

Automated DNA Gel Extractions: Higher Quality Library Preparation using Microfluidics

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Introduction

Current next generation sequencing workflows have numerous manual processes that bottleneck throughput and contribute to process inefficiency. One of the most time consuming tasks is gel-based size-selection during the library generation process. For example, the Illumina standard operating procedures^{1,2} for preparing paired-end libraries calls for running the fragmented sample on an agarose gel, excising a small piece of the separated sample with a scalpel, and then using solid phase extraction to purify the selected nucleic acid. In addition to being labor intensive, this method introduces run-to-run and operator-to-operator variability into the library creation process. Furthermore, the imprecision of this manual excision results in the size selection precision (width of the selected band) being typically no better than $\pm 10\%$ of the median fragment size (e.g. 400 bp ± 40 bp).

Caliper Life Sciences has developed and commercialized instruments that utilize microfluidics^{3,4} to achieve rapid and high resolution electrophoretic separations. While we have focused most of our efforts on analysis applications (e.g. DNA, RNA and protein sizing), we have also previously demonstrated preparative applications, such as fractionation of nucleic acid for cloning⁵. We are now developing a commercial instrument that will simplify and improve nucleic acid fractionation.

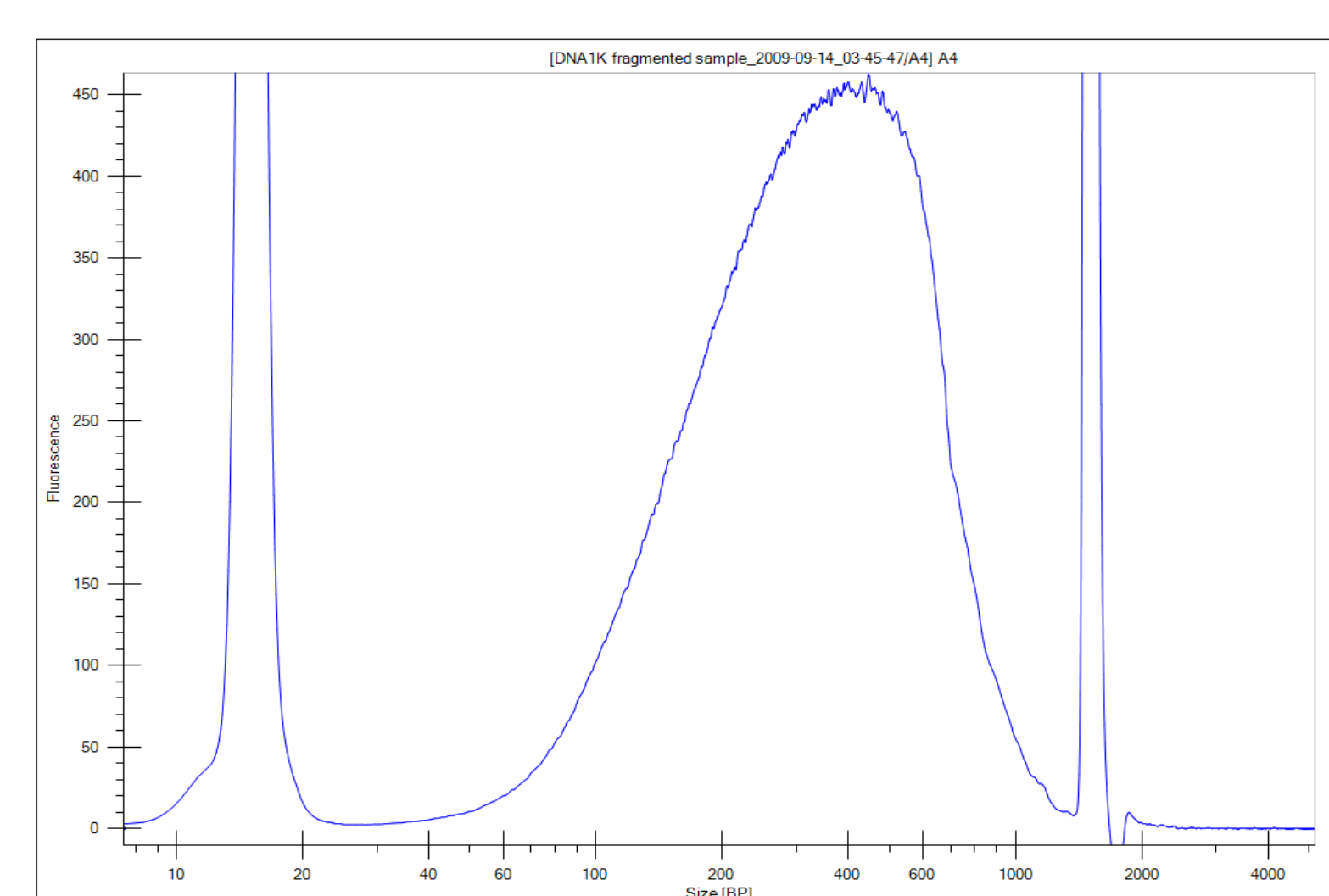
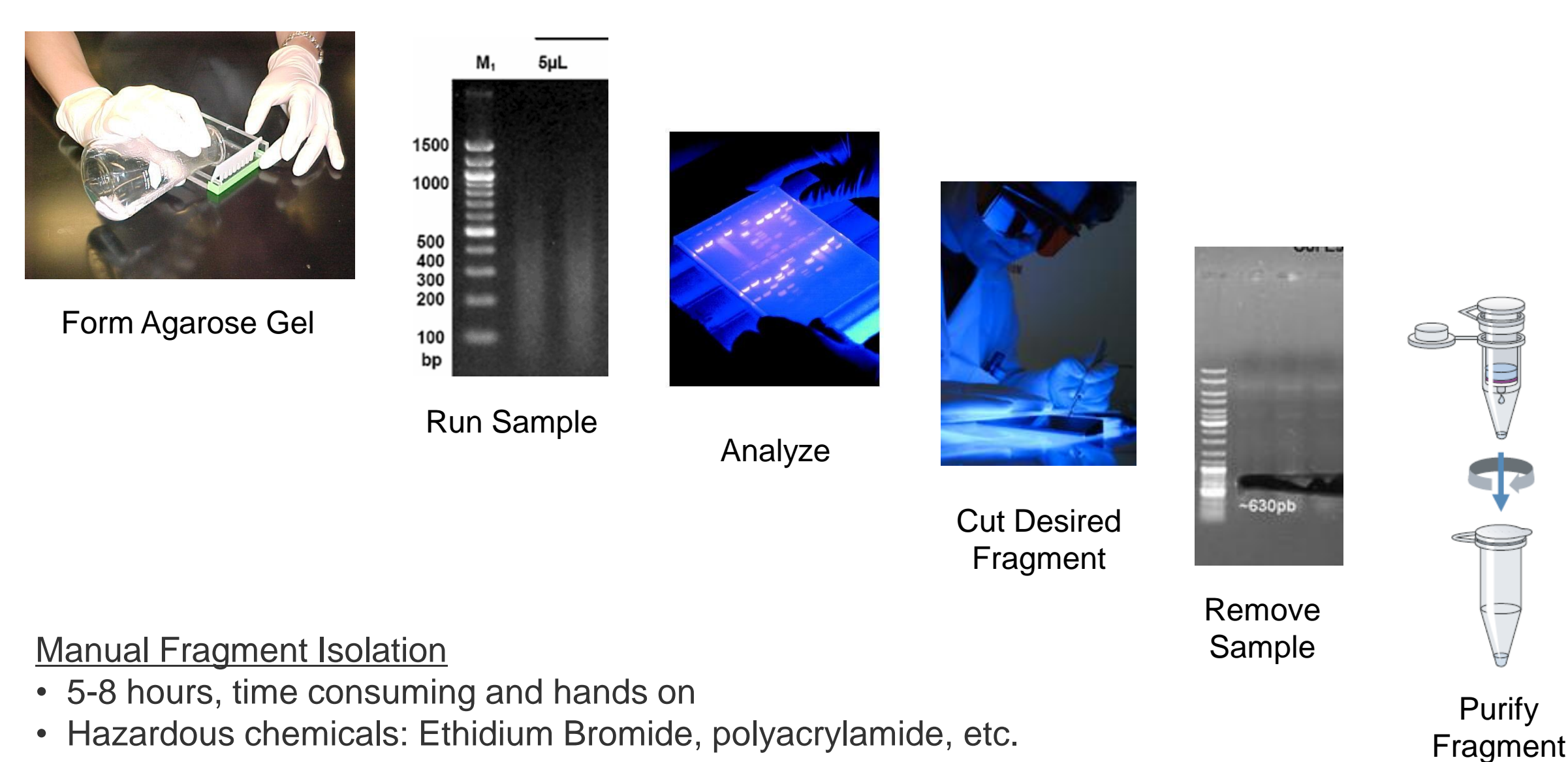


