Setting the Standard for Plasmid DNA Production

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Gene Vaccination and Gene Therapy

Nucleic acid vaccines are still experimental and have been applied to a number of viral, bacterial and parasitic models of disease, as well as to several tumor models. 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. Improvements of gene delivery systems triggered a high number of yearly initiated clinical trials at an average of about 100 during the last 15 years. Therefore Boehringer Ingelheim has developed and continuously improved a best in class production process for plasmid DNA of the highest quality accelerating your path into clinical trials.

Best in class Plasmid Production Technology

- Highest plasmid quality
- Lean to Clinic: in 12 months Drug Product for clinical trials
- Highest product safety
  - proprietary technology for the production of *Antibiotic (AB) Resistance - free miniaturized plasmids*
  - avoidance of animal derived or complex components
- Highest fermentation titers (up to 3,2 g/L)
- Highest plasmid content per g *E. coli* biomass (60mg/g dry cell weight) → improves plasmid purity

Global Contract Manufacturing Excellence in Plasmid DNA Production

We offer fast track services from project start to clinic or commercial to produce highly purified, pharmaceutical grade plasmid DNA meeting all regulatory requirements. By using our worldwide production network we can offer various scales.

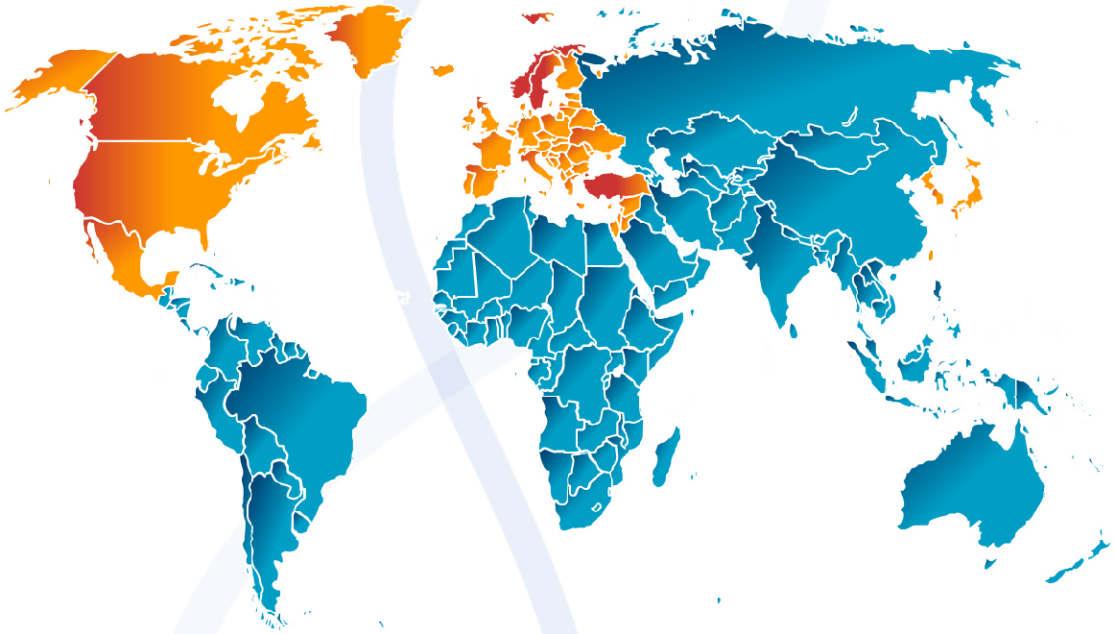
What we offer to our customers:

