

# LabChip GX Touch DNA NGS 3K Assay Comparison

## Microfluidics

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### Introduction

The LabChip® GX Touch delivers rapid electrophoretic separation providing accurate high-throughput nucleic acid quantitation, even at low concentrations using the NGS LabChip DNA NGS 3K Assay. Multiple assays are also available to assess genomic DNA integrity, DNA smear, and DNA/ RNA fragments. The ability to resolve size distribution and quantitation of low concentrated libraries or smear samples allows for accurate normalization, pooling, or quality control during library preparation.

The LabChip DNA NGS 3K Assay was developed for Next-generation Sequencing (NGS) customers to assess size distribution and quantity of extremely low input concentration DNA samples often associated with difficult or low-yielding library preparations. With this assay, the increased sensitivity performance enables robust quality assessment of low concentration libraries with minimal sample consumption (1 µL). In this study, we evaluated the LabChip DNA NGS 3K Assay performance with a similar competitive assay (*Competitor A*). We challenged both assays with the same samples (commercial DNA ladder and Covaris® sheared DNA) and compared assay performance for concentration accuracy, precision, and sensitivity. The LabChip DNA NGS 3K Assay demonstrated higher concentration accuracy and tighter reproducibility.

## Assay Overview and Experimental Setup

The LabChip DNA NGS 3K Assay performance specifications were compared to those of *Competitor A*. Both assays target NGS customers for sample QC prior to sequencing (Table 1). The table shows similar specifications on linearity range, concentration accuracy/precision, and sizing accuracy/precision (all the concentrations listed below are input concentrations).

**Table 1.** Comparison of specification performance of LabChip DNA NGS 3K Assay and *Competitor A* Assay

Specifications	LabChip DNA NGS 3K Assay	<i>Competitor A</i> Assay
Concentration Linearity	Smear: 50 – 5000 pg/μL Fragment: 5 – 500 pg/μL	Smear: 50 – 5000 pg/μL Fragment: 5 – 1000 pg/μL
Sensitivity	Smear: 25 pg/μL Fragment: 2.5 pg/μL	Smear: 50 pg/μL Fragment: 5 pg/μL
Quantification Accuracy	30 %	25 %
Quantification Precision	20 %	15 %

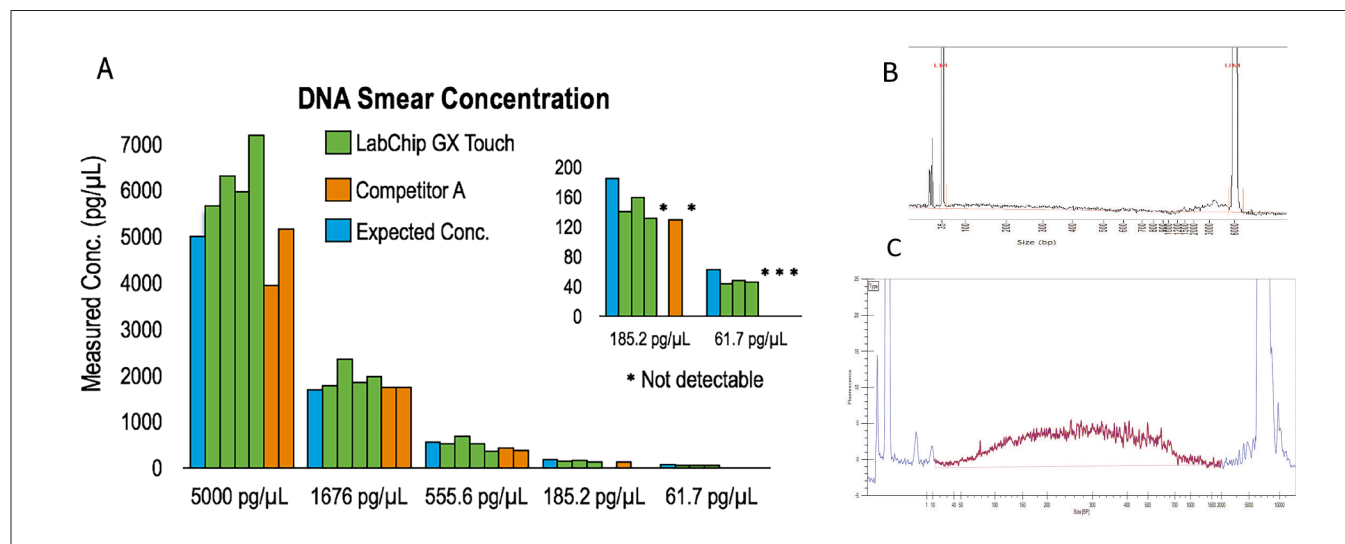
Within this study, three types of samples were tested: a 1:3 titration from 500 to 2 pg/μL of a commercially available ladder, a 1:3 titration from 5000 to 20 pg/μL of a mechanically sheared DNA smear, and eight replicates of a mechanically sheared DNA smear at 1000 pg/μL. DNA sheared in this study

