Utilising Size Selection to Enhance Gene Synthesis in Synthetic Biology Workflows

Biotechnology harnesses cellular and biomolecular processes to develop technologies and products. These products already improve our lives and show great potential for enhancing the health of our planet. In recent years and especially during the COVID-19 pandemic, the critical role of biotechnology and biomanufacturing in developing life-saving diagnostics, therapeutics and vaccines has been demonstrated and looks poised to progress on stratospheric trajectory, if we can successfully refine the technique to suit its myriad applications. Utilising a size selection technology which uses machine vision algorithms to monitor electric mobility and then respond in real time gives us the ability to enrich DNA through size selection with industry-leading precision.

Next-generation size selection instruments enable the dynamic target enrichment of DNA. The core automated size selection functionality is complemented by the ability to perform fragment length analysis and fluorescence assays for next-generation sequencing (NGS) quality control applications. For example, Yourgene Health's Ranger® Technology offers a fast, effective and efficient automated solution for separating DNA molecules based on their size and electrical charge; it uses patent-protected, machine vision algorithms to interpret the gel electrophoresis process in real time.

Overcoming Challenges in Gene Synthesis

Gene synthesis forms the foundation of the new field of synthetic biology. It is also accelerating research in well-established fields by providing critical advantages over more laborious traditional molecular cloning techniques. *De novo* gene synthesis is required when template DNA molecules are not available, such as for codon-optimised sequences. It has been shown that synthetic modified viral sequences produce safer, more effective DNA vaccines. Codon optimisation can increase both the immunogenicity and the therapeutic anti-viral effects induced by DNA vaccines on various targets.

Some of the exciting areas are the clinical, pharmaceutical and other technology sectors struggling with sample purity. When considering the work done in these arenas, everything is highly dependent on the ability to manufacture new drug candidates in novel ways. This relies to an increasing extent on synthetic biology, which is an application that we have really good utility overlap with. Size selection is about enriching and purifying, in other words getting rid of the stuff that you don't want and keeping the high value targets that you do want by differentiating based on size.

Gene synthesis can be challenging. Traditionally, we talk about a process that uses a lot of old bench techniques that have been around for decades. The process is predicated on synthesising a construct which is as pure as possible and

then you've got to clone it. Of the many risks and difficulties associated with the process, this is the largest hurdle in a market which demands complex, quality DNA on increasingly tight turnaround times.

There are many stages in which impurities and error can be introduced early during the building of the construct, and several steps further down the line which jeopardise the chances of isolating your construct, not least when transforming it into a bacterial host. Then you have to let it grow for a while once it's plated out, and finally begin the laborious task of sampling dozens of colonies before you find the exact construct of interest.

In those sectors requiring long DNA constructs on tight turnaround, but which inherently struggle with sample purity, size selection can be used to help clear that hurdle. It's able to clean up a lot of those reactions that end up being heavily polluted with truncation or concatenated products which are concomitant with the target product. Technologies that can cope with a huge range of fragment sizes are especially important in gene synthesis because it is here that short fragment lengths become less relevant, and long fragment recovery really comes into play.

Cloning Workflows Made Effective with Next-Generation Size Selection

There are two key themes for size selection in synthetic biology:

1. Continue to pursue the traditional approach to gene synthesis using a plasmid vector and get a superior hit rate at the end because the clones are purer.
2. Incrementally improve the iterative stages of new enzyme-driven gene synthesis techniques so that longer (high value) building blocks can be stitched together in fewer stages.

Using machine vision algorithms to monitor electric mobility and then respond in real time to intelligently tune the voltage gives us the ability to enrich DNA through size selection with industry-leading 97% precision (Figures 1–3).

Figures 1–3: DNA migration through a gel cassette illustrating synchronised arrival at the extraction wells using Ranger Technology (Yourgene Health).

The user selects their range of desired DNA lengths, anywhere from approximately 50–20,000 bp. The "tightness" of the recovery window can be optimised by customisable reagents and consumables. Dynamic voltage adjustment is then applied across all channels, allowing for the synchronised arrival of the desired fragment sizes at extraction wells. Electrophoresis platforms that monitor the migration of the sample all the way along the lanes, rather than at just a single point across all channels, allow for improved accuracy during the size selection process.

