“High Quality Grade” Plasmid production platform as a scale-up tool for new innovative therapeutics

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INTRODUCTION

The innovative new therapeutic fields based on mRNA or viruses (AAV, lentivirus, retrovirus, adenovirus …) has demonstrated rapid growth in the last decade and offers very promising results in several applications such as vaccines and therapies against cancer, pathogens or rare diseases. The increase in the number of clinical trials is an illustration of the huge development of this field.

mRNA or virus based therapeutics use plasmid as a critical raw material. In contrast to plasmids used in Gene Therapy, mRNA or virus based therapeutics do not necessarily require plasmid DNA produced under in vitro GMP production. This is a very important consideration to reduce the costs of mRNA or virus production processes.

RD-Biotech has developed a specific “High Quality Grade” (HQG) plasmid production platform for the rapid and efficient production of plasmid DNA. The platform ensures the delivery of HQG plasmids for the very high quality of mRNA or viruses required for pharmaceutical applications.

Here we describe how we can help our customers to reduce costs while keeping the highest standards of quality and how we can support customers through increasing the produced quantities to meet the demands of the different phases of the drug development process through to product launch.

MATERIALS & METHODS

First step: Dedicated production optimization process

Preliminary study in order to define the best process (best report between quality and yield) for the “High Quality Grade” plasmid production. Choice of the best *E. coli* strain, culture medium, and culture conditions are tested at this time. Clones for production are selected after minipreps and restriction analysis. Only clones showing good supercoiled profiles, correct poly-A tail (for mRNA production) and restriction profiles can be chosen for the subsequent large scale plasmid production. Pilot productions are also performed in order to validate all specifications of the production.

Second step: “High Quality Grade” master cells bank realization (Selection of the best clone for large scale production, amplification and aliquoting before freezing of the selected clone, dedicated quality controls [Plasmid identity, host cell identity, bacterial and bacteriophage purity,…]).

Third step: “High Quality Grade” plasmid DNA production

“High Quality Grade” plasmid DNA are produced according to the EMA guideline CHMP/BWP/2458/03 for the highest quality standards.

Plasmids are produced from “High Quality Grade” master cells banks.

- with “animal-free” components, "antibiotic-free", single use materials, dedicated rooms and devices
- according to a dedicated process
- with very skilled dedicated people

In order to get the highest quality with no cross contamination, no impurities (RNA, genomic DNA, endotoxins, HCP,…), with high supercoiled rates

Dedicated quality controls (Endotoxins assay, sterility tests,…). Possibility to perform large scale plasmid linearization in “High Quality Grade” conditions.

RESULTS

Production of 100 mg batch of linearized “High Quality Grade” plasmid for GMP mRNA production

RD-Biotech produced 100 mg of “High Quality Grade” plasmid which was linearized under “High Quality Grade” conditions for direct *in vitro* transcription.

We performed a full process for the production of 100 mg linearized “High Quality Grade” plasmid. It started with a dedicated optimization process for plasmid production (selection of bacterial strain, medium for bacteria growth, bacterial clone selection). Master cells bank was prepared and large scale production and linearization of the plasmid were performed.

