

# An inducible fed-batch fermentation process for scale-up and production of DNA vaccines and gene medicines

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## Abstract

It is essential to devise industrial processes whereby plasmid DNA can be manufactured to meet the quality, economy, and scale requirements projected for future gene medicine and DNA vaccine production. To date, most plasmid fermentation media and processes result in low yields, imposing a cost and purity burden on commercialization of plasmid DNA production. Here we report the development of an inducible fed-batch fermentation process that dramatically increases volumetric yield and specific plasmid yield with respect to cell mass, while maintaining or enhancing plasmid integrity. Additionally, this process is well suited for producing unstable plasmids.

This inducible process utilizes commercially available media that we designed specifically for plasmid production. The process consists of an initial biomass accumulation phase, followed by a plasmid accumulation phase. The plasmid is stably maintained at low levels during a period of nutrient restricted growth and reduced temperature (30°C), and then the temperature is increased (37-42°C) to induce plasmid amplification. Typically, the specific plasmid yield increases over a period of up to 8 hours following temperature up-shift.

A set of ten replicate fermentations producing a DNA vaccine plasmid was performed. The total process lasted 38.5 hours, with a 7 hour induction time. The overall plasmid yield averaged 760 mg/L, and the average specific plasmid yield was 8.5 mg/L/OD600. A total DNA analysis by AGE of total cell lysates indicated that plasmid DNA accounted for the majority of the total DNA in the cells at harvest.

A high degree of process robustness and reproducibility has been demonstrated with many different plasmids and with multiple batches of a single plasmid. Fed-batch fermentation yields from 1000-1500 mg plasmid DNA/L fermentation media have been obtained with pUC origin containing plasmids. This five to ten fold increase in plasmid yield dramatically decreases plasmid manufacturing costs, and improves the effectiveness of downstream purification by reducing the fraction of impurities.

## Materials and Methods

### Strains and plasmids

*E. coli* DH5α: F- Φ 80*lacZ*Δ*M15* Δ(*lacZYA*-*argF*) U169 *recA1 endA1 hsdR17*(rk-, mk+) *phoA supE44 λ- thi-1 gyrA96 relA1*

Plasmid gWiz GFP: (Gene Therapy Systems) 5.8 kb, pUC origin, kan<sup>r</sup>

Plasmid pNTC7264-hmPA-EGFP: (Nature Technology Corp, Williams et al. 2005) 6.5 kb, pUC origin, kan<sup>r</sup>

### Fermentation

Dissolved oxygen: 30%  
pH: 6.9-7.1  
Airflow: 1 VVM  
O<sub>2</sub> supplementation increased as required

Concentrated semi-defined glycerol feed was added automatically according to the formula:

$$F(t) = \frac{\mu X_B V_B}{S_j Y_{X/S}} e^{\mu t}$$

F(t) = feed rate, L/h

μ = desired specific growth rate during fed-batch phase, h<sup>-1</sup>

X<sub>B</sub> = biomass concentration at the end of the batch phase, g dry cell weight/L

V<sub>B</sub> = initial liquid volume of culture, L

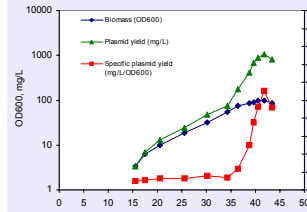
S<sub>j</sub> = limiting substrate concentration in nutrient feed medium, g/L

Y<sub>X/S</sub> = yield coefficient of biomass from substrate, g/g

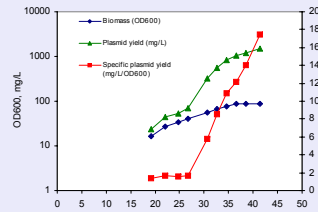
t = time since beginning of fed-batch phase, h

## Results

### Inducible fed-batch fermentation process:



**gWiz GFP 30°C→42°C**  
1070 mg/L



**pNTC7264-mgPA-EGFP 30°C→42°C**  
1500 mg/L

### Feeding strategy

A constant flow rate feeding strategy was tested as an alternative to exponential feeding with two 4L fed-batch fermentations with plasmid pNTC7264-mgPA-EGFP. One was fed exponentially to give a specific growth rate of 0.12 h<sup>-1</sup>, and the other was fed at a constant rate of 20 ml/hr (3 g glycerol/L/h, chosen as the maximum feed rate that would not result in glycerol accumulation):

