HMW DNA Size Selection with the HLS2 instrument: Sequencing with ONT MinIon Ligation Sequencing Kit V14

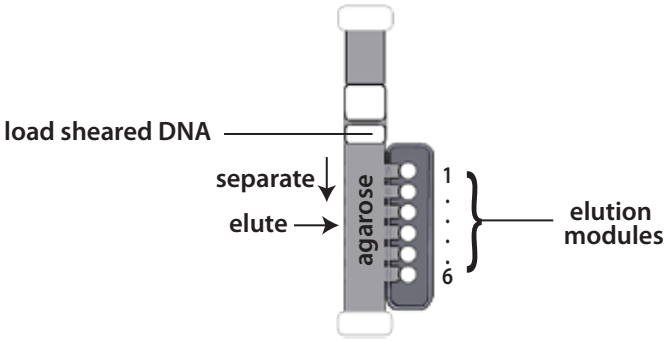
sage science

Application Note: HLS2

Chris Boles, Sage Science, Inc.

Introduction

The HL2 instrument is a electrophoretic device on which DNA can be separated by fragment size, and then collected in liquid buffer into six contiguous elution modules. Using a pre-cast agarose gel cassette, this approach allows users to fractionate a sample into size bins. Using preset pulsed field (PF) wave forms , HMW DNA up to 2MB may be collected.



Schematic of an HL2S gel column (one half of a gel cassette)

HLS2™ High Molecular Weight Library System

Using preset electrophoresis workfolws, users can choose ranges of size selected DNA that best suit experimental design. These workflows are based on PF wave forms that have been tailored to provide either well-seperated DNA sizes within a given range, or alternatively, High-Pass size selection with which DNA is compressed to provide all DNA above a programmed size threshold.

The goal of this study is to evaluate HLS2 size selection used in conjuntion with Oxford Nanopore MinIon sequencing with their Ligation Sequencing Kit V14 (SQK-LSK114 v3) and to suggest size selection protocols to improve N50 readlength metrics.

The Table below lists the preset workflow waveforms available for the HLS2 platform. The asterisks indicate the workflows used for this study.

	Preset workflow name	Run time	Region of good resolution	Onset of HMW compression
*	Size-select 5-100kb sep2.5h	4 hr	5-100kb	100kb
	Size-select 20-200kb sep3h	4.5 hr	20-200kb	240kb
*	Size-select 50-200kb sep4h	5.5 hr	50-200kb	240kb
	Size-select 100-300kb sep3h	4.5 hr	50-240kb	300kb
	Size-select 100-300kb sep4h	5.5 hr	100-300kb	340kb
	Size-select 50-250kb sep8h	9.5 hr	50-250kb	~300kb
	Size-select 340-1000kb sep3h	4.5 hr	340-1000kb	>>1000kb*
	Size-select 600-2000kb sep8h	9.5 hr	600-2000kb	>>2000kb*
*	Size-select High Pass 50kb sep8h.shflow	9.5 hr	N/A	50kb
*	Size-select High Pass 100kb sep8h.shflow	9.5 hr	N/A	100kb

Method for HMW HLS2 Size Selection

The following method, unless otherwise noted, was used in this study.

1. **Input DNA:** Genomic DNA, Female (Promega PN#G1521), concentration by OD260 = 207.1 ng/ul.

2. **Shearing:** Light bead shearing of 100 ul aliquots of the DNA.

   A. In a 2 ml Eppendorf LoBind tube, add 100ul DNA and 1 X 3mm glass beads.
   B. Vortex at 3000 rpm for 2 minutes.

3. **Sample preparation**: 5ug of DNA for loading on the HLS2 cassette (PN# HSS-0004 or HSS-0012).

   A. Add 24 ul of DNA to 36 ul of TE. Add 10 ul of loading solution (provided with cassettes) and mix gently by pippetting up and down with a wide-bore pipet (70ul total volume, 5 ug DNA).

