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Integrated Magneto-Electrophoresis Microfluidic Chip Purification on Library Preparation Device for Preimplantation Genetic Testing for Aneuploidy Detection

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Methods for Optimizing the automated library preparation device

Reagent volume change analysis and organization

The automated device platform utilizes flexible polyethylene tubing for liquid movement and a custom reagent plate which limits the reagent volumes that can be used. Dimensional analysis of the tubing and reagent plate were performed to ensure that reagent volumes were conducive to this scaled down device while also considering evaporation of reagents that may occur on the reagent plate. Off-device DNA library preparations were initially performed based on the dimensional analysis to evaluate the effect of reducing reagent volumes on DNA library yield. One microliter of DNA library sample was run on the Agilent Bioanalyzer 2100 machine with the Agilent DNA 1000 Kit and DNA Chip for On-Chip-Electrophoresis (Agilent Technologies, Santa Clara, CA, USA). After the reduced volumes were optimized, the protocol was analyzed to determine the organization of the reagents in the reagent plate. Since this device utilizes a two-cannula system for all reagent handling, there are no pipette tip changes throughout the whole protocol. This means that the reagent plate organization needed to be set in a way so that a cannula would not interfere with any reagents prematurely that could contaminate the DNA library sample. Additionally, this organization also included determining which cannula should handle which reagents for a successful DNA library preparation.

Analysis and Results for Optimizing the automated library preparation device

Reagent volume change analysis and organization

The PG-Seq kit library preparation involves three main steps: (1) DNA fragmentation, end repair, and adenylation; (2) adapter ligation; and (3) PCR amplification. Steps (1) and (3) of this protocol require reagent heating for proper enzymatic reactions, therefore, these reactions take place on the thermoelectric cooler. It is important that the entire liquid volume fits in the tubing adjacent to the thermoelectric cooler so that it can be heated equally throughout the reactions. The flexible polyethylene tubing in the thermoelectric cooler setup has an inner diameter of 1/16” or 1.6 mm. Using the manufacturers dimensions plus or minus the specified tolerances, the maximum reagent volume that can fit on the 40 ± 0.25 mm wide thermoelectric cooler was calculated to be (80 ± 0.5) µL. Another dimensional constraint to the maximum volumes possible for this device platform was the size of the reagent wells on the custom reagent plate used. These wells were cylindrical in shape with the bottom of the well curved, representing one-half of a sphere. With a radius of 1.75 mm and total height of 8 mm and both dimensions having a tolerance of ± 0.25 mm, the maximum volume that can be contained on the reagent plate was calculated to be (71 ± 20) µL. Therefore, it was determined that the reagent plate well volume would control the maximum volumes used on the device.

One more important consideration used in the reagent volume design process was the amount of evaporation occurring while the reagent plate was open to the atmosphere during the protocol. Strategies to reduce the effects of evaporation include reducing time scales ($t_{reaction} << t_{evaporation}$), changing device geometries to reduce the ratio of exposed areas to volumes, or adding a higher boiling point component to reduce the vapor pressure in the reagent[37]. Another strategy, which was used in this application, is adding sacrificial water around the liquid of interest[37]. This latter strategy was adapted in this case by adding sacrificial nuclease free water to the reagents instead. Initially, 2 µL of sacrificial water was added to reagents to reduce the proportion of reagents that would be evaporated from each well. The amount of sacrificial water added was then adjusted

based on the equation for evaporation rate and the thin-film model of mass transfer between the liquid and bulk vapor phases, which assumes that the liquid concentration changes slowly over time[38]. The time dependent process of this transport is described by Supplemental Equation 1 where the number of moles of component i, N_i, changes over time, t, and this is in balance with the interfacial surface area, A, and molar flux, j_i, of i in moles per area per time. The rate of evaporation will be the flux at the interface.

$$\frac{dN_i}{dt} = -Aj_i \qquad \text{(Supplemental Equation 1)}$$

Therefore, it was determined that reagents being used later in the protocol would receive 3 µL or 4 µL of sacrificial water per reagent well since more evaporation would occur by the time that they are used in the protocol. Nuclease free water was used because it would not contaminate any reagents and as the nuclease free water evaporates it will minimize the proportion of the volume lost from each reagent[37].

The last consideration for determining the final volumes used in the complete automated device procedure was the two buffer exchanges that occur during the DNA purification steps on the microfluidic chip. There are two steps in this workflow where reagents are added together before the DNA library is purified from these reagents. The post-ligation cleanup includes reagents from DNA fragmentation, end repair, and adenylation; adapter ligation; and the first Purification Bead mix. The post-amplification cleanup includes reagents from PCR amplification and the second Purification Bead cleanup. Therefore, the total volume of these mixes plus the sacrificial water needed to fall within the reagent plate maximum allowance of (71 ± 20) µL, also taking into consideration the evaporation that will occur over time.