Delivering Desired DNA Lengths with Greater Precision

Off-targets get produced much more often than desired products: when taking a sample from your construct to clone, it's often not of adequate purity because the processes used to synthesise it are imperfect. Additionally, the presence of numerous concomitant truncation products frequently results in a success rate below 10% in complex synthetic reactions, meaning that fewer than 10% of the bacterial colonies from which the construct is harvested actually contain the desired construct. Size selection can increase that up to as high as 90% (Figure 4). If you can do that, you don't need to check as many colonies before you find your true positive construct. Therefore, the efficiency of one of the most laborious stages of a very time-consuming process is greatly enhanced.

Turbocharging Therapeutic Pipelines

Complicated DNA needs to be turned around on a tight timeline. It normally takes months from the time that it's ordered until the construct is delivered and so this kind of turnover time is incompatible with an R&D environment, particularly in the pharmaceutical industry. If you have an idea about how to make a new therapeutic, you need to have X number of genes made to be able to have them transcribed in order to make the product and, you have to iterate on that cycle many times. But, if it takes months every time you iterate, then it's not conducive to coming up with new therapeutics.

Being able to generate these larger constructs has more economic value for groups like pharmaceutical companies; think about vaccine manufacturers as a topical example. The gene synthesis industry as a whole is really trending towards taking this and trying to turbocharge it to be able to make it work well at scale. However, the huge challenge here is the inefficiency with which large constructs are made. Researchers experienced with molecular cloning know that, despite improvements over the past several decades in recombinant DNA tools, such as enzymes and cloning vectors, getting the clone you want is hardly a fool-proof endeavour. Even the seemingly simple task of isolating a gene using PCR cloning or restriction digestion can be tedious and error-prone depending on the sequence.