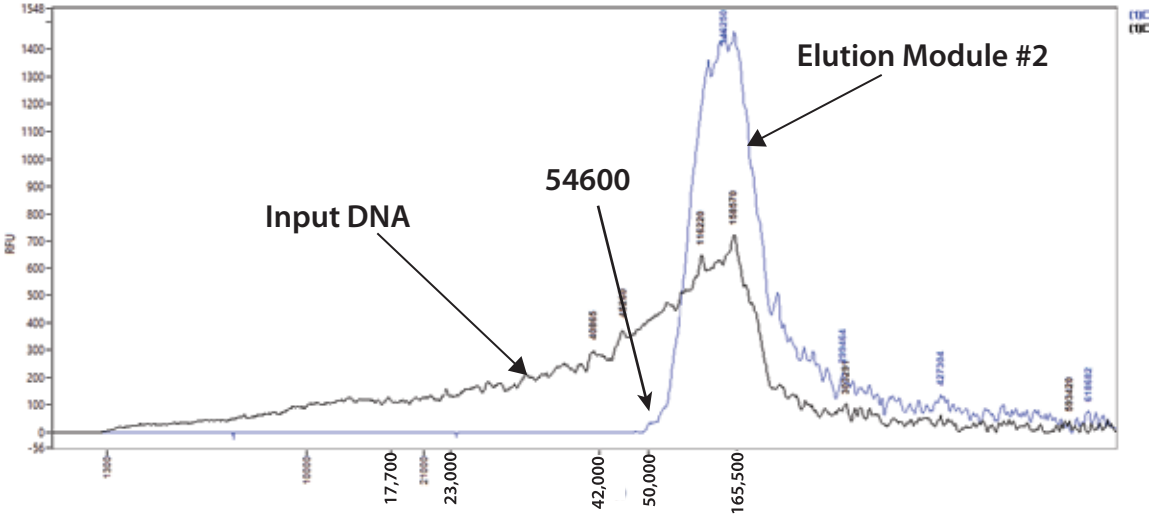
4. **Size selection protocol**: "Size-select 5-100kb sep2.5h" (total runtime = 4 hours)

5. **Analyze fractions**: at the end of the run analyze eluant fractions by Qubit HS.

Qubit analysis of HLS2 size fractions:

Module #	Amount Recovered (ng)	% yield
1	63.8	1%
2	987	20%
3	281.4	6%
4	161	3%
5	39.2	1%
6	26.6	1%
Total	1559	31%

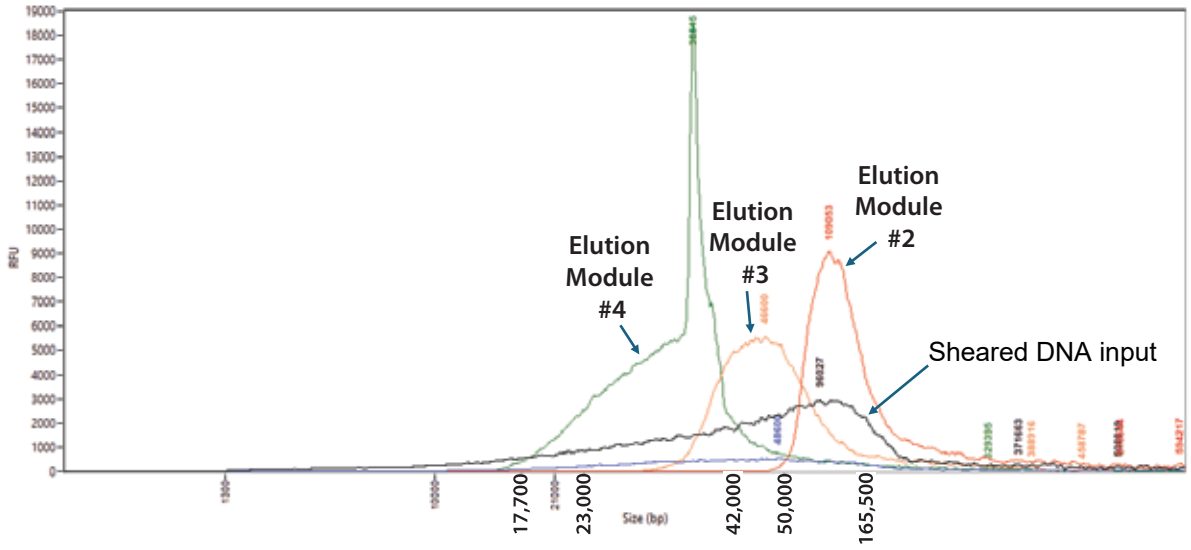
Femto Pulse traces of input DNA and the fraction collected from elution module #2:



