HMW DNA Size Selection with the HLS2 instrument

sage science

Application Note: HLS2

Fragment Ranges between 50kb to 2MB can be collected with programmed protocols.

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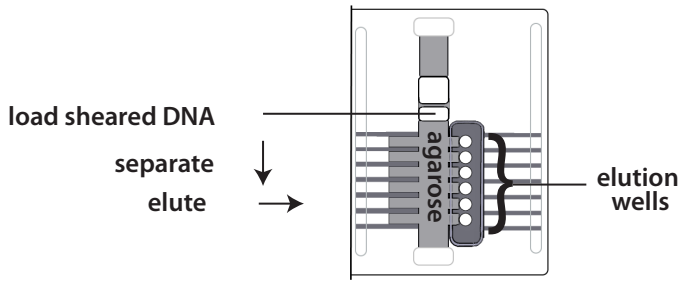
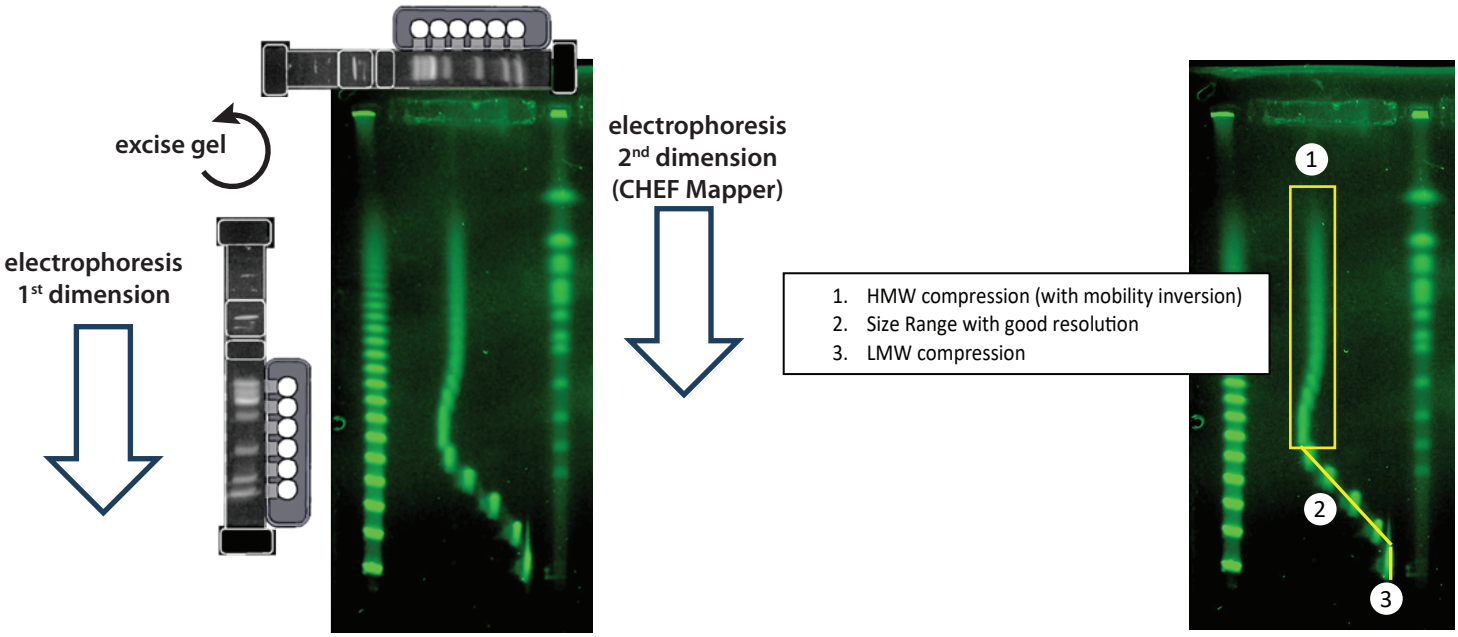
The HL2 instrument is a electrophoretic device on which DNA can be separted by fragment size, and then collected in liquid buffer into six contiguous elution modules. Using a pre-cast agaorse gel cassette, this approach allows users to fractionate a sample into size bins. Using preset pulsed field (PF)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



