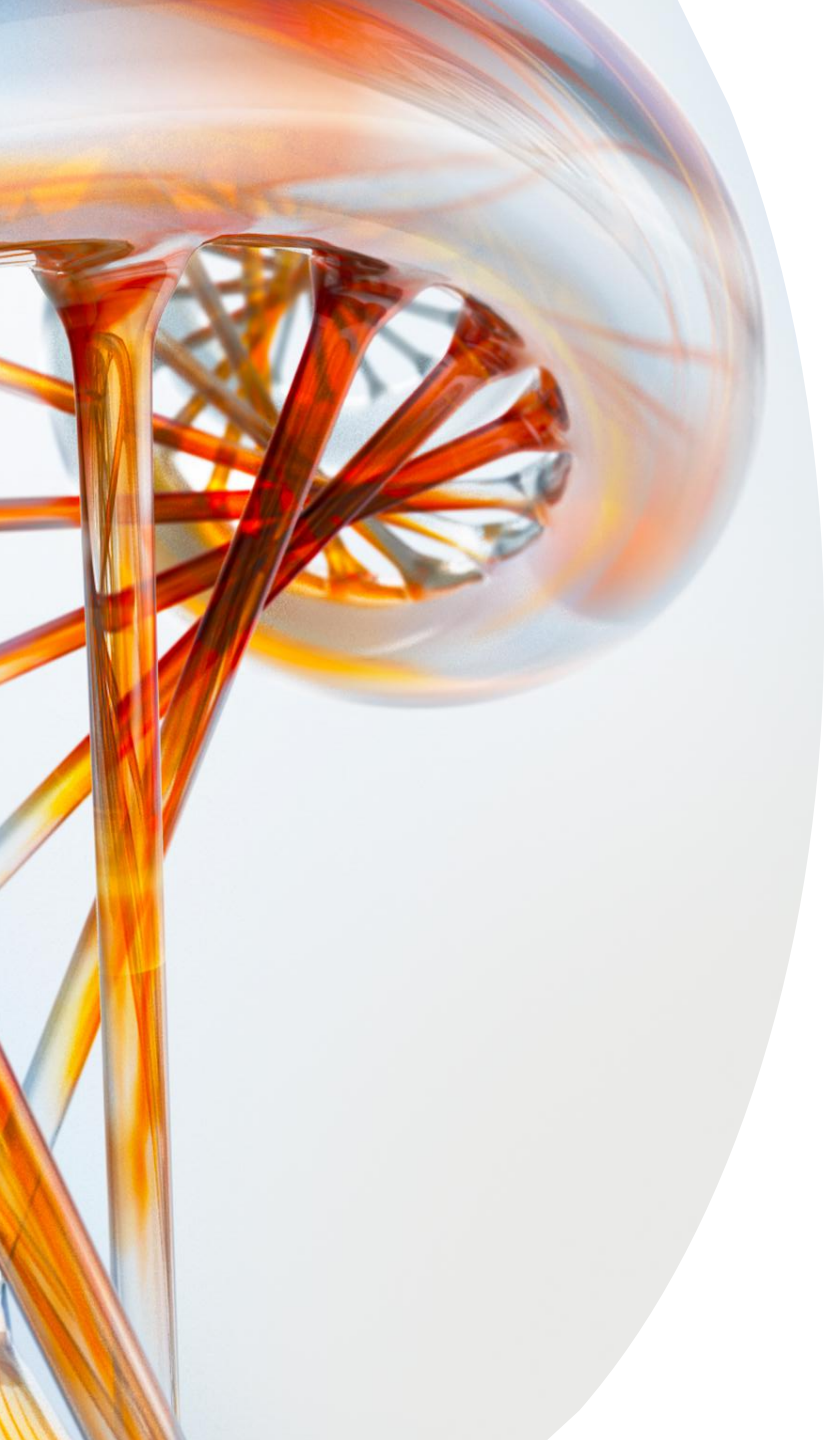

PlasmidFactory®

Advancing AAV Vector Production with High-Integrity DNA Constructs

Dr. Ram Shankar

PlasmidFactory GmbH

The CDMO and service provider for plasmid and Minicircle DNA

Made in Germany

Agenda

- Company introduction
- Different quality grades
- The Minicircle
- Solutions for AAV production
- The 2-plasmid system
- Minicircles for AAV
- The AAV Inverted Terminal Repeats
- Key findings

GMP production facility, PlasmidFactory GmbH, Bielefeld

PlasmidFactory‘s History

2000 | Foundation in Bielefeld, Germany

2005 | License from the German Cancer Research Center

Global exclusive license from the DKFZ for the production and distribution of the 2-Plasmid Transfection system for AAV production

2009 | PF1000

2010 | Launch of first facility for HQ production

2011 | Obtaining the first process patent

Patent for the manufacturing of Minicircles for the production of CAR-T cells

2013 | Obtaining a second process patent

Patent for the manufacturing of Minicircles for the production of AAVs

2016 | Crédit Impôt Recherche certificate

CIR certification until 2018 (renewed 2025-2027)

2017 | PF2000

2018 | Launch of second facility for HQ production

2020 | Takeover of the MIDGE® brand

A minimalistic, immunologically defined gene expression cassette

ITRRESCUE®

2021 | Launch of new ITR/PolyA platform

2022 | Launch of third facility for HQ production

2022 | PF3000

2022 | New investor

Strategic partnering with ARCHIMED, a global investment firm focused on the development of healthcare industries

2026 | Launch of new facility for GMP production

Comprehensive DNA Portfolio

PlasmidFactory®

Custom DNA

- Plasmid DNA
- Minicircle DNA
- MIDGE® Vectors

Tailor-made DNA vectors produced from research to GMP grade.

In-Stock DNA

- Reporter & pEPito plasmids
- AAV plasmids and Minicircles
- Molecular size markers

Ready-to-use plasmids & Minicircles · reporters, AAV tools, markers

Services & Analyses

- Capillary gel electrophoresis (CGE)
- GMP-compliant DNA storage
- Linearized DNA & cloning Services
- ITRPROTECT® / ITRRESCUE® / POLYARESCUE®

Proprietary technologies and QC services · ensuring stability, purity & compliance

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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

3 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

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Solutions for AAV Production

Transitioning from a 3-plasmid to a 2-plasmid system – no compromise on safety while maintaining vector yields

