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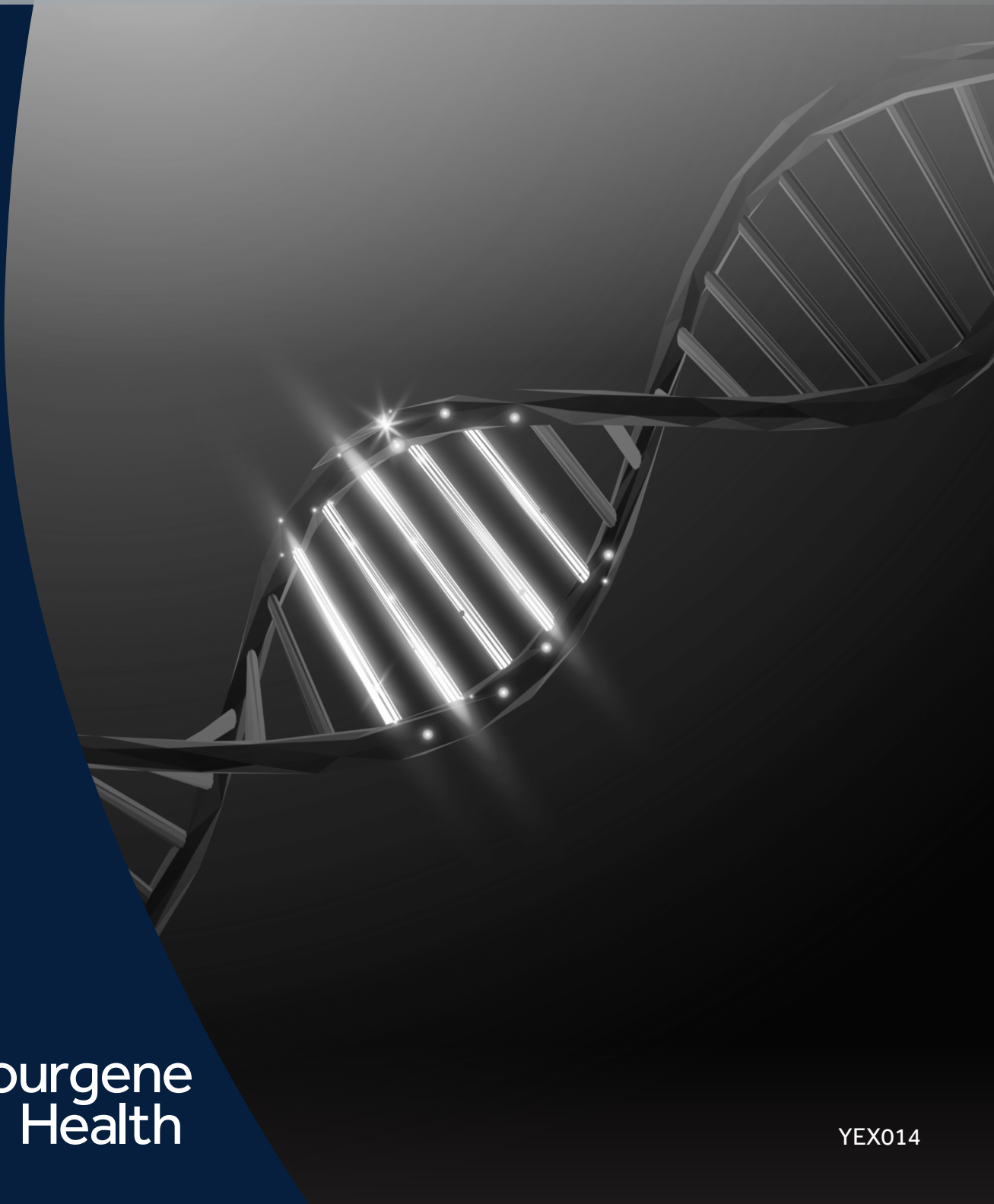
Your Expert

Ranger® Technology Applications: Size Selection for NIPT, Oncology and Gene Synthesis Applications



Yourgene Health

YEX014

In this edition of YourExpert, Yourgene Health interviewed Matthew Nesbitt, one of the developers of Ranger® Technology to discuss how its unique size selection function is helping our collaborators in life sciences and synthetic biology, across various clinical and research applications.

