Boehringer Ingelheim BioXcellence™

Setting the Standard for Plasmid DNA Production.

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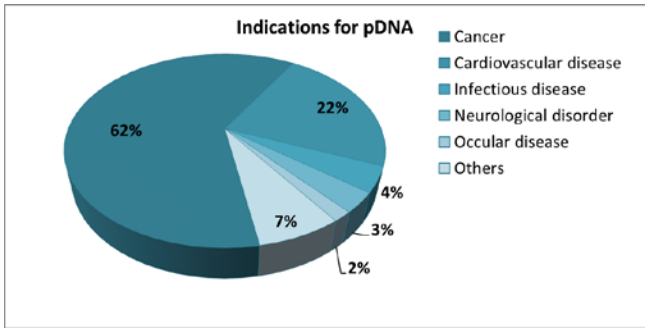
Introduction: Gene Vaccination and Gene Therapy

In the research, development and commercialization of vaccines, improving quality, safety, yield and speed is an on-going challenge. In these areas, gene vaccinations and gene therapies have set new standards. While still experimental, DNA vaccines have been applied to a number of viral, bacterial and parasitic models of disease, as well as to several tumor models. In addition, the advantages of gene vaccines and gene therapy over conventional vaccines and therapies include the ability to induce a wider range of immune response types[1]. Improvements of gene delivery systems triggered a high number of yearly initiated clinical trials at an average of about 100 over the last 15 years. Given the extraordinary potential benefits to our customers and being a pioneer, Boehringer Ingelheim has developed and continuously improved a best in class production process for highest-quality plasmid DNA (pDNA) to leverage this potential.

[1] Phase IV: New Jersey, USA initiated 04.2016/ open/ naked pDNA coding for HPV16 E6-E7 Fusion Protein HPV18 E6-E7 Fusion Protein/i.m. Trial title: A Prospective, Randomized, Double-Blind, Placebo-Controlled Phase III Study of VGX-3100X (DNA Plasmid Vectors Expressing Hpv-16 E6/E7, Hpv-18 E6/E7) Delivered Intramuscularly Followed by Electroporation with Cellectra® 5PSP for the Treatment of HPV-16 and/or Hpv-18-Related Histologic HSIL of the Cervix



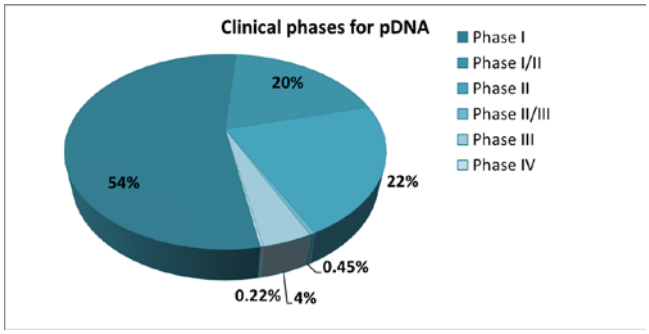


Fig. 1: Status: 19 Oct 18 - Search based on using naked plasmid DNA as vector used in the following site: http://www.abedia.com/wiley/search.php

At a glance: Advantages of our best-in-class plasmid production technology

Higher Quality: Up to 98% monomeric ccc plasmid conformation and highest plasmid content per gram *E. coli* biomass (up to 60 mg/g dry cell weight), improving plasmid purity

Higher Speed: Drug product ready for clinical trials within 12 months

Higher Safety: Proprietary technology for the production of antibiotic-resistance-free miniaturized plasmids and avoidance of animal derived or complex components

Higher Yield: Highest fermentation titers (concentrations up to 3.2 g/L), with Boehringer Ingelheim's *E. coli* host for plasmid production reaching higher fermentation titers than conventional strains in most cases

Our Offer: Global Contract Manufacturing Excellence in pDNA Production

We offer fast-track services from project start to clinic or commercialization to produce highly purified, pharmaceutical-grade pDNA that meets all regulatory requirements. From our specialist site for microbial fermentation in Vienna, Austria, we can offer various scales.