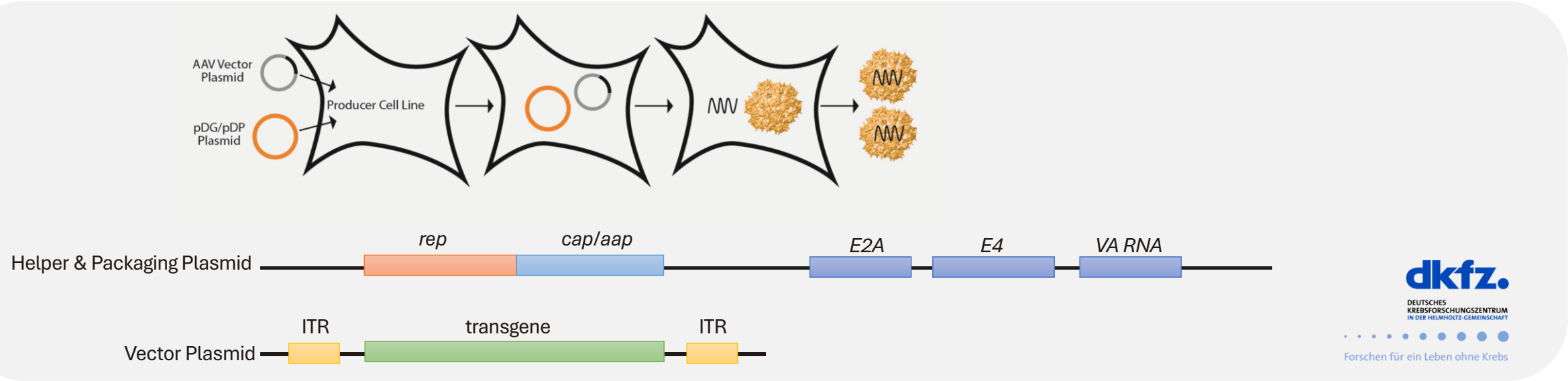
1. Transfer plasmid containing ITRs

2. Helper and Packaging plasmid (serotype-dependent)

3-plasmid system

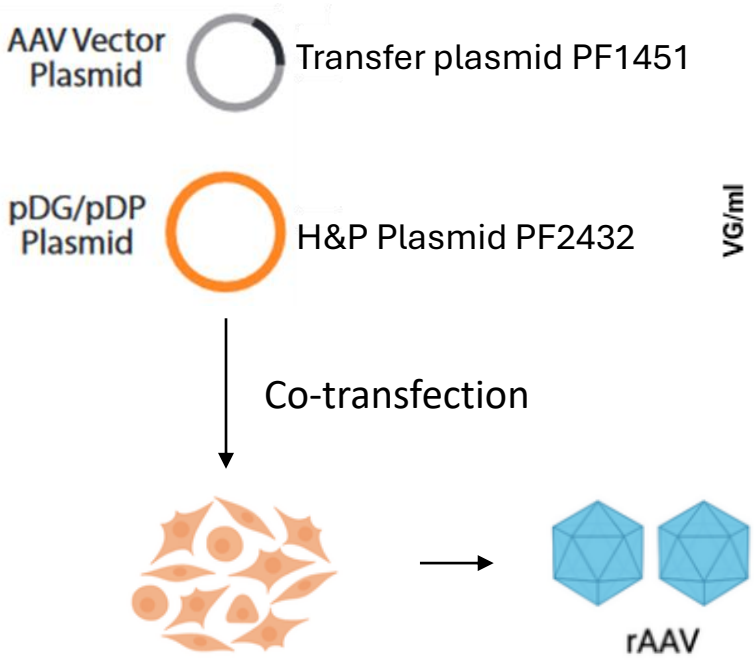
2-plasmid system

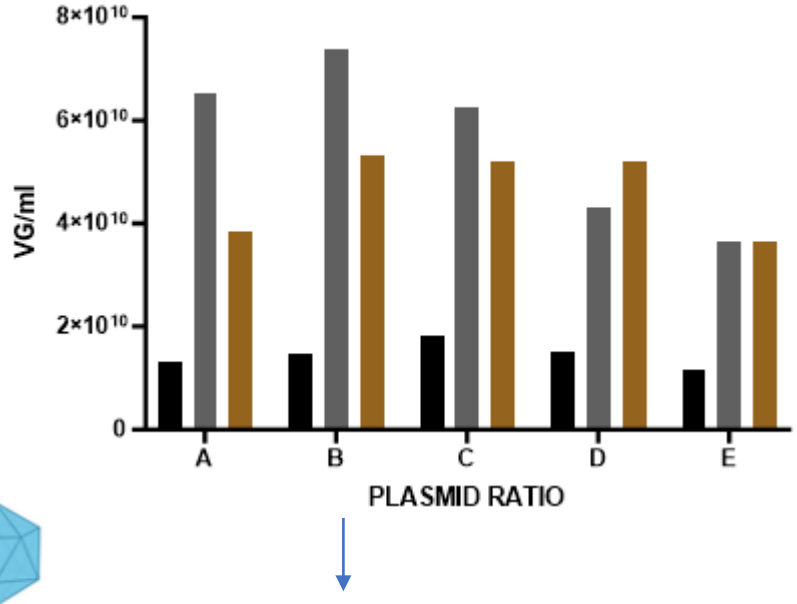
Exclusive 2-Plasmid System



- **Simplified AAV workflow:** Helper + Packaging on one plasmid; fewer components, less complexity
- **Higher efficiency & consistency:** Consistent transfection performance with lower DNA input
- **Cost-effective & GMP-ready:** Reduced DNA production and QC burden, easily scalable to GMP production
- **Future-proof design:** Available as Minicircle and compatible with ITRPROTECT® / ITRRESCUE® technologies

High Vector Genome Titers and Transferability to Bioreactor Cultivation





Optimization of :
i) Plasmid ratio
ii) Plasmid amount
iii) Transfection reagent

Shake flask (30 mL)

VG/ml	VG tot	TU/ml
7.38E+10	2.2E+12	7.61E+07

Bioreactor (2L)

VG/ml	VG tot	TU/ml
1.44E+11	2.88E+14	1.16E+08

Data courtesy of Michaela Gentile, Angelo Raggioli, ReiThera Srl, presented at the 31st ESGCT Congress, Rome.

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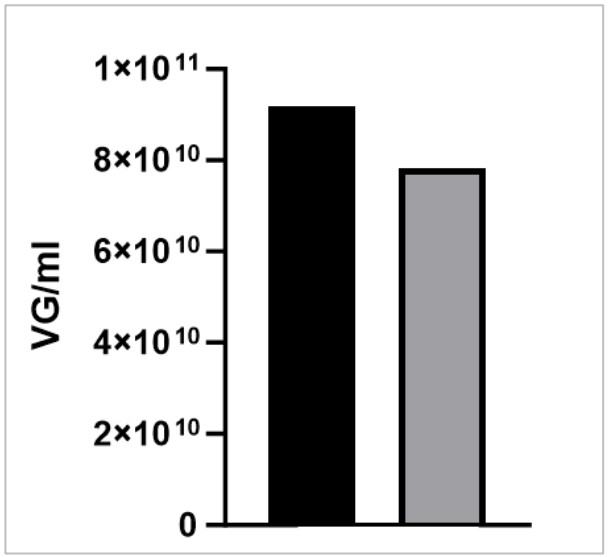
Consistency across Production Systems - Elevate AAV Production in ReiThera's proprietary Cell Line

AAV Vector Plasmid — Transfer plasmid PF1451

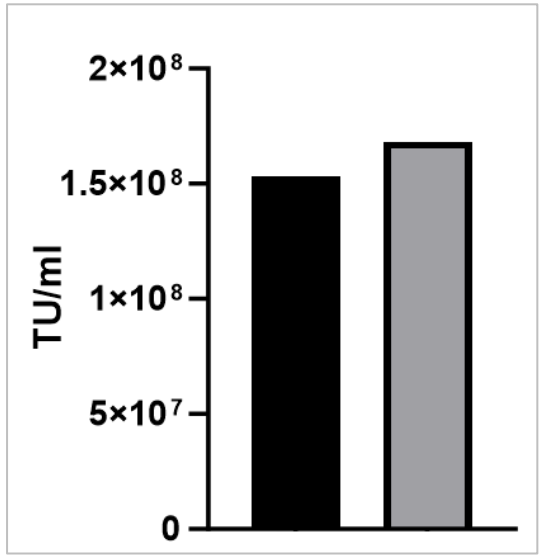
pDG/pDP Plasmid — H&P Plasmid PF2432

2e6 cells/mL
30 mL
DNA 2.5ug /mL

1. Physical titer



2. Infectious titer



HEK293 commercial cell line

ReiCell-AAV

ReiCell-AAV can produce AAV - including AAV2 - at high titers, greater than $7x10^{10}$ vg/mL

Data courtesy of Michaela Gentile, Angelo Raggioli, ReiThera Srl, presented at the 31st ESGCT Congress, Rome.

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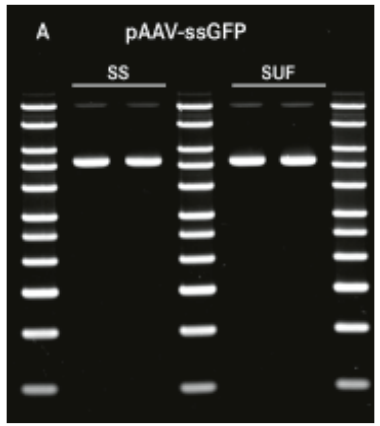
Production in single use fermenter

PlasmidFactory®

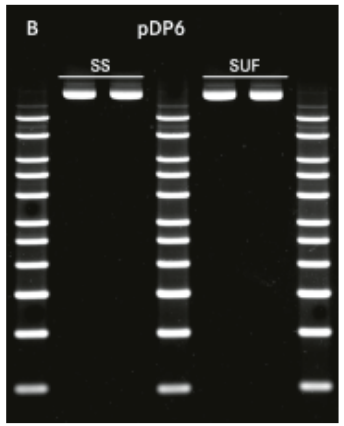
Comparable performance to stainless steel fermenters enabling deployment in GMP environment

