APPLICATION NOTE

Inter run precision analysis of plasmid DNA conformation.

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Introduction

Plasmids are vital for producing viral vector, mRNA, and DNA-based therapies and vaccines. The plasmids used in these therapies have to meet stringent key quality attributes including plasmid isoforms distribution i.e. amount of supercoiled vs linear vs open circular, these requirements have become part of regulatory framework around plasmid production and use (2). There is a gap in analytical methods that can provide isoform quantification with good inter run precision. Inter run precision of plasmid DNA was analyzed using the LabChip Plasmid DNA Assay (part number-CLS160450) on the LabChip™ GXII Touch™ platform. The LabChip plasmid DNA assay can analyze different plasmid forms (supercoiled, linear, and open circular), providing valuable data including area percentage, and sizing for supercoiled and linear isoforms in a single run. This is crucial for downstream applications like cloning or gene expression as it saves time and increases throughput. The platform provides a robust and repeatable method for plasmid analysis, ensuring reproducibility and reliability.

Inter-run precision of supercoiled plasmid DNA for area percentage, and supercoiled and linear sizing using various plasmid samples, across multiple runs performed by different analysts on different instruments was monitored. The goal was to assess the reproducibility of the area percentage with varying sizes of the plasmid DNA isoforms.

The LabChip Plasmid DNA Assay addresses the challenges of measuring both supercoiled and linearized plasmid sizes, eliminating the need for a separate assay kit or cartridge for linear sizing. The kit also enables improved resolution, increased throughput, and improved precision when compared to conventional methods like agarose gel electrophoresis.

This technical note outlines the reproducibility of percent purity and supercoiled and linear size analysis of plasmid DNA isoforms ranging in size from 2.7 kbp to 12.9 kbp.

Assay overview and experimental

Plasmid information

Supercoiled plasmid DNA samples were obtained from New England Biolabs with the following sizes:

- pUC19: 2.7 kbp
- pBR322: 4.4 kbp
- pMAL-c6: 5.2 kbp
- pTXB1: 6.7 kbp

Additional plasmid samples were obtained from Millipore Sigma:

- 8.6 kbp plasmid (Mission® pLKO.1-puro-MV TagRFPTM)
- 12.9 kbp plasmid, TagGFP2 Simplicon® Plasmid (E3L)

The plasmids were diluted to a stock solution of 10 ng/µL using the LabChip Plasmid DNA Assay sample buffer for analysis.

Plasmid digestion protocol

Linearization and open circular reactions were carried out in accordance with the general protocols supplied by the enzyme manufacturer. For plasmid sizes 4.4 kbp, 5.2 kbp, 6.7 kbp, and 8.6 kbp. BamHI (R3136S, New England Biolabs) was used to produce linear isoforms, while Nb.BsmI (R0706S, New England Biolabs) was used to produce open circular isoforms. For the 12.9 kbp plasmid, SwaI (R0604S, New England Biolabs) was utilized to produce the linear isoform. The resulting linearized and open circular plasmids were diluted to prepare stock solutions with concentration 10 ng/µL using sample buffer.

All samples were diluted to 500 pg/µL from an initial concentration of 10 ng/µL. The sizing ladder developed for plasmid DNA kit is comprised of supercoiled plasmids and linear DNA fragments, allowing reliable sizing for samples ranging from 3-13 kbp.

Results and discussion

Supercoiled plasmid DNA percent purity analysis

Analysis was performed on the LabChip GXII Touch platform using the LabChip plasmid DNA assay. The plasmid DNA assay separates plasmids based on their electrophoretic mobilities, resolving supercoiled (SC), linear (L), open circular (OC) and multiple aggregates of the SC species in one assay. The system provides an electropherogram that allows the quantification of SC isoform, which is key for evaluating the purity of the plasmid sample.