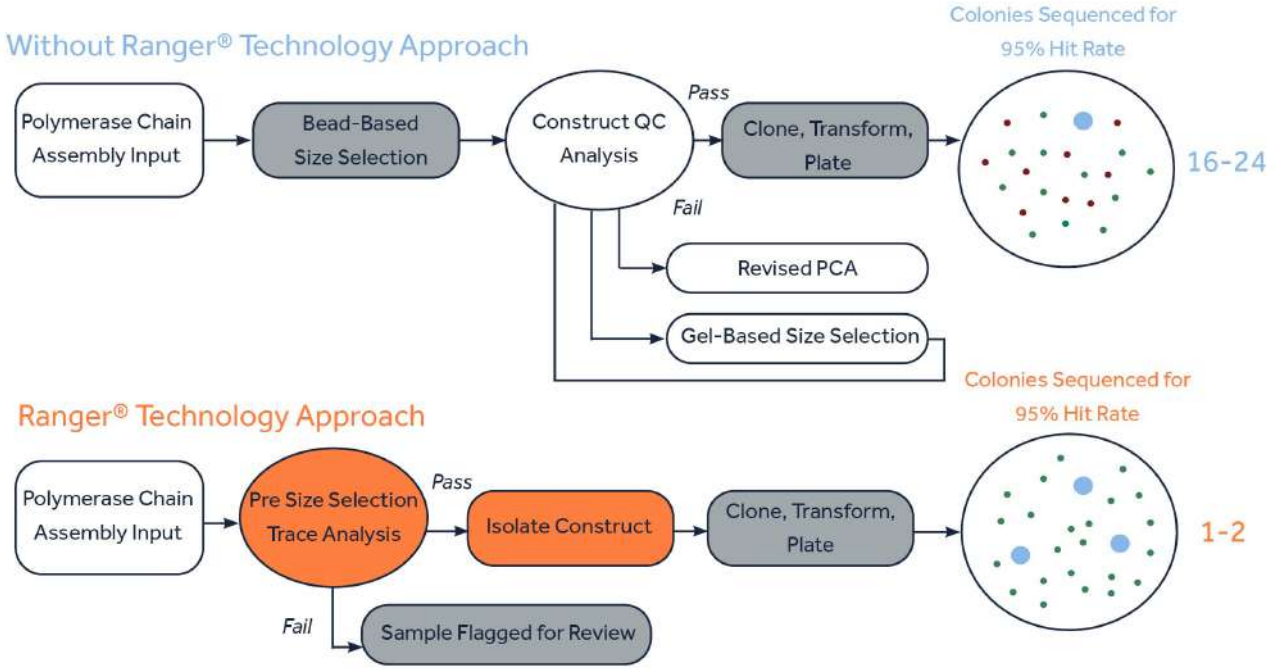


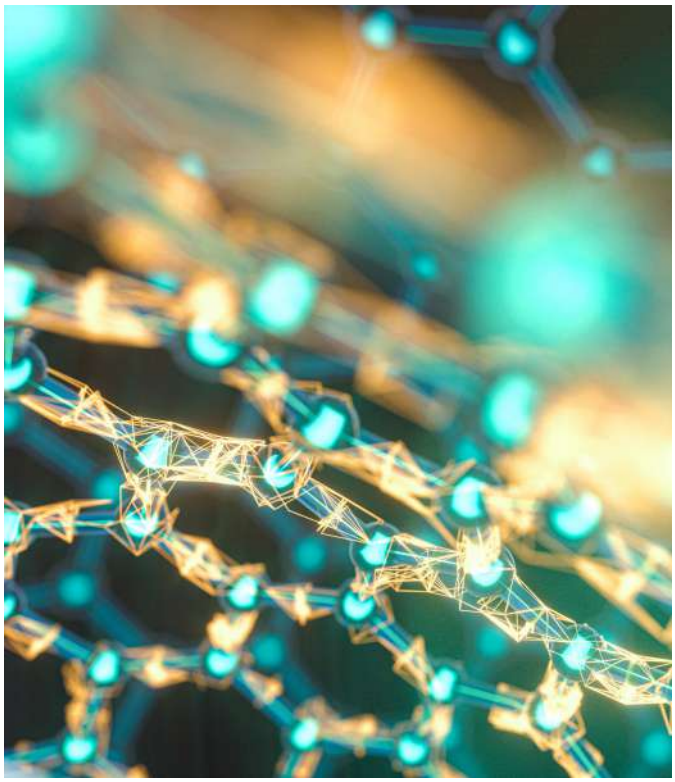
Figure 4: Comparison of gene synthesis with and without next-generation size selection technology. The number of colonies sequenced to identify the target construct is significantly reduced using Ranger Technology (Yourgene Health).

For longer DNA constructs, size selection can help greatly reduce the signal-to-noise issue. In the context of gene synthesis, we can use size selection to get rid of the noise associated with truncation products and recover the full-length construct of interest. Achieving this reduces your sequencing costs, and a range of other costs necessary to identify a synthetic construct that has the desired sequence – a valuable input for manufacturing new therapeutics for pharmaceutical entities. This is perhaps less applicable for simple processes like synthesising short constructs or a simple PCR amplicon, but it is certainly applicable for situations where you require larger and more complicated constructs.

Scaling Synthetic Biology Processes

One of the early adopters of Yourgene's Ranger® Technology is utilising the high-throughput NIMBUS Select platform to reduce the turnaround time for the delivery of their complicated DNA products for the pharmaceutical industry. They deliver gene constructs to their pharma partners who require inputs for their own novel drug pipeline, enabling them to make and test more candidate products than before. Another adopter, a complex DNA firm, utilises our size selection service to affirm the purity of their samples, assuring them of the quality of their constructs between steps and enabling them to make much larger DNA synthetically.

In talking about supplanting industrial products, we also turn our attention beyond biotherapeutics and vaccines to the territory of petrochemical and coming incumbents, such as plastics. While the market economics of that have previously been a little bit questionable, that's less and less the case today. The White House itself released a memo[1] in September 2022 describing how synthetic biology could be used in manufacturing that accounts for about a third of global output, an estimated $30 trillion in terms of value. The White House also laid out plans to initiate programs to increase biomanufacturing and expand opportunities within this sector.



Where speed, complexity and cost matters, next-generation size selection technologies that deliver the highest degree of automation alongside scalable, precise and robust electrophoretic analysis offer clinical and research groups a viable option for the analysis of DNA constructs at high volumes, adding value in various synthetic biology applications to great efficacy.

REFERENCES

1. https://www.whitehouse.gov/briefing-room/presidential-actions/2022/09/12/executive-order-on-advancing-biotechnology-and-biomanufacturing-innovation-for-a-sustainable-safe-and-secure-american-bioeconomy/, visited 28 Jun 2023

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Joanne has been a champion of modernising diagnostics having previously held positions as VP Biodiscovery with Cambridge Epigenetix (now biomodal) and Director of Sequencing and Sample Acquisition for Genomics England. She has acted as an advisor on the DOH Rare Disease Policy board, MHRA Genomics for Diagnosis forum and UK NEQAS – Genomics England Steering Committee and Genomics England sequencing advisory board. Joanne holds a PhD from Cambridge in Molecular and Cellular Biology.

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