Table 3 in the main text outlines the final reagent volumes used in this procedure on-device vs. the standard off-device manual sample preparation volumes provided in the manufacturer's instructions. In the original manual protocol, 50 µL of total volume is used for the DNA fragmentation, end-repair, and adenylation step as well as for the adapter ligation step. For the on-device protocol, the 1:1 ratio of these reagents was maintained by using ~1/3 of the volume of each at 18 µL. The only difference in this ratio being that the barcoded adapters were included in the 50 µL ligation mix volume off-device but not on-device to avoid reducing the already small volume as much as possible. Additionally, off-device there was a 1:1 ratio of reagents to Purification Bead mix for the first wash at 100 µL:100 µL. This was nearly maintained on-device at 38 µL:36 µL but adjusted slightly because the barcoded adapters were not included in the 18 µL reagent volume for the ligation mix. The total volume of these reagents when they were combined was 74 µL which falls within the range determined via dimensional and evaporation analysis. Following the first microfluidic chip purification step, the purified DNA library was eluted into the Resuspension Buffer and combined with PCR primers and PCR master mix for library amplification. The PCR master mix volume was reduced by 5 µL from the manufacturer's instructions to maintain the proportion of the mix to the Resuspension Buffer containing the DNA due to evaporation. The PCR primers were kept at 2 µL because reducing that volume could lead to less precise reagent plate loading. The Purification Bead mix was again 36 µL for this second cleanup because this agreed with the 0.9× bead to reagent volume ratio from the manufacturer's instructions off-device, again accounting for reagent evaporation. The total amount of reagents combine before the second microfluidic chip purification post-amplification was calculated to be 83 µL based on the input reagent volumes. This total amount was again within the desired range of (71± 20) µL.

Both standard and reduced reagent volumes (Table 3) were initially tested off-device through manual preparation before translation onto the automated platform. Results were analyzed using

the Agilent Bioanalyzer 2100 and showed an average with standard deviation of 29.34 ± 2.17 ng/µL of library yield for the large volumes specified by the manufacturer and 38.65 ± 1.59 ng/µL of library yield for the small volumes adjusted to be used on the device. These libraries were prepared from 500 ng and 180 ng of Lambda DNA, respectively (New England Biolabs, Ipswich, MA, USA). In addition to these new volumes being better suited to the dimensions of this device, end users also benefit from cost savings for reagents since most reagent volumes have been reduced.

After the optimal volumes were established for the on-device automated platform, the reagent plate was designed so that the two-cannula system could handle all reagents without any contamination of the final DNA library. One cannula (Cannula 1) was selected to handle both Purification Bead mixes, while Cannula 2 manipulates all purified DNA. The goal was to separate any reagents that could negatively affect or contaminate the prepared sample through residual reagents remaining in the tubing or submerging in an unwanted reagent in the reagent plate prematurely since the cannulas move in parallel. Table 4 in the main text provides the well number and associated reagent used for this assay as well as which cannula manipulated that reagent. The bead mix used in the post-ligation cleanup was optimized to account for the viscous Ligase Buffer Mix used. To ensure all Purification Beads were able to make it through the microfluidic chip, 65% of the mixture was 0.05% TWEEN to provide a surfactant to aid in bead movement. Additionally, 35% of the mixture was Purification Beads but at a doubled concentration to increase the magnetic field of the beads to help improve their movement by the magnet. This was done by taking 70 µL of Purification Beads, collecting them with a magnet, removing 35 µL of the buffer, and resuspending the now concentrated bead mix. No alterations needed to be made to the post-amplification Purification Bead mix. Lastly, wash steps for the tubing were considered to eliminate any unwanted enzymes that were carried over from previous reactions that would negatively affect upcoming reactions in the DNA library preparation process, to improve the quantity and quality. This included rinsing Cannula 1 tubing containing the Fragmentase Enzyme after the fragmentation step to reduce any cross-contamination. The wash buffers 0.5 M EDTA (Thermo Fisher Scientific, Waltham, MA, USA) then nuclease free water (IDTE, Coralville, IA, USA) were utilized as an alternative method for inactivating the Fragmentase Enzyme as opposed to heat. Agilent Bioanalyzer results were critical in performing quality control for the prepared DNA libraries prior to sequencing.