The plasmid samples used for analysis varied in sizes: 2.7 kbp, 5.2 kbp, 8.6 kbp, and 12.9 kbp for the supercoiled (SC) form. Mixed isoforms (supercoiled (SC), linear (L), and open circular (OC)) were prepared in a 1:1:1 ratio for the 4.4 kbp and 6.7 kbp plasmids. Samples were analyzed by three different analysts on three different instruments to generate inter-run precision data. Figure 1 showed reproducibility across different runs for the 5.2 kbp SC plasmid sample. The first peak in the electropherogram represents a free dye peak and is not used for alignment. The second peak corresponds to a 300 bp DNA fragment, which serves as a lower marker to align the ladder and sample peaks for reliable sizing. The major peak corresponds to the supercoiled (SC) form of the plasmid, with a small impurity peak identified as the open circular (OC) isoform.

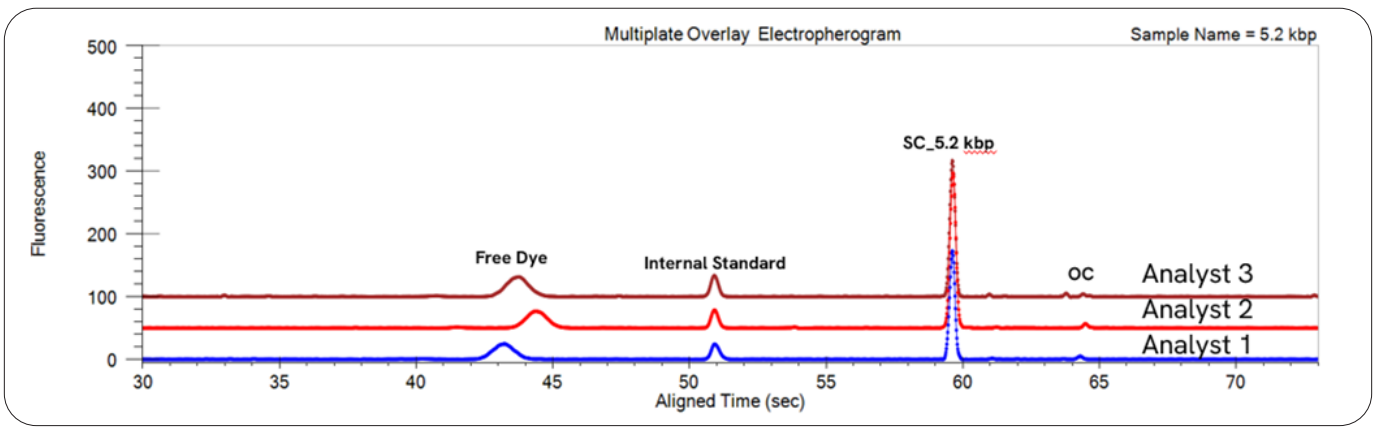

Figure 1: Overlayed electropherograms for 5.2 kbp SC plasmid, demonstrating the assay precision and reproducibility

Six plasmid samples were analyzed on three different instruments by three different analysists. Average percentage purity of each SC isoform was compared across the instruments, as shown in figure 2. The area percentage was consistent for each plasmid between the three different instruments with ~ 5% in CV, as calculated in Table 1.

