

Improving AAV Production with Minicircles



<div Ram Shankar*, Marco Schmeer, Martin Schleef & Dirk Winnemöller, PlasmidFactory GmbH, Germany | *Presenting author | Contact: ram.shankar@plasmidfactory.com

Introduction

AAV vectors are central to gene therapies, yet production remains costly and complex, hampered by inefficient packaging and plasmid-related risks. PlasmidFactory provides innovative DNA technologies, including the Minicircle, to deliver higher titers, cleaner vectors, and faster, more cost-effective AAV production – enabling safer and scalable gene therapies.

Efficient 2-Plasmid System

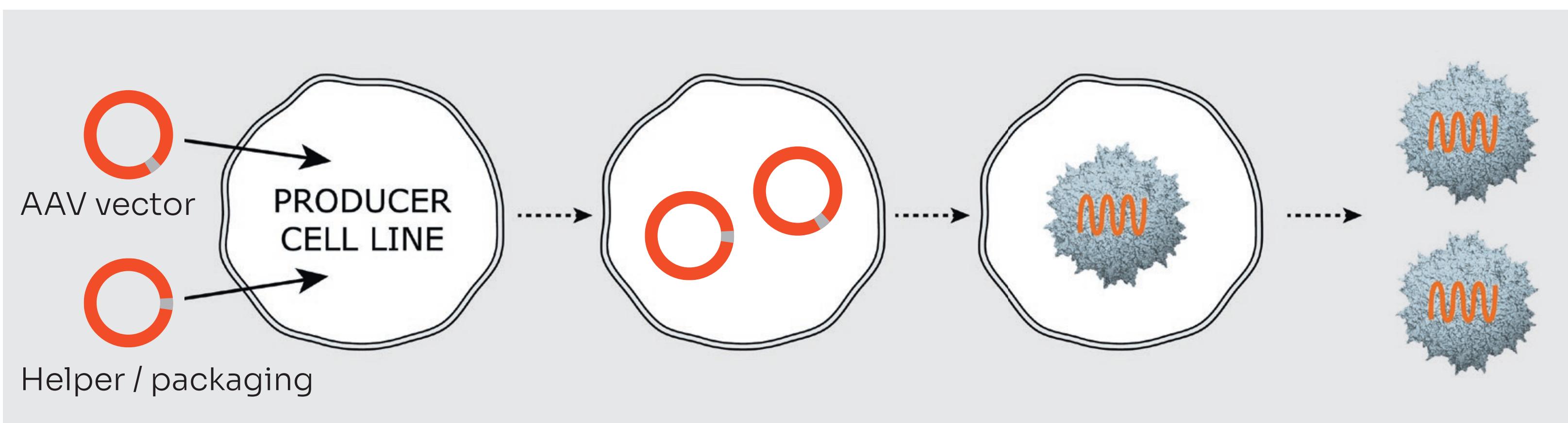


Fig. 1 – The 2-Plasmid System for AAV production.

