

## New SageHLS workflows and stages contained in the 10/31/2017 update.

Sage has developed several new stages for extraction, treatment, and size-selection/collection on the SageHLS.

### New Extraction and treatment stages

The update includes a new long extraction method, “Extraction Method 3hr”. In this new stage the electrophoresis purification is carried out for 3 hrs instead of 1 hr as in the original “Extraction Method 1”. The new longer extraction method is useful for removing partially degraded DNA that is released from non-viable cells during the extraction stage. Use of the 3hr extraction method is especially important for achieving good enrichments in CATCH protocols, since the DNA released from non-viable cells will contribute to the contaminating, non-targeted DNA load in the recovered sample. In HMW DNA extraction protocols, the longer extraction method is important for getting reproducible product size distributions, since the amount and size of degraded DNA from non-viable cells is unpredictable.

The update also includes a simplified Fragmentase treatment stage, “Fragmentase Method – no stop”. In the new treatment stage, stopping the reaction by adding 0.5M EDTA to the sample well is no longer required. We have found that the EDTA stop procedure has little effect on the extent of the Fragmentase digestion, as long as the operator replaces the reagent well contents with HLS Lysis Reagent and proceeds to the collection stage without delay.

### New Collection Stages

Over the summer, Sage has developed a series of electrophoresis protocols that expand the electrophoretic resolution of specific “windows” of DNA size. The new protocols generally produce regions of HMW and LMW mobility compression, which are located in the top and bottom portions of the gel column, respectively. The region between the two compressions is expanded (lengthwise) along the gel column, giving rise to a region of enhanced electrophoretic resolution. For instance, the CATCH 400 collection stage has a HMW compression beginning around 500kb, and a LMW compression for DNA fragments below around 340kb. The 160kb region between 500kb and 340kb has enhanced resolution, which is important for reducing non-target DNA contamination in CATCH experiments.

One caution about the new collection stages, the increased resolution comes at a price in terms of run time. Our optimal results have been achieved using a lower voltage and 8 hr runtimes. Including a 1.5 hr elution time, the total time for the new collection stages will be 9.5 hrs. So, plan on running workflows with these new stages overnight.

In addition, the new electrophoresis protocols can be used in HMW DNA purifications as High-Pass protocols for purification of DNA greater than the size of the low threshold value of the HMW mobility compression. For instance the same electrophoresis protocol used for the CATCH 400 collection stage is also useful for collecting HMW DNA greater than 500kb in size.

New collection stages for CATCH (and effective CATCH range) :

- CATCH 145kb (recommended for targets 100-180kb)
- CATCH 200kb (for targets 145-240kb)
- CATCH 400kb (for targets 340-480kb)

New collection stages for HMW DNA extraction:

High-Pass 242kb

High-Pass 291kb

High-Pass 340kb

High-Pass 500kb

New Workflows:

There are eight new workflows that incorporate the new 3hr Extraction stage. These are labeled with the “vs2” nomenclature. The “vs2” HMW extraction workflows also feature the simpler “Fragmentase Method– no stop” treatment stage.

New workflows for CATCH:

CATCH 100-300kb vs2 (for 100-300kb targets)

CATCH 145 kb vs2 (for 100-180kb targets)

CATCH 200 kb vs2 (for 145-240kb targets)

CATCH 400 kb vs2 (for 340-480kb targets)

New workflows for HMW DNA extraction:

HMW High-Pass 242kb vs2

HMW High-Pass 291kb vs2

HMW High-Pass 340kb vs2

HMW High-Pass 500kb vs2

#### Core instrument software build

There is no update to the system software in this update.

Please contact [support@sagescience.com](mailto:support@sagescience.com) with any questions about this update