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# Quality Control of the Next-Generation Sequencing (NGS) Library through an Integrated Digital Microfluidic Platform

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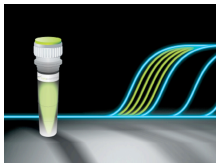
## Quality of the DNA library input dictates degrees of informative sequencing results, and thus amount of genomic and biological knowledge obtained through the high-throughput NGS technology.

Current library QC methods (i.e. Agilent BioAnalyzer, and qPCR) are inefficient, incompatible, and not well-integrated with the library sample preparation workflow. Unnecessarily higher amount of library than needed is often prepared simply for QC purposes, leading to biases in the library sample population.

*BioAnalyzer (requires at least 1 μL)*



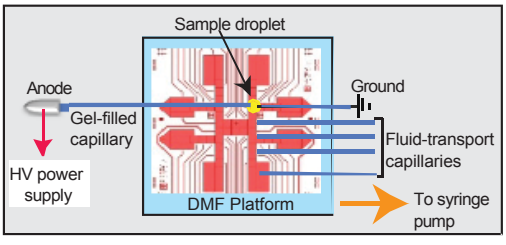
*Kapa qPCR Library Quant Kits (no information on size-distribution)*

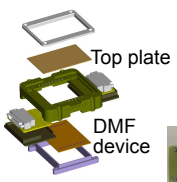
## Integrated droplet-based microfluidic QC platform is developed to streamline sample preparation process.

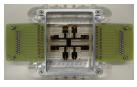
**Goal**: a platform that enables efficient use of sample as well as accurate, full characterization of the library.

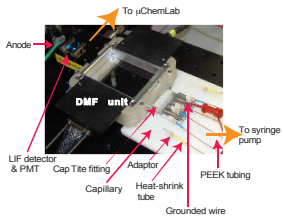
**Approach**: a hub strategy is employed to develop an automated integrated digital microfluidic-capillary electrophoresis (DMF-CE) platform.

- Low-volume and high-sensitivity sample analysis
- Flexible and easily integrated with other DMF-based sample processing modules



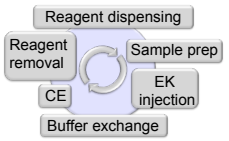



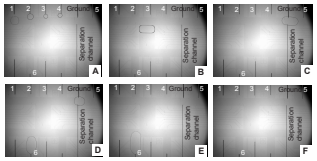





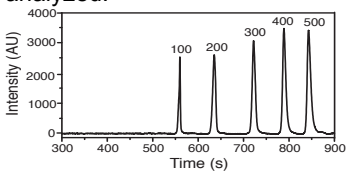

## Reliable and sensitive performance

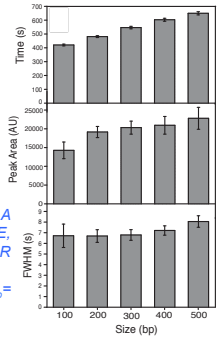
*Automation*: continuously perform multiple runs without manual reagent replacement.

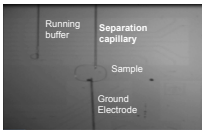


*Repeatability*: robust DMF-capillary interface allows sample droplets to be reproducibly prepared and analyzed.



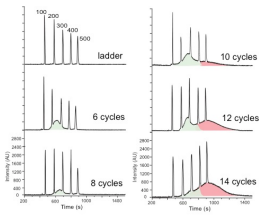

- *100 pg/μL dsDNA ladder in 0.002x TBE, 0.01% Pluronic, SYBR Green I®*
- *$E_{inj}$ = 55 V/cm, $E_{sep}$ = 100 V/cm*

*Sensitivity:*

- Require <1 μL, suitable for small scale library prep
- Sensitivity level of 5-100 pg/μL, appropriate for Illumina sequencing platforms

## *illumina*-compatible library QC & characterization

*Quality Control*: size and concentration determination of NGS library prepared from Nextera™ transposase-fragmented PBMC (peripheral blood mononuclear cells) gDNA using PicoGreen® on a DMF-CE device.




| | DMF-CE | BioAnalyzer |
|---|---|---|
| 6 cycles | 380 pg/μL | 380 pg/μL |
| 8 cycles | 220 pg/μL | 280 pg/μL |

*Over cycling of PCR (as few as 2 rounds) during sample preparation process can result in unfavorable larger fragments (>400bp), biasing the library population distribution.*

**This integrated droplet-based DMF-CE platform provides a significant step toward a completely automated and streamlined NGS library preparation.**

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