PippinHT: Collection of <167 bp DNA for Cell-Free DNA Library Construction

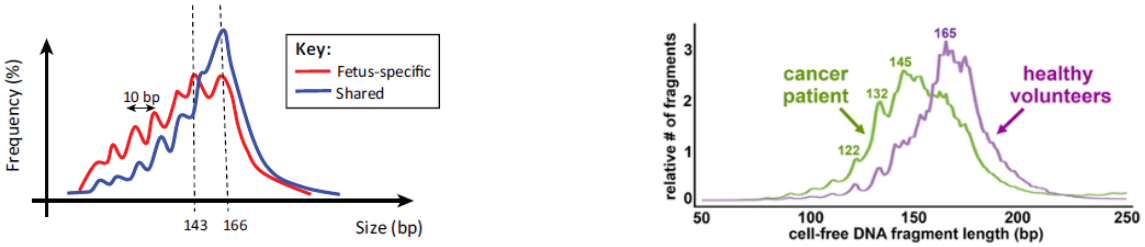
sage science

Application Note: PippinHT

Timed DNA size selection setting may be uses to isolate the <167 bp fraction from cfDNA samples for sequencing library construction with ultra low-input kits.

Introduction

Cell free DNA (cfDNA) isolated from blood plasma allows unprecedented non invasive access to valuable genetic information. The most abundant form of cfDNA shows a characteristic peak of approximately 167 bp, the size of the mononucleosomal unit. Increasing evidence suggest that shorter cfDNA fragments (40 -167bp) retain genetic information about the fetus, tumor associated copy number aberrations and the cellular origin of cfDNA[1,2] .



[1]Jiang and Lo, Trends in Genetics, 2016

[2]Underhill et al. , PLOS Genetics, 2016

Figure 1. Genetic Differentiation in the mononucleosomic fraction in cfDNA Samples

The PippinHT platform performs DNA size selection on up to 24 samples simultaneously. The method uses agarose electrophoresis with a switchable and branched electroelution architecture to electro-elute DNA fractions. The system typically uses the detection of fluorescently labelled oligos that run ahead of the DNA size selection range in order to determine the automated timing of switching of the electrophoresis pathways. Using PippinHT gel cassettes containing 3% agarose (Cat# HTG3010) which allow size selections between 100-250 bp, much of the differentiated mononucleosomal fraction maybe collected using a 100-167 bp setting, or a 100-250 bp setting to collect a larger fraction. Here we provide a method with which users can collect mononucleosomal DNA fragments below beyond the 100 bp size range limit. This is accomplished by omitting the fluorescent internal standard and used manually entered timed settings that are presented here. These fractions can be constructed into sequencing libraries given recent developments of utra-low input DNA library kits provided by several suppliers.