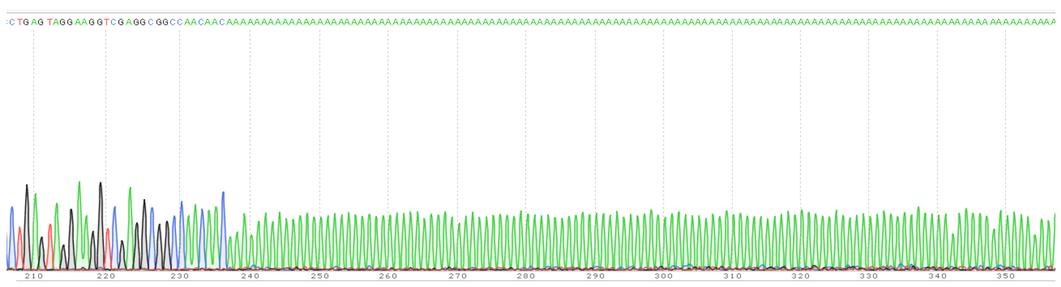
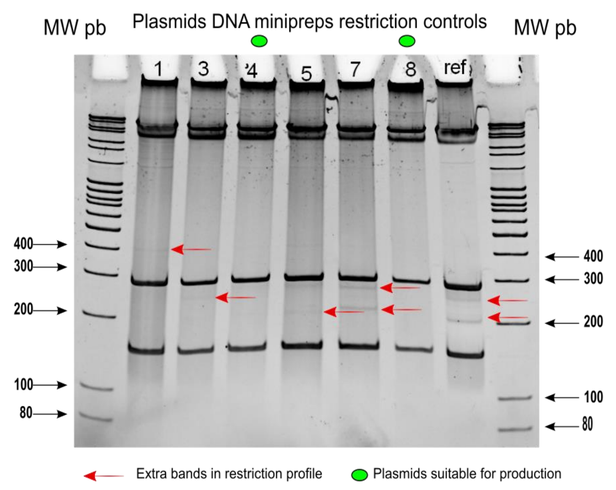


Figure 2: Control of poly-A tail integrity: a very strict sequencing control is performed on each plasmid used for *in vitro* transcription to check the integrity of the poly-A tail.

Figure 1: Dedicated production optimization process: Choice of the best *E. coli* strain, culture medium, and culture conditions are tested at this time. Clones for production are selected after minipreps and restriction analysis. Only clones showing good supercoiled profiles, correct poly-A tail and restriction profiles can be chosen for the subsequent large scale plasmid production.



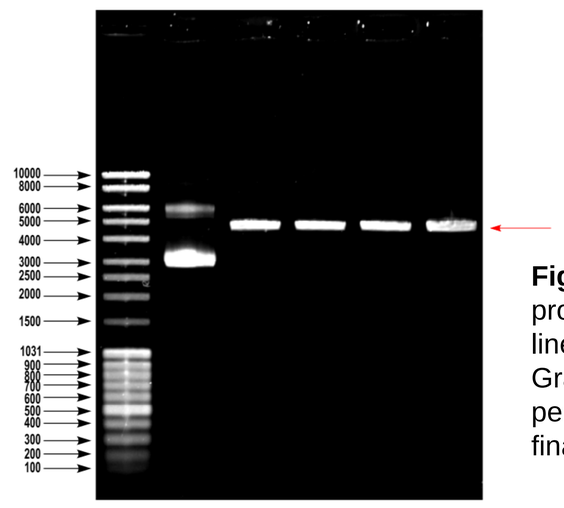


Figure 3: Control of linearized plasmid. After plasmid production, RD-Biotech performed high scale plasmid linearization for direct *in vitro* transcription. After “High Quality Grade" linearization, thorough purification steps are performed to remove enzyme and other contaminants in the final batch of linearized plasmid.

Production of 500 mg of “High Quality Grade” plasmid for GMP AAV production

Classical AAV production needs a large amount of three different plasmids (transgene, packaging and helper sequences) produced in very high quality.

RD-Biotech produced 500 mg of “High Quality Grade” plasmids which were used for transfection.

Dedicated processes were developed in order to obtain the best quality plasmids in rapid time. Each plasmid is strongly controlled at the different steps. The very important points to verify are integrity of plasmids (absence of recombination and fully controlled sequences) and high supercoiled rates.