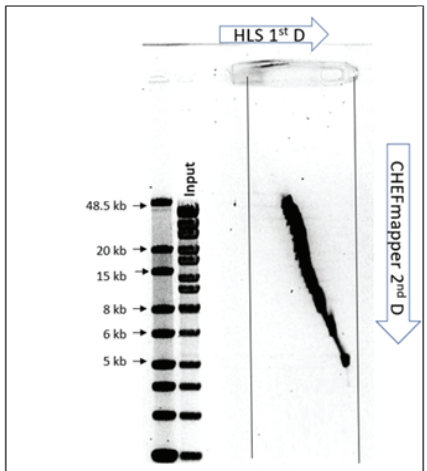
Shematic of an HL2S gel column (one half of a gel cassette)

HLS2™ High Molecular Weight Library System

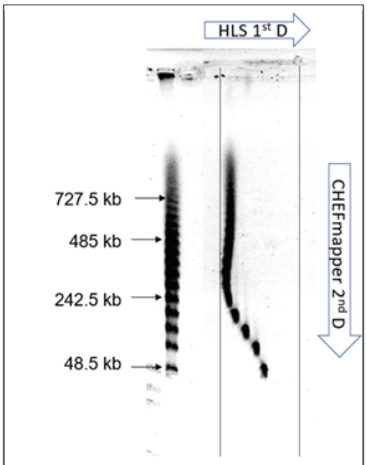
Electrophoretic resolution of large DNA fragments can be improved through the use of field-inversion pulsed field (PF) electrophoresis. However, this style of electrophoresis can result in unexpected elution profiles. For instance, for a given pulsed field program, there will usually be a range with 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.



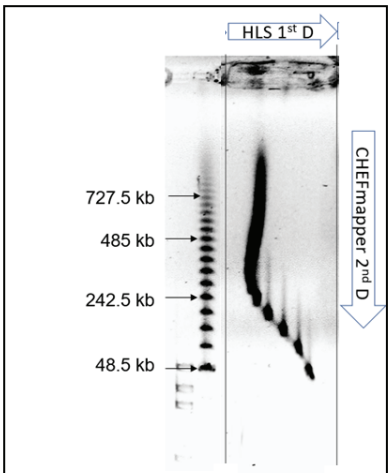
Gel images can be used as a reference to select protocols to collect size ranges that can be collected with good separation. In some applications (i.e. High Pass), collections from HMW compressions may be best.



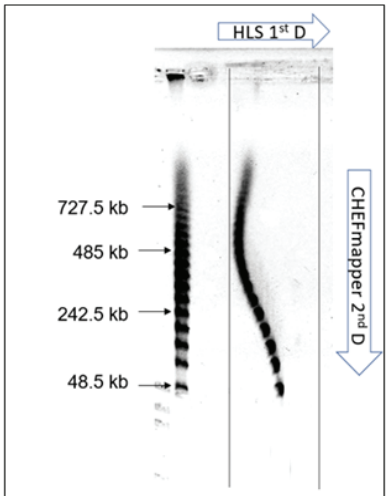
Region of good resolution: 5-100kb
Onset of HMW compression: 100kb



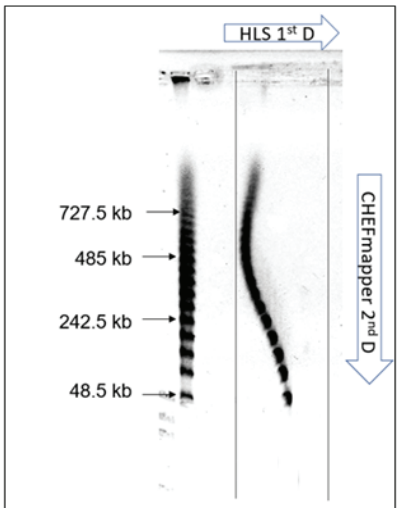
Region of good resolution: 20-200kb
Onset of HMW compression: 240kb



