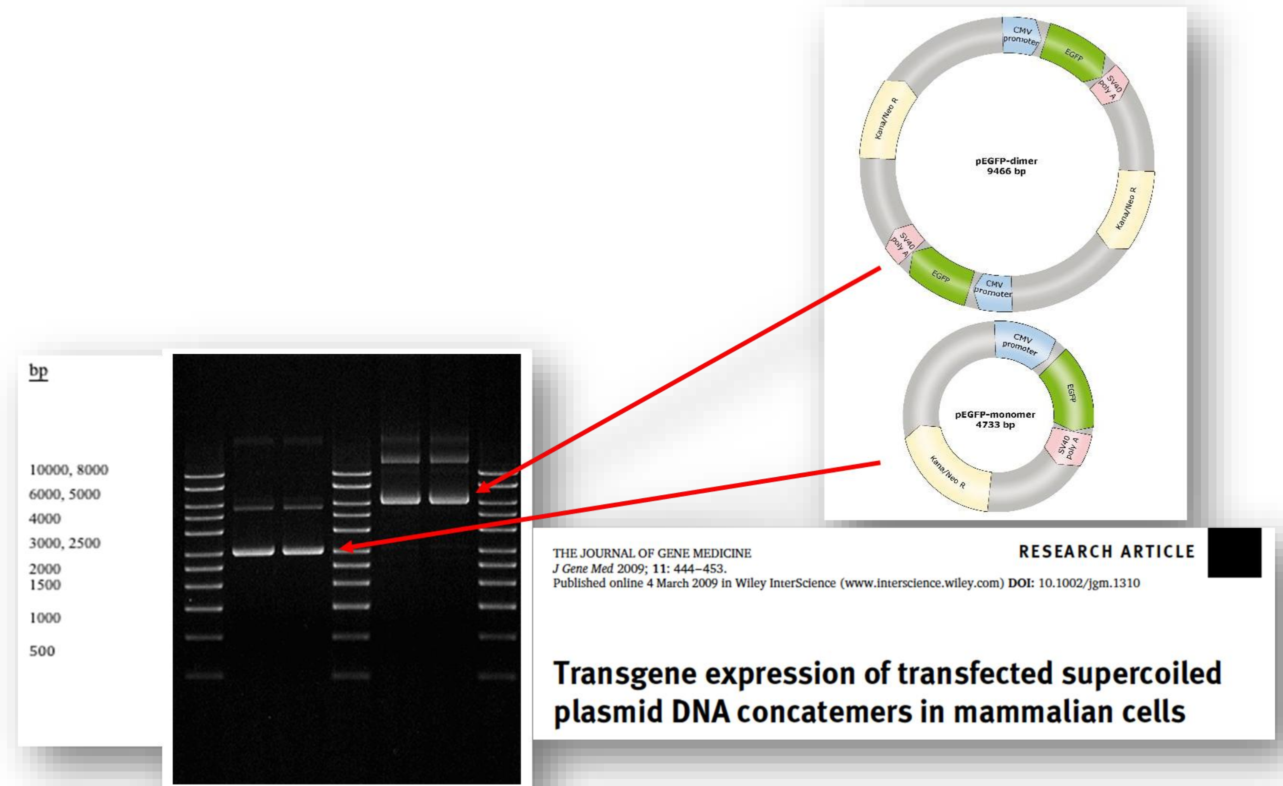
Large plasmids create several challenges:
- Reduced bacterial copy number
- Lower fermentation yield
- More complex impurity profile

Results:
- More difficult purification
- Higher production cost
- Potential variability between batches



Expert process design is therefore essential.

Sequence Features That Affect Production

Certain sequences reduce plasmid stability:

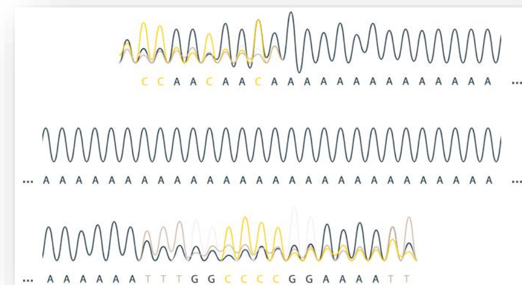
- Inverted terminal repeats (ITRs)
- Long homopolymers (PolyA)
- Hairpin structures
- Repetitive elements

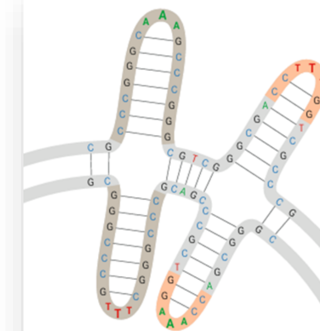
These regions can cause:

- Deletions during replication
- Sequence instability
- Reduced vector performance

Linearization of cutom plasmids & Minicircles

POLYARESCUE®





ITRPROTECT® / **ITR**RESCUE®

PlasmidFactory's proprietary technologies enable amplification of plasmid and Minicircle DNA preserving and repairing the sensitive ITR sequences.

CpG Content

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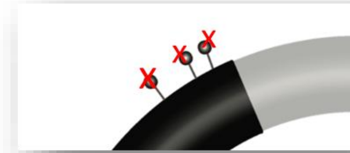
CpG motifs influence plasmid behavior:

- Innate immune activation
- Transcriptional regulation
- Plasmid stability

CpG-optimized or CpG-free plasmids can improve:

- Expression levels
- Tolerability in therapeutic applications

CpG influences the performance of a plasmid



nature biotechnology

CpG-free plasmids confer reduced inflammation and sustained pulmonary gene expression

Stephen C Hyde[1,6,8], Ian A Pringle[1,6,8], Syahril Abdullah[1,6–8], Anna E Lawton[1,6,8], Lee A Davies[1,6], Anusha Varathalingam[1,6], Graciela Nunez-Alonso[1,6], Anne-Marie Green[1,6], Reto P Bazzani[1,6], Stephanie G Sumner-Jones[1,6], Mario Chan[2,6], Hongyu Li[3], Nelson S Yew[4], Seng H Cheng[4], A Christopher Boyd[5,6], Jane C Davies[2,6], Uta Griesenbach[2,6], David J Porteous[5,6], David N Sheppard[3], Felix M Munkonge[2,6], Eric W F W Alton[2,6] & Deborah R Gill[1,6]

Pulmonary delivery of plasmid DNA (pDNA)/cationic liposome complexes is associated with an acute unmethylated CG dinucleotide (CpG)-mediated inflammatory response and brief duration of transgene expression. We demonstrate that retention of even a single CpG in pDNA is sufficient to elicit an inflammatory response, whereas CpG-free pDNA vectors do not. Using a CpG-free pDNA expression vector, we achieved sustained (≥ 56 d) *in vivo* transgene expression in the absence of lung inflammation.

