

Higher Accuracy “Range + T” Size Selection: sage science BluePippin and PippinHT

Application Note: PippinHT/BluePippin

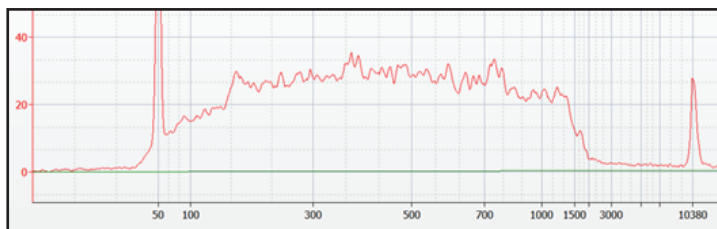
A combination of “Range Mode” and “Time” mode can improve accuracy of DNA size selection

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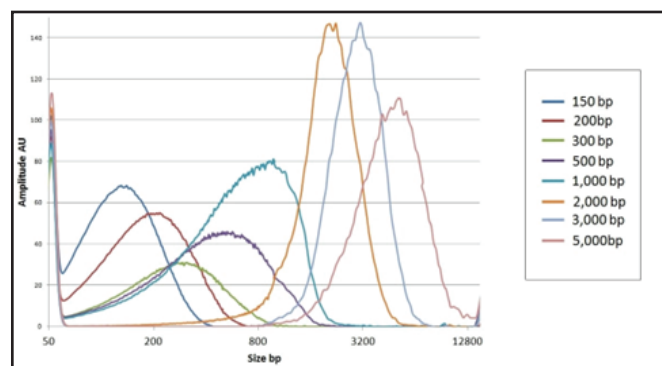
Sage Science has developed a new size selection programming mode; “Range + T”. It is a combination of “Range” mode and “Time” mode. It is a tool for users to obtain more accurate size selection cuts than before if needed. On the PippinHT instrument, it enables a high molecular weight DNA Range cuts where only High-Pass size selections were allowed previously.

Pippin size selection products have a very accurate “start” size selection collections. However, the accuracy of the “end” collections (the higher sized fragments in the collection range) can be affected by the fragment size profile of the input sample. This partially stems from the profile of the sheared DNA input that is used to calibrate the Pippin products. In order to calibrate for the functional range of a gel cassette, pooled E.coli genomic digests are used which have a broad and flat size distribution profile (a). However customer samples often have a narrower, gaussian fragment size distribution when sheared (b). In samples with a Gaussian size distribution, the fragments in the center of the distribution will be more concentrated relative to the fragments in both tails of the size distribution, and consequently migrate slower than the fragments in the tails.

b. Examples of Covaris shearing profiles



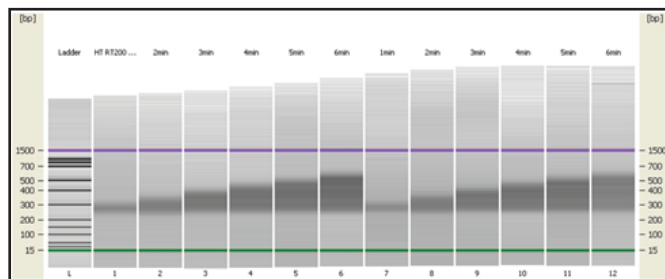
a. An example of a Sage DNA shear used for calibration



b. Examples of Covaris shearing profiles

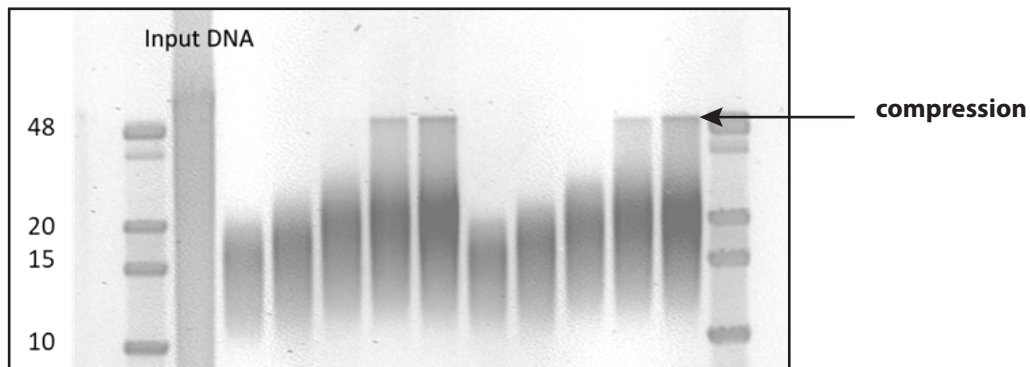
Since Pippin “Range” mode calibrations use samples that have a flat size distribution, the error in estimating DNA size in samples with a Gaussian shape will not be adequately corrected. In addition, “Range” mode will not collect distributions that are narrower than limit that is set by the algorithm for choosing the time to end elution. Furthermore, both problems are aggravated where selections involve DNA sizes greater than 10kb.

For users who require a higher level of accuracy of size selection, we have introduced a new programming mode: “Range + T”. With “Range+T” protocols, the user chooses a DNA size value where product elution should begin, and then chooses an elution time period rather than an ending size value. This requires some method development is required by the user and the user’s sample profiles.



Bioanalyzer analysis, Range + T, PippinHT: 1.5% agarose cassette with a 200 bp start value, plus 1,2, 3, 4, 5, and 6 minutes (in duplicate,)

With HMW DNA, the PippinHT has thus far only been capable of only High-Pass size selection runs. The short agarose columns increased the uncertainty of providing an accurate end values for the size selection range proved impossible for our standard “tight” and “range” modes. Now with a little effort, users can collect defined size ranges -- it is also a good tool to optimize the amount of DNA collected which is often critical for long-read library prep. Guidelines for Time values are provided with protocols. HMW size selections (particularly High Pass) protocols typically include DNA compressions which can be collected if time guidelines are exceeded.



Pulse field gel analysis, Range + T, PippinHT: 0.75% agarose cassette with a 10Kb start value plus 4, 8, 12, 16 and 20 minutes (in duplicate)

Who should use “Range + T”?

“Range + T” provides an additional tool optimizing DNA selection. It is often recommended that users take some time to optimize size selection conditions to best address their application and sample type. Anyone who requires more accuracy and reproducibility than they have been achieving, particularly at the end of the size range, could benefit.