Our services:

- Feasibility studies at laboratory-scale representing the GMP large-scale process: Milligram amounts of highly purified pDNA within 8 weeks
- Production of material for toxicology studies at pilot-scale: gram amounts of highly purified pDNA within 6 months
- Production of clinical material in large-scale GMP facilities: multiple gram amounts of highly purified, GMP-grade pDNA within 12 months
- Whole process chain from transformation of host cell until released drug product for clinical and market supply
    - Cell Banking
    - Process development and Scale-Up
    - Fill & Finish
    - Quality and Regulatory Services

Our outstanding track record:

- More than 35 different plasmids from 2 – 15 kbp at laboratory- and GMP large-scale
- 25 pDNAs produced at GMP large-scale for clinical trials up to phase III
- More than 14 different customers all over the world
- More than 20 plasmids produced in Boehringer Ingelheim *E. coli* host with titers from 0.3 – 3.2 g/L
- 9 plasmids produced in *E. coli* DH5alpha or DH1

Details: Proprietary Production Process for pDNA

The fermentation process:

The Boehringer Ingelheim BioXcellence™ high titer pDNA fermentation process provides highest amounts of pDNA in *E. coli* biomass (Fig. 2). This highly efficient process is characterized by:

- Combination of the Boehringer Ingelheim *E. coli* host and the proprietary fed-batch fermentation process
- Best in class titers - up to 3.2 g/L - using high copy number pUC or similar origins of replication (ORIs)
- Highest plasmid content per gram biomass (up to 60 mg/g dry cell weight) providing highly purified pDNA due to best ratio of pDNA to biomass
- No induction of pDNA formation needed
- Fully defined media without animal-derived or complex components

Consequently, this process achieves the highest product safety and process robustness (independent of supplier of media components).

Depending on your needs, we are able to produce plasmids in alternative hosts, like *E. coli* DH5alpha and DH1 in modified media and with adapted fermentation processes.

Essentially, our process can be used for all plasmids up to 15 kbp using high copy number pUC or similar ORIs.

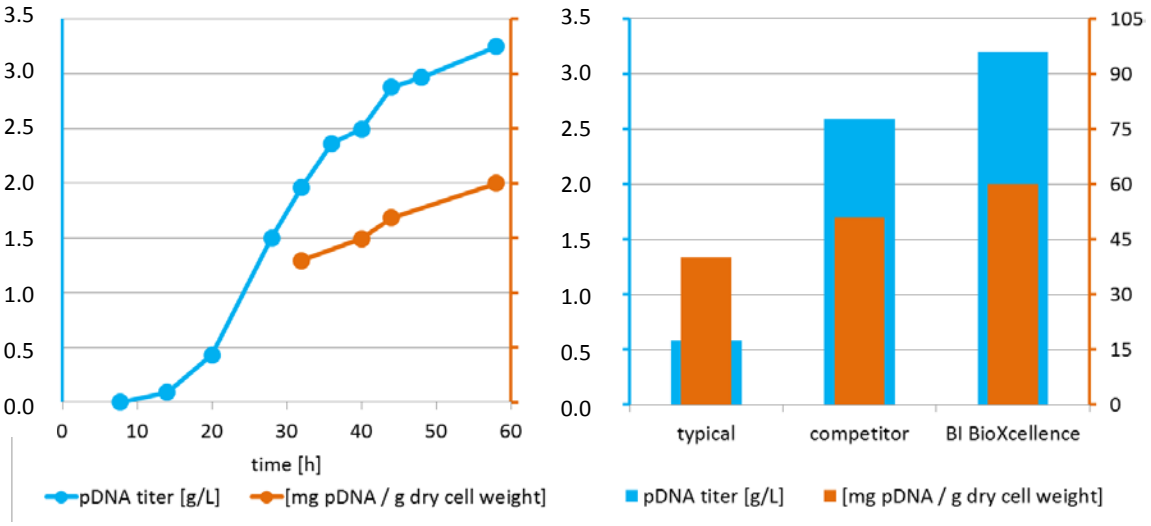


Fig. 2: Boehringer Ingelheim BioXcellence™ fed-batch fermentation process.
Example: Course of pDNA titer and plasmid content per g biomass during fermentation (left side). Superior Boehringer Ingelheim BioXcellence™ fed-batch fermentation process in comparison to typical and competitor processes (right side).

