NovelBiotechnology | WHITEPAPER

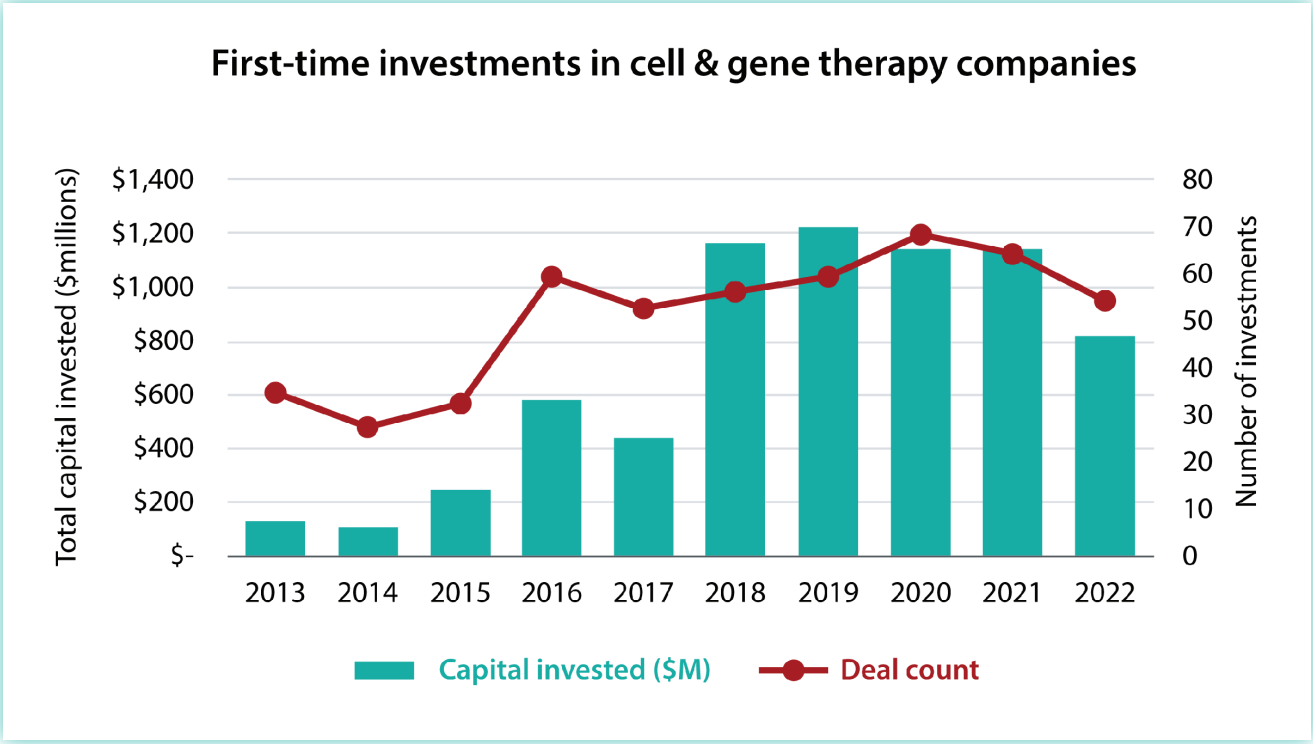
NBx Platform™

A new era begins with the arrival of a bacterial trailblazer, ending *E. coli*'s reign over cloning and plasmid production

Time to say goodbye to *E. coli*?

Plasmid in high demand

Plasmid DNA is the life blood of the biotech industry. Everything from biofuels, through industrial enzymes, to therapeutic proteins, and gene therapies (just to name a few examples) depend upon plasmid DNA as a critical reagent. At the moment, there are over 1,500 ongoing clinical trials in the fields of cell and gene therapy, driven by the industry's steadily increasing investments.[1] Cell and gene therapy-related technologies, such as CRISPR, CAR-T, RNA therapeutics, and nucleic acid-based vaccines, made enormous strides in applications, bringing promise to address multiple diseases. With the rapid advancement and commercialization of these modern therapies comes a massive demand for plasmid DNA, an underlying technology to produce essential tools for cell and gene manipulation. It looks like, however, that the good ol' school of plasmid production in *E. coli* no longer meets the demands of today's construct complexities, yield requirements and speed to market. This technological inadequacy resonates through ongoing feedback around struggles to deliver the needs of the customer and a push in the industry to cell-free applications.



