Large Scale pDNA Manufacturing Process for a Highly Productive E. Coli Strain

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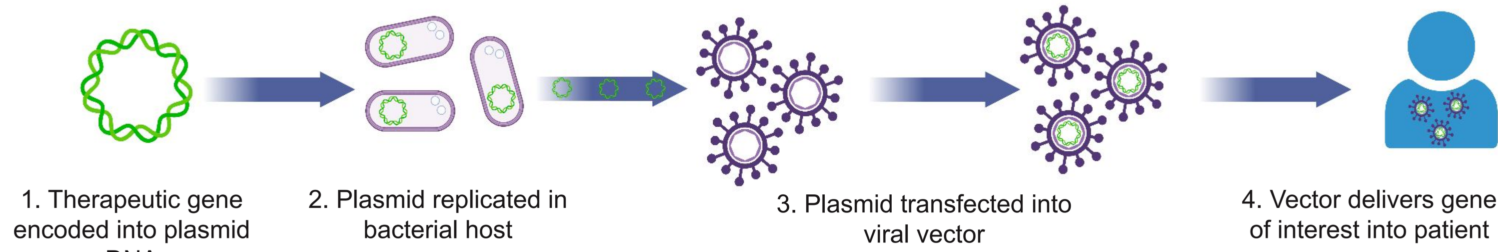
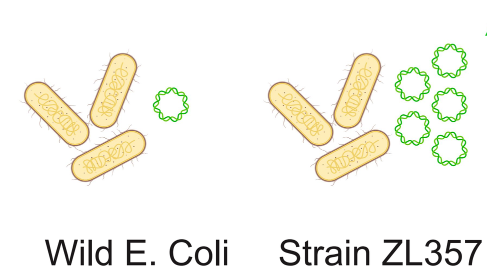
Motivation and Objectives

- Plasmid DNA (**pDNA**) is a key ingredient in manufacturing gene therapies[1].



- The Crook Lab has developed a highly productive E. Coli strain with the potential to lower pDNA manufacturing costs. The strain was named strain **ZL357**.



Project Goals

1. Design a therapeutic-grade plasmid DNA production process that satisfies purity and endotoxin requirements and delivers high process yield.
2. Perform a detailed financial comparison of ZL357-based pDNA production process versus the wild-type strain

Design Objective	Target Specification
Plasmid Configuration	> 90% Supercoiled
Purity (A_{260nm}/A_{280nm})	1.8-2.0
Endotoxin Concentration	<0.5 EU/mL[2]
Output per Year	1 kg pDNA

Technical Overview

1 Batch Fermentation
E. coli cells are grown to high density in nutrient-rich media to maximize pDNA production.

2 Alkaline Lysis
E. coli cells are lysed with an alkaline solution and gentle impeller mixing to release pDNA for downstream purification.

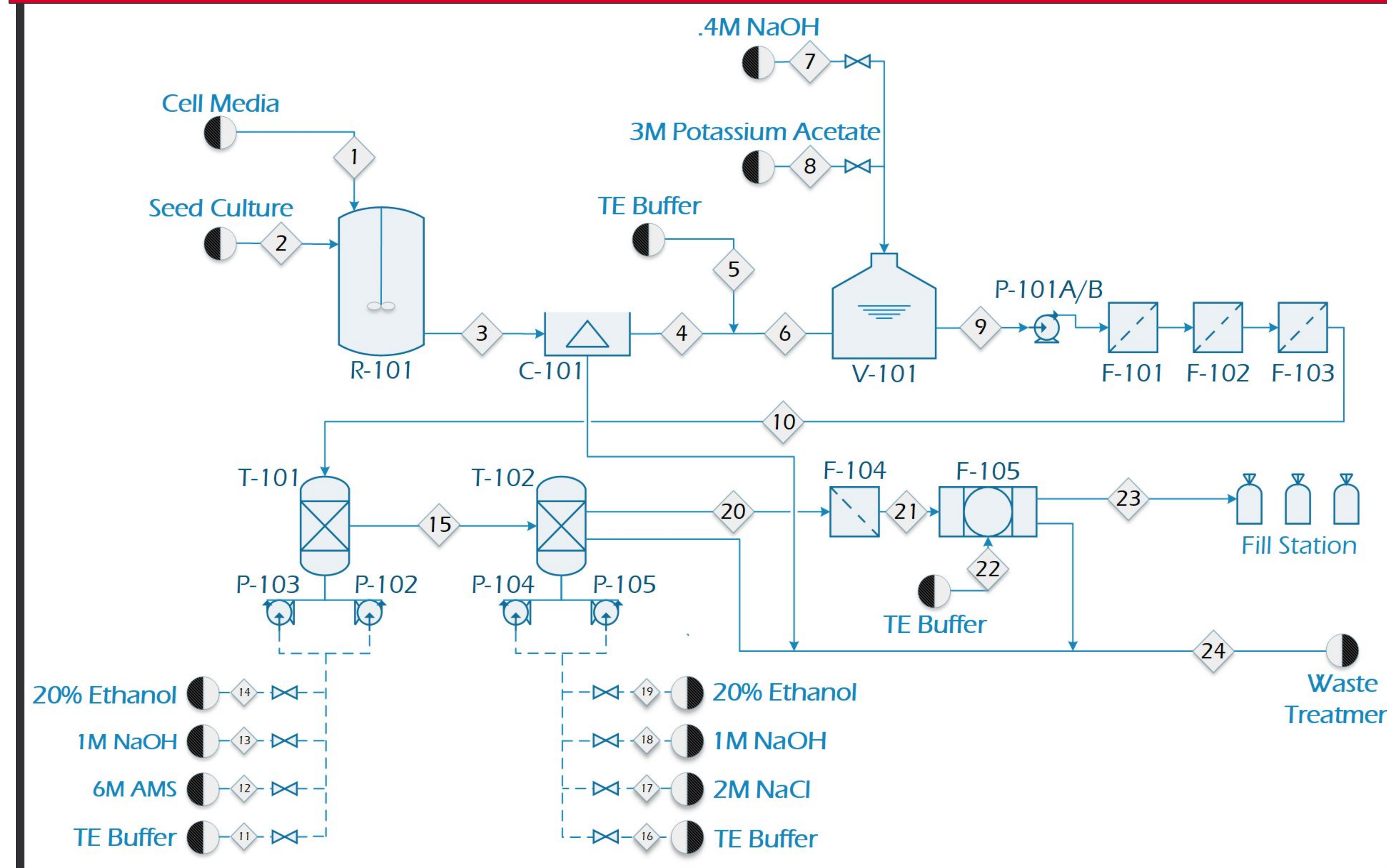
3 Clarification
Lysate is filtered through two depth filters and a sterilizing-grade filter to separate pDNA from cell debris before chromatography.