was prepared using a Covaris® Focused-ultrasonicator. All three types of samples were evaluated in triplicate on both platforms using identical input and diluted samples to minimize batch variability. Samples were prepared according to manufacturer's instructions and performed on their respective instruments using the manufacturer's suggested run protocol. For *Competitor A*, triplicate samples were prepared by adding 6 μL of sample to 66 μL of marker diluent, then aliquoting into three identical sample sets. In contrast, and to challenge the reproducibility of the LabChip DNA NGS 3K Assay, the LabChip GX Touch triplicate samples were each prepared by different users.

## Results

### Titration of Sheared DNA Smears

As shown in Figure 1A, smear concentrations derived from LabChip GX Touch and *Competitor A* are comparable at higher concentrations (> 60 pg/μL). However, some of the DNA smear samples at lower concentrations (< 60 pg/μL), which were detectable on the LabChip GX Touch platform, were not detectable on the *Competitor A* platform (Figure 1B and 1C). We also calculated concentration accuracy as well as precision of the smear samples (see Table 2). The LabChip DNA NGS 3K Assay demonstrated higher concentration accuracy and tighter reproducibility.



**Figure 1.** A. Comparison of the measured concentrations of DNA smears obtained from LabChip GX Touch and *Competitor A* platforms. B) Electropherogram of smear DNA at 61.7 pg/μL from *Competitor A*. The higher signal prior to the upper marker is present in all analyses, regardless of the nature of the sample. C) Electropherogram of smear DNA at 61.7 pg/μL from LabChip GX Touch instrument.

**Table 2.** Comparison of concentration accuracy and precision of the smear DNA samples run on LabChip GX Touch and *Competitor A* platforms (absolute concentration accuracy higher than 30% and %CV higher than 20% are highlighted in red; N/D: not detectable).

Expected Concentration (pg/μL)	LabChip DNA NGS 3K Assay				Competitor A			
	Concentration Accuracy (performance spec = 30%)			Concentration Precision	Concentration Accuracy (performance spec =25%)			Concentration Precision
	1st	2nd	3rd		1st	2nd	3rd	
5000	13%	26%	19%	5.34%	43%	- 21%	3%	32.05%
1676	6%	40%	10%	15.78%	18%	3.7%	4.1%	8.87%
555.6	- 8 %	22%	- 6%	16.29%	-36 %	- 24%	- 31%	26.88%
185.2	- 24%	- 14%	- 29 %	9.83%	N/D	N/D	N/D	N/D
61.7	- 29 %	- 23%	- 26%	4.25%	N/D	N/D	N/D	N/D

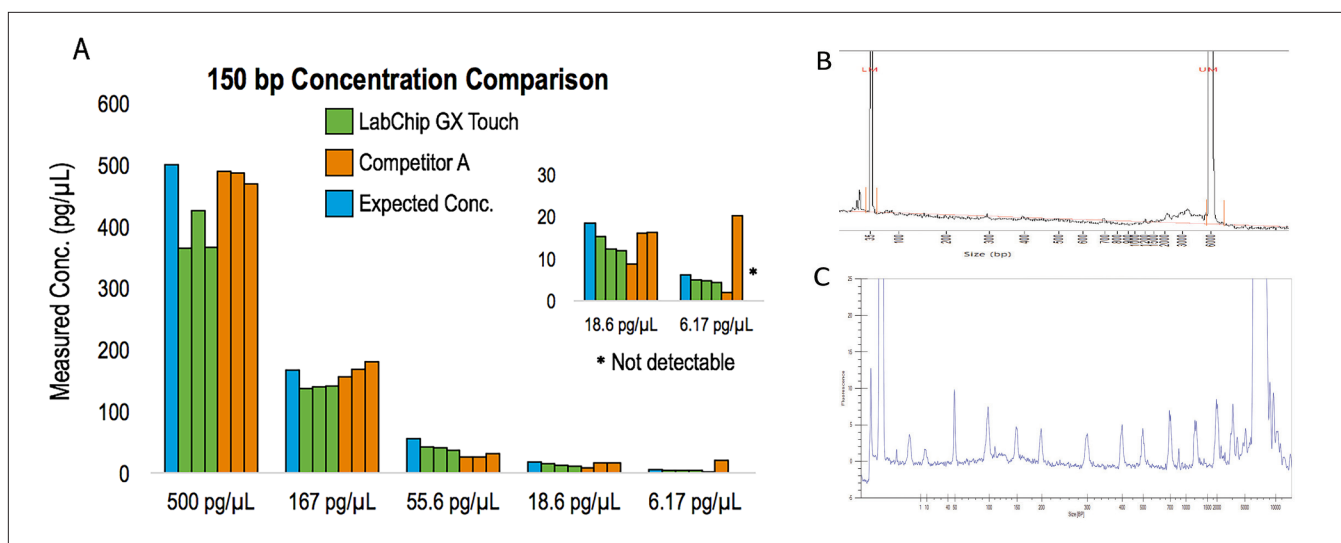


Figure 2. A. Comparison of the measured concentration of DNA smear titration obtained from LabChip GX Touch and Competitor A platforms. B) Electropherogram of commercial ladder at 2 pg/μL from Competitor A. C) Electropherogram of commercial ladder at 2 pg/μL from LabChip GX Touch instrument.