SARTORIUS

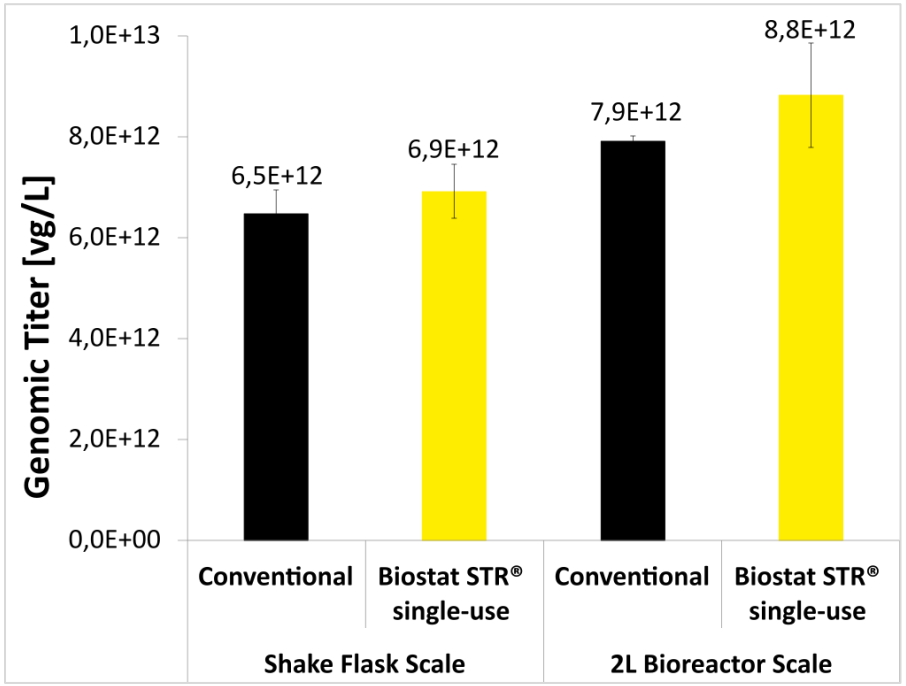
Transfer plasmid



Helper + Packaging plasmid



Application of 2-plasmid system in suspension culture



- Good vector genome titers achieved considering a different transfection reagent was used.

Data courtesy of Alyssa Vetter, Kathrin Teschner, Sandra Klausing and others, Sartorius, presented at the 30th ESGCT Congress, Brussels

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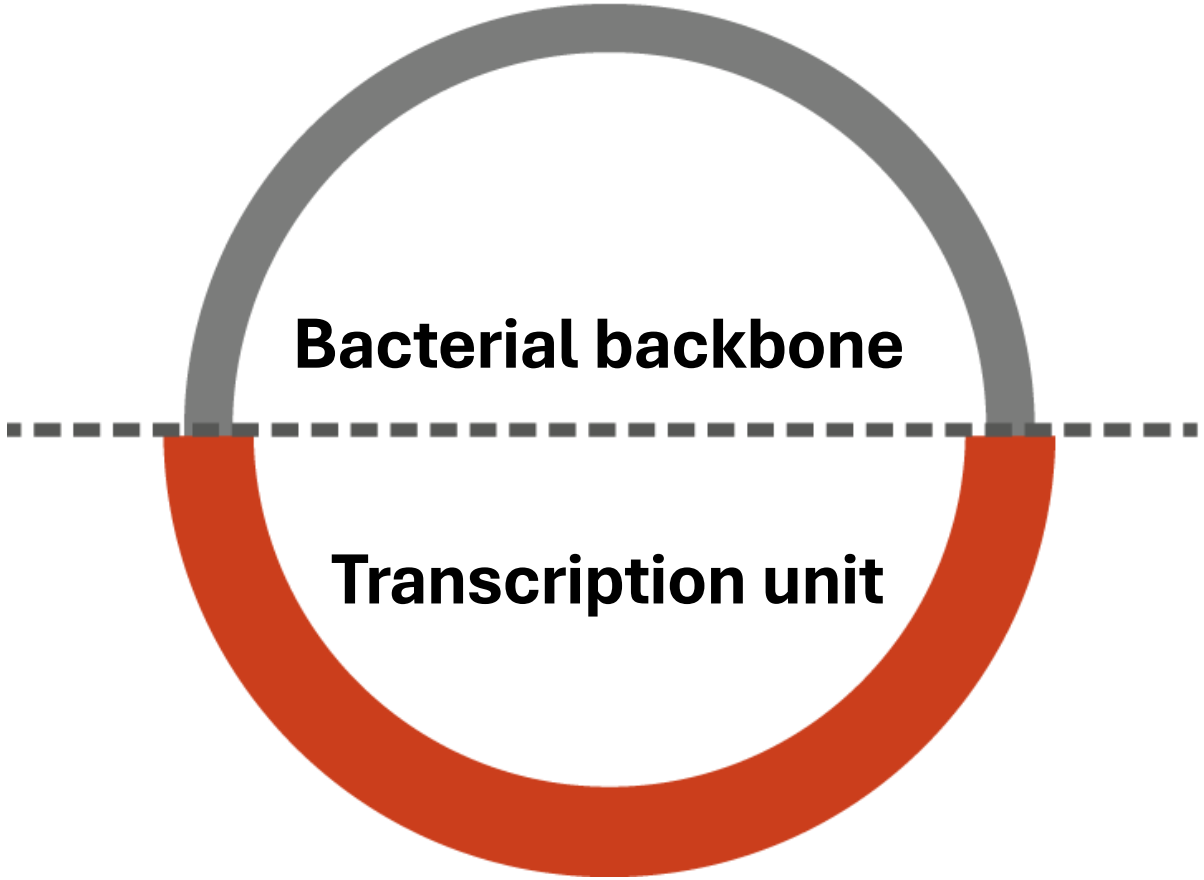
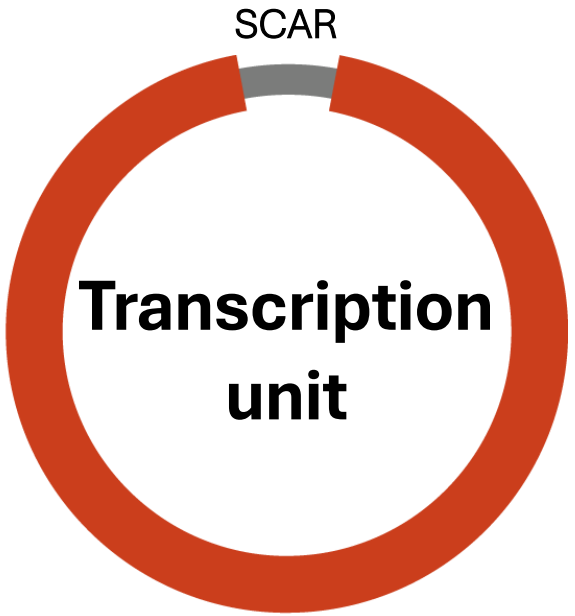
Minicircle vs. Plasmid Comparison