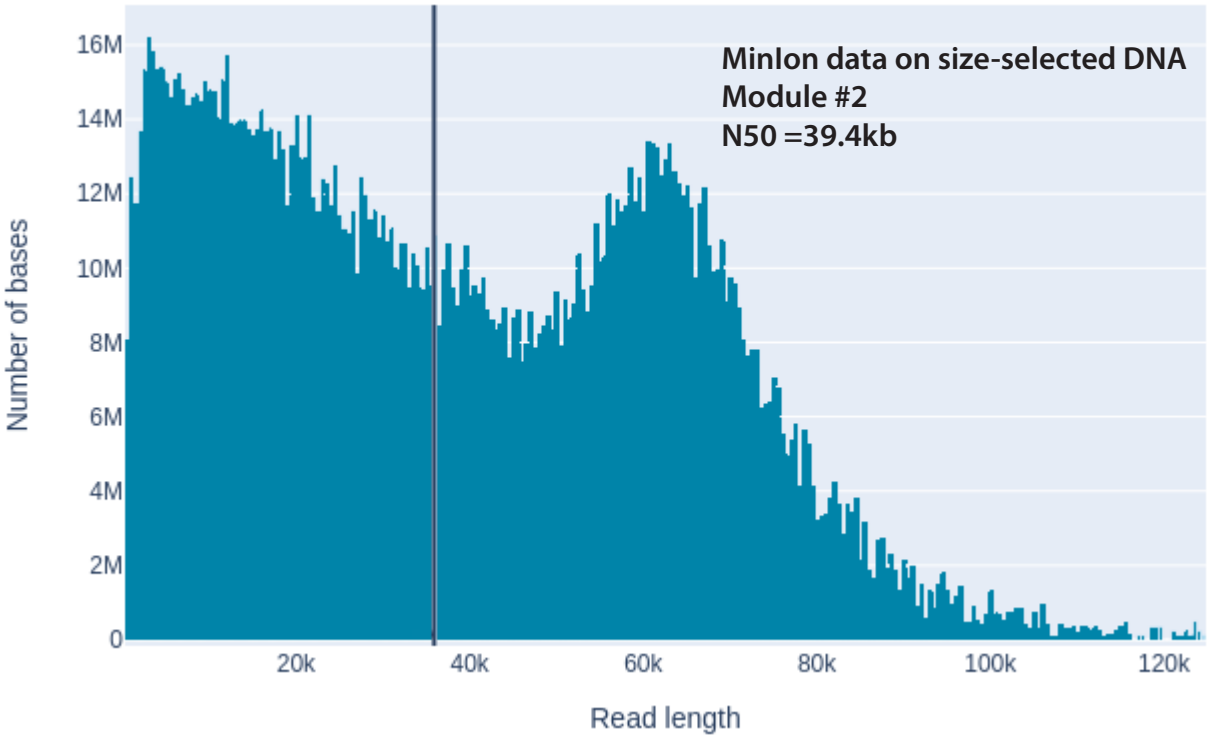
Size-select 5-100kb sep2.5h.shflow

In the flollwing example, DNA was sheared with 3 X 3mm glass beads. The HLS2 size selection workflow "Size-select 5-100kb sep 2.5h" provides good electrophoretic fragment resolution in the size range between 5-100kb. DNA migrates as a compression at sizes above 100kb. With this workflow, DNA sizes above 50 kb can be collected in module #2, inlcuding compressed DNA above 100kb.