Table 3. Comparison of concentration accuracy and precision of the 150 bp fragment run on LabChip GX Touch and Competitor A instruments (absolute concentration accuracy higher than 30% and precision higher than 20% are highlighted in red; N/D: not detectable).

Expected Concentration (pg/μL)	LabChip DNA NGS 3K Assay				Competitor A			
	Concentration Accuracy (performance spec = 30%)			Concentration Precision	Concentration Accuracy (performance spec = 25%)			Concentration Precision
	1st	2nd	3rd		1st	2nd	3rd	
500	-20%	-10%	-20%	7%	-2%	-3%	-6%	2%
167.6	-9%	-5%	-4%	3%	-7%	1%	9%	8%
55.6	-10%	-9%	-15%	4%	-54%	-52%	-42%	13%
18.5	-4%	-12%	-14%	6%	-53%	-13%	-12%	31%
6.17	-7%	-5%	-5%	1%	-66%	229%	N/D	N/D

## Titration of Commercial Ladder

Prior to sequencing, it is imperative to verify if discrete fragments such as adapters or primer/dimers are present in the prepared libraries. We compared the profiles of a 150 bp fragment at concentrations ranging from 500 pg/μL to 2 pg/μL. Figure 2A illustrates greater variation in concentration accuracy in the Competitor A assay profile when the concentration of the samples goes below 18 pg/μL. Figures 2B and 2C show that the LabChip DNA NGS 3K Assay yields a clear signal at 2 pg/μL, whereas discrete peaks are not observed for the Competitor A assay profile.

## Replicates of DNA Smear at 1000 pg/μL

Reproducibility of the DNA NGS 3K Assay and Competitor A assay was evaluated with eight replicate samples, performed in triplicate. As shown in Figure 3, the run-to-run and sip-to-sip variability on the DNA NGS 3K Assay is lower than the Competitor A assay; 12% CV vs. 15% CV, respectively. Moreover, the average measured concentration of the 1000 pg/μL DNA smear on LabChip GX Touch is 930 pg/μL, which is more accurate than the 840 pg/μL measured using the Competitor A assay in its respective platform.

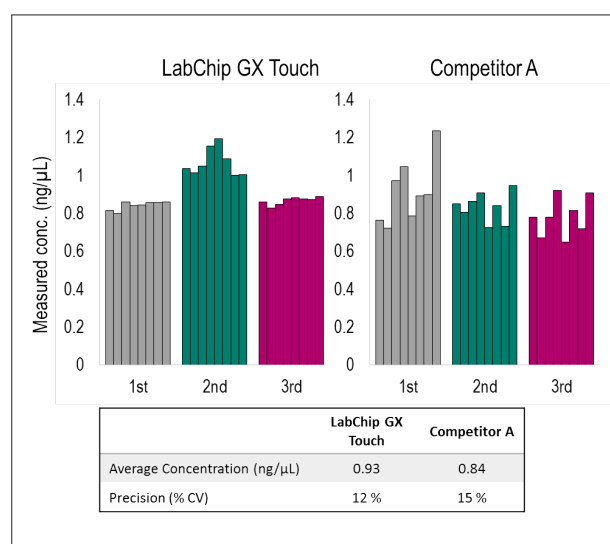


Figure 3. A. Comparison of replicate runs (n=8) of DNA smear at 1000 pg/μL on both the LabChip GX Touch and Competitor A platform.

## Conclusions

In this technical note, we have demonstrated that the LabChip DNA NGS 3K Assay on the LabChip GX Touch platform provides benchmark performance for quality assessment of extremely-low concentration samples. Performance was shown to be comparable or superior to a competitive assay on its respective platform in several key areas: concentration accuracy and precision, reproducibility and assay sensitivity. Sizing accuracy and precision are also comparable (data not shown; available upon request). With a sample volume requirement of only 1 microliter, the LabChip DNA NGS 3K Assay provides precise quantitative concentration measurements, essential where DNA quantities are limited. This assay is ideal for the QC/QA of NGS libraries, and where libraries are usually at low concentrations, such as ChIP-Seq, ultra-low Input and PCR-free protocols.

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