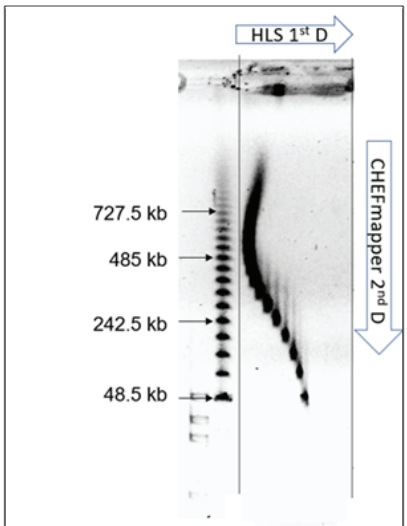
Region of good resolution: 50-200kb
Onset of HMW compression: 240kb



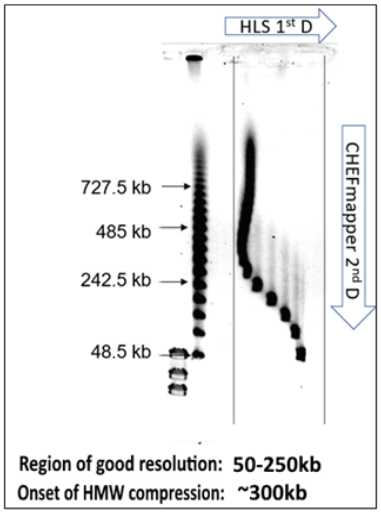
Region of good resolution: 50-240kb
Onset of HMW compression: 300kb



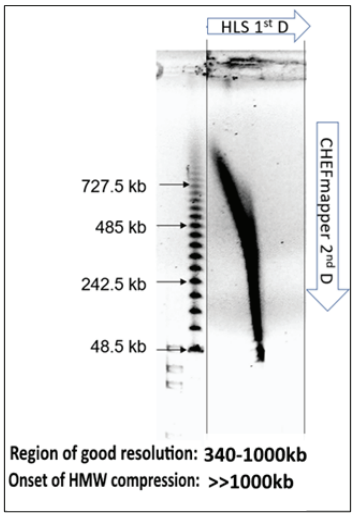
Region of good resolution: 50-240kb
Onset of HMW compression: 300kb



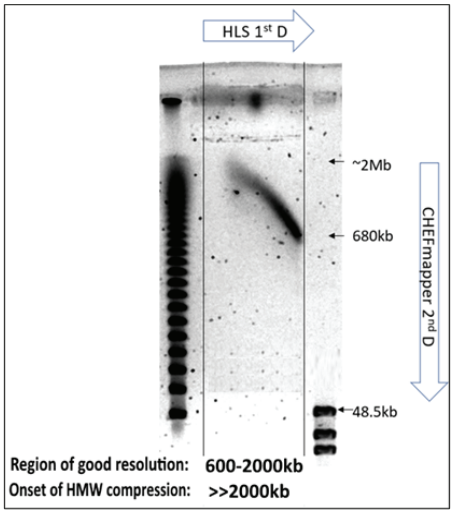
Region of good resolution: 100-300kb
Onset of HMW compression: 340kb



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	Region of good resolution	Onset of HMW compression	Voltage	Waveform**
Size-select 5-100kb sep2.5h	90m	5-100kb	100kb	80V	25 10 25 10 5 2 21
Size-select 20-200kb sep3h	90m	20-200kb	240kb	55V	150 50 30 10 3 1 81
Size-select 50-200kb sep4h	90m	50-200kb	240kb	55V	150 50 30 10 3 1 81
Size-select 100-300kb sep3h	90m	50-240kb	300kb	55V	150 50 60 20 6 2 81
Size-select 100-300kb sep4h	90m	100-300kb	340kb	55V	150 50 60 20 6 2 81
Size-select 50-250kb sep8h	90m	50-250kb	~300kb	37V	1000 333 240 80 0 0 101
Size-select 340-1000kb sep3h	90m	340-1000kb	>>1000kb*	55V	3000 1000 2550 850 0 0 24
Size-select 600-2000kb sep8h	90m	600-2000kb	>>2000kb*	55V	3000 1000 2550 850 0 0 24

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

** Waveform definitions (all in milliseconds): initial F time, initial R time, initial F increment, initial R increment, incr. to F incr., incr. to R incr., number of F/R cycles until return to initial conditions.