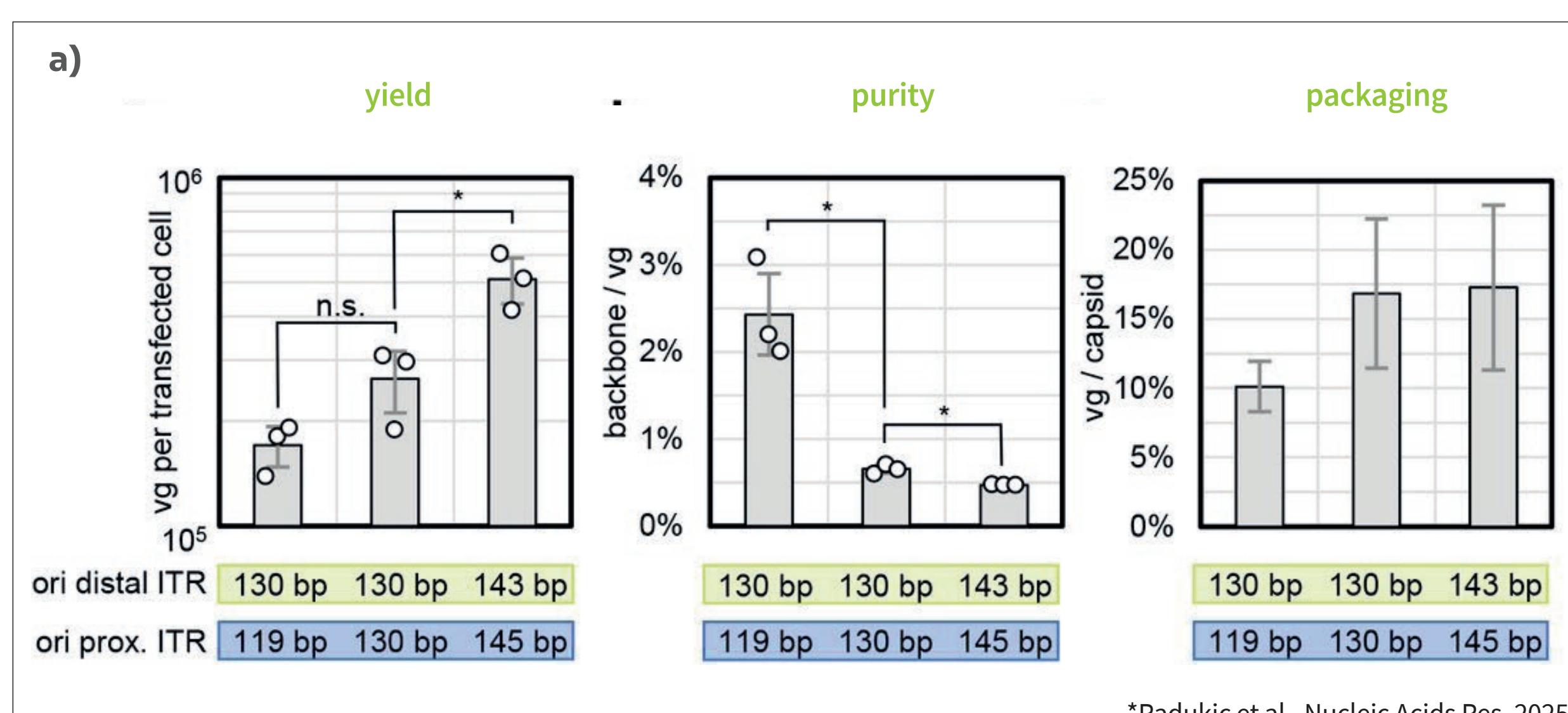
PlasmidFactory's exclusive 2-plasmid-system, licensed from DKFZ, combines helper and packaging functions on a single construct, reducing the requirement from three plasmids to two. This streamlines transfection, lowers production complexity, and enables more efficient and cost-effective AAV manufacturing at scale.

✓ Simpler transfection ✓ Reduced costs ✓ Validated system

Intact ITRs

Fig. 2 – ITR integrity drives vector quality.

(a) Intact AAV ITRs are essential for efficient genome rescue and replication. Plasmids with full-length ITRs generate higher titers, reduced backbone mispackaging, and more full capsids compared to truncated or deleted ITRs. (b) PlasmidFactory's ITRPROTECT & ITRRESCUE® technologies ensure stable, intact ITRs during plasmid production, thereby securing reliable yields, genomic purity, and consistent AAV performance.



b) ITRPROTECT/ITRRESCUE®

The diagram illustrates the ITRPROTECT/ITRRESCUE® mechanism. It shows a circular RNA molecule with two internal transcribed regions (ITRs) highlighted in orange. The top ITR is labeled 'ITRPROTECT' and the bottom ITR is labeled 'ITRRESCUE'. The genome is composed of repeating units of the sequence: C G C C C G G G G C A A G C C C G G G C G A C C C T T T G G T T C G C C C G. The ITRs are positioned at the 5' and 3' ends of the genome, and they contain specific sequence motifs: 'A A' in the top ITR and 'T T' in the bottom ITR. The rest of the genome is represented by grey segments.