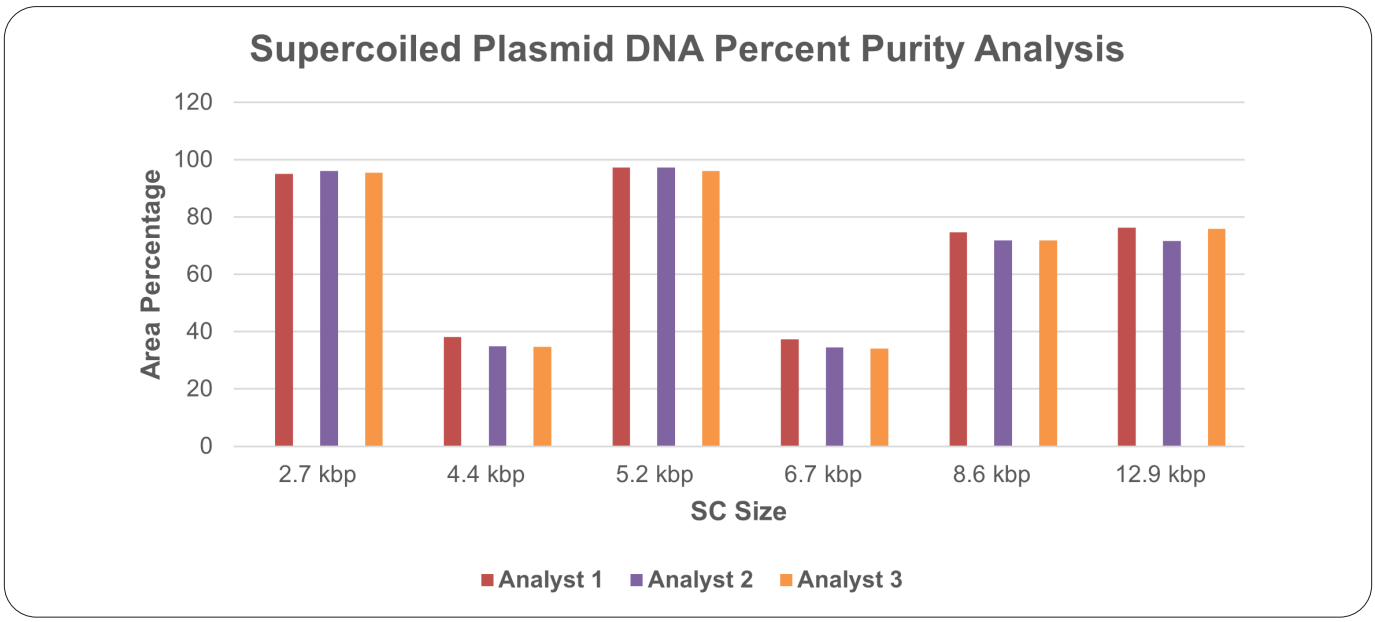

Figure 2: The bar graph comparing the average area percentage of the plasmid DNA samples (2.7, 4.4, 5.2, 6.7, 8.6, 12.9 kbp), as well as the mixed plasmid DNA isoforms (4.4 and 6.7 kbp).

Table 1: Calculated average area percentage, standard deviation, and percentage CV for plasmid DNA isoforms among three analysts using three different Lab Chip GXII Touch platform.

Area percentage	2.7 kbp	4.4 kbp	5.2 kbp	6.7 kbp	8.6 kbp	12.9 kbp
Analyst 1	95.1	38.09	97.33	37.28	74.65	76.37
Analyst 2	96.1	34.95	97.33	34.58	71.89	71.55
Analyst 3	95.52	34.81	96.06	34.19	71.9	75.88
Average	95.57	35.95	96.91	35.35	72.81	74.60
Std Dev	0.50	1.85	0.73	1.68	1.59	2.65
% CV	0.53	5.16	0.76	4.76	2.18	3.56

Supercoiled plasmid DNA size:

The sizing reported by the LabChip GXII Touch platform was consistent with expected size of supercoiled plasmid samples with linearity coefficient of > 0.99, Figure 3.

