Ultra-HMW DNA Size Selection with the HLS2 instrument and a >50kb High-Pass protocol

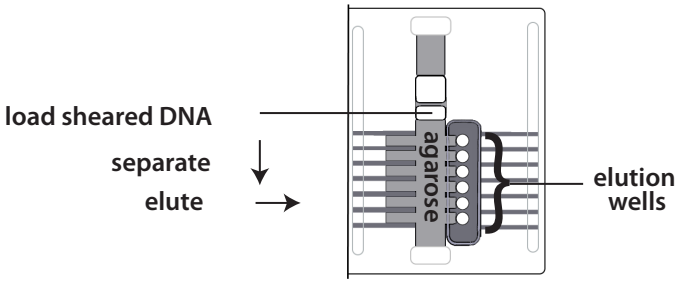
sage science

Application Note: HLS2

Chris Boles and Bryan Spencer, Sage Science, Inc.

Introduction

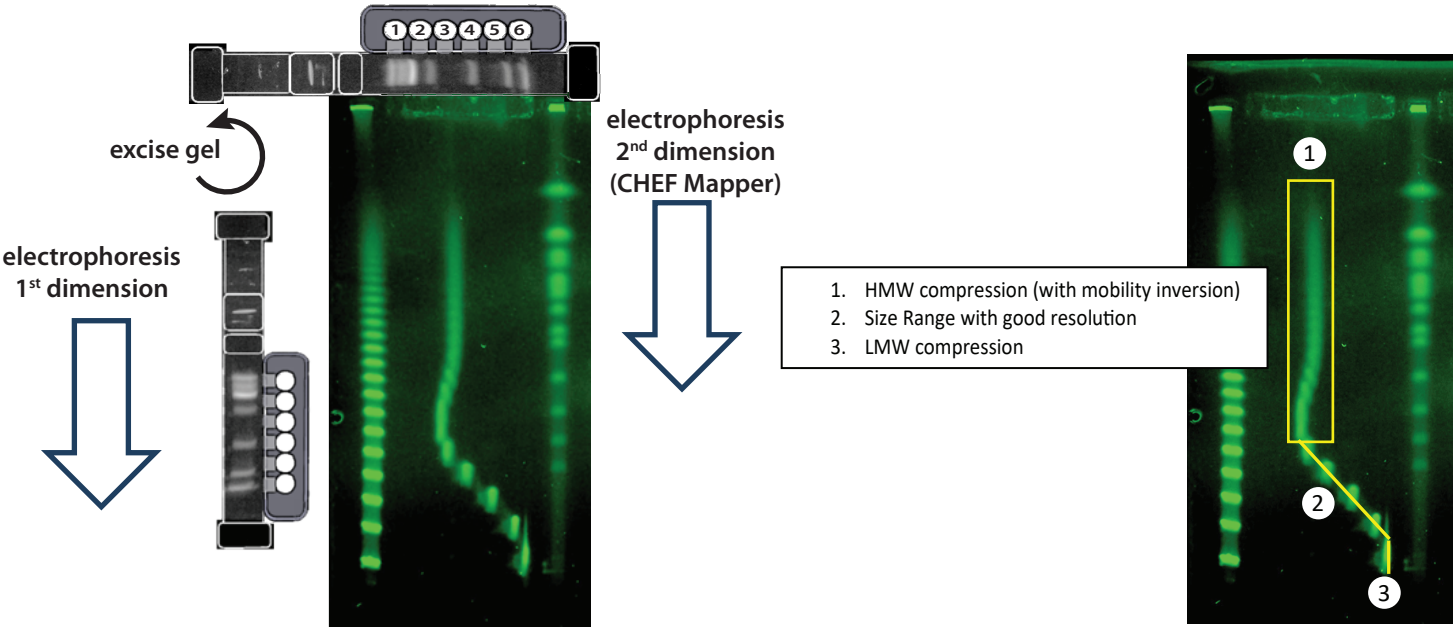
The HL2 instrument is a electrophoretic device on which DNA can be separted by fragment size, and then collected in liquid buffer into six contiguous elution modules. Using a pre-cast agaorse gel cassette, this approach allows users to fractionate a sample into size bins. Using preset pulsed field (PF) wave forms , HMW DNA up to 2MB may be collected. Here we describe how the protocols are used and provide a method for the collection of >50kb DNA fragments from a genomic sample.