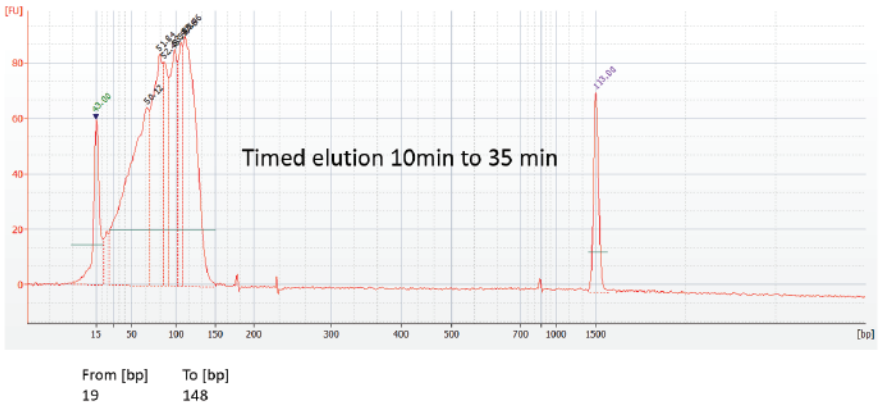
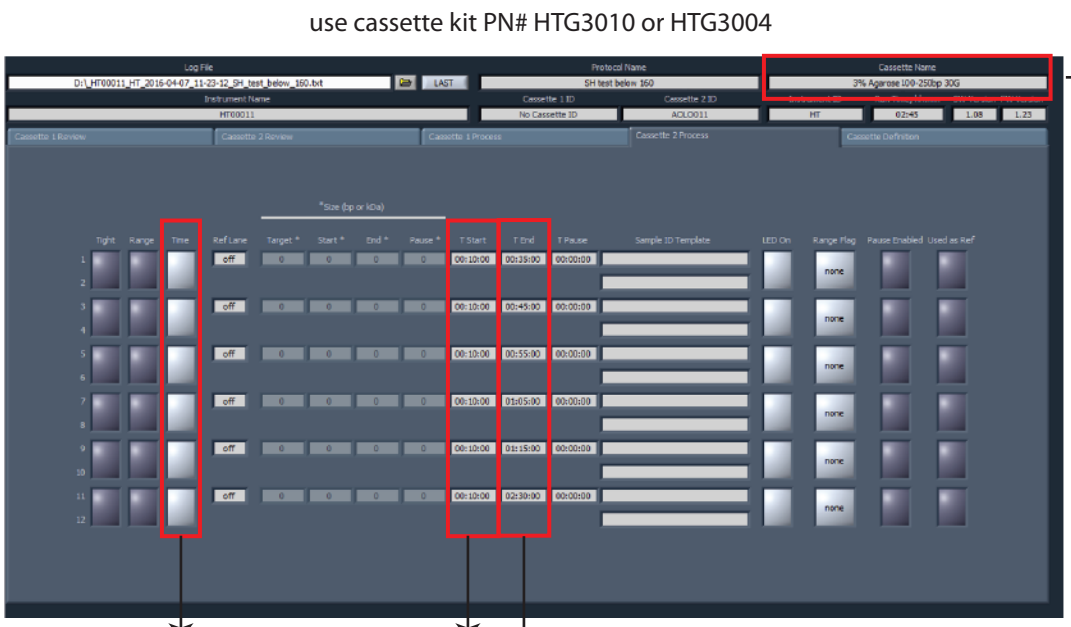


Figure 2. BioAnalyzer Trace of a Size Selected Sheared DNA Sample Using a Timed Collection, 0 -150 bp

Timed Software Settings in PippinHT Software

The Protocol Editor section of the PippinHT software interface (**Figure 3**) allows the programming of timed-switching rather than automated-switching with the use of fluorescently labelled standards. It is important to omit the labelled internal standard (Marker 30G) from the sample preparation and to instead use marker-free Loading Solution (included in the kit) as a densifying agent. In the Protocol Editor the "Time" buttons must be pressed, and 10 minutes must be entered into the "TStart" fields to begin collection of the leading edge of the sample. The value entered into the "TEnd" will determine the upper range of the collection.



bp	T End
120	31:42
130	32:36
140	33:36
150	34:36
160	35:36
170	36:36
180	37:36
190	38:36
200	39:36
210	40:30
220	41:30
230	42:30
240	43:30
250	44:30

* Add loading solution to cfDNA input samples. Do not add Marker 30G.

Figure 3. Steps for Programming the Collection of Mononucleosomic cfDNA fractions using Timed Mode

[1]Peiyong Jiang and Y.M. Dennis Lo, *The Long and Short of Circulating Cell-Free DNA and the Ins and Outs of Molecular Diagnostics.* Trends in Genetics DOI:10.1016/j.tig.2016.03.009 June 2016, Vol. 32, No. 6

[2]Hunter R. Underhill et al., *Fragment Length of Circulating Tumor DNA.* PLOS Genetics | DOI:10.1371/journal.pgen.1006162 J uly 18, 2016