- Feasibility Studies in laboratory scale representing the GMP large scale process: mg amounts of highly purified plasmid DNA within 8 weeks
- Production of material for toxicology studies in pilot scale: g amounts of highly purified plasmid DNA within 6 months
- Production of clinical material in large scale GMP facilities: multiple g amounts of highly purified, GMP-grade plasmid DNA 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 30 different plasmids from 2 – 15 kbp in laboratory and GMP large scale
- 20 produced in GMP large scale for clinical trials up to phase III
- More than 10 different customers all over the world
- 20 plasmids produced in Boehringer Ingelheim *E. coli* host with titers from 0,3 – 3,2 g/L
- 8 plasmids produced in *E. coli* DH5 alpha or DH1

Our worldwide customers:



Proprietary Production Process for Plasmid DNA

The Fermentation Process

The Boehringer Ingelheim BioXcellence™ high titer plasmid DNA fermentation process provides highest amounts of plasmid DNA in *E. coli* biomass. 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
- Highest plasmid content per g biomass (up to 60 mg/g dry cell weight) providing highly purified plasmid DNA due to best ratio of pDNA to biomass
- No induction of plasmid DNA formation needed
- Fully defined media without animal derived or complex components

Therefore this process achieves highest product safety and process robustness (independent on supplier of media components).

Depending on your needs we are able to produce plasmids in alternative hosts, like DH5 alpha and DH1 in modified media and adapted fermentation processes.

Your benefit:

Our process can be used for all plasmids using high copy number pUC or similar origins of replication up to 15 kbp.

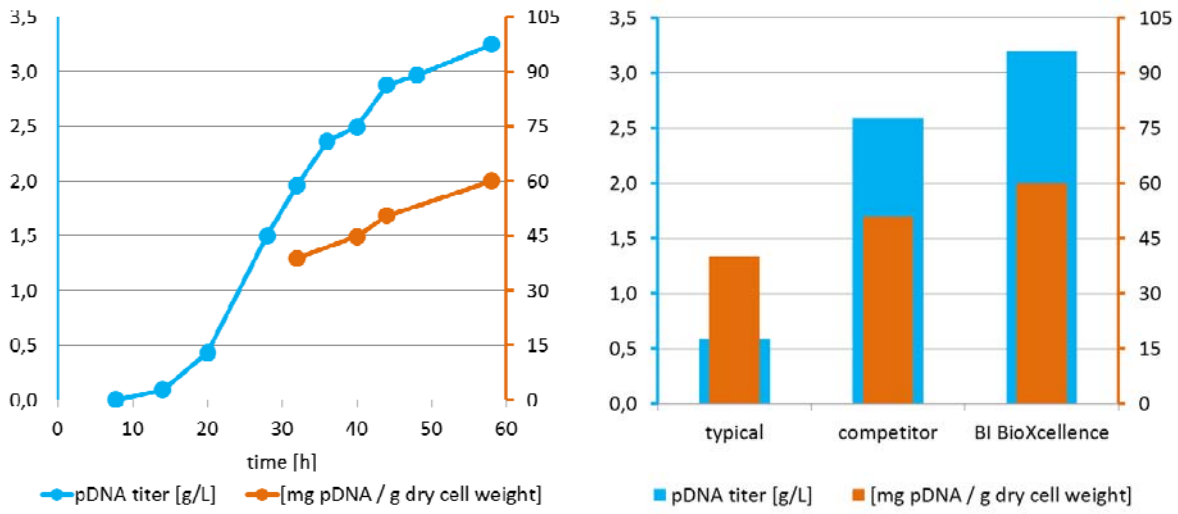


Fig. 1: *Boehringer Ingelheim BioXcellence™ fed batch fermentation process; right side: superior Boehringer Ingelheim BioXcellence™ fed-batch fermentation process in comparison to typical and competitor processes.*

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 get highly purified plasmid DNA with very low endotoxin content suitable for gene vaccination and gene therapy.

