APPLICATION NOTE

Targeted NeoNGS using minimal DBS input: 2 x 3.2 mm punch.

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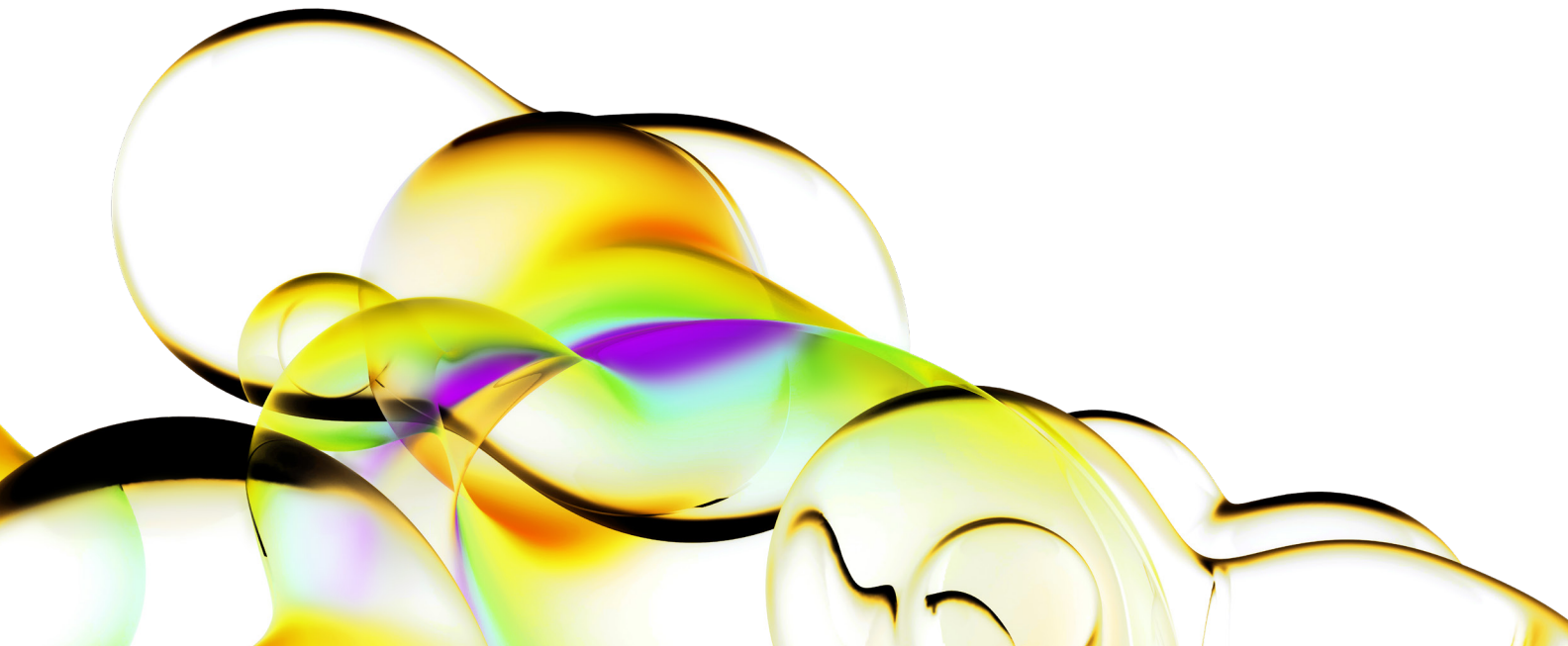
Revvity

Introduction

Genomic newborn testing is an evolving field, with targeted next generation sequencing (NGS) increasingly being explored to enable early detection of inherited disorders from minimally invasive samples. Dried blood spots (DBS) remain the standard sample format for newborn screening programs worldwide due to their simplicity of collection, stability during storage, and compatibility with high throughput workflows.

Efficient recovery of high quality genomic DNA (gDNA) from standard punch sizes (3.2 mm) is critical for preserving valuable samples while supporting scalable sequencing workflows. In particular, reducing the number of punches required for extraction can improve sample utilization and enable repeat testing when needed. Automated extraction instruments further enhance reproducibility and throughput, which are essential in screening environments.

In this application note, we demonstrate that only two 3.2 mm DBS punches are sufficient to consistently generate more than 100 ng of high quality gDNA using the chemagic™ 360 automated DNA extraction instrument. The resulting gDNA meets the integrity requirements for targeted NGS library preparation, supporting robust and reproducible sequencing performance. This workflow offers an efficient and scalable solution for genomic newborn screening applications while minimizing sample consumption.



Automated DNA extraction from DBS

Protocol

DBS samples were processed by excising two 3.2 mm discs per extraction into 96-well deep-well plates using a DBS puncher. The gDNA was isolated using the DNA Blood Spot 3 Kit H96 (CMG-1130) in accordance with the kit manual. Lysis was performed in a thermoshaker at 1100 rpm using a lysis buffer supplemented with Proteinase K and freshly prepared DTT to ensure efficient protein digestion and disulfide bond reduction. Downstream purification and magnetic bead–based DNA isolation were conducted on the chemagic 360 automated platform.