Figure 1. Typical genomic smear as visualized on a Caliper GX system.

Figure 1 shows an electropherogram of a typical smear of fragmented DNA. As part of most next generation sequencing workflows, a band of material must be selected from such a smear. Typically this is done by excision of a band from a slab gel (see Figure 2). This process is tedious and subject to operator-to-operator and run-to-run variation. We aim to replace this process using a LabChip[®] device that will automate and make the fraction collection process more reproducible and efficient.

Current Fragment Isolation Workflow



Manual Fragment Isolation

- 5-8 hours, time consuming and hands on
- Hazardous chemicals: Ethidium Bromide, polyacrylamide, etc.
- Not reproducible and inaccurate
- High samples loss

Figure 2. Typical fractionation workflow. First a gel is formed, the sample is separated and then analyzed. Next a desired band is excised followed by purification of the excised material.

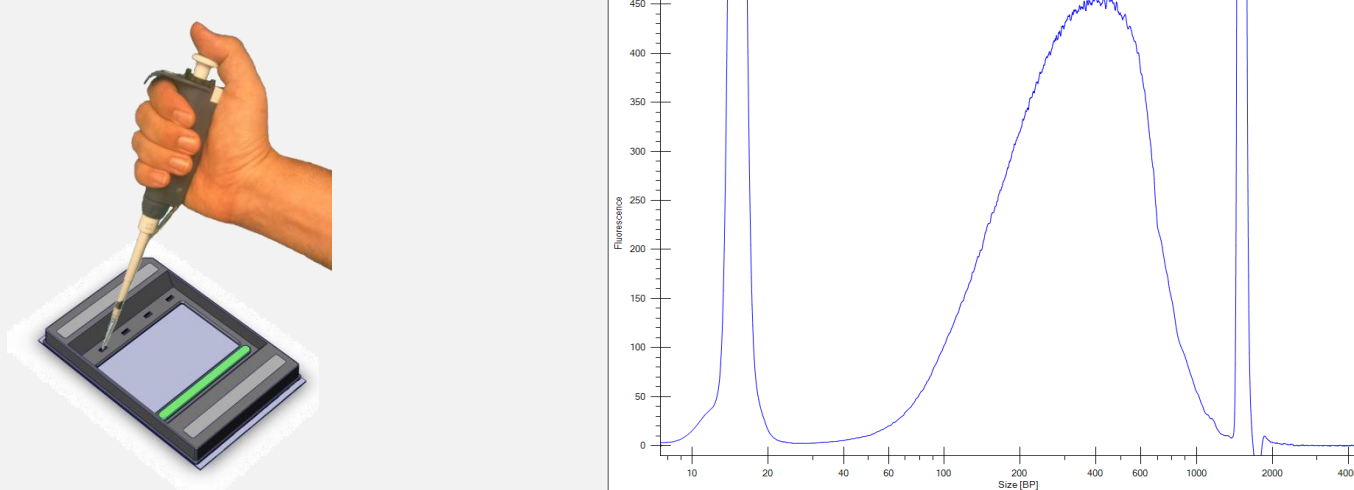
Materials, Methods, and Channel Design

Channel Design

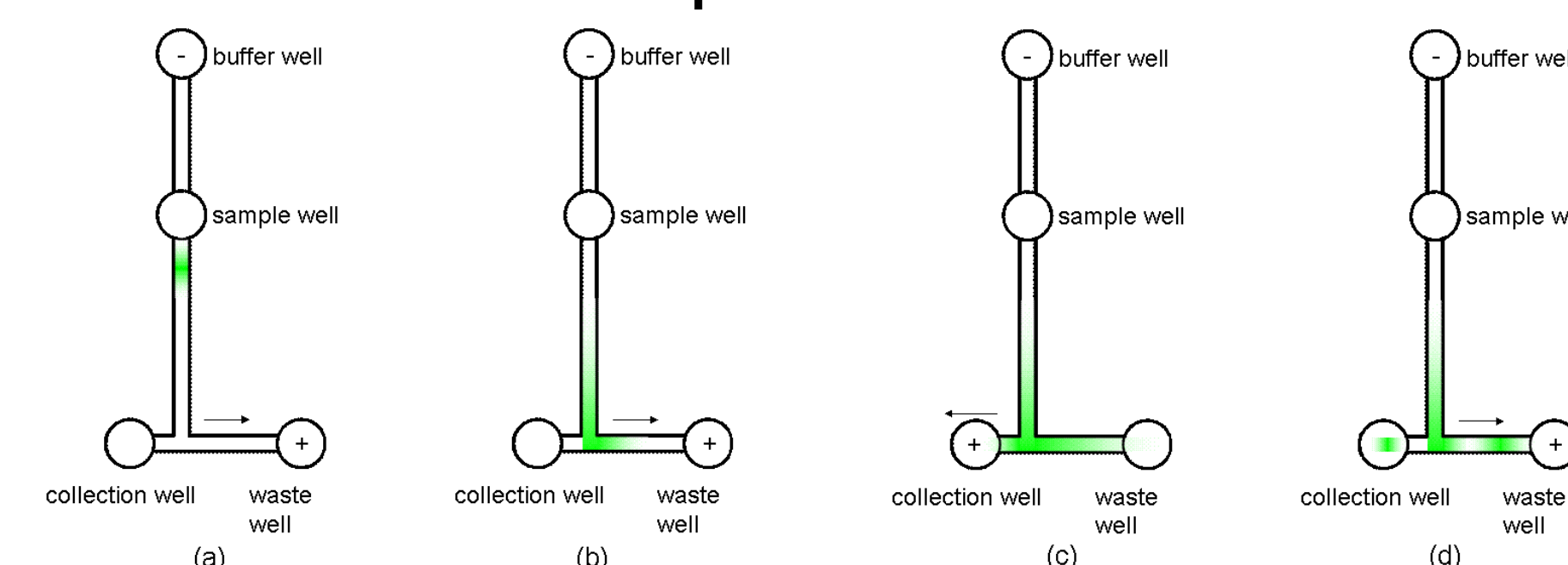
The LabChip fractionation device uses intersecting microfluidic channels to isolate a fragment of DNA from a smear. Figure 3 describes the operation of the device.