Plasmid DNA market shows strong growth projection of 25% annually until 2030 and beyond, valued at USD 3 Billion by 2030 (Credit: Pitchbook).

Old tools are inadequate

Contrary to how dramatically DNA sequencing and synthesis have improved from the initial applications in the 1970s, plasmids are still produced in the same *E. coli* K-12 strains as host organisms. Plasmid production can be tailored to specific research or therapeutic needs. For example, large numbers of plasmids can be produced in a high-throughput manner at small scale to facilitate screening in R&D optimization studies. Or, on the other hand, manufactured in large quantities in large bioreactors for pre-clinical in vivo studies or clinical trials.

It's an example of an engineering mentality: "If it ain't broke, don't fix it." However, this approach eliminates the curiosity to look for better solutions and we believe that there is an opportunity for innovation at the level of host organisms for molecular cloning and plasmid DNA production.
Like in protein expression, shouldn't we have alternatives, especially if it's cheaper, faster, and safer? It is difficult to change a paradigm unless the novel solution can be adopted seamlessly and produce much better results.

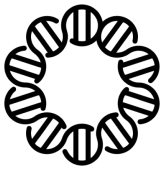
Matthew Weinstock, our CTO has addressed the historical significance of how we got where we are today with the use of *E. coli* as well as the significant challenges associated with plasmid DNA manufacturing in our previous white paper series. To summarize, the major pain points of the prevailing technology are as follows:

- **Low yields**
- **Cloning failure rates with complex and large DNA constructs or those toxic to *E. coli***
- **Removal of endotoxins in the final product**
- **Manufacturing lead times including backlogs accrued by the existing providers**
- **Intellectual property concerns regarding shipping designs to providers outside North America and Europe**

NBX Platform™ to the rescue

Novel Biotechnology introduces the world's first-ever bacterial challenger to *E. coli* for cloning and plasmid production. Our NBx Platform™ is a new bacterial organism that can be managed in bio-safety laboratory 1 (BSL1) settings to produce plasmid with low endotoxin levels (100 - 400 x lower than *E. coli* in tested examples) and can handle difficult and complex sequences successfully. Our drop-in, seamless replacement for an *E. coli*-based pipeline is fully compatible with standard transformation methods, plasmid replication origins, selection markers, and isolation procedures. Moreover, it can use standard USP & DSP equipment and comes with chemically defined and rich media for use in high throughput gene synthesis or high volume pDNA production applications. Finally, the NBx Platform™ offers a significant increase in the plasmid yield.

The NBx Platform™ is a proprietary, high-fidelity, microbial system optimized for the production of plasmid DNA.