Despite the limited input material, the workflow reproducibly yielded gDNA quantities sufficient for downstream NGS, supporting robust library construction from two DBS punches.

Library preparation was performed on a subset of DNA extracts using the NEXTFLEX® Neo NGS RUO Panel 1 kit, requiring 100 ng input DNA. Sequencing was carried out on the Element AVITI™ instrument using standard operating parameters.

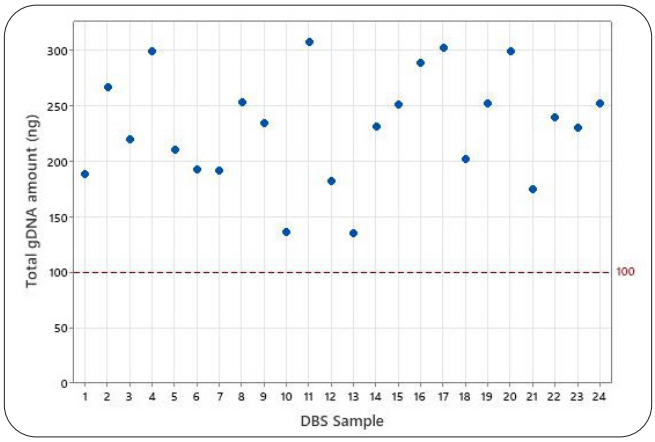


Figure1: Total gDNA yields obtained from 2 x 3.2 mm DBS punches using the DNA Blood Spot 3 Kit H96 (CMG-1130) on the chemagic 360.

Table 1: DNA yields were obtained from 24 newborn DBS samples, each using two 3.2 mm punches, followed by extraction with the DNA Blood Spot 3 Kit H96 (CMG-1130)* on the automated chemagic 360 instrument.

Sample	Concentration (ng/µl)	Total amount of gDNA
1	3.77	189
2	5.33	267
3	4.4	220
4	5.97	299
5	4.21	211
6	3.84	192
7	3.83	192
8	5.06	253
9	4.69	235
10	2.72	136
11	6.14	307
12	3.64	182
13	2.71	136
14	4.62	231
15	5.01	251
16	5.77	289
17	6.04	302
18	4.04	202
19	5.04	252
20	5.97	299
21	3.49	175
22	4.78	239
23	4.6	230
24	5.03	252

Targeted NeoNGS using minimal DBS input: 2 x 3.2 mm punch.

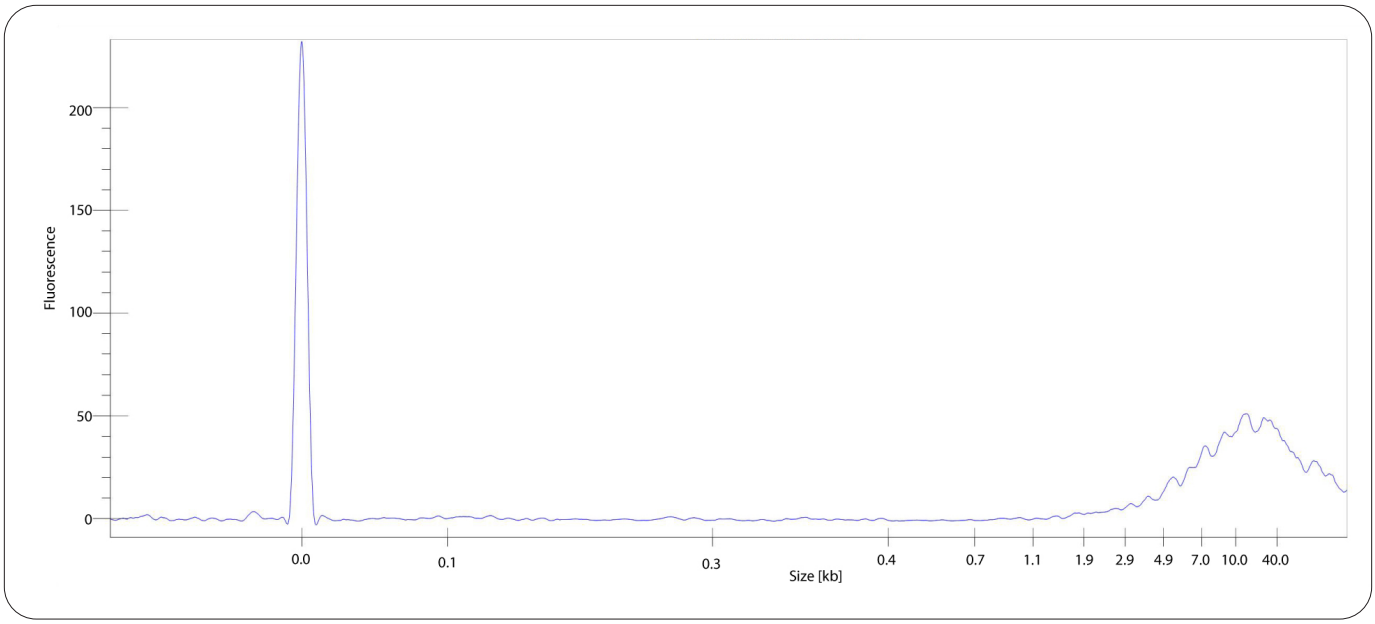


Figure 2: Representative electropherogram of gDNA extracted from DBS using the chemagic 360 instrument, analyzed on the LabChip GX Touch HT with the DNA 1K / 12K / Hi Sensitivity Assay LabChip™ (760517) and Genomic DNA Reagent Kit (CLS760685).