The purification process:

Based on our *E. coli* biomass with the highest plasmid DNA content (up to 60 mg/g dry cell weight) all host-related impurities are efficiently separated by several process steps to obtain highly purified plasmid DNA (Fig. 3) with very low endotoxin content suitable for gene vaccination and gene therapy.

The process is characterized by:

- Absence of enzymes (such as RNase), detergents and organic solvents
- 2 – 3 chromatography purification steps
- Ultrafiltration for concentration of plasmid up to 10 g/L
- Proprietary process and equipment for alkaline lysis of the host cells
- Improved separation of cell debris, genomic DNA and host related proteins and other impurities

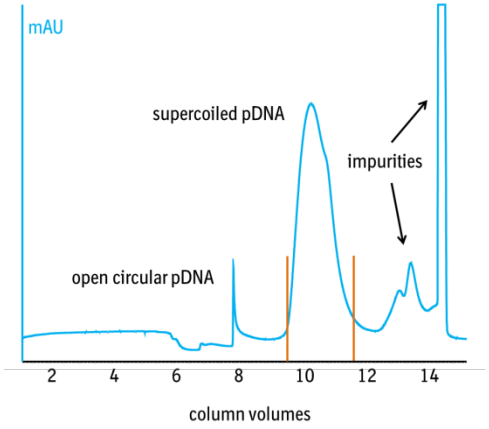


Fig. 3: Separation of impurities and open circular pDNA from supercoiled pDNA by hydrophobic interaction chromatography (HIC).

Production of antibiotic-resistance-free plasmids

Boehringer Ingelheim has developed its proprietary antibiotic-resistance-free selection system. It meets regulatory guidance recommending the absence of antibiotic resistance genes in plasmids used for gene therapy and vaccination.
This technology allows the production of antibiotic-resistance-free miniaturized plasmids with pUC / ColE1 ORIs from laboratory to large scale GMP facilities:

- Highest product safety: No antibiotic resistance genes
- Higher productivity / fermentation titer
- Higher plasmid potency / transfection rate

Selection mechanism

As depicted in figure 4, the selection is based on an *E. coli* host strain, the genome of which has been genetically modified. Without plasmid, the expression of a repressor protein (TetR) represses the transcription of a gene essential for cell wall formation (*murA*). Therefore, the host's growth is inhibited. In the presence of plasmid (Fig. 5), RNA I originating from the ColE1 / pUC ORI binds to the antisense RNA (AS-RNA) region located between the promoter and the coding sequence of the TetR repressor. Thus, the

expression of the essential *murA* gene is not further repressed and the plasmid-carrying cells survive.

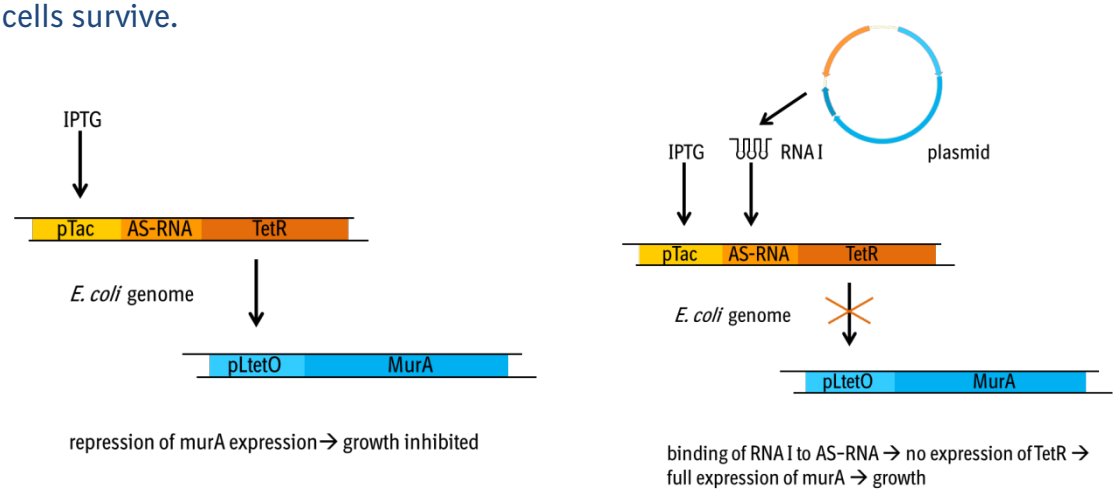


Fig. 4: Mechanism of the Boehringer Ingelheim BioXcellence™ antibiotic-free selection system