The process is characterized by:

- Absence of enzymes (like 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

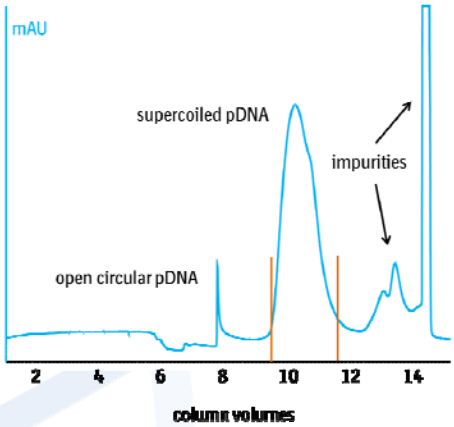


Fig. 2: *Separation of impurities and open circular plasmid DNA from supercoiled plasmid DNA by hydrophobic interaction chromatography (HIC)*

Production of Antibiotic (AB) Resistance - free Plasmids

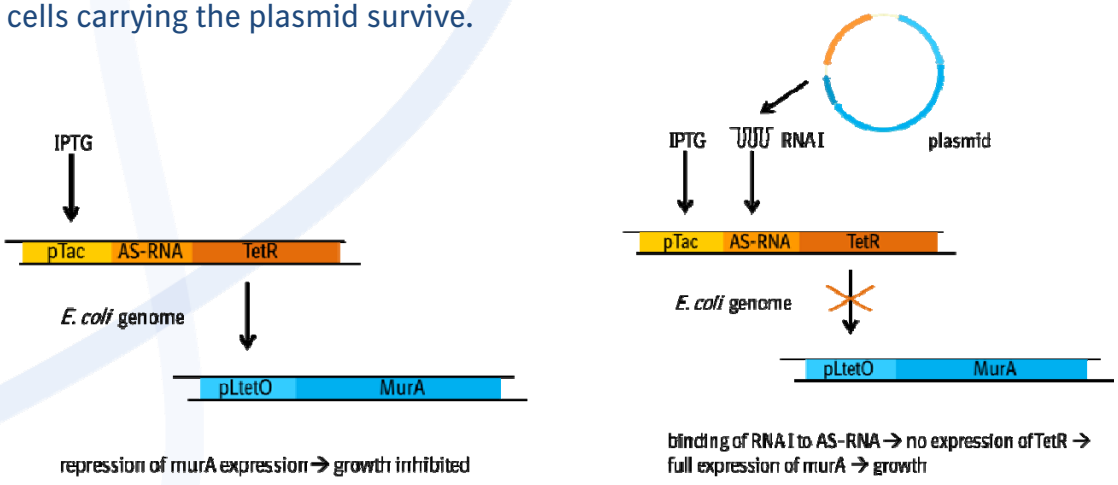
Boehringer Ingelheim has developed its proprietary leading AB Resistance - free selection system. It meets regulatory guidance recommending the absence of antibiotic resistance genes in plasmids used for gene therapy and vaccination.

This technology allows the production of AB Resistance - free miniaturized plasmids with pUC / ColE1 origins of replication from laboratory to large scale GMP facilities:

- Highest product safety: reduced genome, no AB Resistance genes (Fig. 4)
- Higher productivity / fermentation titer
- Higher plasmid potency /transfection rate

Selection mechanism (Fig. 3):

The selection is based on an *E. coli* host strain which has been genetically modified in its genome. Without plasmid the expression of a repressor (TetR) represses the production of a gene essential in cell wall formation (murA). Therefore the host growth is inhibited. In the presence of plasmid RNA I from ColE1 / pUC origin of replication binds to the antisense RNA (AS-RNA) between the promoter and the TetR repressor. Therefore the expression of the essential murA gene is not further repressed and the cells carrying the plasmid survive.