✓ Better yield ✓ Higher purity ✓ Increased packaging

The Minicircle Advantage

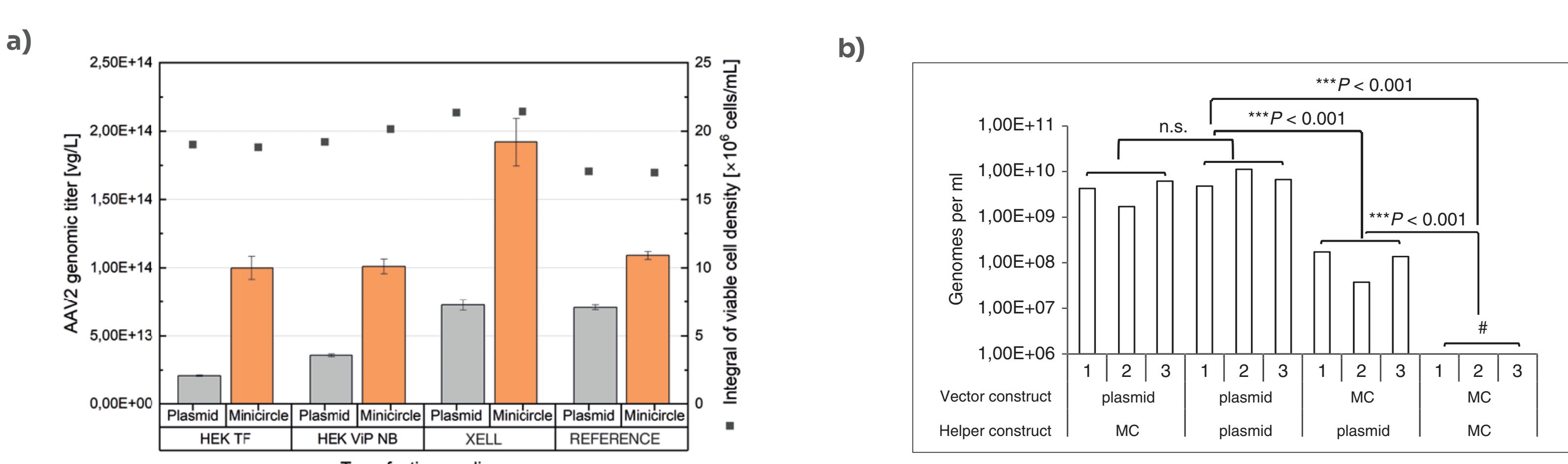


Fig. 3 – Minicircle DNA boosts AAV productivity, purity, and transduction.

(a) AAV titers measured 72 h post-transfection were 3–4× higher with minicircle DNA (MC-AAV-ssGFP + pDG, both PlasmidFactory) vs. plasmid DNA (pAAV-ssGFP + pDG, both PlasmidFactory). (b) Replacing both vector and helper plasmids with Minicircles eliminated detectable antibiotic resistance sequences from AAV preparations, resulting in bacterial backbone-free, cleaner vectors, enabling up to 30× higher transduction efficiency.