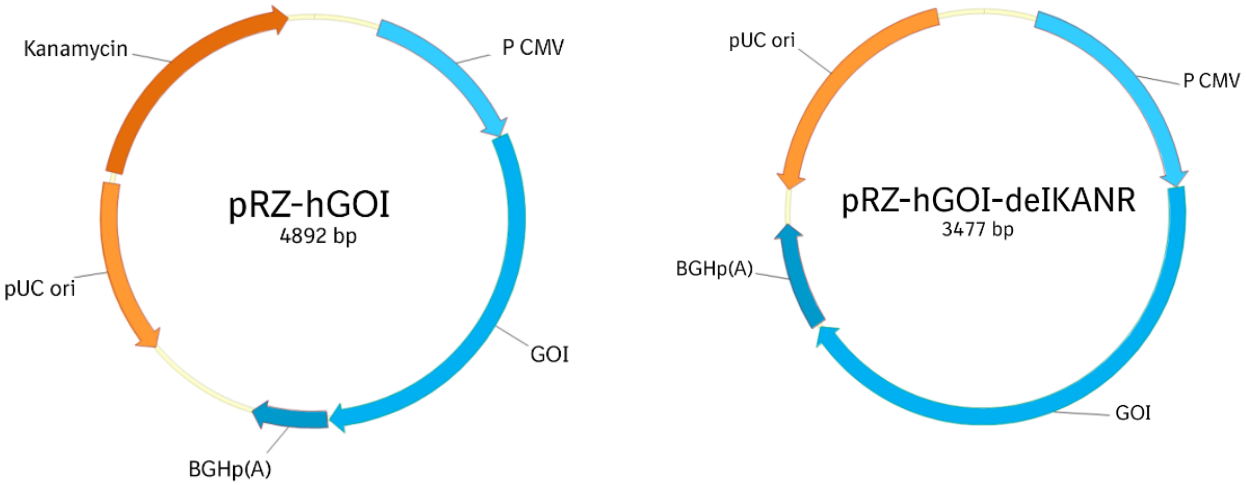


Fig. 5: Antibiotic-resistance-free therapeutic plasmid (right side) can be produced by using BI´s proprietary genetically modified E. coli host. For that purpose the kanamycin resistance gene can simply be excised from a typical therapeutic plasmid (left side) resulting in a significantly smaller plasmid. This, additionally, results in higher fermentation titers and an increased transfection rate. The insert consists of the viral CMV promoter (P CMV), the therapeutic gene of interest (GOI) and a sequence encoding a poly A tag (BGHp(A)).

Quality Control

In order to monitor all stages of the process for plasmid DNA quality and levels of contaminants, we have developed fast and sensitive validated analytical methods based on AIEX-HPLC (Fig. 6) and agarose gel electrophoresis (AGE). We offer all methods for detection of the levels of contaminating genomic DNA, RNA, proteins, endotoxins and methods for further characterization of the drug substance and drug product.

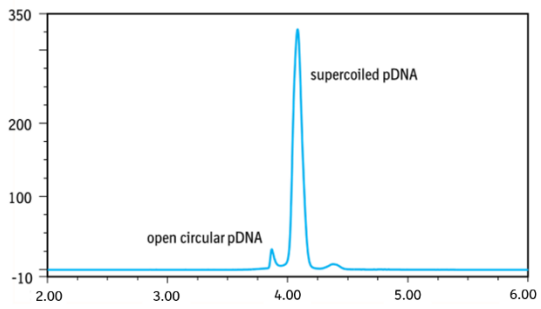


Fig. 6: AIEX (anion exchange) HPLC chromatogram of purified plasmid DNA

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