Agilent Femto Pulse traces of the collected DNA and input DNA:



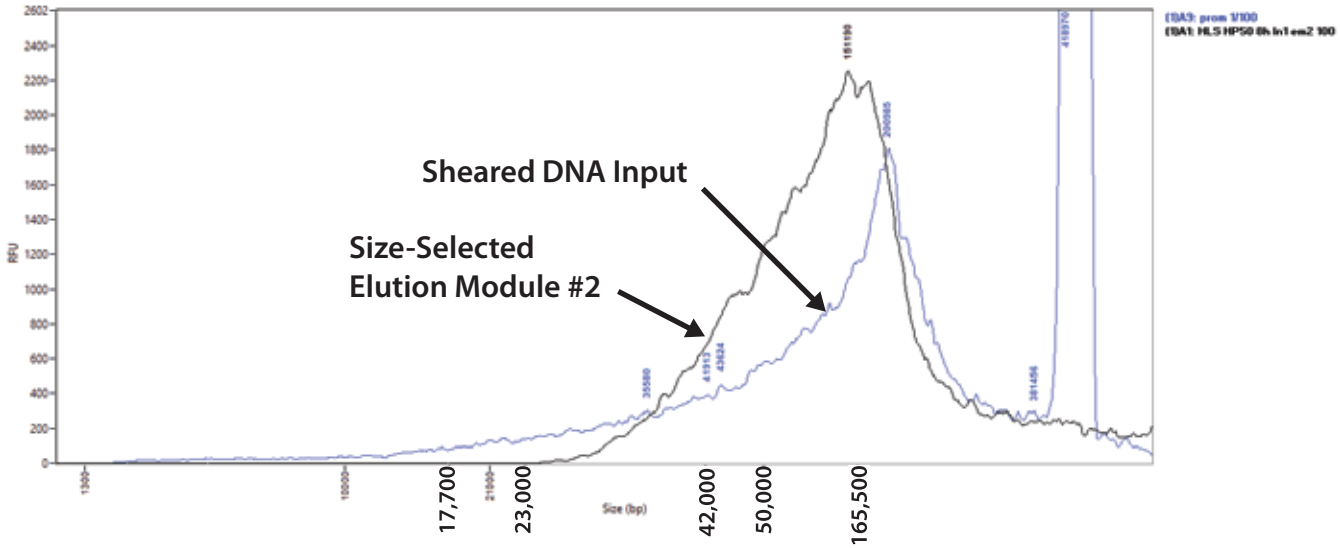
The MinIon sequencing histogram for DNA sequenced from elution module #2:



Size-select High Pass 50kb sep8h.shflow

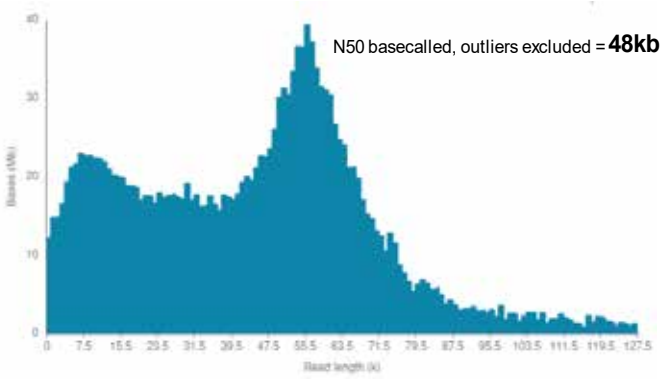
The HLS2 size selection workflow "Size-select High Pass 50kb sep8h" is programmed to provide a DNA compression around 50kb.