PlasmidFactory®



Conventional plasmid



Minicircle DNA

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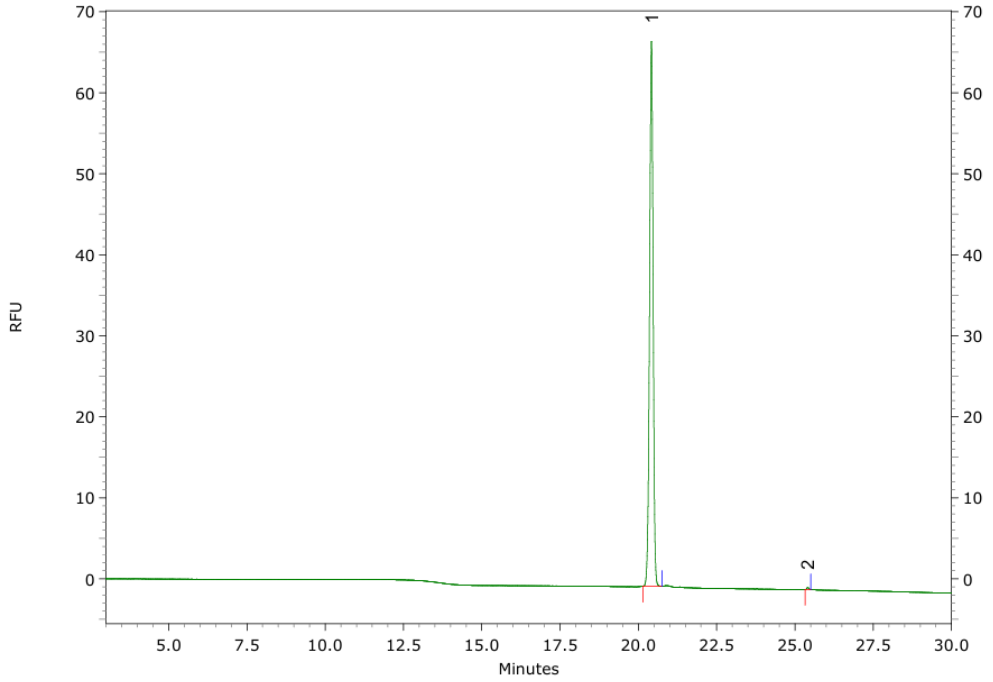
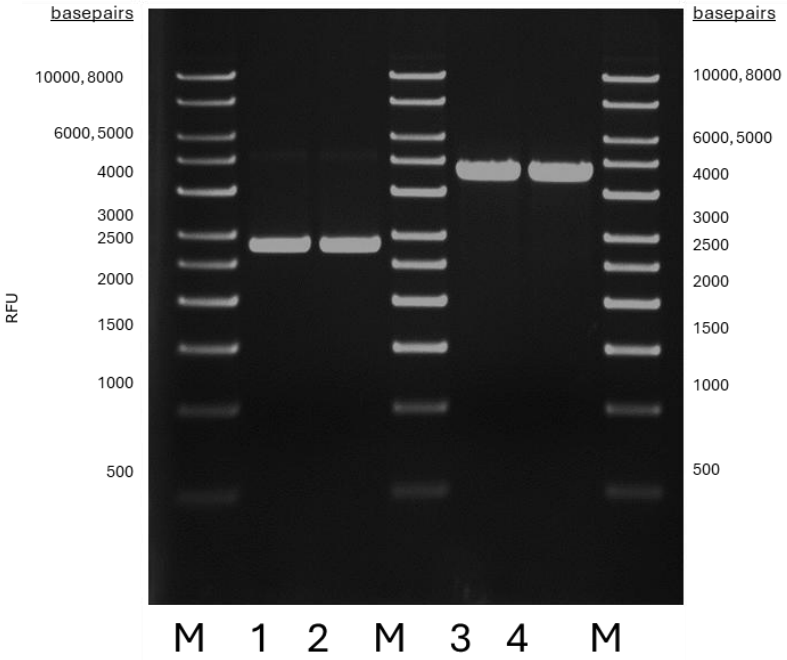
AAV Minicircles: Highest Purity

QC (CGE)



P1:supercoiled Minicircle (ccc; 99.8%)
***P2**: open circle (oc) Minicircle (0.2%)*

QC (AGE)



Lane M: 1 kbp ladder (PlasmidFactory, Item no. MSM-865-50), 300 ng
Lane 1: MC.AAV-ssGFP, 250 ng
Lane 2: MC.AAV-ssGFP, 250 ng
Lane M: 1 kbp ladder (PlasmidFactory, Item no. MSM-865-50), 300 ng
Lane 3: MC.AAV-ssGFP, PacI digestion, 250 ng
Lane 4: MC.AAV-ssGFP, PacI digestion, 250 ng
Lane M: 1 kbp ladder (PlasmidFactory, Item no. MSM-865-50), 300 ng

- **Exceptional purity:** Strong ccc Minicircle signal, with no detectable genomic DNA.
- **Consistent quality:** Single clear band (AGE) and dominant ccc peak (CGE), confirming minimal open-circle forms.
- **Regulatory confidence:** Clean profiles support GMP compliance and safer downstream applications.

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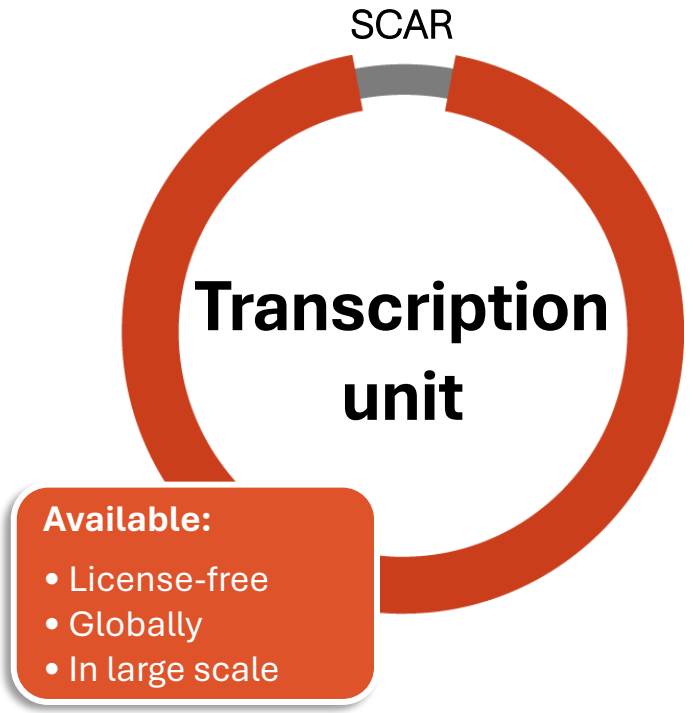
Minicircle Benefits at a Glance

PlasmidFactory®

Plasmid backbone sequences removed

- Small, monomeric, supercoiled
- 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 back-packaging during AAV production

Successful in several clinical trials & manufacturing



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Solutions for AAV Production

Converting one of the constructs into a Minicircle – increased vector genome yields

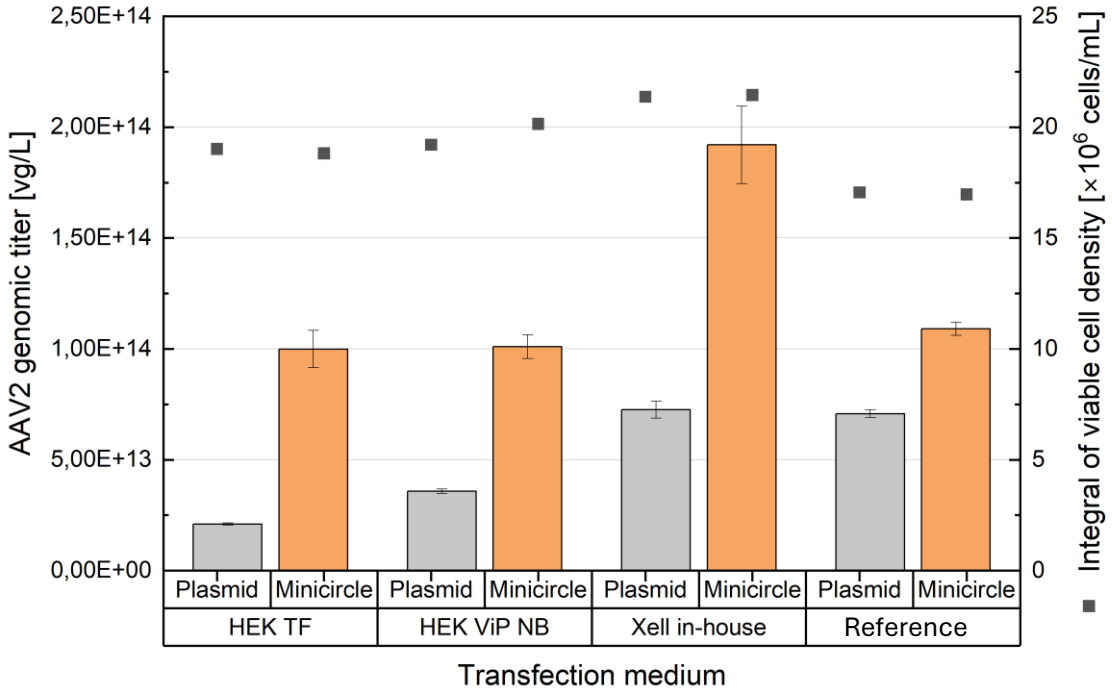
1. Transfer **Minicircle** containing ITRs

2. Helper and Packaging plasmid

Plasmid/plasmid

Minicircle/plasmid

Minicircles: Increase AAV Titers



- **Marked increase in viral titers:** 3 to 4-fold
- **Consistent effect** across different cell culture media
- **Lower downstream burden and COGS:** More efficient AAV manufacturing
- **Elevated yield** not related to increase in viable cell density