4 Purification
Chromatography is used in two steps—anion exchange removes non-nucleic acids, and hydrophobic interaction separates supercoiled pDNA from other isoforms.

5 Polishing
Tangential flow filtration is used for ultrafiltration to concentrate pDNA and diafiltration for buffer exchange, preparing the product for storage.

6 Final Fill
The purified pDNA is aliquoted into prepared vials and maintained at -20°C for long-term storage.

Process Flow Diagram



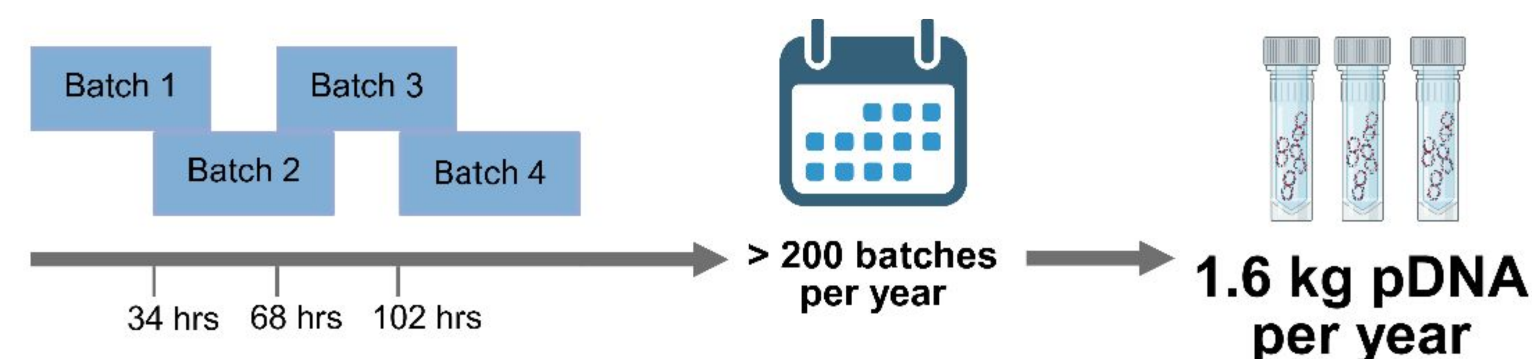
Process Performance

8 g pDNA per batch

✓ 94% Supercoiled Form
✓ Endotoxin free
✓ Research Grade Quality

48 hours per batch

- To maximize throughput, production can be staggered, with new batches initiated every 34 hours—the rate-limiting duration of fermentation. As earlier batches move downstream, staggered starts enable overlapping operations without bottlenecks. This strategy significantly boosts annual output.



