Integrated method for extraction of HMW DNA and preparation of genomic sequencing libraries using agarose gels

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Introduction

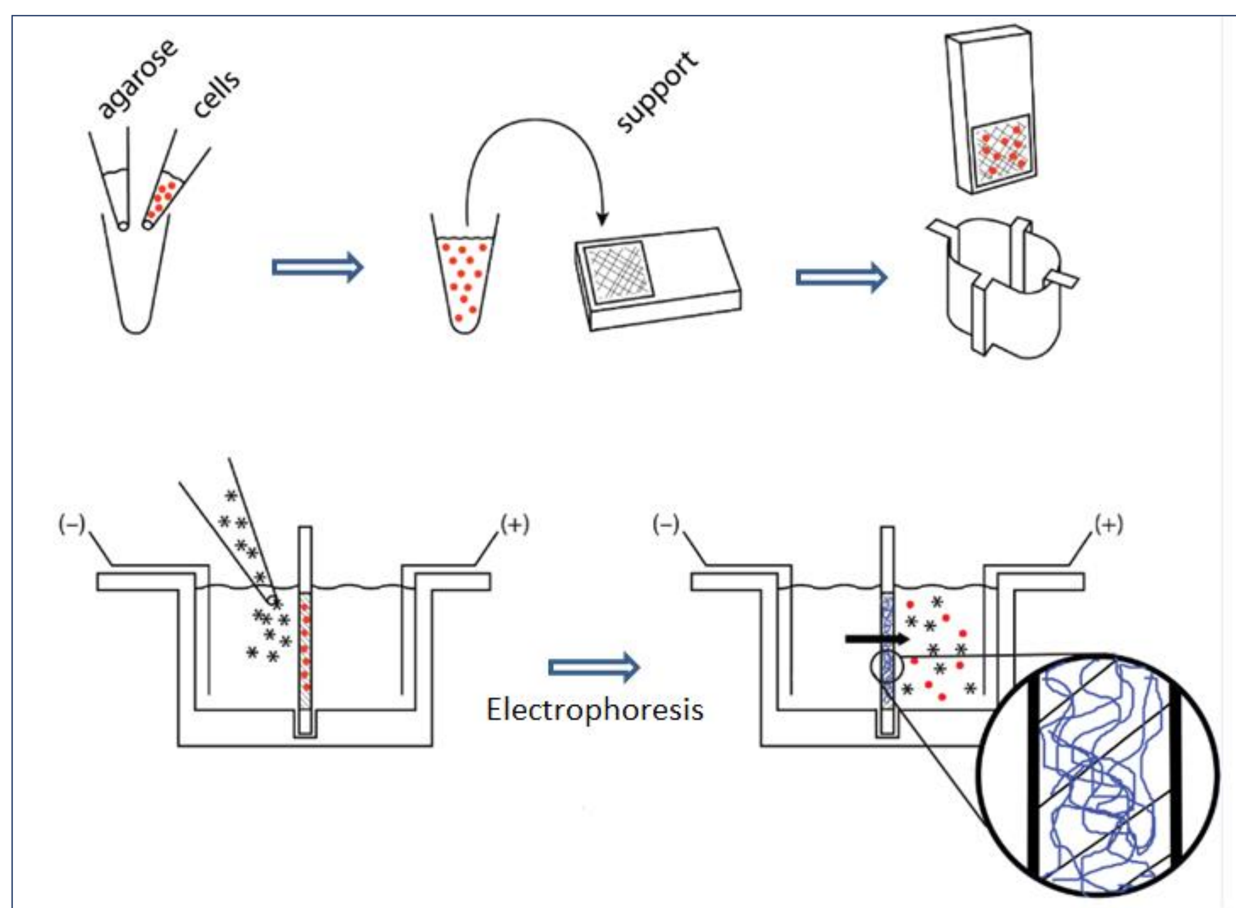
Several new DNA sequencing methods can provide read lengths greater than 10,000 base pairs (bp). This trend has motivated researchers to reevaluate standard DNA sample prep methods which usually produce genomic DNA fragments of only up to 50,000 bp in length. To this end, we have developed a new workflow and equipment for rapid purification of high-molecular-weight (HMW) DNA (>50,000 bp) from whole blood and cell suspensions. The system can also be adapted to perform both DNA extraction and transposase-mediated sequencing library preparation in a fully integrated, automated process.