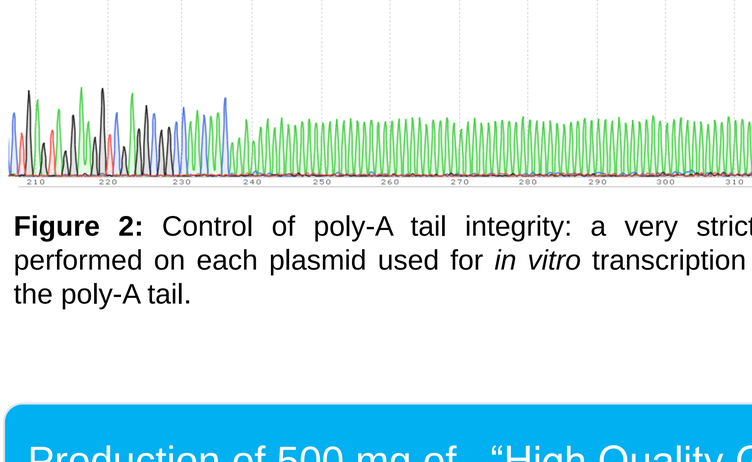


Figure 5: Control of plasmid integrity
Example of agarose gel electrophoresis, which permits to verify the absence of recombination (no extra bands in ND) and the plasmid identity by enzymatic restrictions.

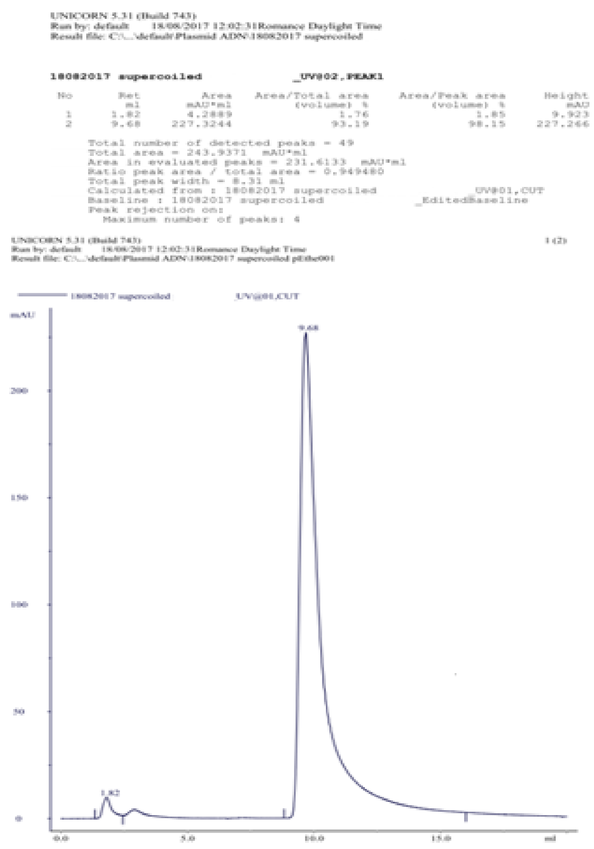


Figure 4: Control of the supercoiled rate
Example of chromatography step for determination of supercoiled plasmid DNA rate. The quality control indicates that 98,3 % of plasmid DNA is supercoiled.

Examples of Quality controls, other dedicated controls on demand		
Parameter	Method	Specification
Appearance	Visual inspection	Clear colorless solution
Concentration	UV absorption	1 mg/ml +/- 10%
Absorption *ratio* 260/280 nm	UV absorption	1.8 - 2.0
Supercoiled rate determination	Chromatography	> 90%
E. coli DNA	qPCR	< 5%
Proteins	Micro BCA	< 5 µg/mg DNA
E. coli RNA	Agarose gel electrophoresis	Not detectable
Identity	Sequencing	100% alignment with control
Identity	Restriction digestion	Expected bands/peaks
Endotoxin content	EP 2.6.14	< 10 EU/mg DNA
Bioburden	EP 2.6.12	< 5 CFU/mg DNA
Mycoplasma detection	qPCR	Not detectable
pH measurement	pHmeter	Report result
Osmolality	Physico-chemical analysis	Not detectable

Table 1: Typical required specifications for “High Quality Grade” plasmids used for AAV productions

CONCLUSION

RD-Biotech has developed a very efficient and optimized platform dedicated for the production of “High Quality Grade” plasmid DNA for mRNA or virus based therapeutics and other applications according to the authorities requirements. This platform is specifically designed to support our customers during all phases of mRNA or virus based drug development or marketing. Our technical know-how combined with our flexibility and rigorous process allow us to offer “High Quality Grade” plasmids in a very short time and very affordable price. This is crucial in the field of new therapies since costs and delays have to be reduced as much as possible.