LabChip Approach Automated Separation, Analysis & Purification

1. Load Sample



2. Separate and Collect – on chip



3. Remove Sample

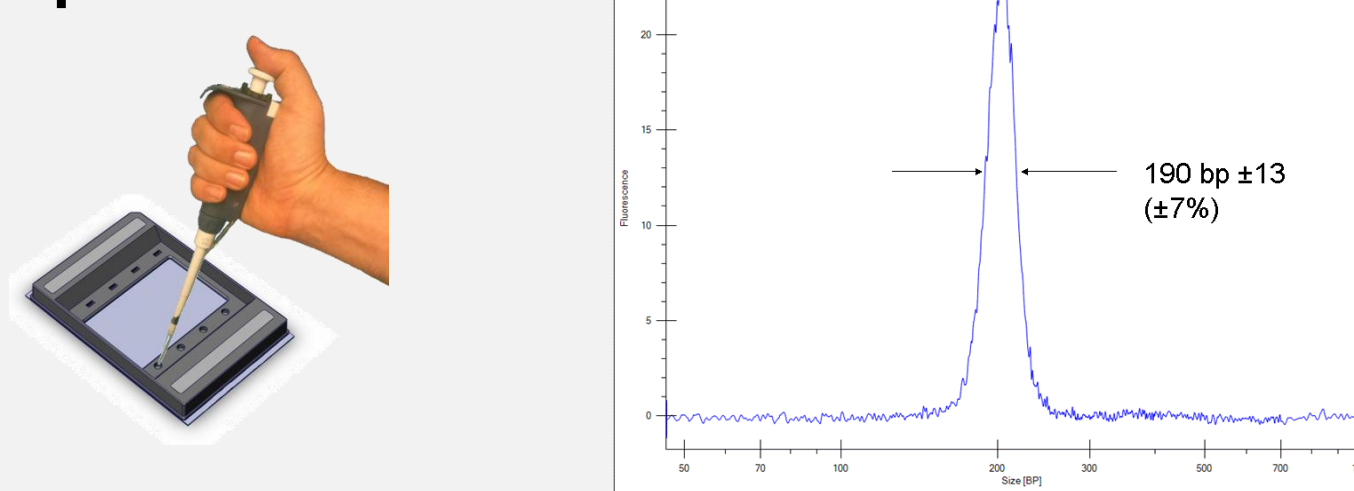


Figure 3. Workflow of LabChip[®] fractionation device. In step 1, the fragmented sample is loaded onto the chip. Step 2 comprises separation and fragment collection. In step 2a, separation begins. In step 2b, nucleic acid is routed towards the waste well. In step 2c, voltage is applied between the buffer well and collection well, driving nucleic acid towards the collection well. In step 2d, voltage is again applied between the buffer well and the waste well, leaving a small band isolated in the collection well. In step 3, the user removes the isolated fragment from the collection well.