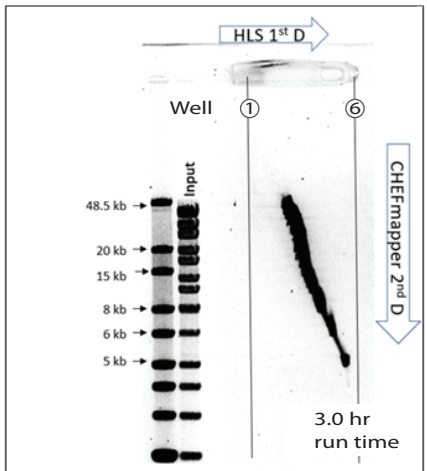
Schematic of an HL2S gel column (one half of a gel cassette)

HLS2™ High Molecular Weight Library System

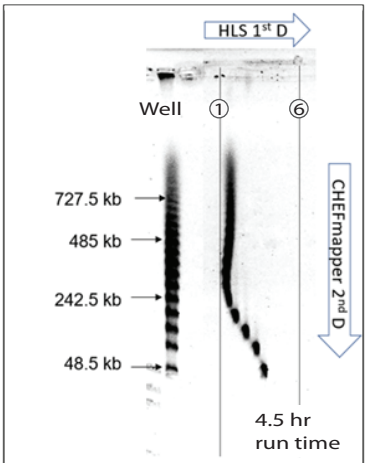
Electrophoretic resolution of large DNA fragments can be improved through the use of field-inversion pulsed field (PF) electrophoresis. However, this style of electrophoresis can result in unexpected elution profiles. For instance, for a given pulsed field program, there will usually be a range with good electrophoretic resolution, bracketed by high and low molecular weight compression regions where there is little or no resolution, as shown below. To develop useful PF conditions, we have developed a two-dimensional analytical electrophoresis procedure in which a sample of phage lambda DNA concatemers is electrophoresed in an HLS lane without elution. The cassette is cut open and the HLS gel lane is removed and cast into the sample well of a high-resolution Bio-Rad CHEFmapper gel. The CHEFmapper gel is run under conditions where linear separations of DNA up to 2Mb in size can be accomplished. This procedure allows us to unambiguously determine the range of linear DNA resolution along with the positions of HMW and LMW compression.



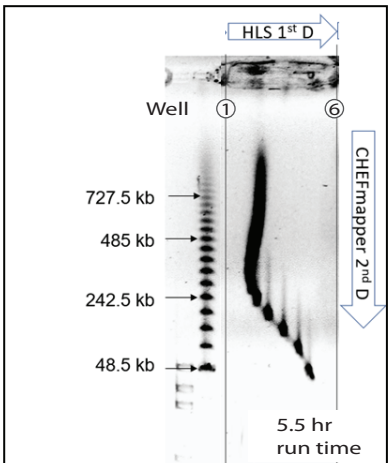
Gel images can be used as a reference to select protocols to collect size ranges that can be collected with good separation. In some applications (i.e. High Pass), collections from HMW compressions may be best.



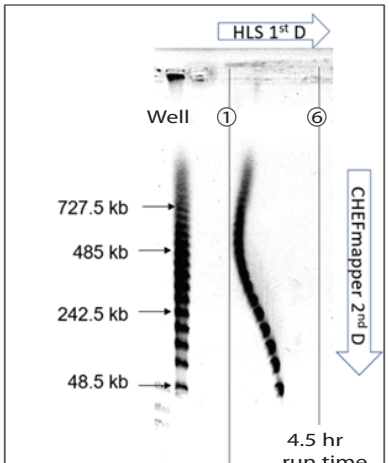
Region of good resolution: **5-100kb**
Onset of HMW compression: **100kb**



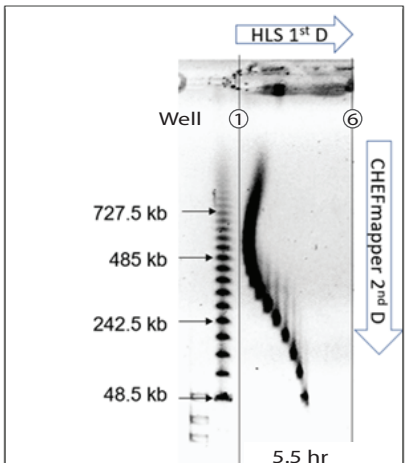
Region of good resolution: **20-200kb**
Onset of HMW compression: **240kb**



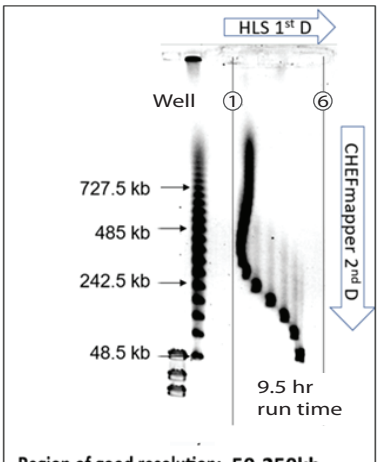
Region of good resolution: **50-200kb**
Onset of HMW compression: **240kb**



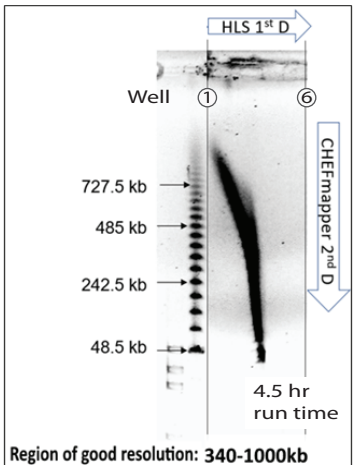
Region of good resolution: **50-240kb**
Onset of HMW compression: **300kb**