Chemical and Biological Safety

- Work will be conducted under Biosafety Level 1 (BSL-1) containment[3]
- Biohazardous waste will be autoclaved prior to disposal
- Chemical disposal will follow OSHA standards, including neutralization or dilution where required

Financial Analysis

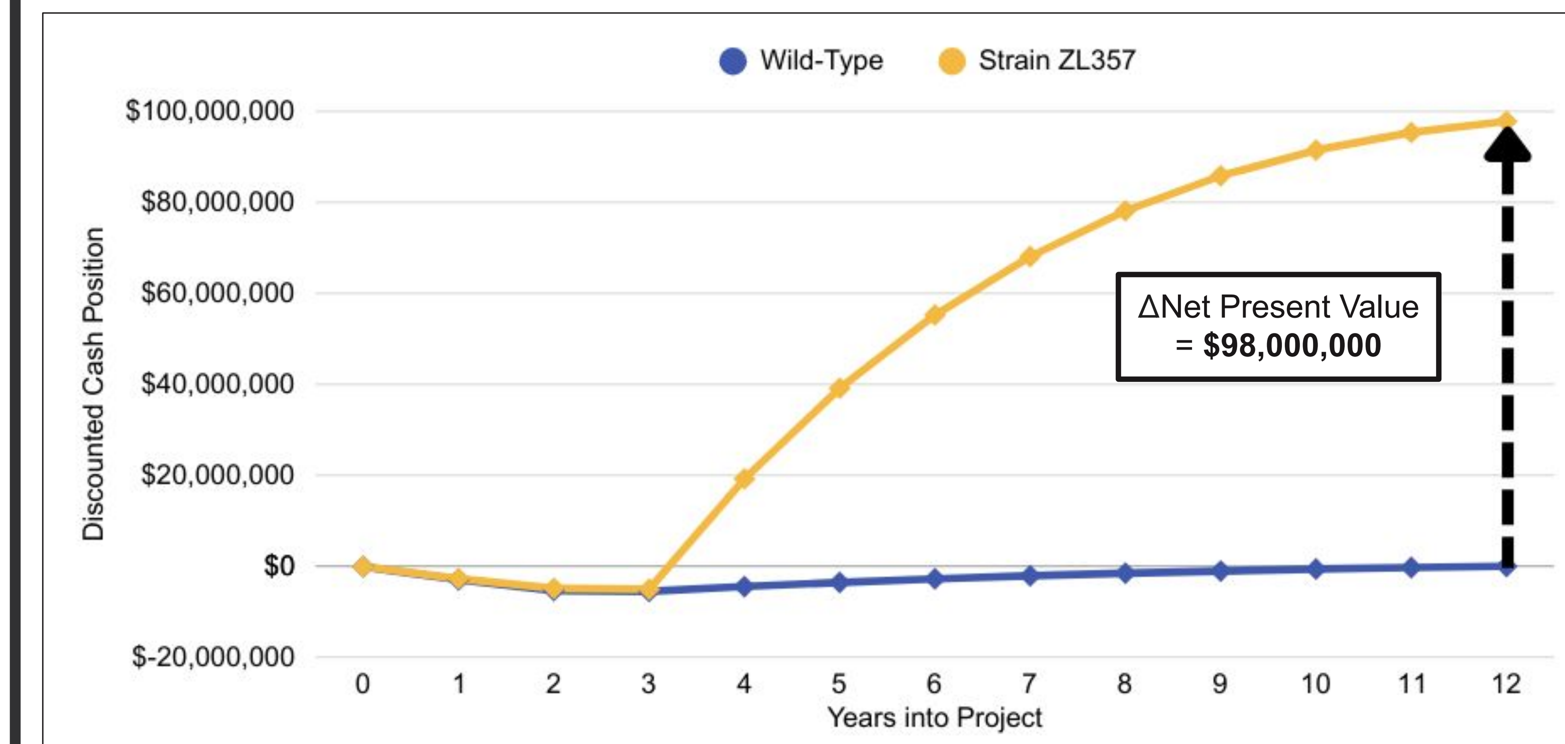
- Table 1 highlights the impact of the Strain ZL357 on key economic metrics, demonstrating reductions in capital investments, annual production costs, and the minimum viable selling price relative to the wild-type strain.

Metric	Wild Strain	ZL357
Fixed Capital Investment	$6,431,430	$5,869,830
Yearly Cost of Manufacturing	$48,334,860	$14,293,900
Minimum Sell Price (Per Gram pDNA)	$25,980	$7,950

Table 1. Economic Evaluation of Plasmid DNA Production

- Fermentation of Strain ZL357 requires considerably less media to achieve pDNA output commensurate with the wild-type, driving down manufacturing costs.

- The graph below presents the cumulative discounted cash flows for the two E. coli strains, demonstrating a net present value difference of $98 million in favor of Strain ZL357 over a 12-year period



Cumulative discounted cash flow comparison of two E. coli strains. Discounted cash position determined from capital expenses, manufacturing costs, and revenue based on the minimum sale price of Wild-Type pDNA. Discount rate of 10% applied to account for the time-value of money.

Key Takeaways

1. High-throughput manufacture of pDNA using E. coli strain ZL357 is technologically and economically feasible.
2. Strain ZL357 improves pDNA yield without introducing downstream production bottlenecks.
3. The consumable and capital savings offered by strain ZL357 present strong economic advantages over wild-type E. coli. Commercialization of this process **is recommended.**

Acknowledgements and References

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