*AAV titer was measured via qPCR with an AriaMx system (Agilent Technologies, USA) 72 h after transfection. Cells were lysed with three consecutive freeze/thaw-cycles. The samples were treated with DNase I (Thermo Scientific, USA) and Proteinase K (Thermo Scientific, USA), according to an internal protocol. Cycling conditions were chosen according to the manufacturers protocol. Transfection was carried out using FectoVIR®-AAV (Polyplus, France, now part of Sartorius). "**Plasmid DNA" consisted of pAAV-ssGFP and pDG (both PlasmidFactory, Germany)** in a molar ratio of 1:1.3. "**Minicircle DNA" comprises MC.AAV-ssGFP and pDG (both PlasmidFactory, Germany**) in a molar ratio of 1:1.3. pAAV-ssGFP and MC.AAV-ssGFP were used in the same molar ratio. DNA concentration was 1 µg per 1 million cells at the time of transfection.*

Data courtesy of Niklas Kraemer et al., ESGCT Annual Congress 2021

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Solutions for AAV Production

Converting both constructs into Minicircles – significant increase in vector purity

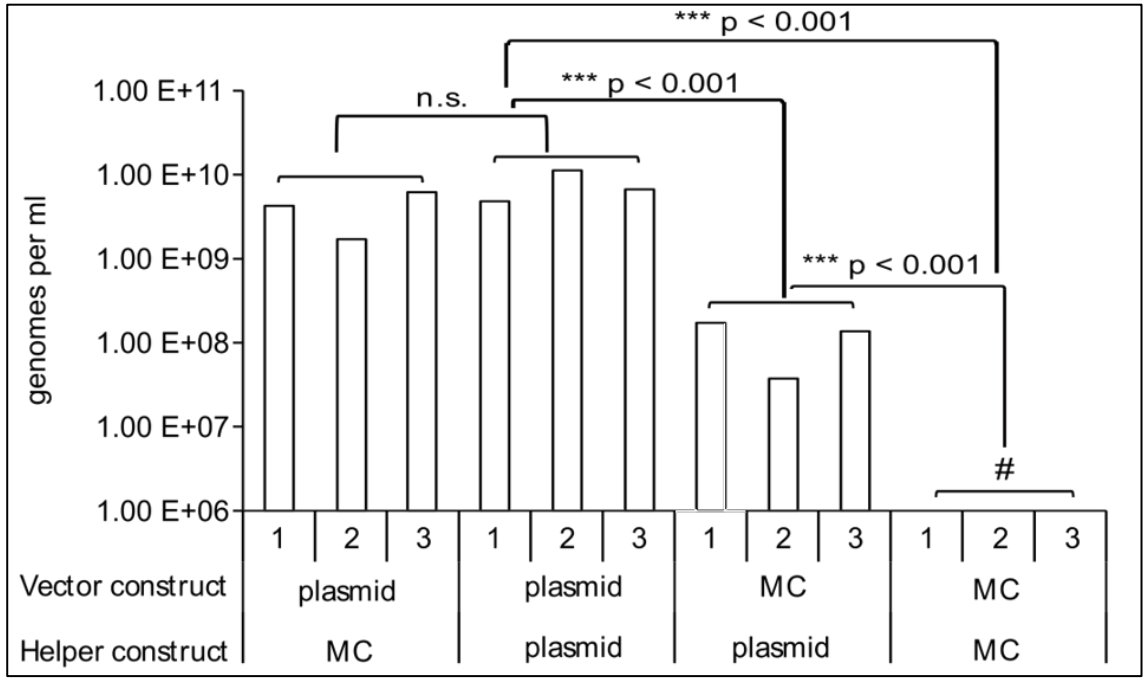
1. Transfer **Minicircle** containing ITRs

2. Helper and Packaging **Minicircle**

Minicircle/plasmid

Minicircle/Minicircle

Minicircles: Improved AAV Vector Purity



Replacing the vector plasmid by Minicircle significantly decreases the amount of encapsidated DNA particles
Replacing both plasmids by Minicircles results in decrease of backbone sequence detection to below limit of quantification

- **No bacterial backbone or antibiotic resistance genes:** cleaner vectors, lower immunogenicity
- **No back-packaging:** higher vector purity and product consistency
- **Improved transduction efficiency:** up to 30× higher for scAAV
- **Maintained productivity:** comparable yields and capsid numbers
- **Enhanced safety profile:** suitable for clinical and GMP use

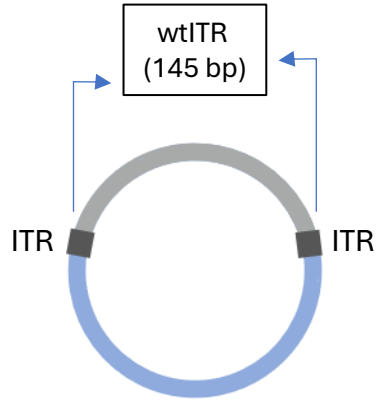
Schnödt et al., Mol. Ther.-Nucl. Acids 2016

Solutions for AAV Production

PlasmidFactory®

Throwing the spotlight on ITR sequence integrity: pioneering research by academic collaborators

Transfer plasmid containing **wild-type length ITRs**



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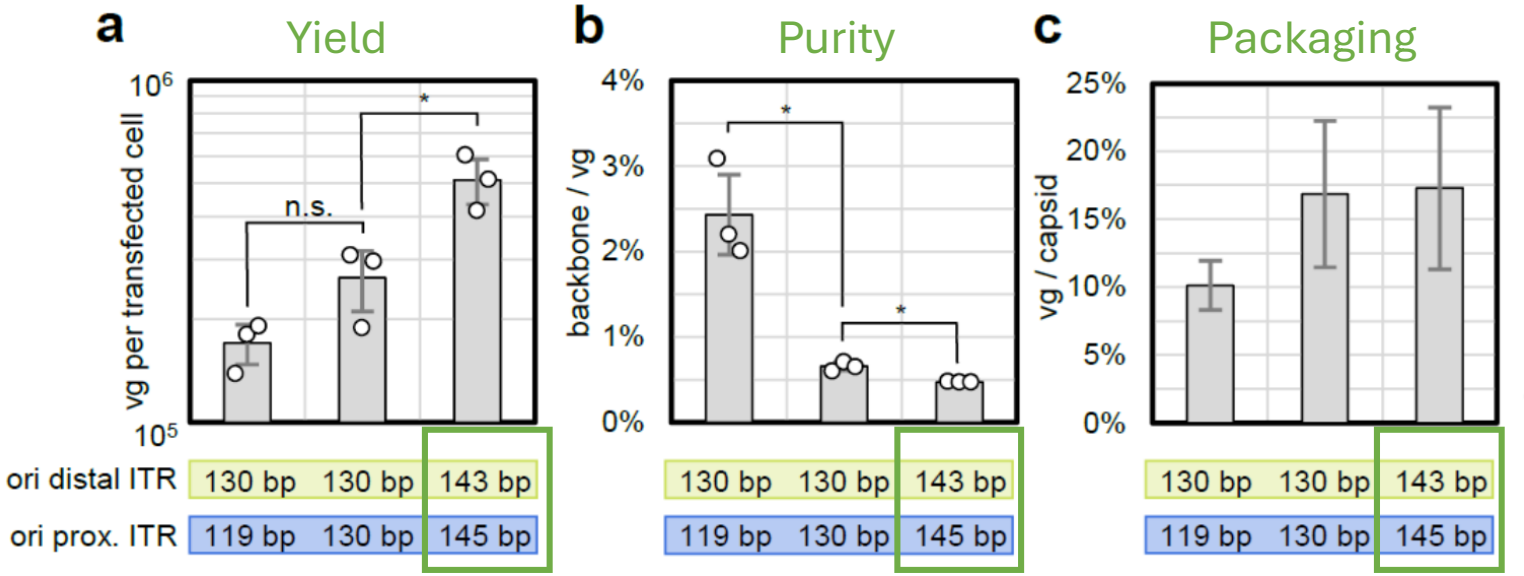
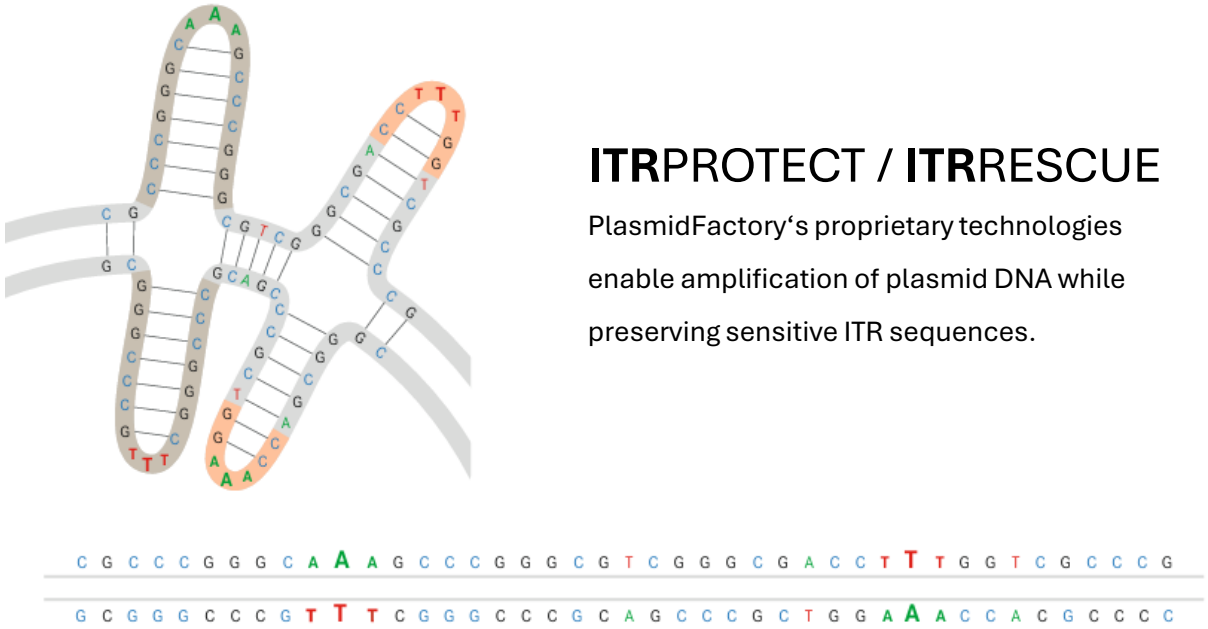
Intact ITRs for More Efficient AAV Production