Selection Markers

Traditional plasmids contain antibiotic resistance genes

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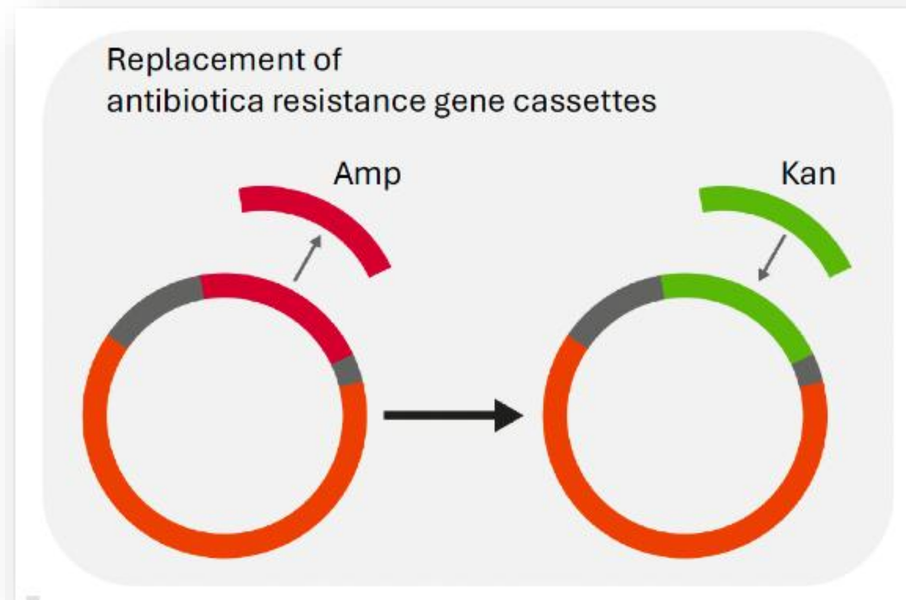
Implications:

- Regulatory concerns
- Metabolic burden on host cells
- Increased vector size



Alternative systems and optimized designs can improve vector performance.

Consequences for CGT Development

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Inconsistent DNA quality can lead to:

- Irreproducible research results
- Difficulties in technology transfer
- Delayed clinical development

Worst case:

- Late-stage surprises in clinical development

GMP production facility, PlasmidFactory GmbH, Bielefeld

DNA quality must be addressed early in development.

Our Approach

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PlasmidFactory addresses these challenges through:

- Deep expertise in difficult plasmid sequences
- Optimized amplification strategies
- Advanced analytics

Our goal:

- Consistent DNA quality from discovery to GMP

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Manufacturing of High Quality DNA

Key steps from fermentation to purified plasmid DNA

Quality starts with Process Design

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DNA quality is not just final QC

It depends on:

- Well-defined raw materials
- Validated equipment
- Controlled processes
- Trained personnel

Pilot Run

- process parameters
- productivity
- specifications

E. coli Cell Bank

- K12 safety strain
- transformation
- research cell bank
- master cell bank
- working cell bank

Consistency of manufacturing is the key determinant of quality.

Plasmid Fermentation Strategy

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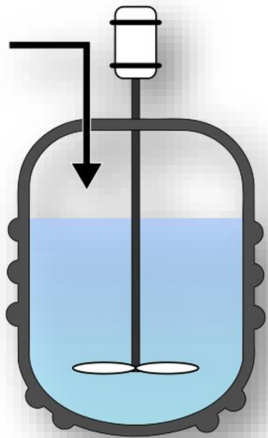
Plasmid production uses fed-batch fermentation

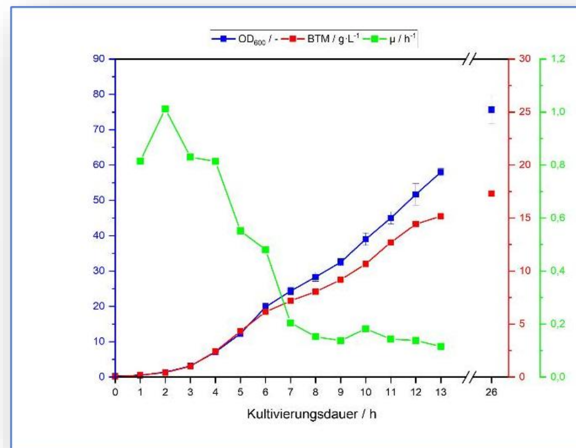
Advantages:

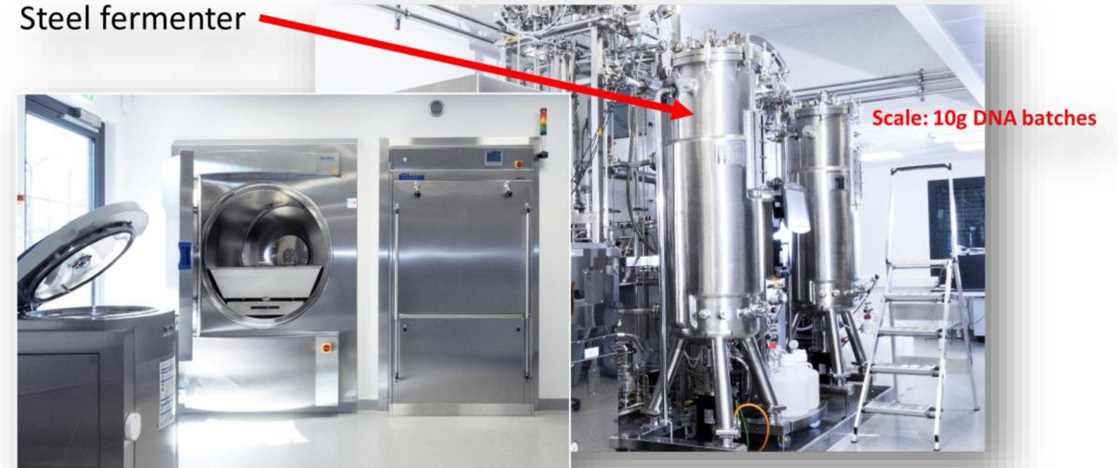
- Scalable production
- Controlled growth conditions
- High plasmid yield

Large Scale Fermentation

- bio-reactor
- monitoring of operating parameters
- reproducible process





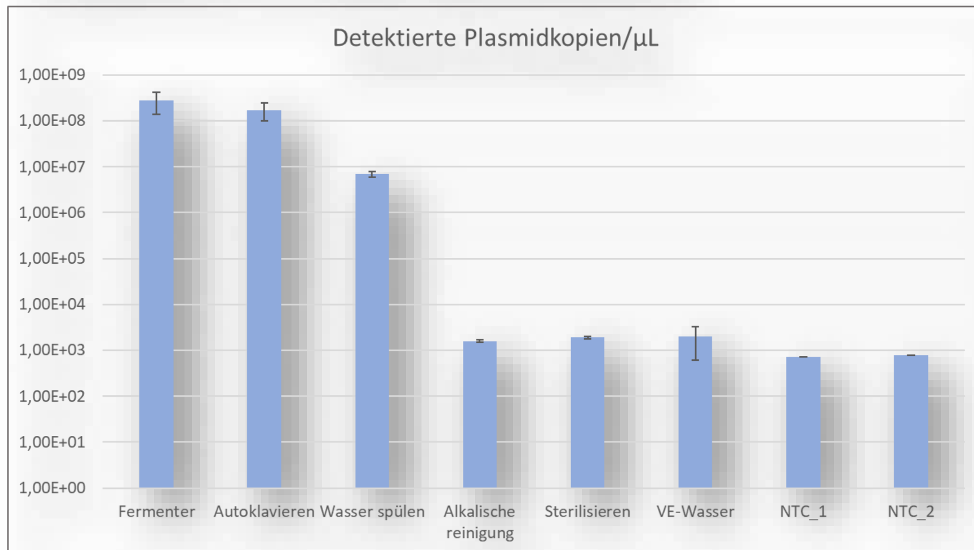


Industrial batches can reach multi-gram DNA scale.

