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Robust and high yielding platform process for the plasmid DNA production

Syngene

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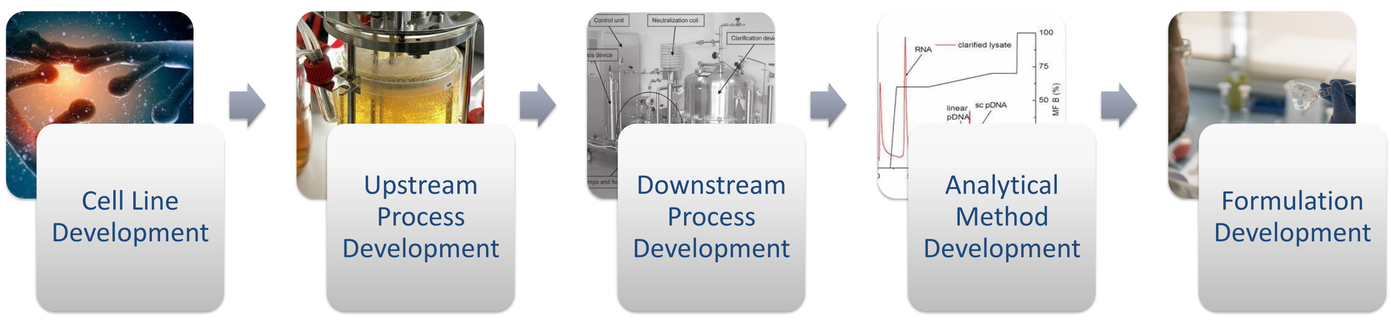
Introduction

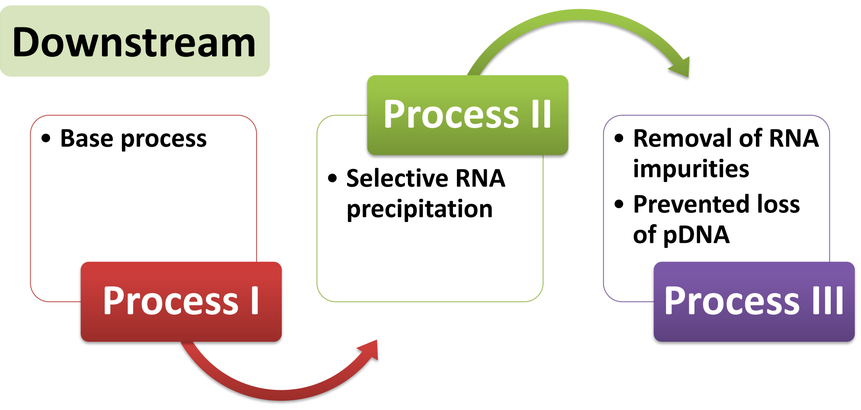
Plasmid DNA is a new generation biotechnology product (gene medicines and DNA vaccines) that is just beginning to enter the marketplace. Plasmid DNA vectors may find application as preventive or therapeutic DNA vaccines for viral, bacterial, parasitic diseases or for other indications such as cancer and gene therapy products [1]. However, large scale production of pDNA is challenging, due to effect of production parameters on the extent of plasmid supercoiling, generational loss and instability [2]. Syngene scientists were able to overcome these challenges with their extensive experience and novel ideas, resulting in a pDNA production platform process with high pDNA yield and purity. Microbial Upstream team at Syngene has developed a high cell density fed-batch fermentation platform process to produce pDNA at high titers (>1 g/L), while downstream scientists have overcome the challenges by implementing a custom-made cell lysis vessel, which allowed to control critical parameters such as lysis pH, agitation, and temperature. The Process Development was supported by state-of-the-art analytical capability at Syngene.

Process

Overview



Downstream



Upstream

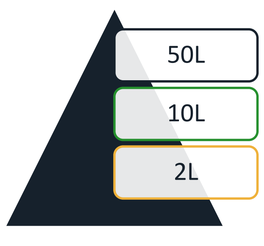
-Seed : Lean media
-Batch: Semi-defined media

Seed Stage

- Shake flask scale (1L)
-Transfer O.D (1.5-2.5)

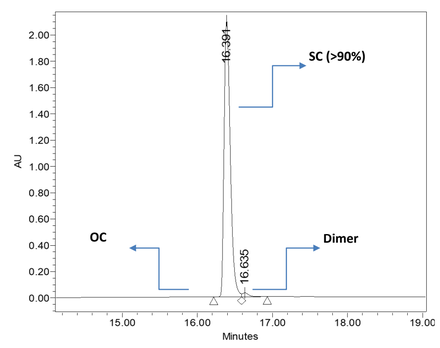
Fermenter Stage

-Fed-batch process
- Feeding strategy: pH Stat
- Duration of batch - 30h
-Cell density achieved ~140 $O.D_{600}$