Methods

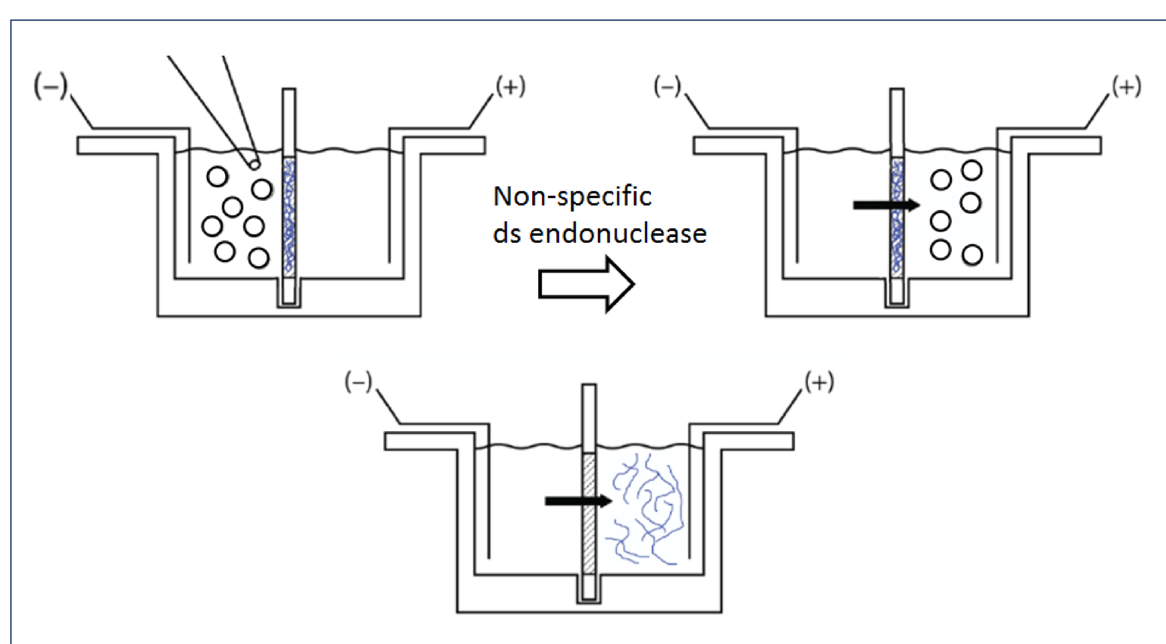
Our method is loosely based on the original agarose-based sample preparation method of Schwartz and Cantor (1984). We embed cell suspensions in agarose, followed by lysis and enzymatic processing steps which are carried out in the sample-embedded gel. However, we dramatically accelerate the process by using electrophoresis to remove contaminants, and to exchange DNA processing reagents. Electrophoretic processing is enabled by the fact that the genomic DNA produced in our process is many megabases (mb) in size, and cannot be electrophoresed out of the sample gel. After purification and processing, the DNA is lightly and randomly cleaved with a non-specific nuclease, allowing the processed DNA sample to be electroeluted from the gel into a liquid-filled elution chamber.

Sage HLS System Concept

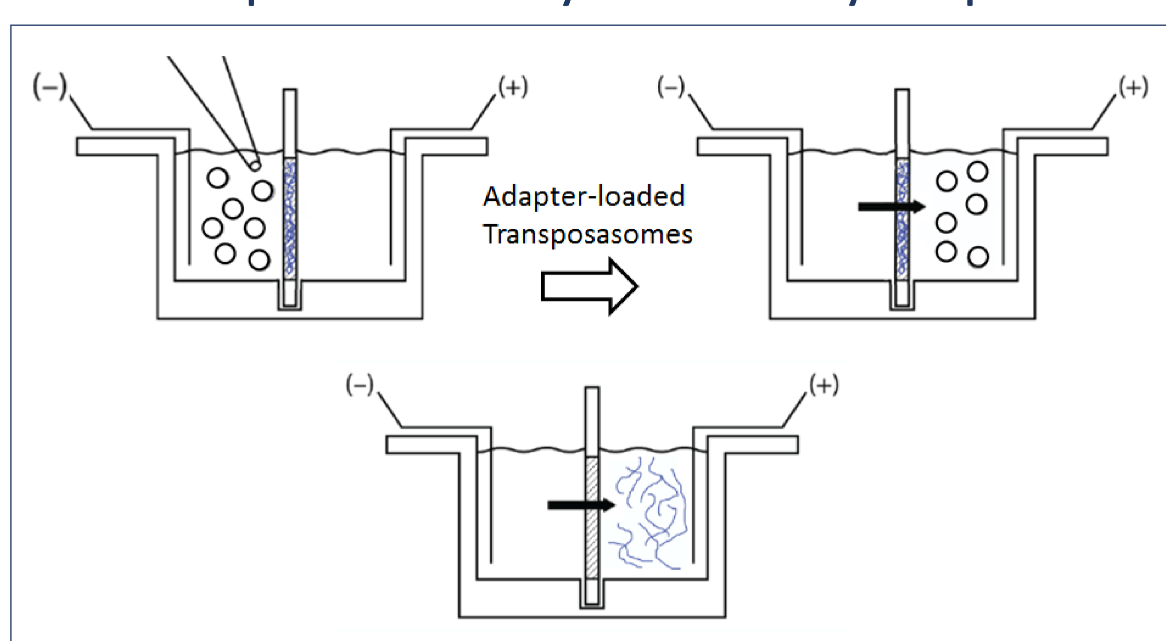
1. Accelerated DNA Purification using electrophoresis