- **Higher yields** with plasmids carrying full-length ITRs vs. truncated ITRs
- **Greater genomic purity**, with fewer backbone contaminants
- **Improved packaging**, yielding more full capsids and better genome-to-capsid ratios
- **Functional advantage**, as intact ITRs produce AAVs with stronger transduction

Radukic et al., Mol. Ther.-Nucl. Acids 2025

Keeping ITRs Intact: **ITR**PROTECT® **& ITR**RESCUE®



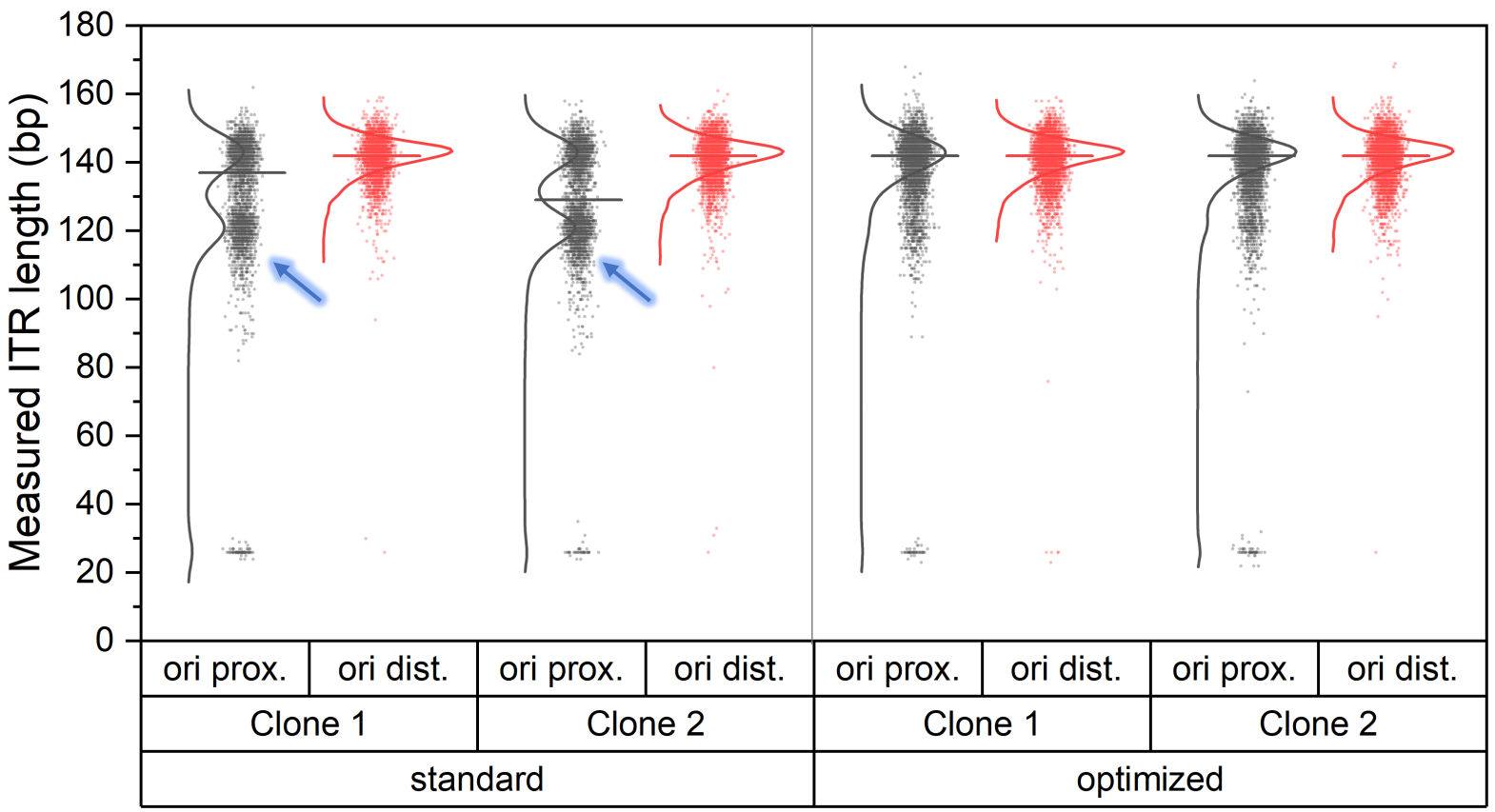
ITRPROTECT / **ITR**RESCUE

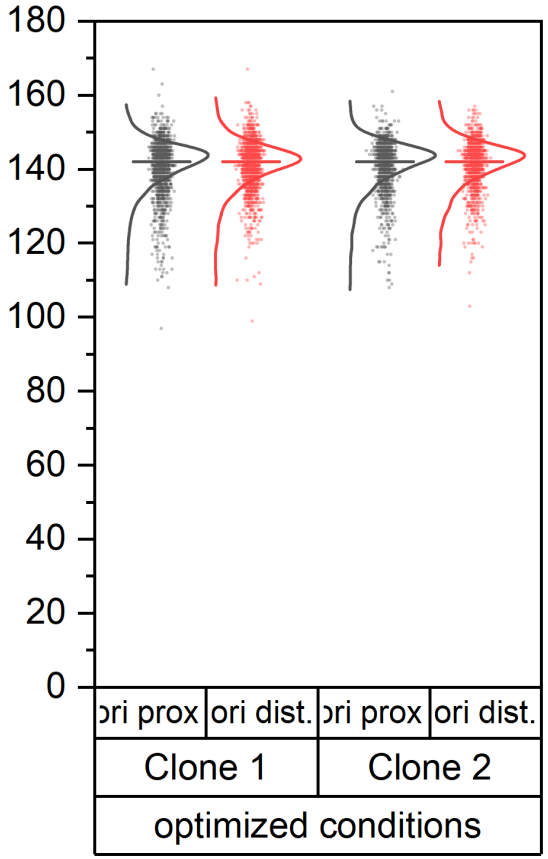
PlasmidFactory‘s proprietary technologies enable amplification of plasmid DNA while preserving sensitive ITR sequences.