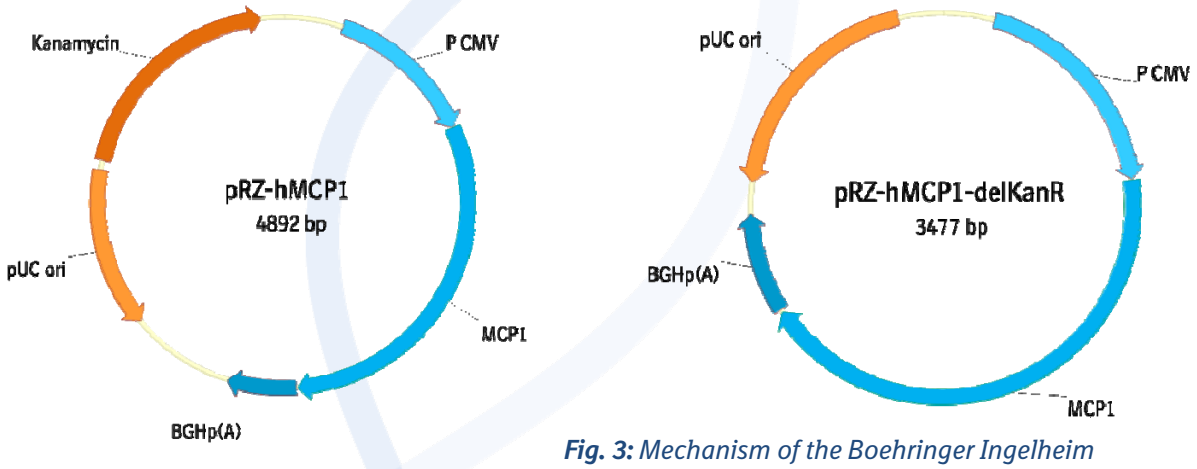


Fig. 3: *Mechanism of the Boehringer Ingelheim BioXcellence™ antibiotic-free selection system:*

Fig. 4: *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 increased transfection rate. Insert consists of the viral CMV promoter (P CMV), the therapeutic gene (MCP-1 in this example) and 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. 5) and 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.

We have also developed a proprietary fast and sensitive method for the identification of pattern of supercoiling of plasmid DNA topoisomeres based on HPLC (Fig 6).

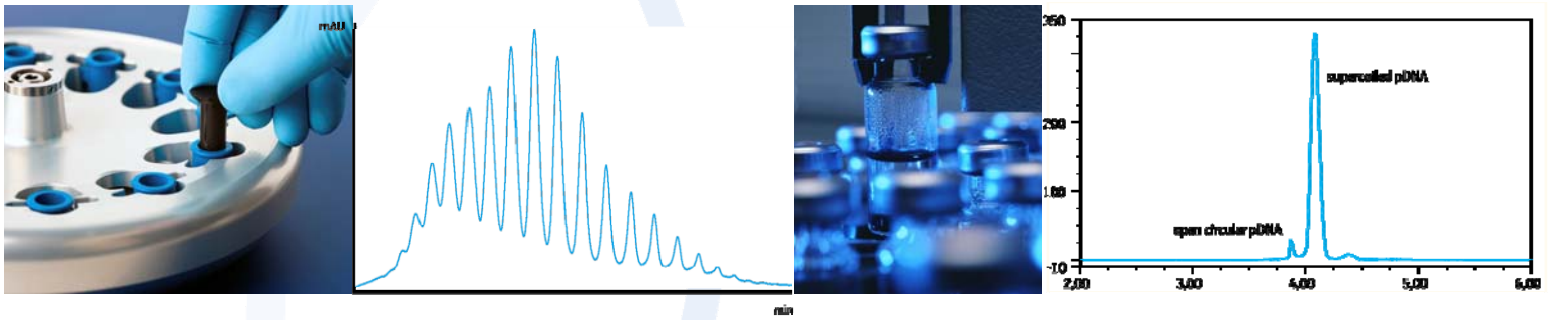


Fig 5 (left): *Separation of pDNA topoisomeres by Boehringer Ingelheims´s proprietary HPLC method*
Fig. 6 (right): *AIEX (anion exchange) HPLC chromatogram of purified plasmid DNA*

References and publications

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