Plasmid Fermentation Strategy

Steel or single-use

A question of cleaning validation!

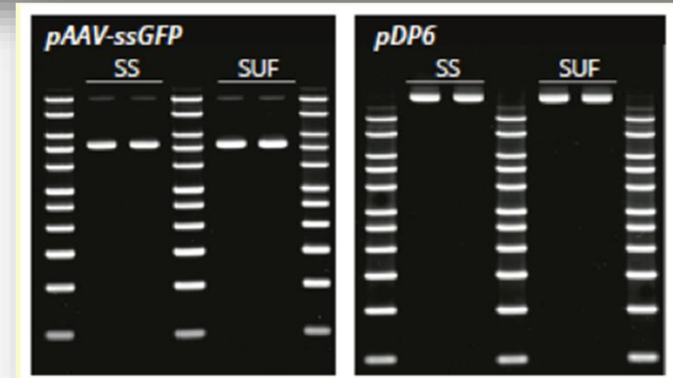


R. Shankar et al. 2020 (PlasmidFactory, not published)

Fermentation in Single-Use Fermenters for plasmid and minicircle DNA production

F Weber[1], C Buschmann[1], R Shankar[1], R Baier[1], C Ramke[2], R Klementz[2], M Leupold[2], J Rupprecht[2], A Vetter[3], K Teschner[3], S Klausing[3], M Schmeer[1], M Schleef[1]

1: PlasmidFactory GmbH, Meisenstrasse 96, 33607 Bielefeld, Germany | 2: Sartorius Stedim Biotech GmbH, August-Spindler-Straße 11, 37079 Göttingen, Germany | 3: Sartorius Xell GmbH, Waldweg 21, 33758 Schloss Holte-Stukenbrock, Germany



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SARTORIUS

Cell Lysis and Clarification

Efficient lysis is required to release plasmid DNA

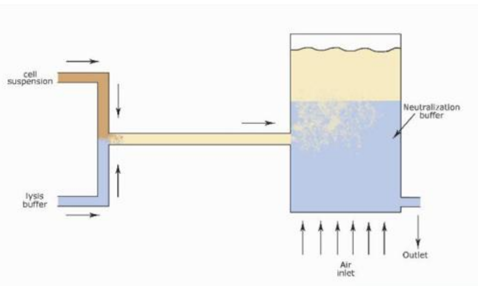
Key considerations:

- DNA integrity
- Removal of host cell impurities
- Process scalability

Cell Harvest and Alkaline Lysis

- bulk biomass
- cleared lysate

In-Line Lysis to replace bottle shaking



(12) **United States Patent**
Voss et al.

(10) Patent No.: **US 7,842,481 B2**
(45) Date of Patent: **Nov. 30, 2010**

(54) METHOD FOR PRODUCING EXTRA-CHROMOSOMAL NUCLEIC ACID MOLECULES

(75) Inventors: **Carsten Voss**, Lage (DE); **Erwin Flaschel**, Bielefeld (DE)

(73) Assignee: **Plasmid Factory GmbH + Co. KG**, Bielefeld (DE)

(56) References Cited

U.S. PATENT DOCUMENTS

5,407,426 A * 4/1995 Spears 604/24
2005/0026177 A1* 2/2005 Urthaler et al. 435/6
2005/0079534 A1* 4/2005 Warthen et al. 435/6

FOREIGN PATENT DOCUMENTS

WO WO 03102184 A1 * 12/2003

Modern processes often use in-line lysis systems.

Purification Strategy & Impurity Removal

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Purification typically combines:
- Capture chromatography
- Polishing chromatography
- Tangential flow filtration

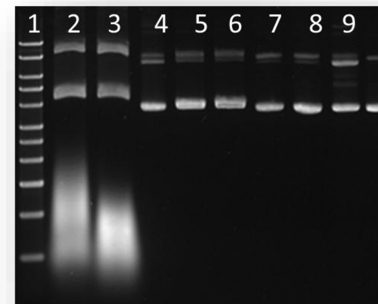
Major impurities include:
- Chromosomal DNA
- RNA
- Host cell proteins
- Endotoxin

Optimized purification strategies ensure:
- High purity
- Consistent batch quality

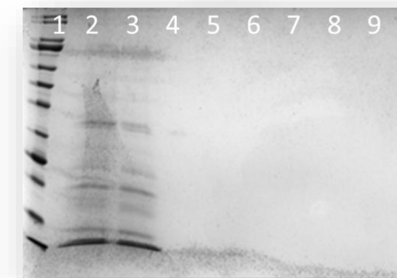
Chromatography
- procedure depends on required quality
- removes undesired plasmid forms and chromosomal DNA

Fill and Finish
- purified plasmid DNA
- aseptic filling

Agarose gelelectrophoresis

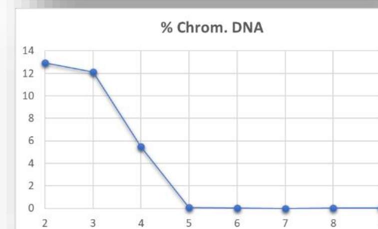


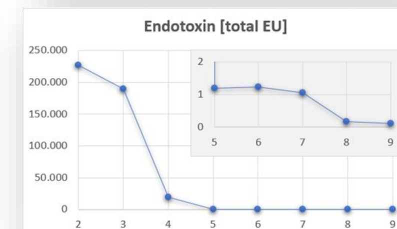
SDS-PAGE



6.1 kb plasmid

1: Size standard
2: Lysate
3: Lysate filtered
4: Eluate Capturing chromatography
5: Eluate Polishing chromatography
6: Eluate Polishing 2 chromatography
7: Eluate Polishing EF chromatography
8: Desalted by TFF
9: Filtered product





I. Schmitt, R. Shankar, et al., not published

Goal: High purity plasmid DNA suitable for therapeutic applications.

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Quality Control & Product Robustness

Ensuring consistency from research to GMP

Robustness of the Manufacturing Process

Leading to reliable cultivation processes – the basis for pure DNA.