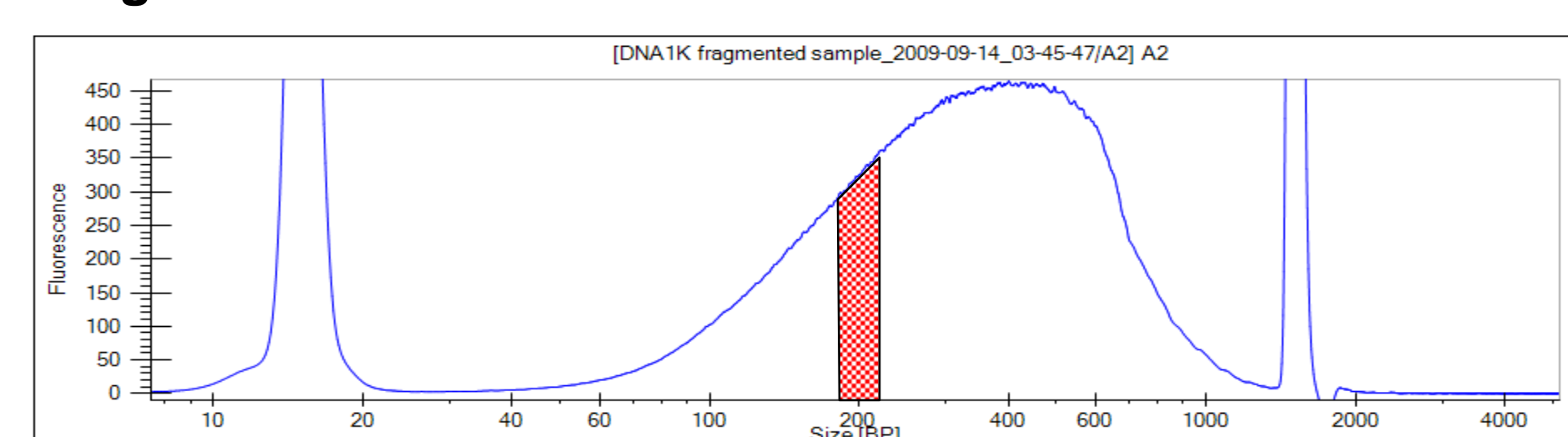


Figure 4. A depiction of an electropherogram highlighting the region defining the isolated sample.

Materials

Caliper LabChip[®] fractionation prototype containing sieving matrix, separation buffers and DNA staining dye

Human Genomic DNA purchased from Biochain (www.biochain.com) and fragmented in-house.

50% Glycerol in water, 99% Glycerol for molecular biology (Sigma)

1X Tris-Acetate-EDTA (TAE) buffer

Caliper LabChip[®] GX instrument, DNA 1K chip and reagent kit

Stanford Research Systems, Inc. Model PS 350/5000V-25W, High voltage supply

Methods

Approximately 4 μ L of fragmented DNA was mixed with 1 μ L a glycerol containing sample buffer and loaded into the sample well (see schematic in Figure 3). Approximately 15 μ L of TAE buffer was added to the collection well. The chip was connected to the high voltage power supply and custom designed circuitry. Voltage was applied to begin separation of the fragmented DNA and migration towards the waste well. At the appropriate time (depending on the desired fragment size), voltage was switched to the collection well causing migration of a plug of sample to the collection well. After an appropriate period of time (depending on the desired band width) the voltage was switched back to the original configuration.

Results and Discussion

Figure 5 shows typical fragments after collection from the LabChip[®] fractionation device. In this case, the isolated fragments have an apparent size of 113 bp, 186 bp, and 305 bp, and have a standard deviation of 5 bp ($\pm 4.2\%$), 12 bp ($\pm 6.7\%$), and 19 bp ($\pm 6.3\%$), respectively. By comparison, bands extracted from a gel typically are no tighter than $\pm 10\%$. Thus, the LabChip[®] fractionation device offers the benefit of tighter size distribution. The commercial system will also offer the benefits of improved convenience and reproducibility.

Figure 6 is a plot of the extracted band size versus band width for 14 different fractionations. The slope of the fit curve is approximately 6% showing that narrow extracted bands can be achieved over a range of approximately 100-500 bp.