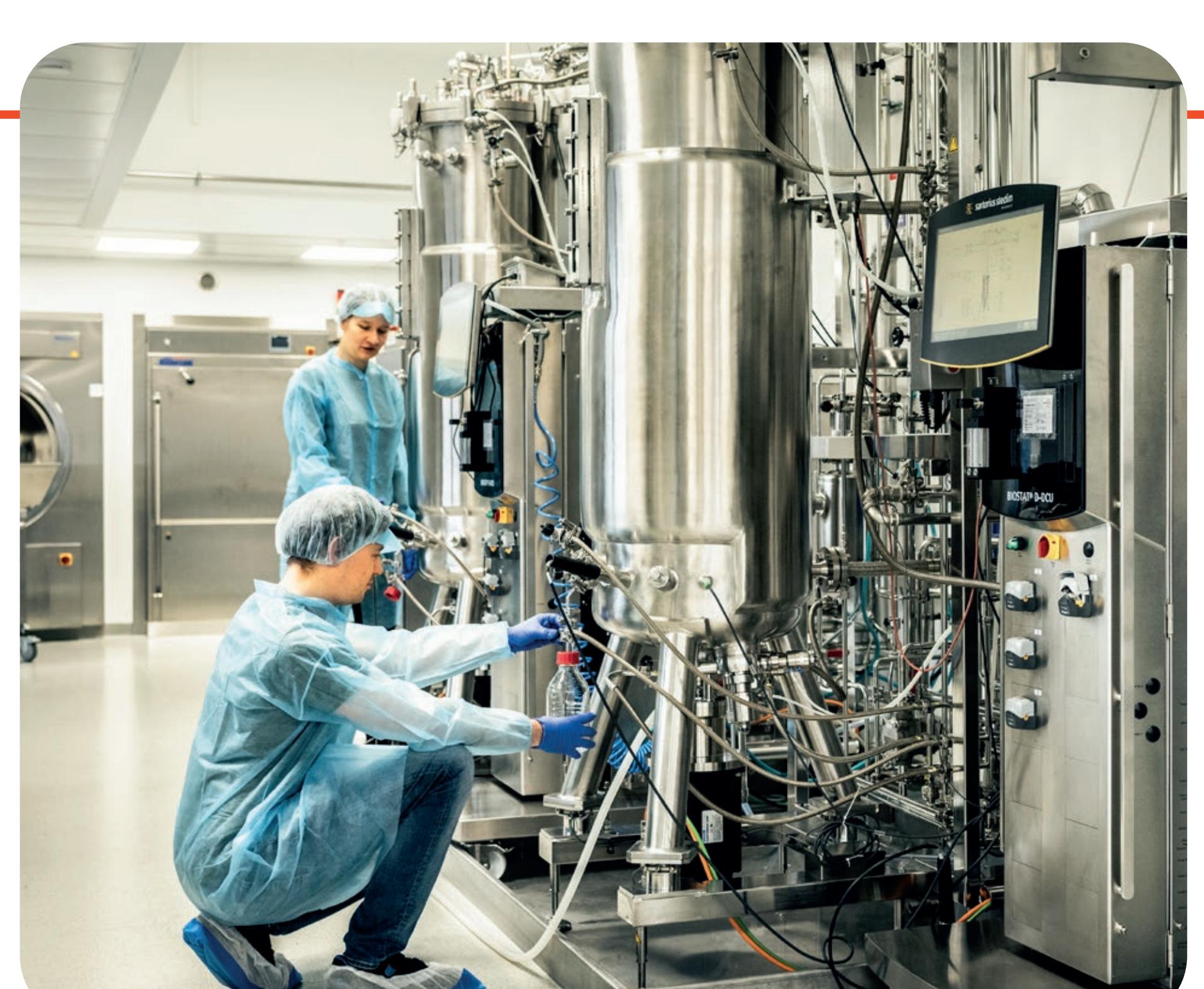
✓ No backbone packaging ✓ Lower immunogenicity ✓ Regulatory ready

Conclusion & Outlook

PlasmidFactory drives AAV production with DNA solutions that unite today's reliability and tomorrow's innovation.

- The **2-Plasmid System** streamlines production and reduces complexity.
- **ITRRESCUE®** protects ITRs, delivering higher yields, purity, and consistency.
- **Minicircle DNA** enables bacterial backbone-free vectors with up to 4 \times higher titers and 30 \times stronger transduction, eliminating backbone packaging and meeting future regulatory needs

These advances enable **faster, safer, cleaner AAV manufacturing** from research to GMP. PlasmidFactory's expertise and unique portfolio make it the trusted partner for advancing gene therapies toward safer and more accessible patient treatments.



 Faster, safer and cleaner AAV manufacturing