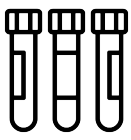


Up to 20X Higher Yield

Half the Time

Higher Quality than *E.coli*

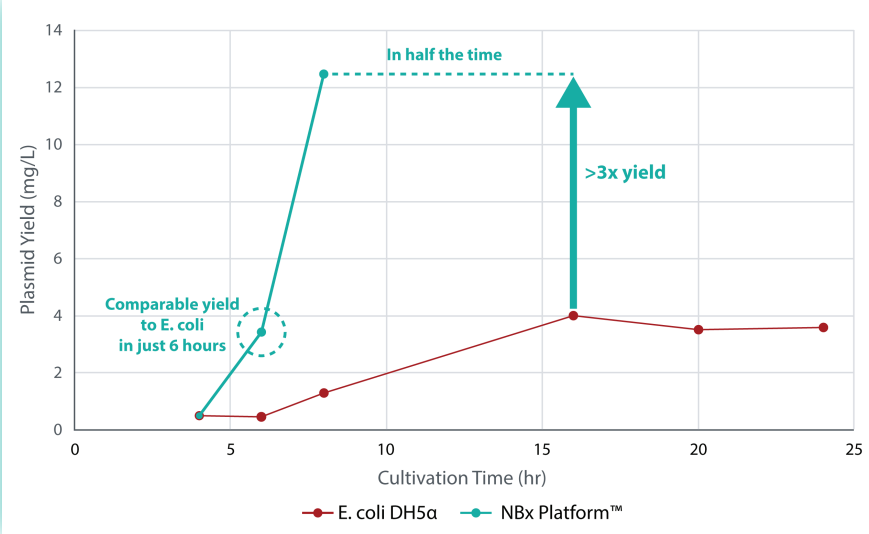
Lower Endotoxin than *E.coli*



Fully Compatible with *E.coli* infrastructure

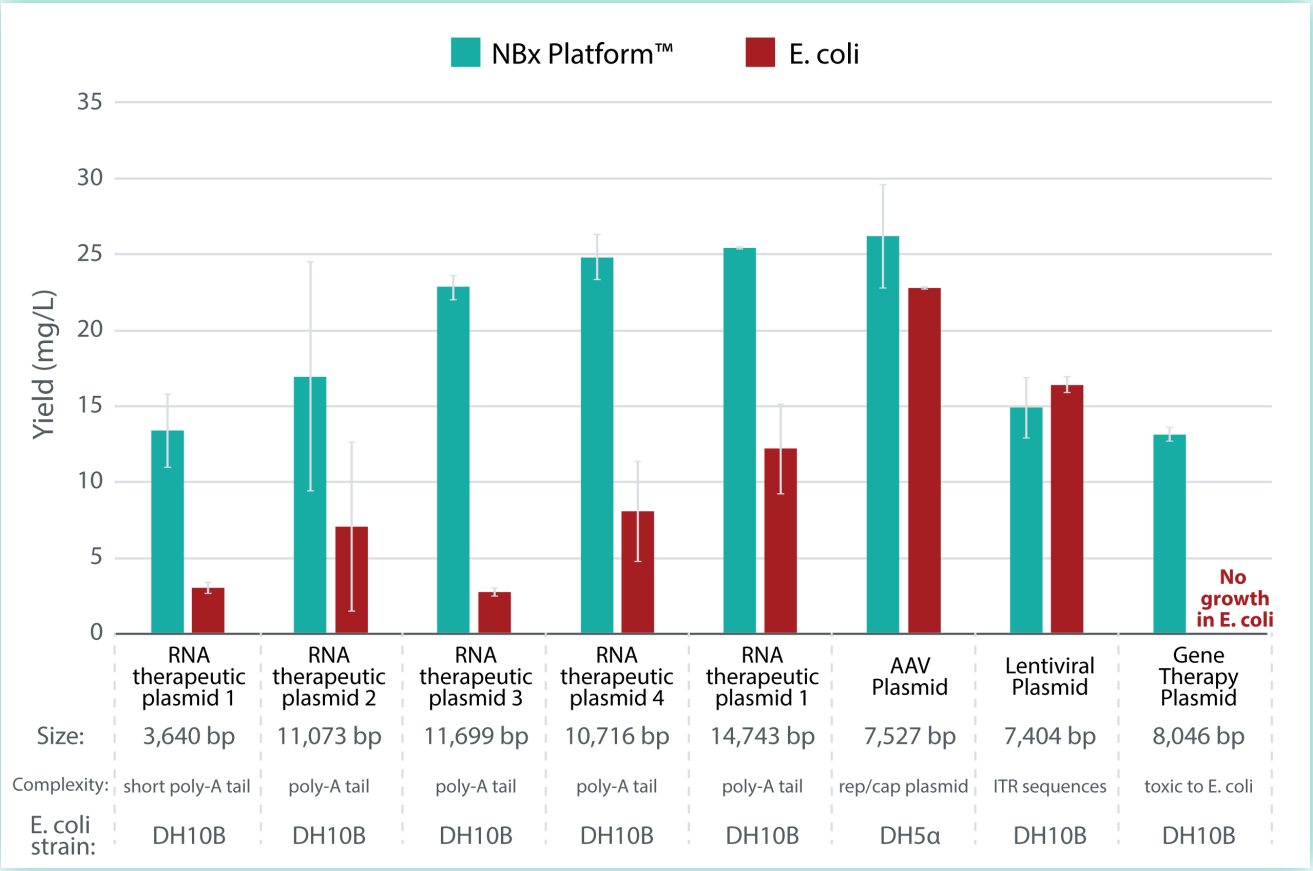
Scalable to Large Scale pDNA Production

Let's look at some technology evaluation studies conducted by Novel Biotechnology scientists to compare the plasmid yield from NBx™ with the *E. coli* DH5α strain. In the graph below, both organisms were transformed with pUC19 plasmid and cultivated in shake flasks at 37 °C with agitation at 200 RPM. *E. coli* was cultivated in a classic LB medium, whereas NBx™ was in an LB-like proprietary media solution. As shown in the graph to the right, the NBx Platform™ offers a 3-fold yield increase in plasmid yield relative to *E. coli* in half the time.