2a. Post-purification fragmentation and electroelution by nuclease



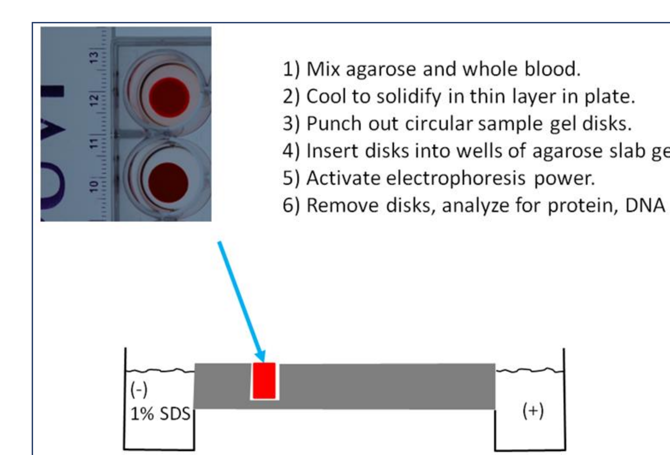
2b. Post-purification library construction by transposase



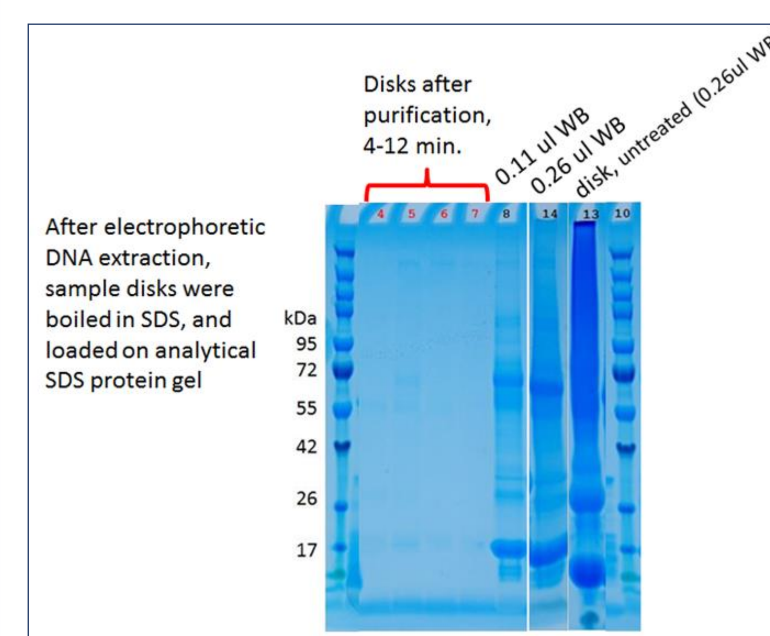
Proof of Concept

Mammalian whole blood samples were mixed with molten agarose and allowed to gel in petri dishes. Discs of agarose embedded blood were placed in the wells of horizontal agarose slab gels with SDS buffer in the upper buffer chamber and electrophoresed for 4-12 minutes. The SDS electrophoreses into the sample discs, lysing the cells, and facilitating electrophoretic purification of protein and lipids from the discs. After purification electrophoresis, the discs were boiled in SDS buffer and analyzed on SDS protein gels to evaluation removal of protein. A replicate set of purified discs were analyzed for DNA content, by inserting them into horizontal agarose gels and subjecting them to pulsed fields suitable for resolving DNA up to 165 kb in length. To some of the purified discs a small amount of non-specific nuclease was added to release the HMW DNA trapped in the purified discs.