Matthew has expertise in automated, scalable solutions for electrophoresis and over 16 years' experience in sample preparation.

Matthew Nesbitt

Co-Creator of Ranger® Technology

Yourgene's Product Specialist Director

Introduction to Ranger® Technology

Yourgene Health uses Ranger® Technology in our next-generation size selection instruments to deliver dynamic target enrichment of DNA.

Ranger® Technology has so far been deployed to power multiple solutions across our instrument portfolio, with applications in next-generation sequencing (NGS), non-invasive prenatal testing (NIPT), oncology and beyond.

What is Ranger® Technology?

Ranger® Technology is about the hardware. Importantly, we have different incarnations of Ranger® Instrumentation depending on throughput requirements. The key feature that all our platforms share is the quality of the end result. We are proud to demonstrate industry-leading repeatability, accuracy and intrinsic recovery in a single instrument.

When we first started, we knew that we intended to do electrophoresis at scale, and that it would be helpful to have some of the upstream hands-on activities taken care of too, in order to streamline the workflow optimally. The instrument which has the capacity to handle these tasks while processing at scale is the NIMBUS Select; as a bonus, it automates both the loading of cassettes and is also a liquid handler. So the whole process is automated, and we can run up to 96 samples in a single run.

"...groups want to take advantage of...industry-leading repeatability, accuracy and intrinsic recovery."

We had a lot of success with that, but also found a lot of interest was coming from smaller groups, academic labs for instance, or start-up companies. They wanted to take advantage of the result quality that they knew existed with a NIMBUS Select, but they didn't have the capital or the requirement for a fully automated, scalable solution. So, we took all the key hardware and we put it into a modular box, which is where our LightBench® instruments came in; they feature the same camera, the lights, the capabilities and the performance – minus the liquid handling.

When you work with the LightBench®, liquid handling is provided either by your research technician or by a third-party instrument. We intentionally developed this platform to be easily integrated with off-the-shelf liquid handling workstations and for this reason we designed the LightBench® to be SiLA 21 compliant.

I try to convey the idea that any Ranger® instrument is a really smart box, capable of simplifying complicated lab workflows by taking care of multiple different types of protocols in parallel.

Three things are possible with these instruments:

- **Fragment Length Analysis:** a quick-turnaround option for quality control applications including next generation sequencing. Fragment distribution analysis allows us to focus on confirming double stranded DNA is suitable for downstream processes. Ranger® is a strong competitor compared with others in this space:

Electropherogram Trace Generation

Parameter	Specification	Additional Information
Allowable Size Range	50 bp - 20 kbp+	Different gel percentages permit analysis across different regions of this total range
Sizing Accuracy	5-20% of nominal size	Depends on the fragment size and the reagent kit selection. When working with the recommended size-range for each gel type, sizing accuracy is typically within 5%
Lower Detection Limit	0.5 ng for constructs 5 ng for smears	Depends on the reagent kit and the run conditions. Resolution is defined as the minimum difference in fragment length between the start or end of a size selection window and the peak of the closest off-target fragment that ensures at least 90% rejection of said off-target fragment
No of Samples / Run	24 - 48	This is a reagent kit parameter
Run Time	Typically 20 - 40 minutes	Depends on fragment size and reagent kit selection. Low voltage analysis of larger fragment (>20 kb) can take significantly longer depeneding on the settings
Intrinsic Recovery Effiency	70 - 80 %	Dependent on run conditions, particularly the relationship between the target range and the extraction volume
Mass Calculation Accuracy	With in-gel calculation (No plate-based quant): + 40% With plate-based quant: + 20%**	The accuracy of the plate-based quant is primarily dependent on pipetting accuracy during sample-plate setup

**Resolution assumes the use of a size-selection cassette with 30 mm channel length. Size-selection cassettes with shorter channel lengths will have correspondingly lower resolution.*

*** with CG-14000 product series*

Figure 1: Specifications for Electropherogram Trace Generation

- **Fluorescent Assays:** when it comes to optical quantification of DNA using fluorescence, we are also very competitive:

Plate-Based Quantification

Parameter	Specification	Additional Information
Lower Detection Limit	0.2ng	When using the prescribed Pico-Green-based assay. Other Fluorophores may provide increased sensitivity
Acuracy	+/- 15%	Depending on pipetting accuracy and the use of replicates, this number can be brought down

**Resolution assumes the use of a size-selection cassette with 30 mm channel length. Size-selection cassettes with shorter channel lengths will have correspondingly lower resolution.*

Figure 2: Specifications for Plate-Based Quantification

- **Size Selection:** the cornerstone of the Ranger® revolution. Our unique function with which we are seeing critical, measurable real-world impact in various next generation sequencing and cloning applications as well.

Size Selection Applications

Parameter	Specification	Additional Information
Allowable Size Range	0 - 20 kbp+	Different gel percentages allow size-selection targets across different regions of this total range
Loading Capacity	≤ 1 kb: 500 ng – 2 µg 2 - 5 kb: 250 ng – 1.5 µg 10 kb: 500 ng – 1 µg 20 kb+: 250 – 500 ng	Exact loading capacity depends on the size range of interest and the gel percentage chosen
Resolution	10 – 20%*	Depends on the reagent kit and the run conditions. Resolution is defined as the minimum difference in fragment length between the start or end of a size selection window and the peak of the closest off-target fragment that ensures at least 90% rejection of said off-target fragment
No of Samples / Run	12 (CGI-Format) 8 (SBS-Format)	Depends on reagent kit selection
Run Time	1 – 4 hrs	Depends on optimisation of reagent kit selection and required resolution
Intrinsic Recovery Effiency	70 - 80 %	Dependent on run conditions, particularly the relationship between the target range and the extraction volume

**Resolution assumes the use of a size-selection cassette with 30 mm channel length. Size-selection cassettes with shorter channel lengths will have correspondingly lower resolution.*

Figure 3: Specifications for Size Selection Applications

Can you briefly explain how Size Selection works with Ranger®?

Using machine vision to monitor electric mobility and then respond in real time gives us the ability to enrich DNA through size selection with industry-leading precision.

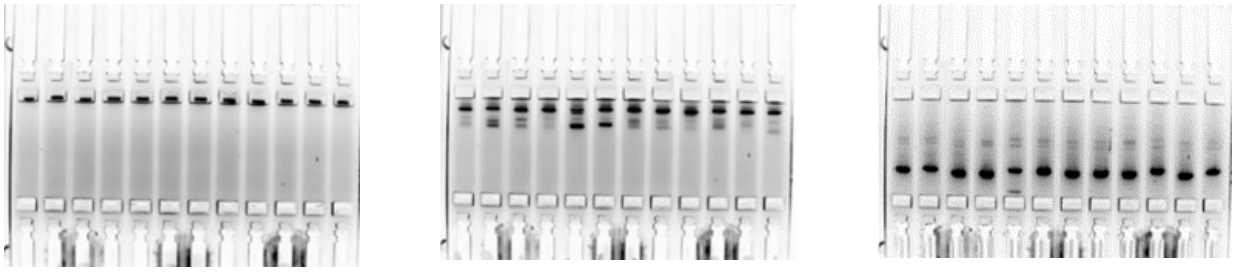


Figure 4: DNA migration through a gel cassette illustrating synchronised arrival at the extraction wells.

The user selects their range of desired DNA lengths, anywhere from approximately 50 bp – 20,000 bp. The "tightness" of the recovery window can be optimised by our customisable range of 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.

Unlike other electrophoresis platforms, our Ranger® instruments monitor the migration of the sample all the way along the lanes, rather than at just a single point across all channels.

We are also doing this in a way that simplifies set up; when most customers implement assays of this type using competitor instruments, they always have to set calibration lanes in addition to their sample lane, plus a marker. We don't use calibration lanes as we don't need them to be able to deliver the same quality of results, so that simplifies our assays.

How does Size Selection lend itself to applications like NIPT?