Fermenter Scales

Analytical Methods For pDNA



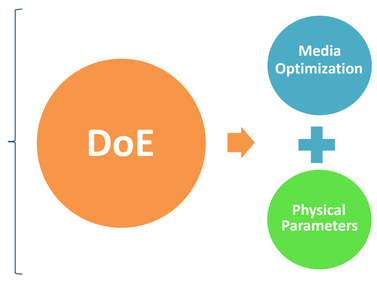
AEX: Purity analysis of pDNA batch

Forms	%
OC	<0.2
SC	97
Dimer	2.8

Results

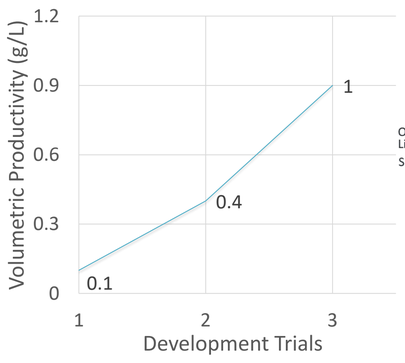
Upstream

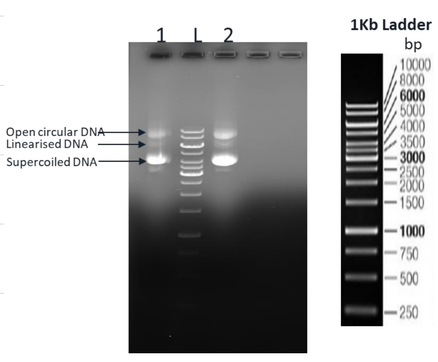
DEVELOPMENT CRITERIA



PRODUCTIVITY

- ✓ Growth Profile
- ✓ Wet Cell Weight
- ✓ Titer (g/L)





~0.1 g/L >> 0.4 g/L >>> 1 g/L;
↑6X-9X Productivity Enhancements

Downstream

DSP FLOW DIAGRAM

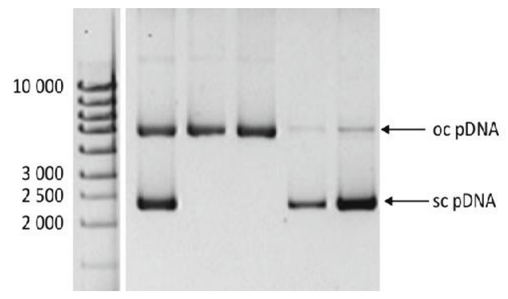
5% Cell suspension in solution A
↓
Equal volume of soln. B followed by 5 min incubation @8-10°C
↓
Added soln. C (30min @8-10°C)
↓
Centrifugation
↓
$CaCl_2$ precipitation
↓
UFDF
↓
Anion Exchange Chromatography
↓
Affinity Chromatography
↓
Gel filtration Chromatography

AUTOMATED LYSIS SYSTEM (ALS)

Benefits of ALS:

1) Automated lysis system helps in performing continuous lysis with less sheer under controlled condition of critical parameters during lysis.
2) ALS provides good yield of supercoiled pDNA.

AGAROSE GEL ELECTROPHORESIS



Attributes	Percentage
Recovery	> 25%
Purity	> 90%

Plasmid DNA Downstream Platform Process:

1) Syngene has developed a scalable platform process for the plasmid DNA production.
2) The developed process worked equally well with different plasmid sizes (2-14 kb).
3) Process resulted in yield of 25-40% enriched with the supercoiled form of plasmid DNA

Conclusion

After successful planning and experimentation, Syngene scientists were able to develop a platform process for plasmid DNA production, with high titers > 1 g/L at Upstream and >25% recovery at Downstream. The platform process has been successfully tested across 2L, 10L and 50L production scales, for both high and low copy number plasmids. Syngene pDNA platform process has served and continues to serve the needs of a diversity of Syngene clients.

Acknowledgement

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References

1. Mark A. Eiteman and Elliot Altman, Overcoming acetate in *Escherichia coli* recombinant protein fermentations, 2006, TRENDS in Biotechnology Vol.24 No.11
2. James A. Williams, Aaron E. Carnes, Clague P. Hodgson, Plasmid DNA vaccine vector design - Impact on efficacy, safety and upstream production, 2009, Biotechnology Advances, 27, 353–370