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PlasmidFactory's production process is robust and produces reliable quality on a large scale

OD_{600} Fermentation

25
20
15
10
5
0
May 2020
June 2021
SOP specification

Total Amount of Biomass (in gram)

1000
800
600
400
200
0
May 2020
June 2021
Reference

Monitoring Plasmid Topology

Plasmid topology strongly influences biological activity

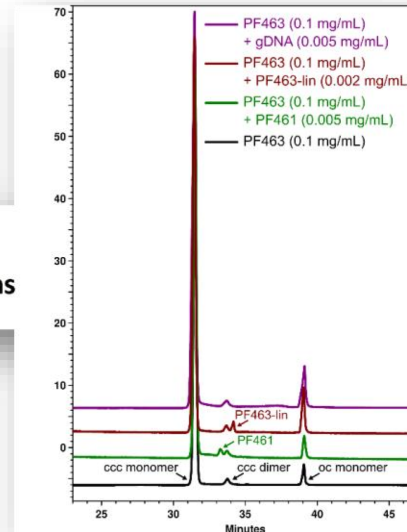
Typical forms:

- Supercoiled
- Open circular
- Linear

Validation of an analytical capillary gel electrophoresis method for the relative quantification of plasmid DNA topoisoforms
A Burgardt, M Strakeljahn, R Shankar, M Wiedemann & M Schleef

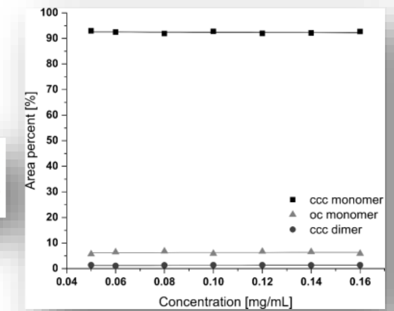
CGE Specificity

Validation of an analytical capillary gel electrophoresis method for the relative quantification of plasmid DNA topoisoforms
A Burgardt, M Strakeljahn, R Shankar, M Wiedemann & M Schleef



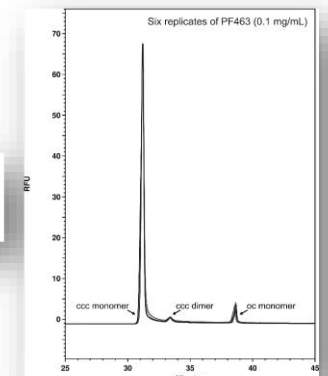
CGE Linearity

Validation of an analytical capillary gel electrophoresis method for the relative quantification of plasmid DNA topoisoforms
A Burgardt, M Strakeljahn, R Shankar, M Wiedemann & M Schleef



CGE Repeatability

Validation of an analytical capillary gel electrophoresis method for the relative quantification of plasmid DNA topoisoforms
A Burgardt, M Strakeljahn, R Shankar, M Wiedemann & M Schleef



Analytical methods such as capillary gel electrophoresis (CGE) allow precise monitoring.

Product & Freeze/Thaw Stability

Plasmid DNA is generally stable under appropriate conditions

Examples:

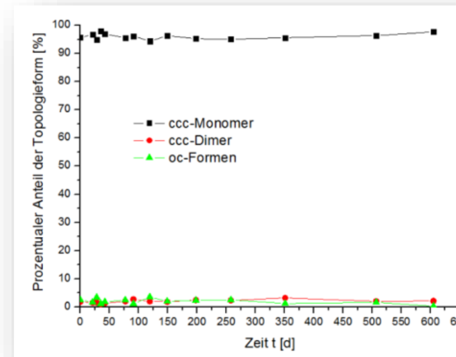
- Storage in WFI at suitable concentration
- Storage at −20 °C for long-term preservation

Repeated freeze-thaw cycles have limited impact on plasmid integrity.

This robustness is important for:

- Distribution
- Storage
- Routine laboratory use
- Clinical applications

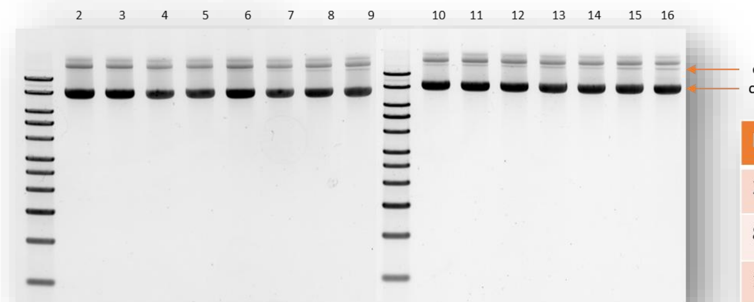
Long term stability of plasmid DNA



Plasmid DNA in WFI
at 1,0 mg mL^{-1}
Storage at -20 °C

Impact of freeze-thaw-cycles on plasmid DNA ≥10 kb

Number of cycles over 2 moths



Number of cycles	ccc peak [%]	oc peak [%]
2	98,8	1,1
8	98,5	1,6
16	99,1	0,9

Long-term stability supports reliable use in research and clinical applications.

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Advanced DNA Vector Technologies

Minicircle DNA and next-generation vector formats

Beyond Classical Plasmids

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To address limitations of conventional plasmids, alternative DNA formats are used:

Main vector concepts:

✓ **Plasmid DNA (circular, with backbone):**
- Standard format for gene delivery
- Flexible, proven, but includes bacterial elements

✓ **Backbone-free circular DNA (Minicircle):**
- Circular expression cassette only
- No bacterial sequences, optimized for expression

Additional approaches:

✓ **Synthetic and enzymatic linear DNA (e.g. dbDNA, MIDGE®):**
- Backbone-free and cell-free production possible
- Covalently closed linear formats improve stability
- Limitations in scalability, handling and expression performance depending on format

✓ **Other "mini“ or "nano“ plasmids:**
- Reduced bacterial backbone
- Partial improvement, but not fully backbone-free

Alternative formats to plasmids can improve safety and efficiency.

Linear DNA: MIDGE® Vectors

MIDGE (Minimalistic Immunogenically Defined Gene Expression) vectors are linear DNA constructs without bacterial backbone elements.

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Key characteristics:

- Linear DNA expression cassette
- Absence of bacterial sequences
- Reduced vector size compared to plasmids

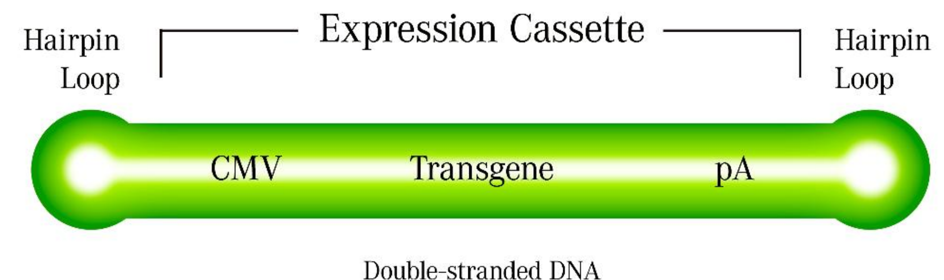
Potential advantages include:

- Lower immunogenicity
- Improved safety profile
- Simplified vector design

Potential disadvantages (incl. dbDNA):

- No enzyme-free
- Not supercoiled

Linear DNA (MIDGE) produced *in vitro* by use of enzymes?