Using Ranger® Technology allows NIPT applications to preferentially select for shorter fragments more likely to be of fetal than maternal origin. Being able to enhance the proportion of fetally-derived cfDNA means a reduction in the number of screening failures, significantly reducing the anxiety for expectant parents and greatly improving the efficiency of NIPT services.

The use of size selection has the potential to salvage samples that have been stored for prolonged periods in low-cost, EDTA BCTs, by discarding white blood cell DNA fragments which effectively dilute the fetal fraction (FF).

The size selection utility of Ranger® Technology when used in combination with cell free DNA (cfDNA) applications wasn't appreciated by us in the early days. Once we understood what some of these Ranger® NGS libraries were intended for, we realised that there were fundamental quirks of biology that loaned themselves perfectly to size selection.

In the case of NIPT, NGS is utilised in screening during pregnancy to determine the fetal risk of chromosomal abnormalities from samples containing cell-free DNA (cfDNA) present in maternal plasma. These cfDNA fragments are typically <200 bp in size and are of both maternal and placental (fetal) origin; the proportion of fetally-derived cfDNA in the maternal blood sample is known as fetal fraction (FF).

It is one of these biological phenomenons that we are able to take advantage of; fetally-derived cfDNA is shorter than cfDNA of maternal origin. Being able to preferentially select for shorter fragments more likely to be of fetal than maternal origin has given greater clinical utility to NIPT assays. To be specific, it has generated more valid results from samples that would previously have had insufficient fetal fraction and subsequently reduced the number of screening failures, improving overall patient outcomes.

"The use of Ranger® in NIPT...enriches fetal fraction and...greatly improves the feasibility of EDTA beyond 8 hours."

S ize selection has another benefit in NIPT. Over time, white blood cells in the blood sample degrade, releasing additional maternal DNA into solution and causing a relative dilution of the fetal fraction.

While costly blood collection tubes (BCTs) commonly used for NIPT stabilise the sample for up to 14 days, more common, lower-cost BCTs which lack these stabilisation reagents are not recommended for blood storage beyond 8 hours.

Following a promising proof-of-concept study we have shown that Ranger® can help dispense with the need for costly BCTs. Combining an EDTA tube and size selection, we can enrich that biomarker fraction back to the level that is needed to ensure robust, reproducible and reliable NIPT results.

Scan the QR code on the right to view our publication poster

Yourgene® are now exploring an extension to the on-label use of EDTA tubes in their own NIPT workflows, effectively broadening the choice of BCTs that clinicians can use beyond Streck®. This significantly improves the feasibility of EDTA tube usage for NIPT investigations, particularly in circumstances where delayed transport of samples between collection site and analysis location may occur.

Scan the QR code to learn more about Ranger® for Clinical NIPT applications with the LightBench® Detect **(Low Throughput) and the NIMBUS Select (High-Throughput)**

How is this applicable in Oncology?

The impact of including Ranger® Technology size selection in these workflows is a tumour fraction two- to three-fold higher, which can translate to a significant increase in the signal from a particular mutation, known to be associated with cancer. This has great benefits reducing signal-to-noise in NGS investigations, especially in the toughest cancers to crack, the non-shedding types, allowing detection of signal from a blood draw and opening up potential for non-invasive diagnosis and monitoring applications.

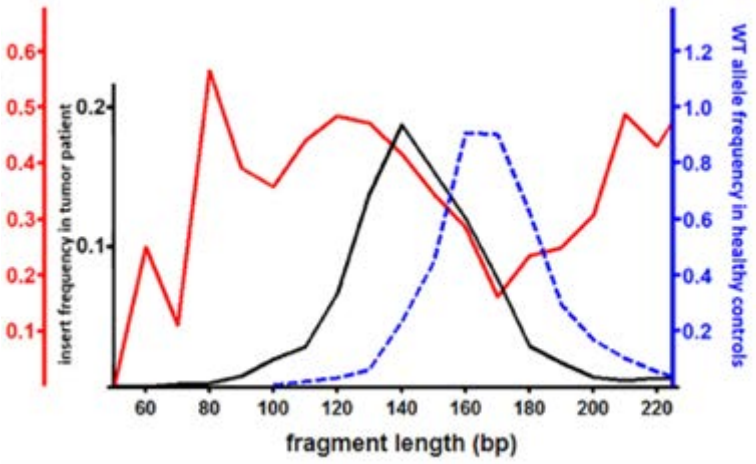
We know that early diagnosis enables an opportunity for early intervention. We also know that total circulating tumour-derived DNA (ctDNA) content correlates with advancing disease, which makes liquid biopsy an attractive option for diagnosis and monitoring by minimally invasive means.