- **Protects fragile ITRs:** Reliable amplification of intact repeats, avoiding truncation and yield loss
- **Verified by NGS:** Confirms intact ITRs in every batch for consistent, regulatory-ready quality

Keeping ITRs Intact: **ITR**PROTECT® **& ITR**RESCUE®

PlasmidFactory®





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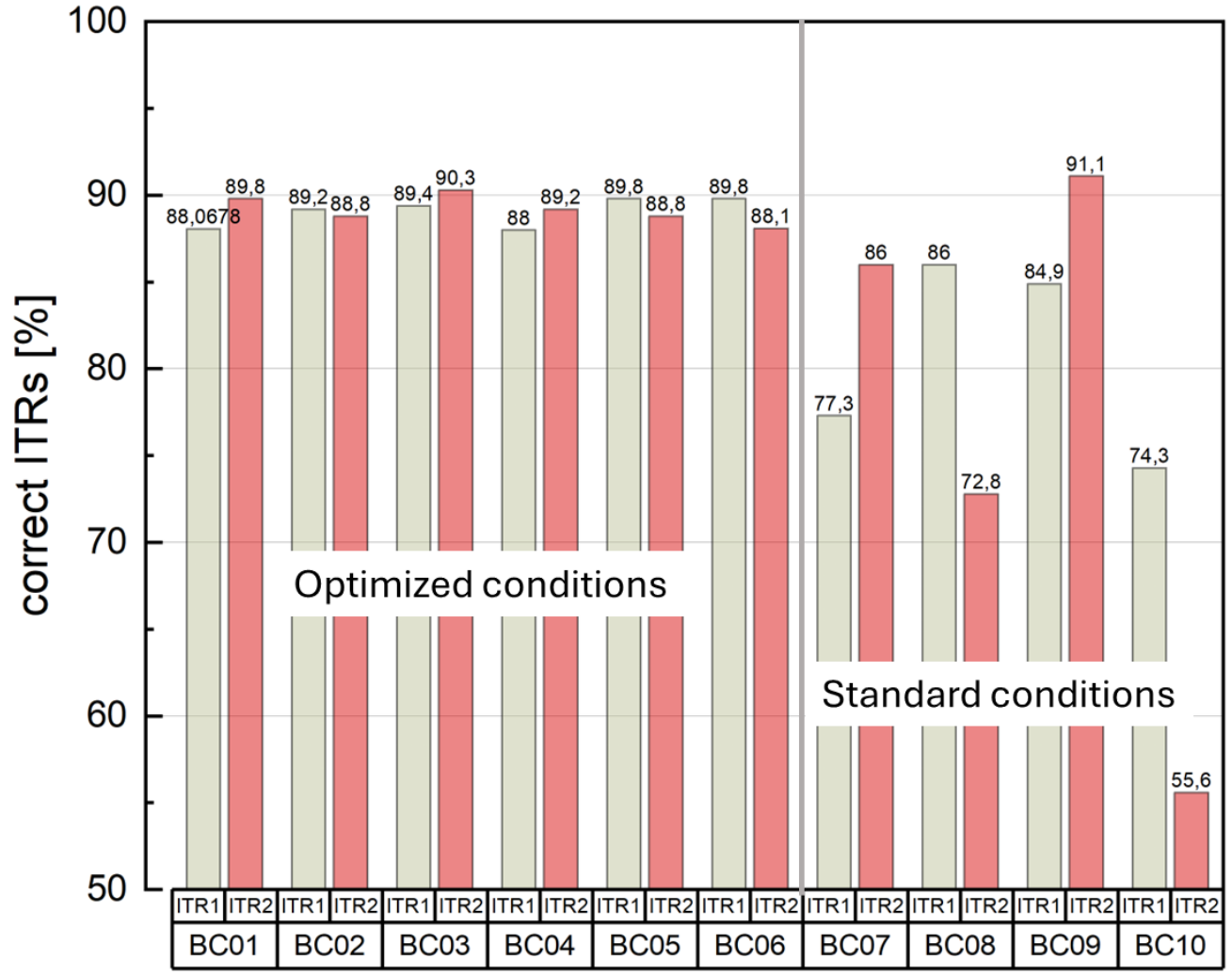
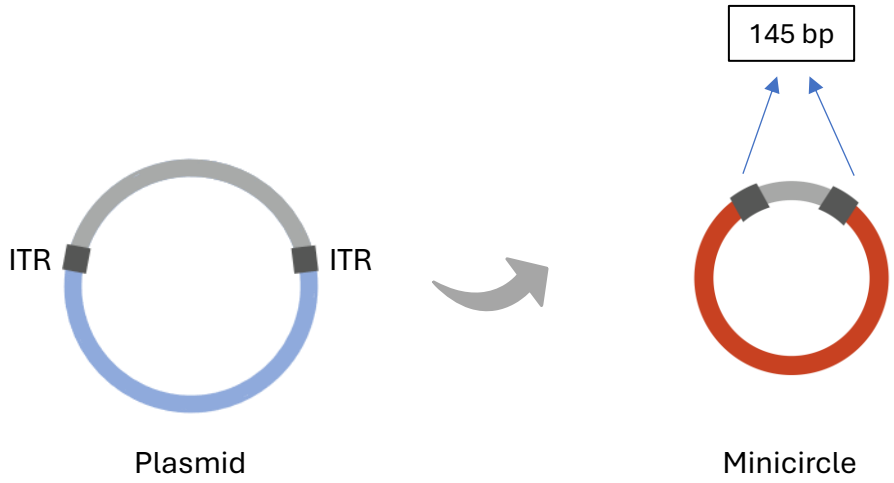
Keeping ITRs Intact: **ITR**PROTECT® **& ITR**RESCUE®



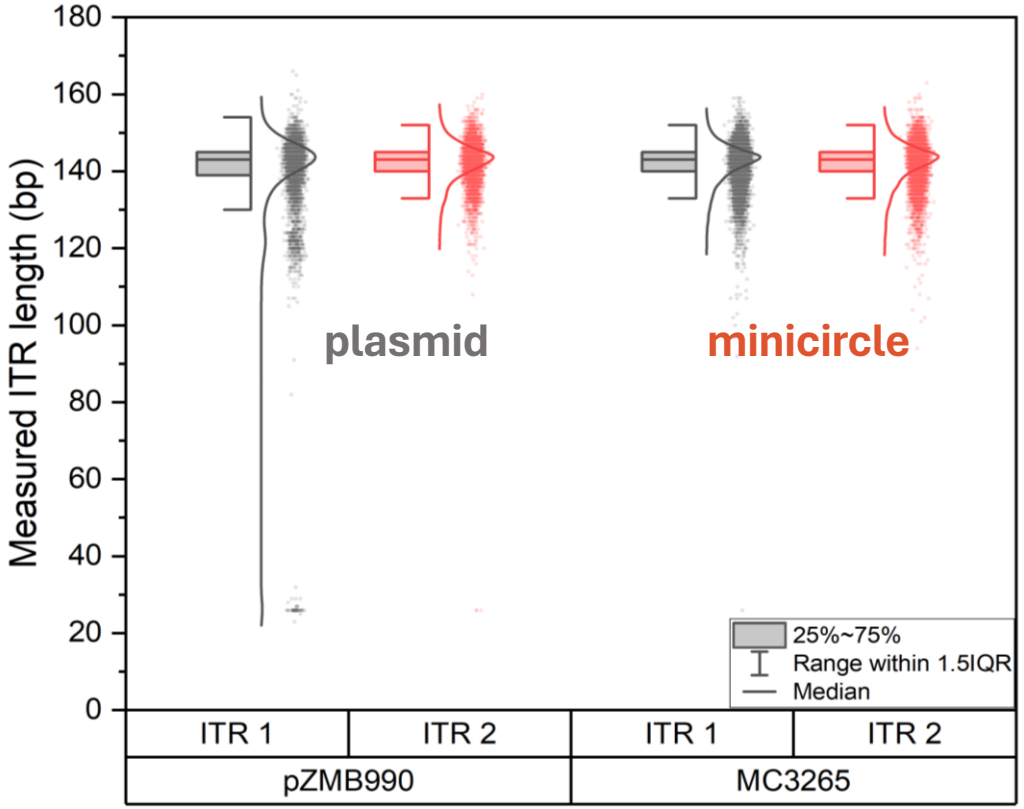
Solutions for AAV Production

Combining wtITR technology with Minicircles: cumulative effect observed in key parameters