MIDGE - a registered trademark of PlasmidFactory

Minicircle: Advantages at a Glance

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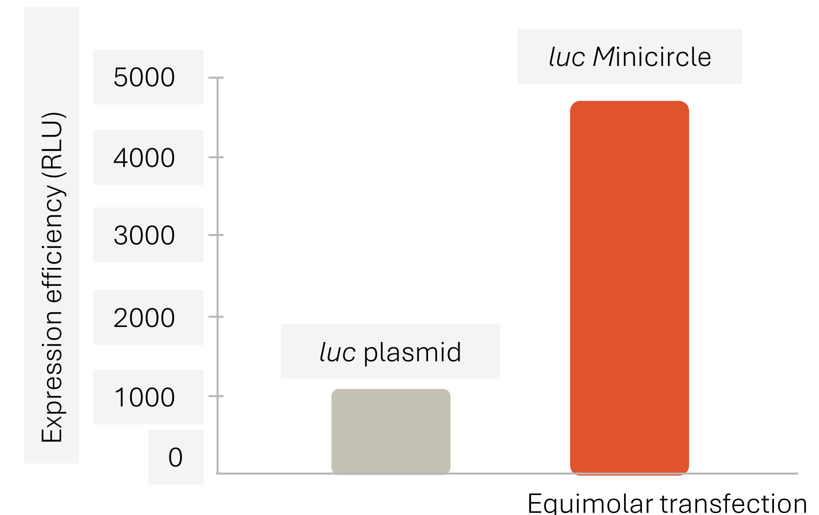
Bacterial backbone-free Minicircles

- No antibiotic resistance or other bacterial markers
- Less immunogenicity
- Lower DNA toxicity
- Highter transfection efficiency
- Reduced transgene silencing
- Stronger, more stable gene expression
- Almost no cargo size restriction
- Improved yield – minimized costs
- No backpacking in AAV production
- Ideal for virus-free gene transfers

Successful in several clinical trials



Kobelt et al, 2012

Minicircle info

Minicircle Technology: Proprietary Production Method

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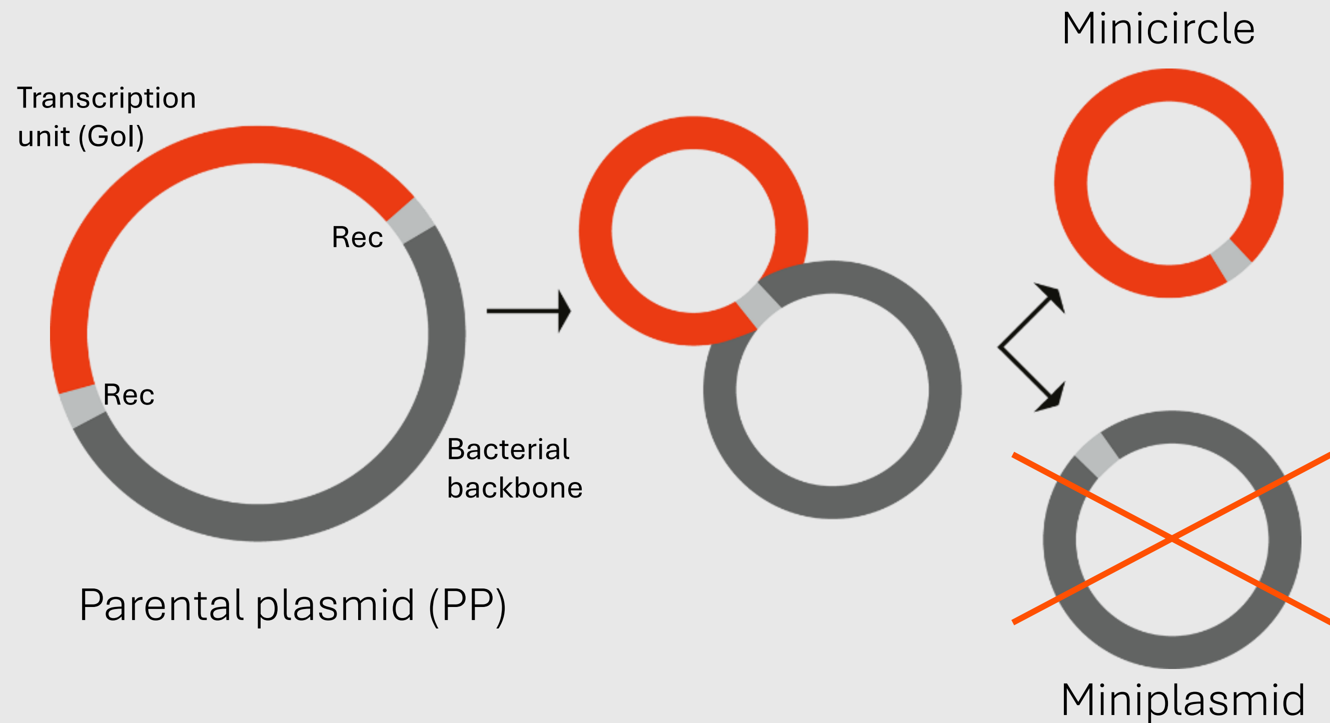
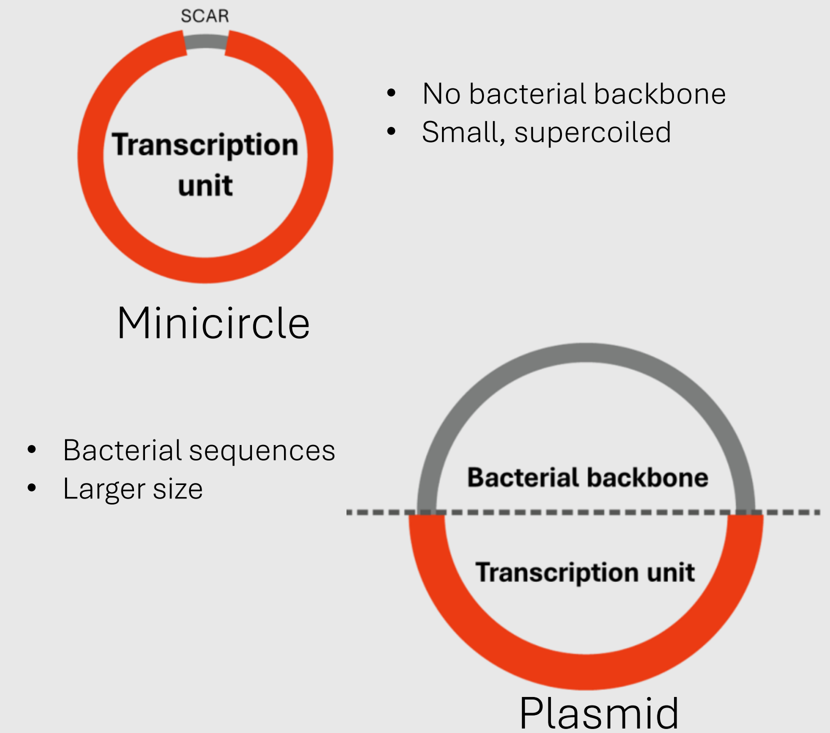
Minimalistic DNA vector reduced to the gene of interest (GoI)