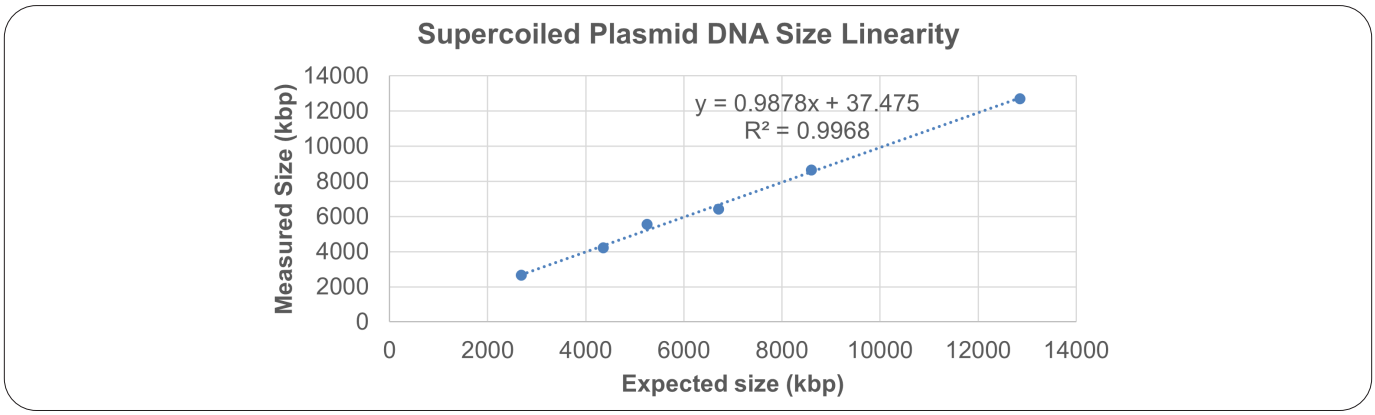

Figure 3: The correlation between supercoiled DNA measured size and expected size with linearity coefficient R^2 >0.99.

Six supercoiled plasmid samples were analyzed on three different instruments by the three different analysts. The average size of each SC isoform was compared across the three instruments, as shown in Figure 4. The size was consistent for each plasmid between the three different instruments with less than 3% of CV, and less than 6% error from the expected size, as shown in Table 2.

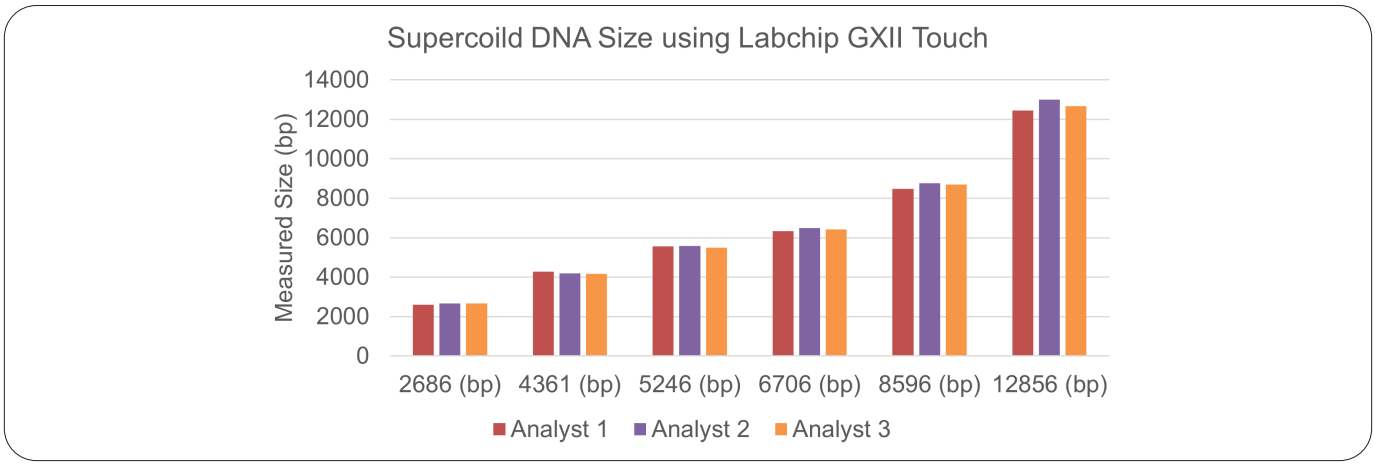

Figure 4: Bar graph comparison of average SC size for 2.7, 4.4, 5.2, 6.7, 8.6 and 12.9 kbp plasmid DNA samples between three analysts and three instruments.

Table 2: Calculated average of SC plasmid DNA size, standard deviation, and percentage CV for plasmid DNA isoforms among three analysts using three different Lab Chip GXII Touch.