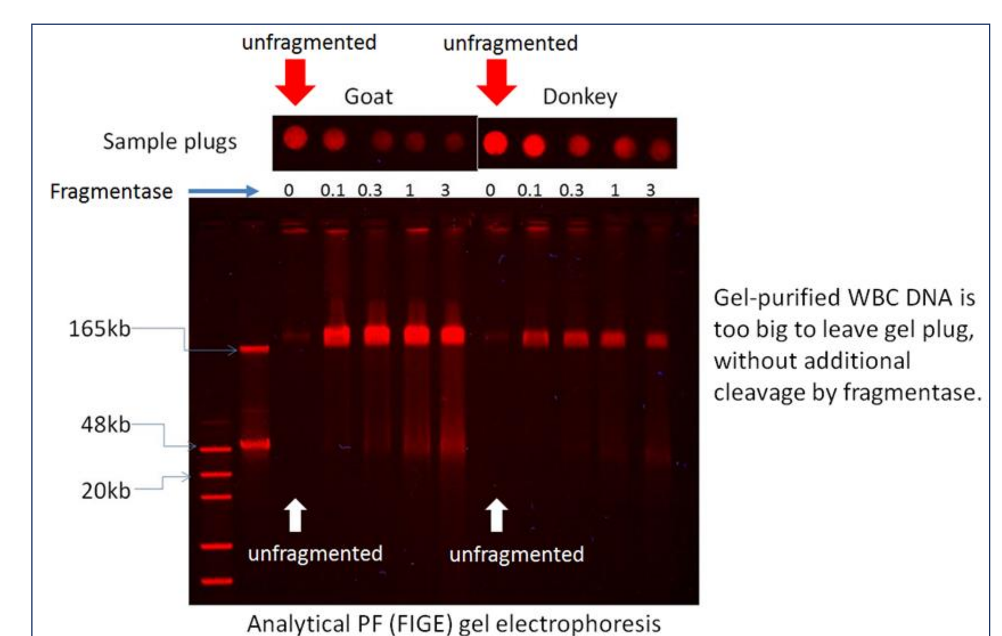
Model extraction system



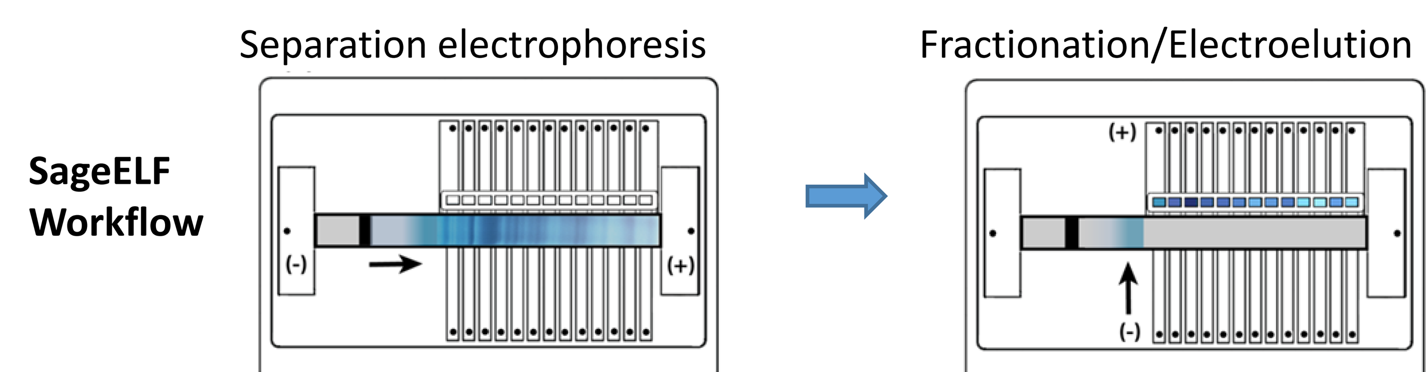
Protein content of purified discs

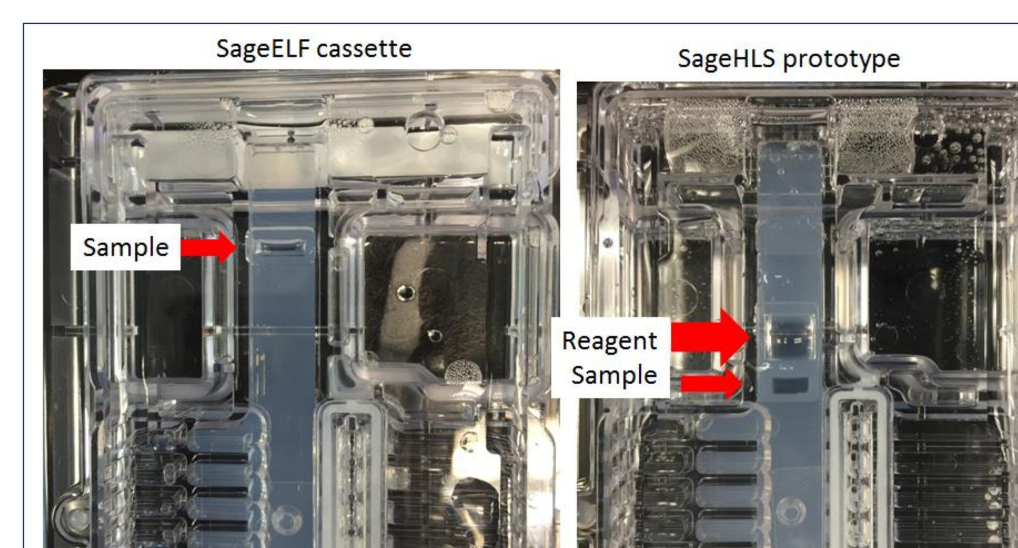


DNA content of purified discs



Sage HLS prototype based on SageELF size selection cassette

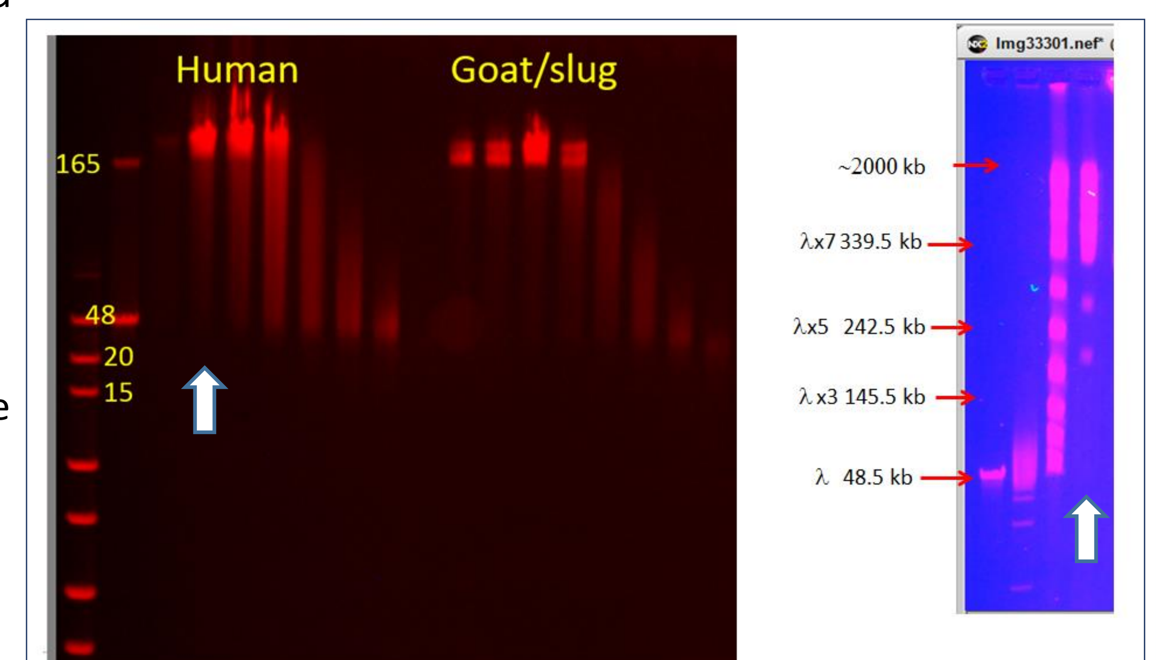




In the Sage HLS prototype, the gel-embedded sample is cast in the sample well, while lysis reagents are loaded in reagent well. For fragmentation or transposase-mediated library prep, the reagent well is emptied and refilled with nuclease or transposase reagents for the second electrophoresis step. Size fractionation and electroelution also takes place in the second electrophoresis step.

Rapid DNA extraction, fragmentation, elution in Sage HLS prototype

Human cultured cells and goat whole blood were extracted and fragmented with a restriction enzyme in a SageHLS prototype. Elution fractions 1-8 (L to R) from each HLS cassette were analyzed on a PF analytical gel. The gel at left has a HMW compression at 165kb. The sample indicated with the white arrow was analyzed in a 2nd gel with a higher size range, demonstrating that DNA up to 2 mb was recovered in lane 2 of the human HLS cassette. Similar results have been obtained using adapter-loaded transposase for fragmentation.



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