Minicircle Purification & Quality Control

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Cultivation:

- Recombinase expression
- Unidirectional
- Extremely fast

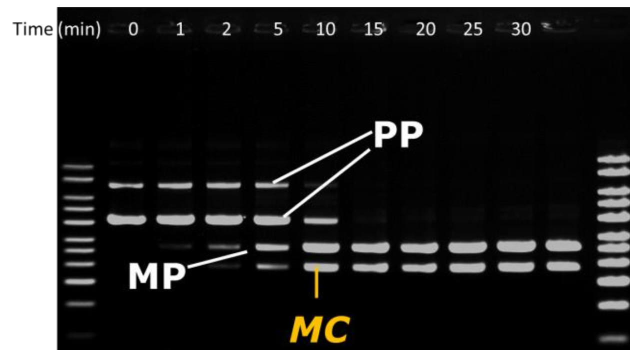
Purification:

- Separation of Minicircle and parental plasmid
- Chromatography-based process (e.g. affinity)
- Removal of backbone and process-related impurities

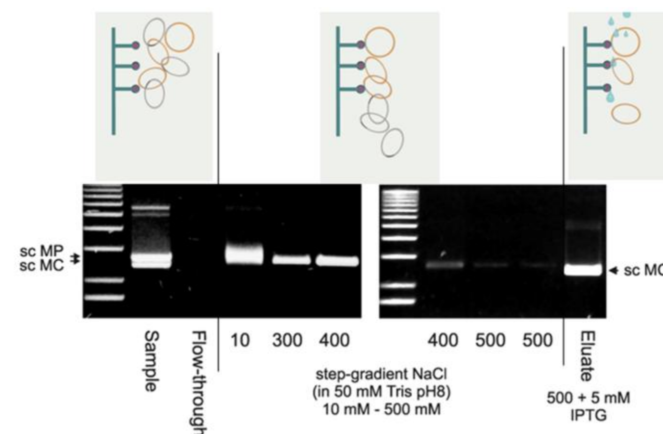
Quality control:

- Analysis by capillary gel electrophoresis (CGE)
- Verification of monomeric supercoiled topology
- Confirmation of purity and residual MP removal

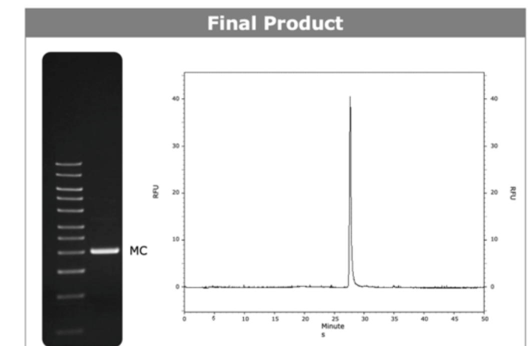
Arabinose induction of recombination:
Extremely fast process with high efficiency



Separating MP and MC:
Affinity chromatography



Product Quality determined by CGE:
onomeric, supercoiled, removal of chromosomal DNA



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

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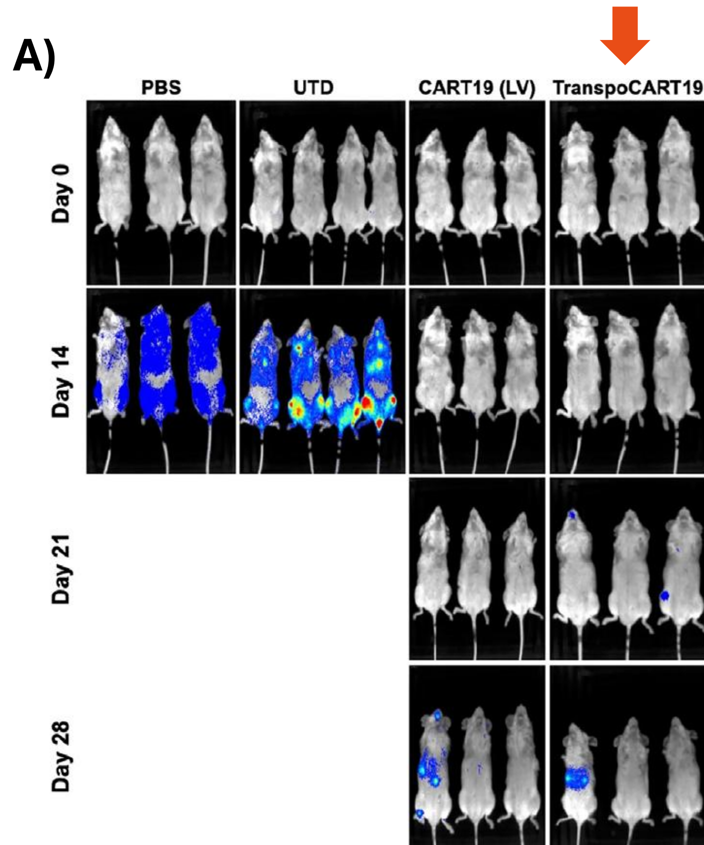

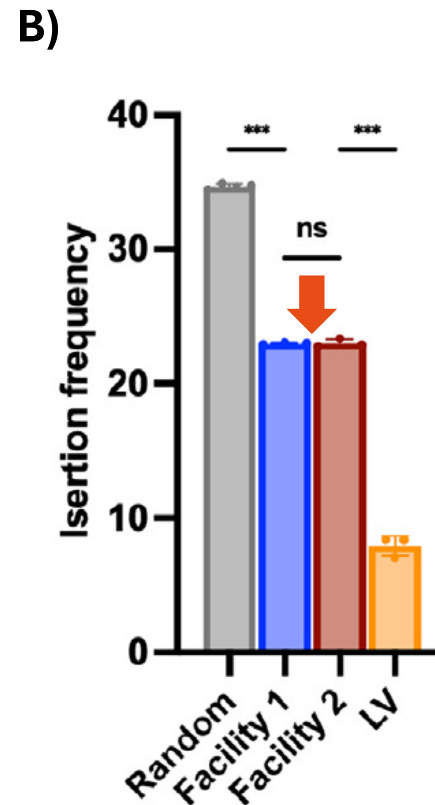

Díez *et al.*, 2025, Mol Ther Methods Clin Dev

- 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)