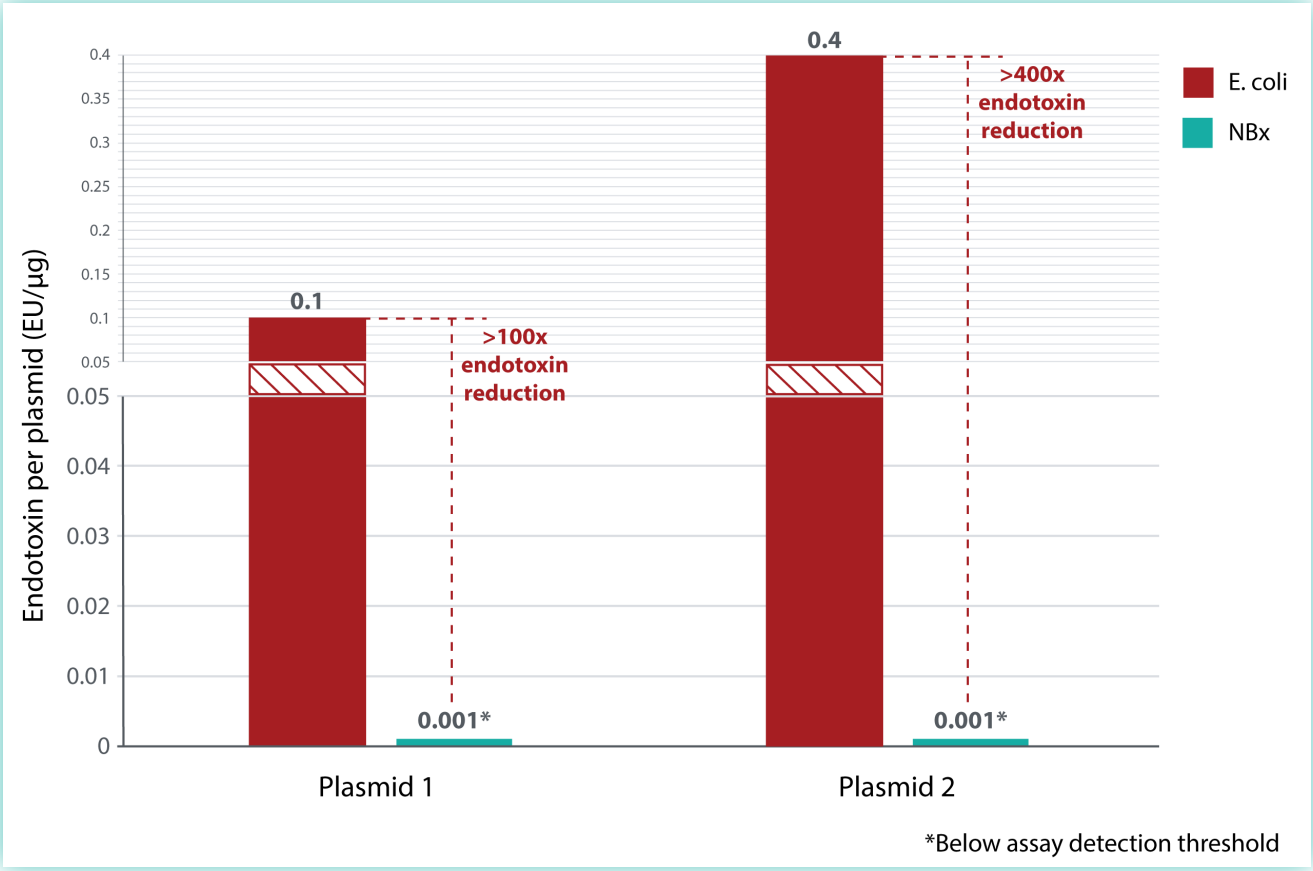
NBx Platform™ shows more than 3x increase in plasmid yield in half the time when compared to *E. coli* DH5α.

Despite the omnipresence of *E.coli* strains in the plasmid biomanufacturing, not every DNA construct can be successfully produced in the traditional platform. Plasmid size, troublesome sequences such poly-A tails or ITRs can significantly impair yield or even prevent the construct being amplified due to toxicity toward the host organism. Our scientists tested such challenging constructs in NBx Platform™ and were able to prove that the technology delivers on these complex constructs, significantly increasing plasmid yield and and producing constructs that were not manufacturable in *E. coli*.