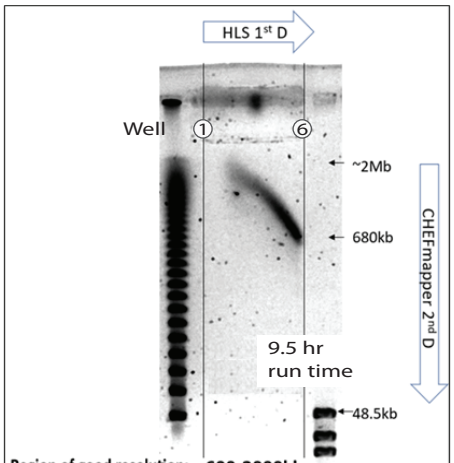
Region of good resolution: **100-300kb**
Onset of HMW compression: **340kb**



Region of good resolution: **50-250kb**
Onset of HMW compression: **~300kb**



Region of good resolution: **340-1000kb**
Onset of HMW compression: **>>1000kb**



Region of good resolution: **600-2000kb**
Onset of HMW compression: **>>2000kb**

Summary of HLS2 HMW size slection protocols

Workflow name	Elution time	Total run time	Region of good resolution	Onset of HMW compression	Voltage
Size-select 5-100kb sep2.5h	90m	3.0 hr	5-100kb	100kb	80V
Size-select 20-200kb sep3h	90m	4.5 hr	20-200kb	240kb	55V
Size-select 50-200kb sep4h	90m	5.5 hr	50-200kb	240kb	55V
Size-select 100-300kb sep3h	90m	4.5 hr	50-240kb	300kb	55V
Size-select 100-300kb sep4h	90m	5.5 hr	100-300kb	340kb	55V
Size-select 50-250kb sep8h	90m	9.5 hr	50-250kb	~300kb	37V
Size-select 340-1000kb sep3h	90m	4.5 hr	340-1000kb	>>1000kb*	55V
Size-select 600-2000kb sep8h	90m	9.5 hr	600-2000kb	>>2000kb*	55V

* These stages are not useful for high-pass size selections because HMW compression is not seen with this stage up to >2Mb.

A method for collection of >50kb DNA

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).

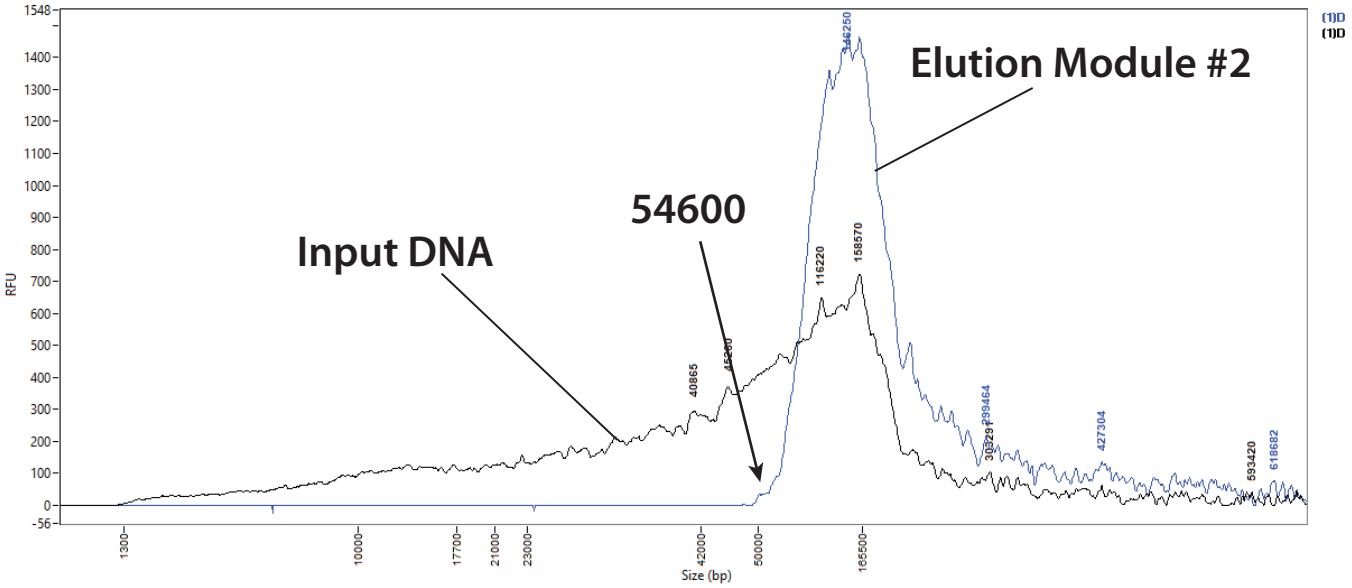
4. **Size selection protocol**: Use "Size-select 5-100kb sep2.5h" (total runtime = 4 hours)

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

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%

Qubit analysis of HLS2 size fractions for >50kb collections (module #2).

Femtopulse Analysis >50kb DNA collection



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