GMCT Webinar

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

Minicircles in AAV Production for higher Vector Purity and increased Titers

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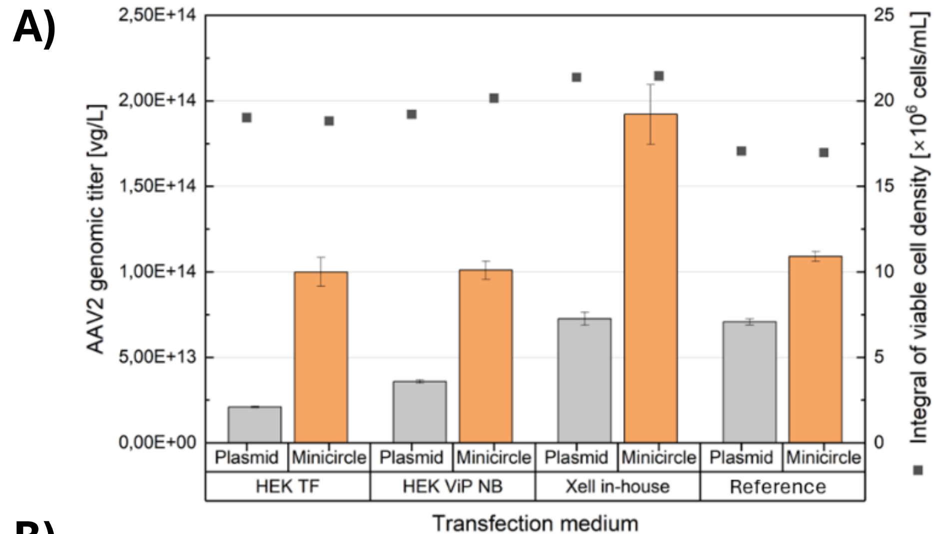
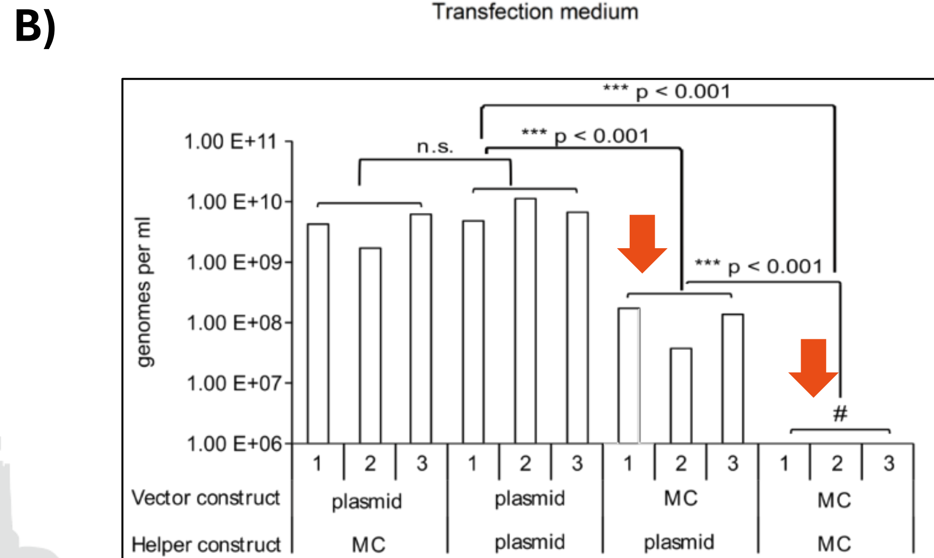
A)



B)



Kraemer et al., ESGCT Annual Congress 2021
Schnödt et al., Mol. Ther.-Nucl. Acids 2016

- **Marked increase in viral titers**: 3 to 4-fold (A)
- **No back-packaging:** higher vector purity and product consistency (B)
- **Improved transduction efficiency**: up to 30× higher (scAAV; data not shown)
- **Cleaner & safer** – lower immunogenicity, less silencing, up to GMP Grade (data not shown)

Article title: DNA Minicircle Technology Improves Purity of Adeno-associated Viral Vector Preparations

AAV webinar

Keeping ITRs Intact: ITRPROTECT® & ITRRESCUE®

Preserved ITRs for better yield, purity and packaging efficiency

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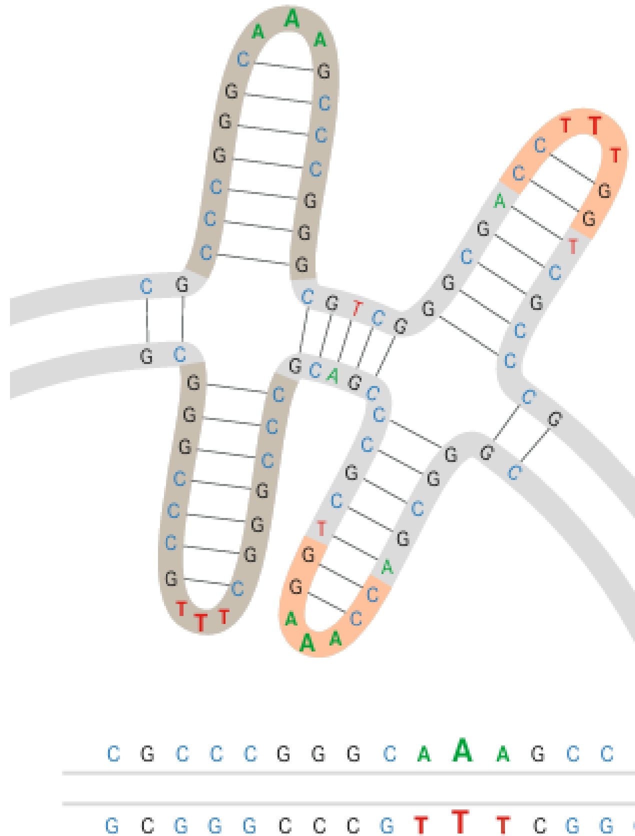
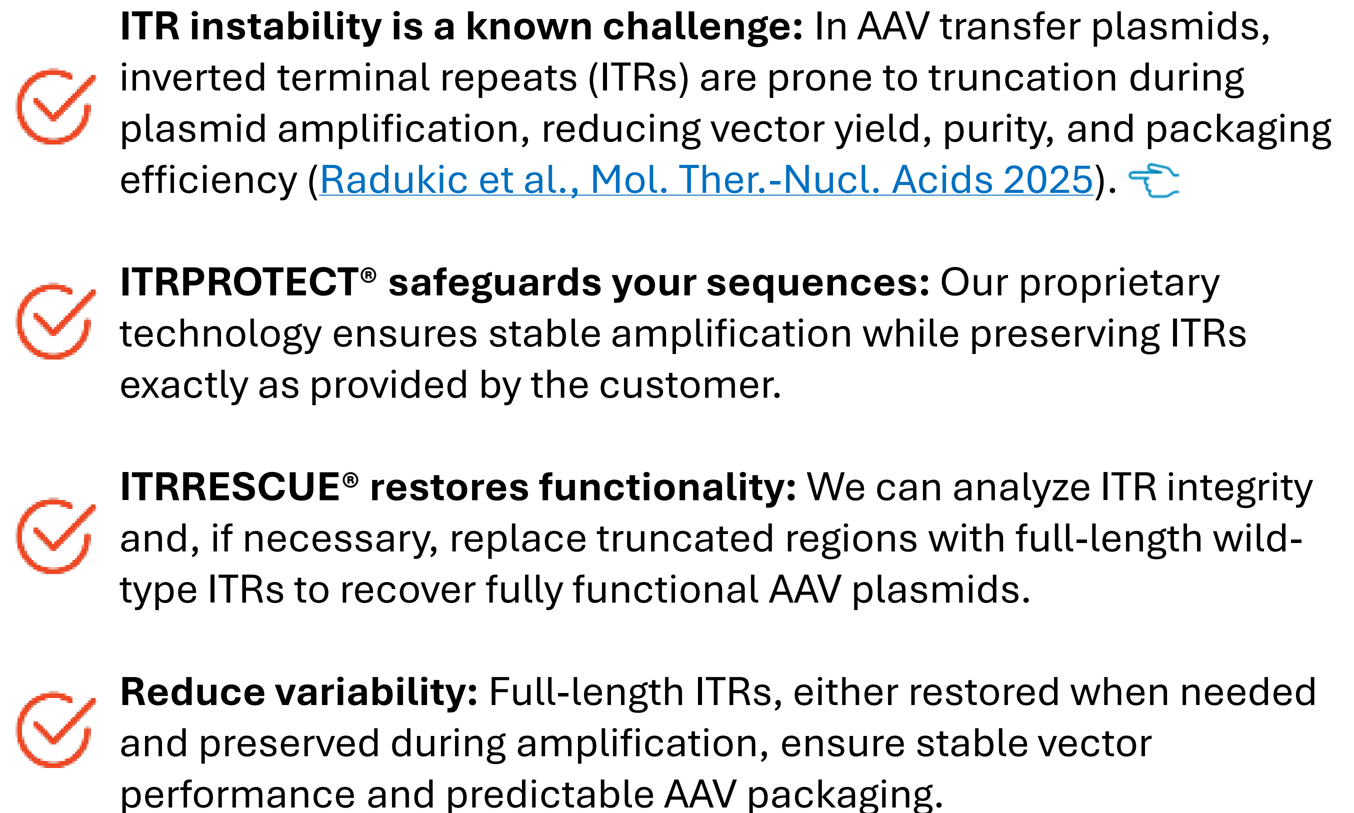