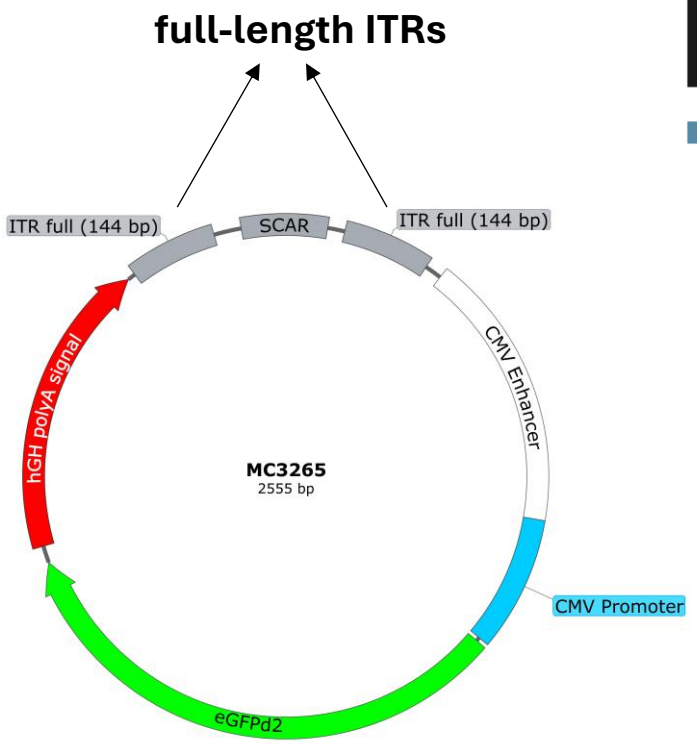
Transfer **Minicircle** containing **wild-type length ITRs**



Minicircles: Preserved ITR Length and Integrity

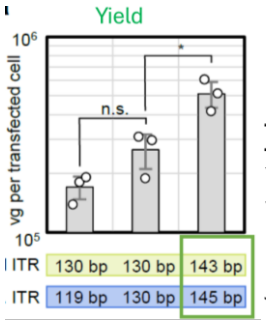


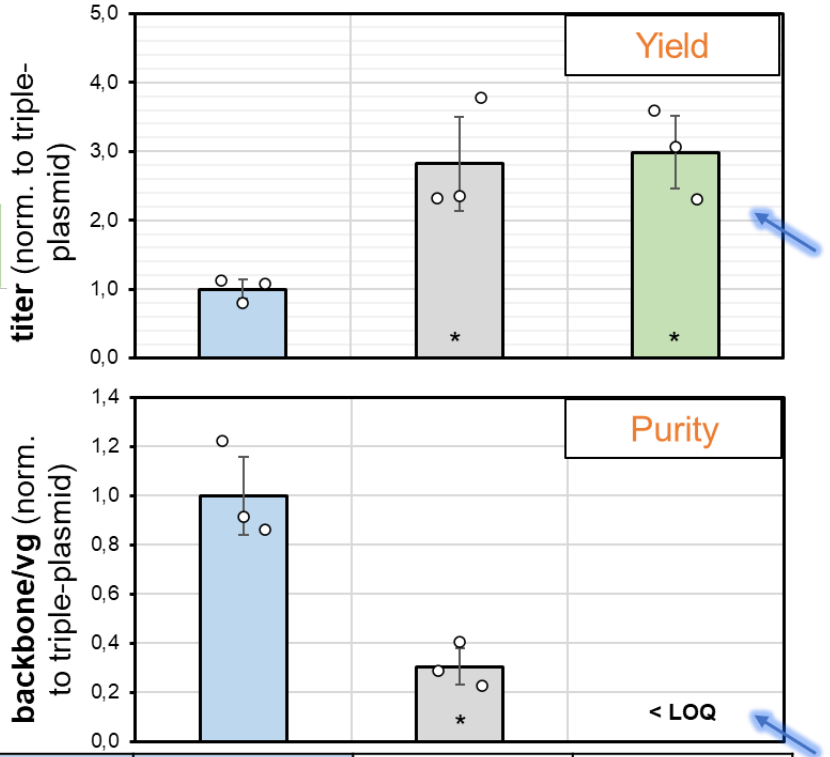
NGS data proved ITR integrity



Minicircles with intact ITRs boost yield, purity, and efficiency

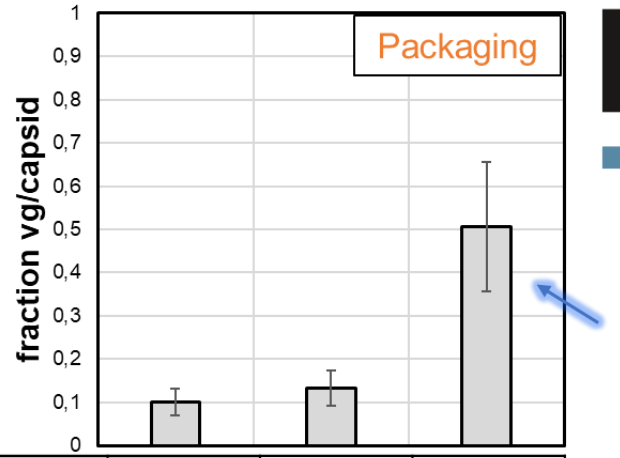
PlasmidFactory®





Full-ITR **Plasmid**	x		
RepCap **Plasmid**	x	x	
Helper **Plasmid**	x	x	
Full-ITR **Minicircle**		x	x
RepCap **Minicircle (v2)**			x
Helper **Minicircle**			x

* p<0.05, LOQ = 0.001



Full-ITR **Plasmid**	x		
RepCap **Plasmid**	x	x	
Helper **Plasmid**	x	x	
Full-ITR **Minicircle**		x	x
RepCap **Minicircle**			x
Helper **Minicircle**			x

UNIVERSITÄT BIELEFELD
Faculty of Technology

- **Higher titers:** Equal or superior yields vs. plasmids for greater productivity
- **Lower mispackaging:** Cleaner vectors with fewer unwanted DNA fragments
- **Better capsid loading:** More genomes per capsid for better vector quality

→ **GMP-ready for high-quality AAV production**

Radukic, et al. ESGCT Annual Congress 2025

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AAV Production: From Standard to Innovation

	Old Standard	Innovative PlasmidFactory Solution
AAV production architecture	**3-Plasmid System** – requires three separate plasmids → higher risk of incomplete transfection and imbalanced helper ratios.	**2-Plasmid System** – *Rep/Cap* + helper genes combined in one plasmid → simpler, more reliable transfection with lower DNA input and cost.
DNA template type	**Classic plasmids** – contain prokaryotic backbone and antibiotic-resistance sequences.	**Minicircle DNA** – no bacterial backbone or antibiotic markers; only functional AAV elements.
Product purity	Backbone DNA often co-packaged into AAV capsids (0.5–2.9 % in ssAAV, up to 26 % in scAAV).	No prokaryotic DNA carry-over → cleaner, safer AAV vectors.
Vector quality & efficiency	Risk of impurities, lower transduction efficiency, higher downstream burden. Patient risk.	Higher fraction of functional particles, improved purity, and reduced downstream processing. Future proof and regulatory-ready.
Transfer plasmids	Truncated and deletion carrying ITR sequences have worked in the past, however potential for improvement clearly demonstrated	**Wild-type length ITRs** improve yield, purity and packaging efficiency. Combination with **PlasmidFactory's Minicircle** technology shoots up key parameters even further – **unsurpassed technological advantage.**

Our AAV Offerings

PlasmidFactory®

In-stock plasmids and Minicircles

Helper and packaging plasmid	AAV serotypes
pDM	wt rep2/cap2
pDP1	rep2/cap1
pDP2	rep2/cap2
pDP3	rep2/cap3
pDP4	rep4/cap4
pDP5	rep2/cap5
pDP6	rep2/cap6
pDP8	rep2/cap8
pDP9	rep2/cap9

*also available as minicircles

Vector constructs	AAV types
pAAV-ITR-CMV-GFP	ss
pAAV-ITR-CMV-GFP	sc
MC.AAV-ITR-CMV-GFP	ss
MC.AAV-ITR-CMV-GFP	sc

Vector construct with wildtype-length ITRs	AAV types
pAAV-ITR-CMV-GFP	ss
MC.AAV-ITR-CMV-GFP	ss

- Customized *cap* modified plasmids
- Custom ITR-plasmids

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Key Findings

PlasmidFactory's team at your service

- **Maximizing AAV vector production goals:** high variety of plasmid and Minicircle constructs tailored to customer needs
- **Application data from collaborators:** tangible benefits for improved efficiency
- **Reliability backed by experience**: industry pioneer with a solid 25 year track record
- **Scalable production up to GMP Grade:** to accomodate changing customer needs

PlasmidFactory®

Thank you for your interest!

PlasmidFactory.com

PlasmidFactory 25 YEARS

PlasmidFactory GmbH
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