However, the accurate detection of low Variant Allele Frequencies (VAF) remains a principal obstacle to widespread application of cfDNA in clinical oncology.

"Enter Ranger®...for accurate detection of ctDNA at low VAFs."

Tumour fraction and median VAF in the very early stages (when chances of positive outcome are best) is extremely low, due to its dilution by abundant normal circulating cfDNA. To meaningfully implement circulating ctDNA diagnostics for early disease detection, you have to be able to reliably identify VAFs as low as <1%.

When taking into account the additional accumulation of potential false positive errors by sequencing and PCR, the reliable identification of true tumour variants at low VAF in NGS workflows seems insurmountable.



Enter Ranger® Size Selection once again. The phenomenon of size variation in biomarker fragments depending on their origin also extends to liquid biopsy work, perfect for oncology applications.

There is mounting evidence that demonstrates DNA molecules originating from tumors have a different size profile to normal background cfDNA, and because of this, you can use electrophoretic size selection to enrich for those circulating tumor DNA fragments in exactly the same way as you do in NIPT.

Figure 5: Distribution of DNA fragments by size. Those originating from a tumour (red) vs wild type / background origin (blue). The aggregate of both (black).

Figure 5 shows a distribution profile of whole blood (black). Two clearly-defined profiles are identified when sequenced: tumour-derived fragments (red) versus normal controls (blue). If you were to size select this sample such that you reject most of the bigger fragments and keep most of the smaller fragments, an enrichment of the tumour fraction is the net effect.

Scan the QR code to learn more about Ranger® Technology for Oncology Applications with the LightBench® (Low Throughput) and the NIMBUS Select (High-Throughput)

How does Size Selection differ in Gene Synthesis applications?

In those sectors which require long DNA constructs on tight turnaround, but which inherently struggle with sample purity, Ranger® Technology can be used to help clear that hurdle. The reason for that is because 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, and it does it by size selection.

One of the many beauties of Ranger® Size Selection capabilities is that we are able to cope with a huge range of fragment sizes. This is especially important in Gene Synthesis because it is here that short fragment lengths become less relevant, and long fragment recovery really comes into play.

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.

"Where purity of long constructs matter, Ranger® Size Selection can help"

Being able to generate these larger constructs has more economic value for groups like pharmaceutical companies – think about vaccine manufacturers as an example. 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.

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.

We've got lots of customers that have demand for this function and who are coming to the conclusion that Ranger® Technology is the best and most viable option, because the volume required can only be satisfied by Ranger® Technology solutions. The additional flexibility of being able to utilise our size selection service in Vancouver as a project-based proof of concept to demonstrate the capabilities of Ranger® and allow customers to test this before they commit to any capex spend has helped us build confidence in the sector.

Scan the QR code to learn more about Ranger® Technology for Gene Synthesis Applications with the LightBench® (Low Throughput) and the NIMBUS Select (High-Throughput)

How would you summarise the performance of Ranger® Size Selection compared to its competitors?

Where speed, complexity and cost matters, we are allowing clinical and research groups to take advantage of electrophoretic size. We offer the highest degree of automation and we add value in various applications to great scale and efficacy.

It is important to acknowledge that there are other instruments which are established in this category, such as the PippinHT. It offers a size selection option, although it works on a different basis. It doesn't take pictures of the entire gel, they have what's known as a line scanner that continuously monitors a single coordinate of a gel cassette.

While they are both good systems, this method does suffer from some deficiencies that don't allow it to track mobility changes that are happening throughout the run, which is one of the key advantages of Ranger® Technology.

About YOURGENE HEALTH

Yourgene Health is an international molecular diagnostics group which develops integrated genomic technologies and services *enabling genomic medicine*

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