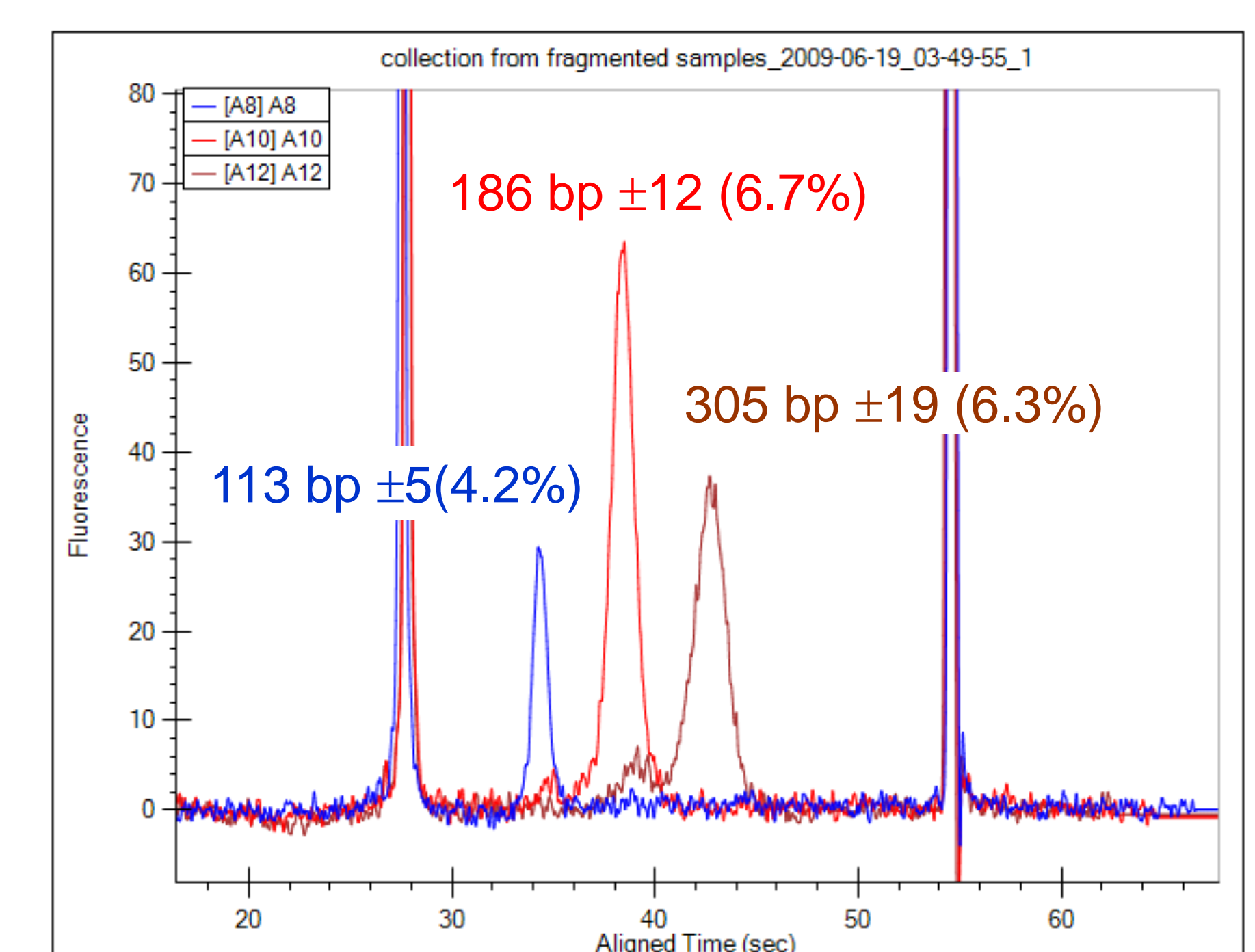


Figure 5. Typical fractionated DNA band as measured on a Caliper LabChip[®] GX instrument