- **ITR instability is a known challenge:** In AAV transfer plasmids, inverted terminal repeats (ITRs) are prone to truncation during plasmid amplification, reducing vector yield, purity, and packaging efficiency (Radukic et al., Mol. Ther.-Nucl. Acids 2025).
- **ITRPROTECT® safeguards your sequences:** Our proprietary technology ensures stable amplification while preserving ITRs exactly as provided by the customer.
- **ITRRESCUE® restores functionality:** We can analyze ITR integrity and, if necessary, replace truncated regions with full-length wild-type ITRs to recover fully functional AAV plasmids.
- **Reduce variability:** Full-length ITRs, either restored when needed and preserved during amplification, ensure stable vector performance and predictable AAV packaging.

PlasmidFactory®

PlasmidFactory‘s Expertise

Supporting CGT programs from early research to clinical development

Your trusted CDMO Partner

Why chose PlasmidFactory

- 25+ years of DNA manufacturing expertise
- Independent, flexible & trusted European CDMO
- Scalable production: Research → High Quality → GMP
- Experts in cell & gene therapy and vaccination apps
- Experts for difficult sequences (ITRs, large plasmids, poly(A), repetitive & CpG-rich regions
- Proprietary technologies: Minicircle, ITRPROTECT®, ITRRESCUE®, POLYARESCUE®, MIDGE®
- 3,500+ plasmid & Minicircle projects delivered
- 99.9% success rate, proven in clinical trials

Company info

Available Quality Grades

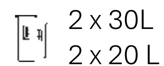
Scientific Quality (SQ) Grade

Advanced research standard

- 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

- Bioreactor fermentation



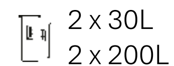
High Quality (HQ) Grade

Highest non-GMP standard

- 2 HQ facilities
- According to EMA guidelines
- Production scale 10 mg – 10 g**
- Also available as "HQ in GMP“ Grade

Key feature

- Complete traceability



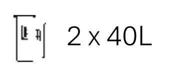
GMP Grade

Clinical & commercial compliance

- Dedicated state-of-the art GMP facility
- GMP / FDA-compliant
- End-to-end single-use processes
- Late-stage clinical trials and commercial supply

Key feature

- Single use equipment



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

GMP Manufacturing You Can Trust

Designed for highest plasmid & Minicircle DNA quality requirements

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- process
- 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

GMP info

Minicircle DNA: Clinical Footprint

Snapshot of registered and planned clinical trials using Minicircle for cell engineering

Registered clinical trials

2020 **CARAMBA-1 - SLAMF7 CAR-T (Multiple Myeloma)** Recruitment ended

- Phase I/IIa (FiH) • EUCT 2024-512643-23-00 • NCT04499339
- Virus-free Sleeping Beauty gene transfer using Minicircle DNA + transposase mRNA
- Opened Jun 2020 • 33 participants • DE site (multi-country approvals reported in EU project updates)

2024 **TranspoCART19 - CD19 CAR-T (r/r B-cell Lymphoma)** Recruiting

- Phase I/IIa • EUCT 2024-514544-90-00 • NCT06378190
- Non-viral Sleeping Beauty transposon delivered as a Minicircle + SB100X mRNA
- Start Mar 2024 • 27 participants • 9 sites (Spain)

2025 **LION-1 - ROR1 CAR-T (ROR1^{+} tumors)** Recruiting

- Phase I (FiH) • EUCT 2024-512019-36-00 • NCT pending (CTIS)
- Start Apr 2025 • 46 participants • 1 site (Germany)
- Includes hematologic + solid tumors (e.g., MCL, CLL, TNBC, OC, ACC)

Studies in preparation

Oncology (engineered cells)

- ROR2 CAR-T (Uniklinikum Würzburg)
- FLT3 CAR-T (Uniklinikum Würzburg)
- SLAM/F7 program (T-CurX)

Autoimmune / gene therapy

- Nimvec™ AM510 (Amarna; Type 1 Diabetes)

Undisclosed / confidential

- At least 4 additional clinical studies in preparation

Summary & Take-Home Message

PlasmidFactory®

- **Successful cell & gene therapy development requires:**
  - High-quality vector design
  - Robust manufacturing processes
  - Validated analytics & QC
- **PlasmidFactory supports this with:**
  - Expertise
  - Innovation
  - Full GMP grade manufacturing

Thank you for your interest!

Feel free to contact:
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