SC size	2686 (bp)	4361 (bp)	5246 (bp)	6706 (bp)	8596 (bp)	12856 (bp)
Analyst 1	2592	4284	5555	6336	8484	12453
Analyst 2	2669	4182	5589	6485	8769	12999
Analyst 3	2657	4177	5490	6417	8694	12662
Average	2639	4214	5545	6413	8649	12705
Std Dev	41.4	60.4	50.3	74.6	147.7	275.5
% Error	1.7	3.4	5.7	4.4	0.6	1.2
% CV	1.6	1.4	0.9	1.2	1.7	2.2

Linearized plasmid DNA size analysis:

The sizing reported for linearized plasmid DNA samples by the LabChip GXII Touch platform was consistent with expected size of linearized plasmid samples with linearity coefficient of > 0.99, as shown in Figure 5. Mixed isoforms were used to determine the sizing from the range of 2.7 kbp to 12.9 kbp.

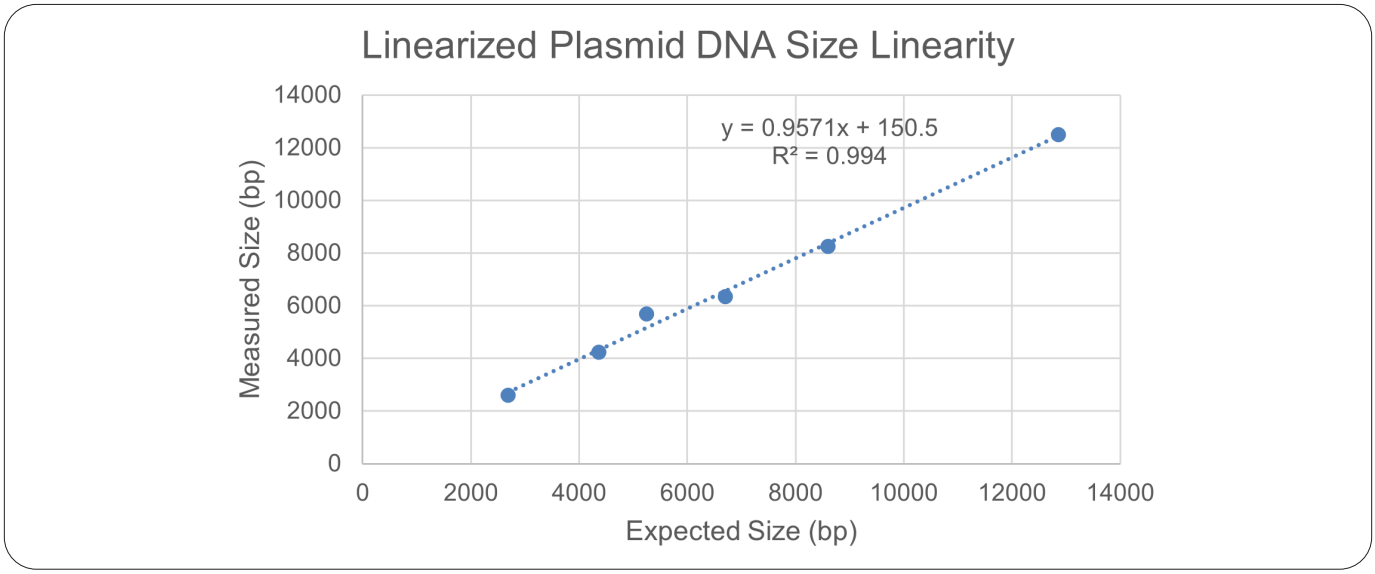

Figure 5: The correlation between linearized pDNA measured size and expected size with linearity coefficient R^2 >0.99.

From the 6 plasmid sizes, three linearized plasmid samples were analyzed on three different instruments by the three different analysts, and the average size of each linear isoform was compared across the instruments, as shown in Figure 6. The average size was consistent for each plasmid between the three different instruments with less than 4% of CV, and less than 8% error from expected size, as shown in Table 3.