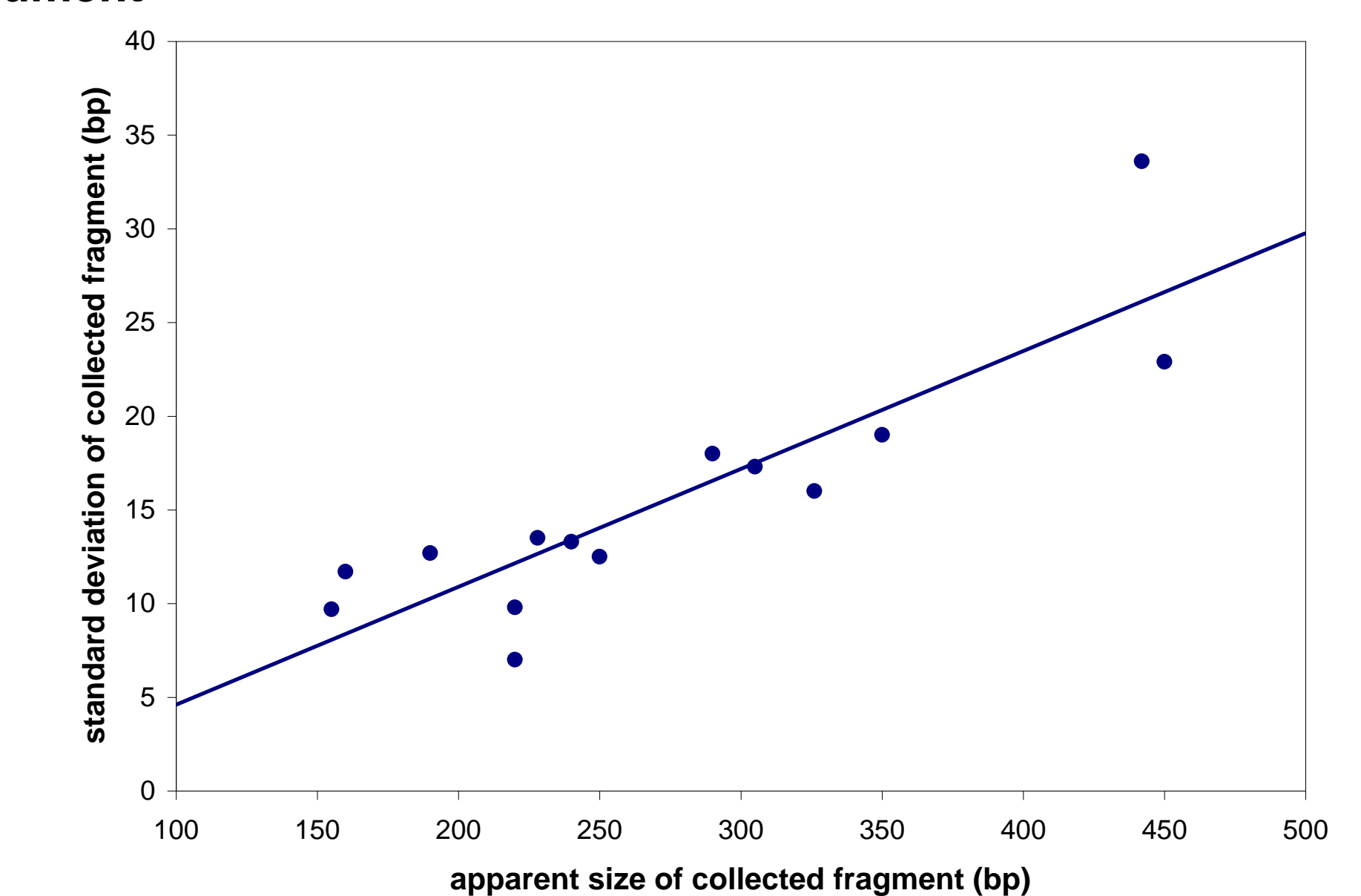


Figure 6. Plot of extracted band width versus apparent size of the collected fragment (as determined by a Caliper LabChip[®] GX instrument).

Conclusion

The LabChip[®] fractionation device uses a microfluidic network to extract a target band during separation and route the selected material to a collection well. Using this process we have successfully isolated narrow bands in the range of 100-500 bp from a fragmented DNA sample. The isolated nucleic acid is PCR-compatible and the fractionation operation is complete in 30-60 minutes.

References

1. Sample Preparation for Genomic DNA Paired End Libraries, www.illumina.com
2. Quail et al., "A large genome center's improvements to the Illumina sequencing system," *Nature Methods*, 2008, 5(12), 1005-1010.
3. Reyes et al., "Micro Total Analysis Systems. 1. Introduction, Theory and Technology," *Anal. Chem.*, 2002, 74(12), 2623-2636.
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5. Minalla et al., "Automated DNA Fraction Collection on Glass Microchips," *Micro Total Analysis Systems* 2002, volume 2, 946-948.