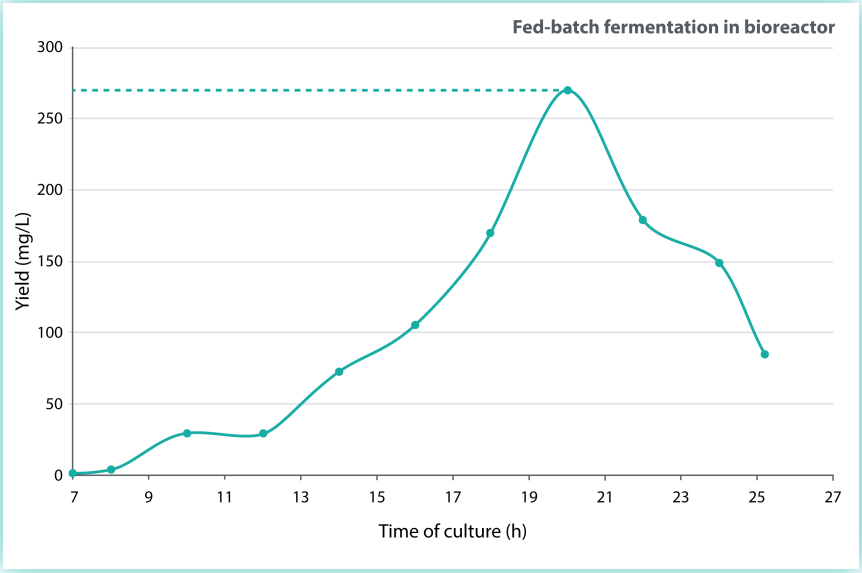
NBx Platform™ not only outperforms *E. coli* in difficult construct's yield, but allows for amplification of plasmids toxic to the traditional host.

Because the gram-negative bacterial membrane is covered in antigenic lipopolysaccharide (LPS), all the therapeutic reagents produced in such hosts need to undergo testing to ensure the removal of these endotoxins. Otherwise, LPS can trigger an immune response in humans via the TLR4 receptor. Novel Biotechnology developed the NBx Platform™ as a safer alternative to, engineering a breakthrough organism to provide a pure product with significantly reduced endotoxin content.



The endotoxin contamination was evaluated via LAL cartridge assay after plasmid purification through commercial Endosafe Megaprep kit (Qiagen).

The NBx Platform™ is currently being evaluated for scalability. Novel Biotechnology is partnering with the National Research Council Canada to develop a scalable manufacturing process for large-scale plasmid production. in preliminary runs, the platformed achieved a yield of 270 mg/L in a chemically defined process demonstrating that this new bug can thrive in the confines of an industrial bioreactor. Ongoing work aims to further improve the yield.



NBx Platform™ NBx is compatible with fed-batch bioreactors.

The yield of plasmid in large-scale biomanufacturing directly influences production efficiency and production cost. Less media, lab operation and maintenance, and time is needed to achieve a comparable yield with NBx Platform™ relative to traditional methods. Thus, ultimately, optimizing plasmid yield is a key factor in ensuring the economic viability and success of large-scale biomanufacturing operations.

Summary

The landscape of cell and gene therapy is rapidly evolving, driven by a surge in clinical trials and substantial investments. The demand for plasmid DNA, a fundamental element in these cutting-edge therapies, is projected to grow exponentially, presenting challenges and opportunities. While traditional *E. coli* - based production has been the standard, Novel Biotechnology's bacterial NBx Platform™ emerges as a groundbreaking alternative. With superior yields, reduced endotoxin contamination, and compatibility with existing methods and equipment, the NBx Platform™ demonstrates its potential to revolutionize plasmid DNA manufacturing in a number of ways. The promising results from technology evaluation studies and ongoing scalability assessments position the NBx Platform™ as a frontrunner in meeting the escalating demands of the dynamic cell and gene therapy landscape in the following ways:

- **Increasing yields and shortening production runs**
- **Reducing rework and turn-around times for high throughput gene manufacturers**
- **Significant reduction in capital requirements for expansion by switching to the higher yield production of NBx Platform™**

As we embrace innovation, Novel Biotechnology invites the industry to consider a seamless transition to a faster, safer, and more efficient era in plasmid DNA production using the NBx Platform™ Technology.

NovelBiotechnology

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References:

[1] Lohr,Adam. "2023's Market Outlook For Cell And Gene Therapies." cellandgame.com, 14 February 2023, https://www.cellandgene.com/doc/s-market-outlook-for-cell-and-gene-therapies-0001

[2] Stadler J, Lemmens R, Nyhammar T. Plasmid DNA purification. J Gene Med. 2004 Feb;6 Suppl 1:S54-66. doi: 10.1002/jgm.512. PMID: 14978751.