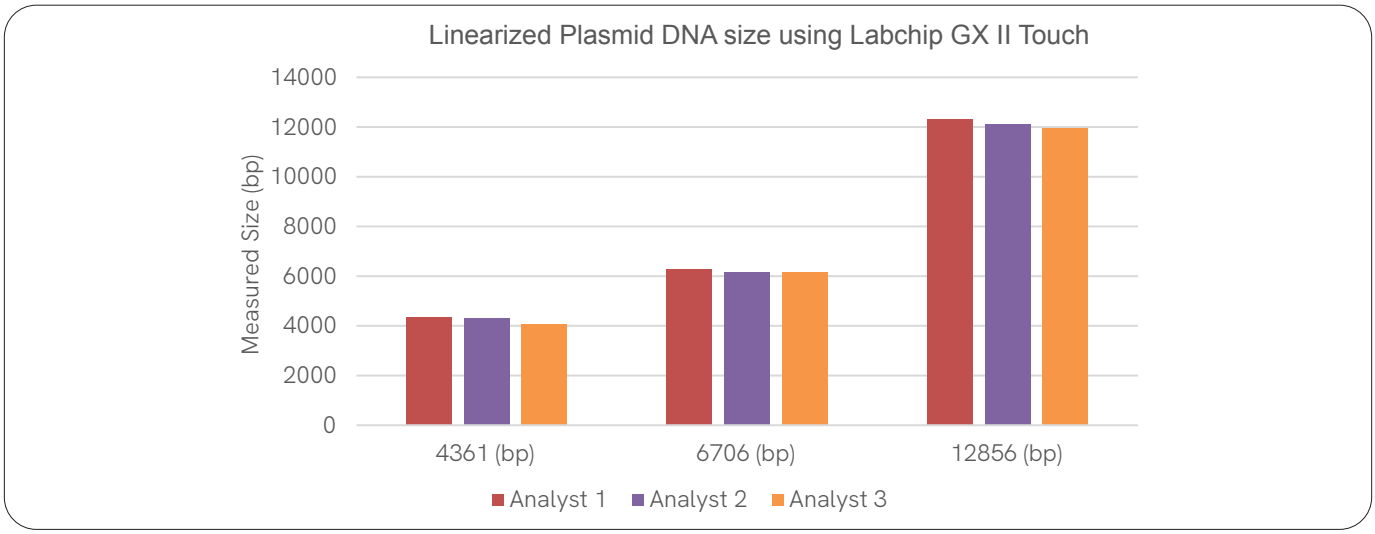

Figure 6: Bar graph comparing average size of linearized plasmid DNA samples (4.4, 6.7, and 12.9 kbp) among three analysts and three instruments.

Table 3: Calculated average of linearized plasmid DNA size, standard deviation, and percentage CV for plasmid DNA isoforms among three analysts using three different Lab Chip GXII Touch.

Linear Size	4361 (bp)	6706 (bp)	12856 (bp)
Analyst 1	4340	6260	12297
Analyst 2	4306	6144	12101
Analyst 3	4074	6157	11959
Average L Size (bp)	4240	6187	12119
Std Dev	145	64	170
% Error	2.8	7.7	5.7
%CV	3.4	1.0	1.4

Conclusion:

The LabChip GXII Touch platform provides remarkable inter-run precision for analyzing area percentage and sizing of both supercoiled and linear plasmid DNA in a single run.

- The platform automates the analysis process, saving time, minimizing human errors, and increasing throughput for plasmid DNA analysis.
- The LabChip plasmid DNA Assay offers one step sample preparation, and a single run to obtain information on percentage purity and sizing for supercoiled and linearized DNA samples.
- The area percentage of supercoiled plasmid samples shown ~ 5% inter run precision which proves reliability of day to day runs.
- By providing reliable results for area percentage and sizing for both supercoiled and linear plasmids in a single run, this platform is highly valuable for high-throughput labs that need to assess plasmid quality and integrity with high precision.

References

1. Technical Note: Plasmid DNA analysis in less than 2 minutes per samples, Revvity 2025.
2. US Food and Drug Administration. Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. Guidance for Industry, November 2008