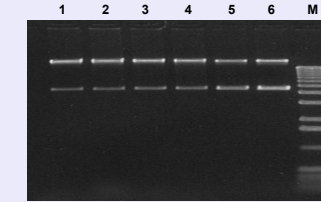
Feeding strategy	Cell density	Overall plasmid yield	Specific plasmid yield	Fermentation time	Productivity
Constant 3 g glycerol/L/h	OD <sub>600</sub> 64	620 mg/L	9.7 mg/L/OD <sub>600</sub>	43.8 h	14 mg/L/h
Exponential μ = 0.12 h <sup>-1</sup>	OD <sub>600</sub> 86	1500 mg/L	17.5 mg/L/OD <sub>600</sub>	41.4 h	36 mg/L/h

### Reproducibility

The reproducibility of the inducible process was evaluated utilizing a single Vaccine Research Center (VRC) DNA vaccine plasmid (VRC 5737) in ten 10 L fermentation runs, all induced between OD<sub>600</sub> 50 and 55. The results demonstrate high process consistency. No significant differences in specific plasmid yield were observed between 6 and 8 hours of induction.

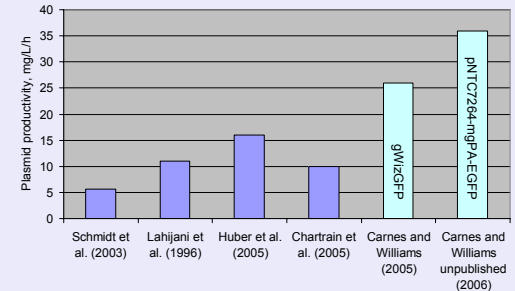
Run #	Induction OD <sub>600</sub>	Final OD <sub>600</sub>	Overall plasmid yield (mg/L)	Specific plasmid yield (mg/L/OD <sub>600</sub> )	Temp shift (hours)	Begin harvest (hours)	Time at 42°C (hours)
1	54	97	820	8.5	31:38	38:45	7:07
2	51	92	751	8.2	31:24	37:40	6:16
3	50	93	783	8.4	31:50	38:27	6:37
4	51	90	851	9.4	32:15	40:16	8:01
5	53	93	829	8.6	32:49	39:45	6:56
6	53	83	737	8.9	31:59	39:16	7:17
7	53	73	814	11.2	30:43	38:11	7:28
8	55	82	814	9.9	32:47	39:33	6:46
9	51	91	848	9.3	32:22	39:20	6:58
10	53	89	928	10.5	31:06	38:16	7:10

Data from ten 10L fed-batch fermentations with plasmid VRC 5737 showing process consistency.



- 1) Shake flask inoculum
- 2) Batch phase, 30°C, OD<sub>600</sub> = 4
- 3) Fed-batch phase, 30°C, OD<sub>600</sub> = 18
- 4) Fed-batch phase, 30°C, OD<sub>600</sub> = 45
- 5) Fed-batch phase, 42°C, OD<sub>600</sub> = 70
- 6) Fed-batch phase, 42°C, OD<sub>600</sub> = 83
- M) 1kb DNA ladder (Invitrogen)

Total DNA analysis from sample time points of a VRC 5737 *E. coli* DH5α fermentation with a 30→42°C temperature shift. The lower band is supercoiled monomer plasmid, the larger band is genomic DNA and supercoiled dimer plasmid. Plasmid DNA accounted for 61% of total DNA of *E. coli* at harvest.



Plasmid productivity of various high yield fermentation processes from literature and patent publications, determined by dividing volumetric plasmid yield by fermentation time. The light bars indicate productivity from the process described in this poster.

## Conclusions

- The combination of optimized media, reduced temperature, and nutrient limited growth during biomass accumulation results in plasmid yields up to 1500 mg/L.
- High specific plasmid yield increases final purity and downstream purification efficiency
- Nutrient limited fed-batch with exponential feeding gave higher plasmid yield and productivity than the constant feed rate tested.
- High process consistency
- High productivity→reduced production time
- This process has also been used to successfully produce plasmids containing unstable sequences (e.e. inverted or direct repeats for shRNA therapies and for viral vectors such as AAV and HIV).
- Dramatically decreased plasmid manufacturing costs

### Acknowledgements

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