Agilent Femto Pulse traces of the collected DNA and input DNA:

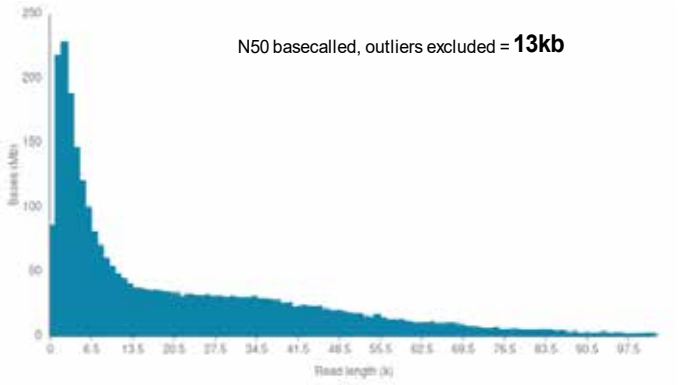


The MinIon sequencing histogram for DNA sequenced from elution module #2 and using non-size selected input DNA:

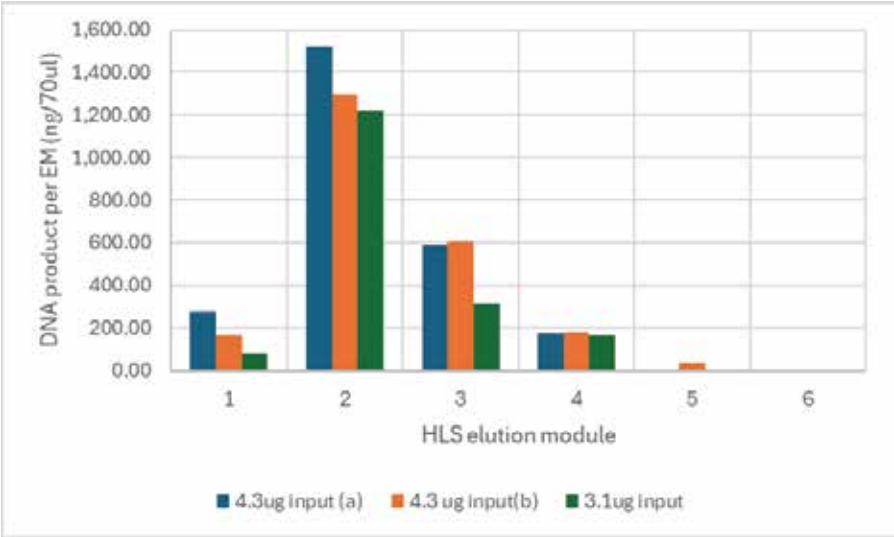
After size-selection, EM2



Before size-selection



A summary of size selection yield from elution modules (4290 ng input and 3075 ng input) from the "Size-select High Pass 50kb sep8h.shflow" workflow:



Summary of HLS recovery data

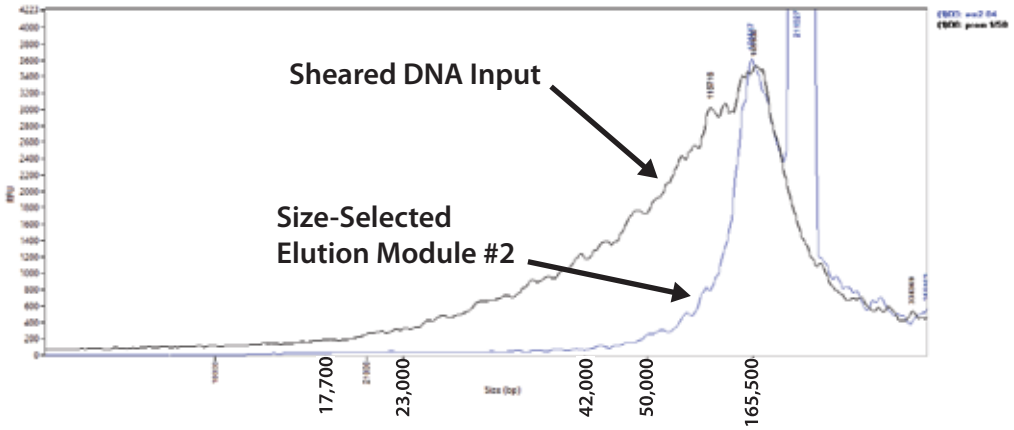
Input loads of 4290ng and 3075ng were used.

Elution Module #	4290ng(a)	total ng/70ul	%input	4290ng(b)	total ng/70ul	%input	3075ng	total ng/70ul	%input
1	3.91	273.70	6%	2.33	163.10	4%	1.15	80.50	3%
2	**21.70**	**1,519.00**	**35%**	**18.50**	**1,295.00**	**30%**	**17.40**	**1,218.00**	**40%**
3	8.44	590.80	14%	8.68	607.60	14%	4.48	313.60	10%
4	2.48	173.60	4%	2.56	179.20	4%	2.34	163.80	5%
5	ND			0.56	38.92	1%	ND		
6	ND			ND			ND		
	Total =	2,557.10	60%	Total =	2,283.82	53%	Total =	1,775.90	58%

ND = not detectable by Qubit BR kit

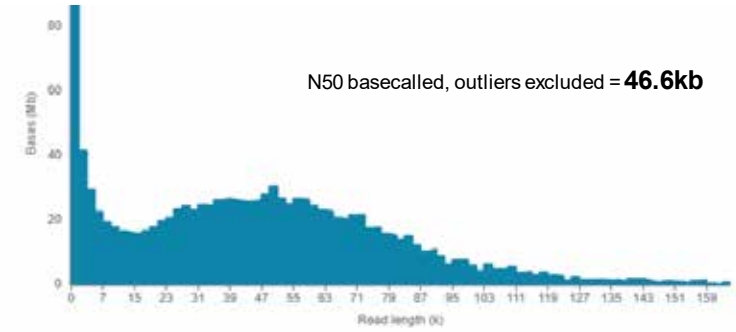
Size-select High Pass 100kb sep8h.shflow

The HLS2 size selection workflow "Size-select High Pass 100kb sep8h" is programmed to provide a DNA compression around 100kb.

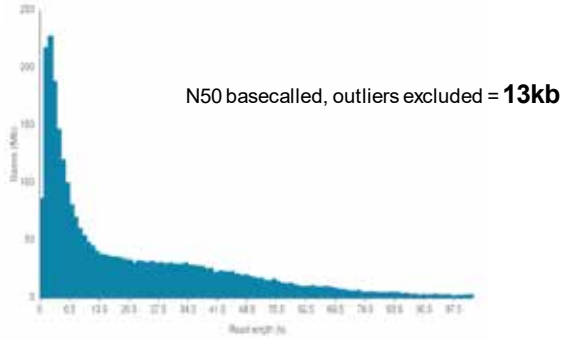


The MinIon sequencing histogram for DNA sequenced from elution module #2 and using non-size selected input DNA:

After size-selection, EM2

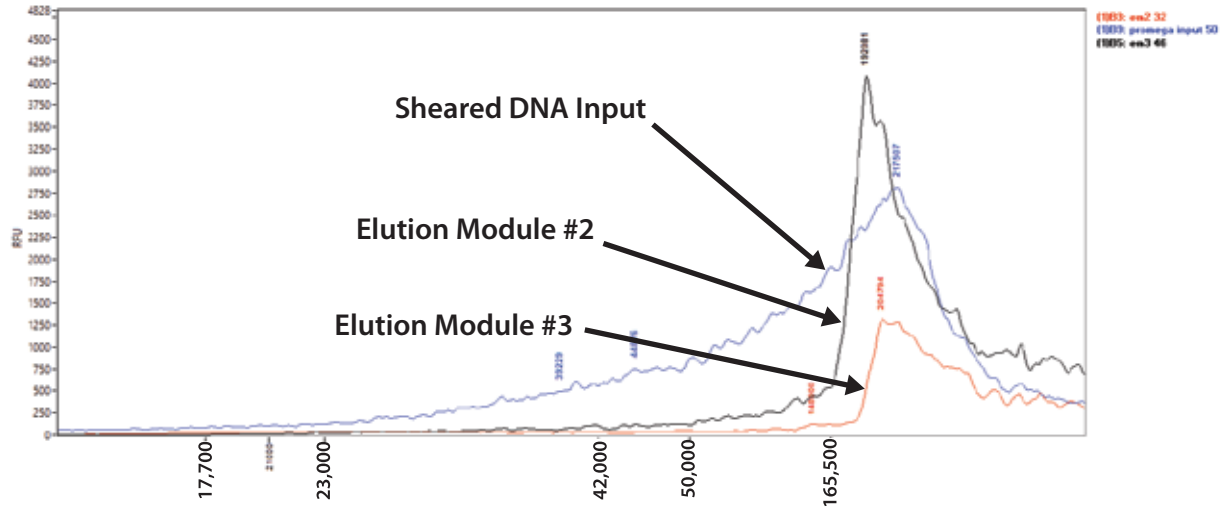


Input DNA (before size-selection)



Size-select 50-250kb sep8h.shflow (not sequenced)

The "Size-select 50-250kb sep8h.shflow" workflow has and onset of HMW DNA compression around 240kb. In this instance, combining elution wells 2 & 3 provided 1 ug of HMW with significant rduction of DNA <50kb.



Conclusions

The HLS2 system can be used to size-select HMW DNA for the purpose of eliminating lower molecular weight fragments to improve average readlengths for long-read sequencing platforms. Sage Science's BluePippin platform, which is often used for this purpose, uses tapered agarose gel columns to separate and resolve DNA. While feature improves accuracy and speed of DNA size selection, this geometry is not ideal for high resolution or accuracy for size selections above 50kb. The rectangular geometry of the HLS2 system provides substantially better resolution under pulsed-field electrophoresis for DNA >50kb inlcuding ultra-high molecular weight DNA (hundreds of kb). The HLS2 has the same DNA input requirements as the BluePippin (max. 5 ug), and the same sample capacity (4 lanes per run).

Studies Citing HLS Size Selection

Huang, Z., et al. 2023. Evolutionary analysis of a complete chicken genome. PNAS 12 (8) e2216641120. https://doi.org/10.1073/pnas.2216641120.

Morita, S., et al. 2023. The draft genome sequence of the Japanese rhinoceros beetle *Trypoxylus dichotomus septentrion* towards an understanding of horn formation Sci Rep 13, 8735 (2023). https://doi.org/10.1038/s41598-023-35246-w

Xing Guo, et al. 2023. The genome of *Acorus deciphers* insights into early monocot evolution. Nat Commun 14, 3662 (2023). https://doi.org/10.1038/s41467-023-38836-4

Kun Li, et al. 2023. Genetic Diagnosis of Facioscapulohumeral Muscular Dystrophy Type 1 Using Rare Variant Linkage Analysis and Long Read Genome Sequencing. medRxiv preprint. https://doi.org/10.1101/2023.06.05.23290975

Q Wang, et. al. 2023. Draft genome of the oriental garden lizard (*Calotes versicolor*). Front. Genet., 20 February 2023 Sec. Livestock Genomics Volume 14 - 2023 https://doi.org/10.3389/fgene.2023.1091544

Fengjiao Ma, et al. 2023. Gap-free genome assembly of anadromous *Coilia nasus.* Sci Data 10, 360 (2023). https://doi.org/10.1038/s41597-023-02278-w