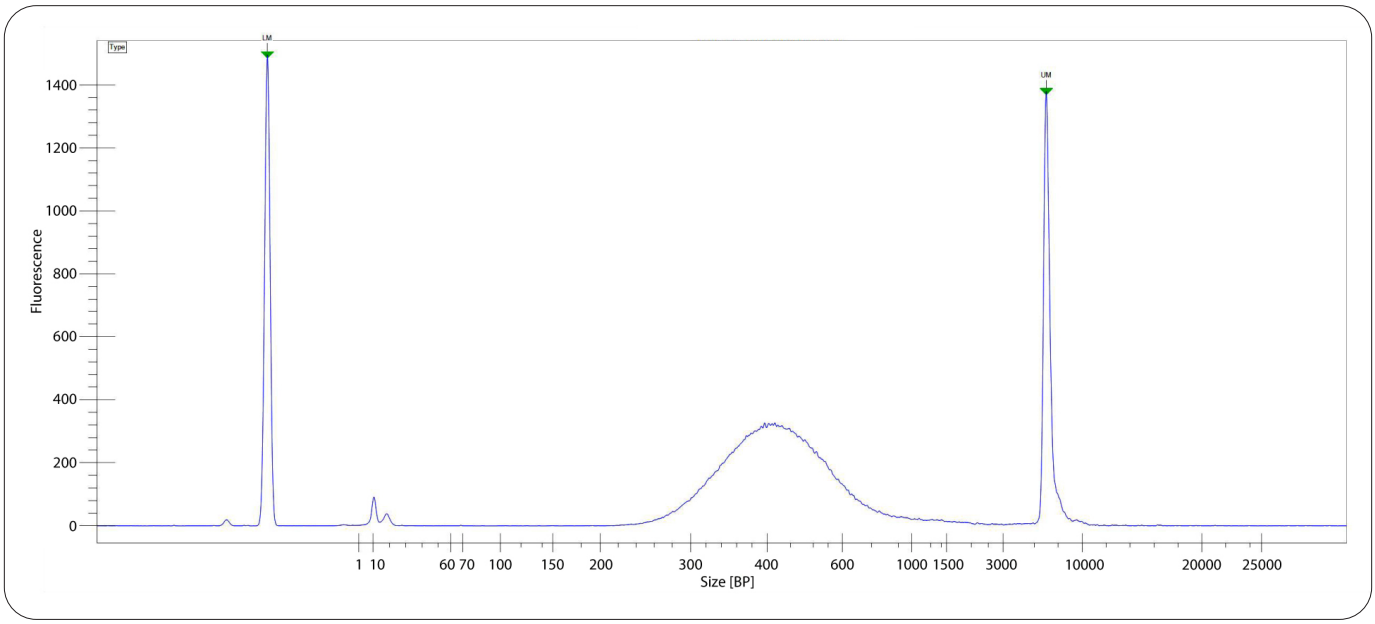


Figure 3. Representative electropherogram of a sequencing library analyzed on the LabChip GX Touch HT using the DNA HiSens Reagent Kit (CLS760672).

Data summary and alignment

The presented data demonstrate consistent and robust recovery of gDNA from DBS using two 3.2 mm punches processed on the chemagic 360. Across the evaluated samples, gDNA yields consistently exceeded 100 ng, confirming that this minimal input is sufficient to meet the DNA requirements for downstream targeted NGS workflow.

Quality metrics shown in the data indicate that the extracted gDNA is of high integrity, with values falling within acceptable range for NGS library preparation. No systematic degradation or inhibition effects were observed, supporting the suitability of the gDNA for enzymatic library construction and amplification. Importantly, yield variability between samples remains low, highlighting the reproducibility of the automated extraction process.

Sequencing performance data further aligns with these findings, demonstrating successful targeted NGS library generation from the extracted gDNA. Libraries generated from two 3.2 mm punches have Q30 score >96 % with mean Q score around Q42 (Table 2).

Table 2: Sequencing yield and QC metrics from libraries prepared with the NEXTFLEX Neo NGS Panel 1 kit using DNA isolated from DBS samples using the chemagic 360 instrument.

Sample	Yield (Gb)	% Q30	Mean Q score
1	3.44	96.46	42.80
2	3.35	96.40	42.82
3	3.27	96.60	42.88
4	3.83	96.22	42.71
5	3.19	94.37	41.74
6	3.18	96.39	42.79
7	3.26	96.53	42.84

Overall, the data confirm that two 3.2 mm punches are sufficient to consistently yield >100 ng of high quality gDNA, enabling efficient, scalable, and sample conserving workflows. When combined with the chemagic™ 360 platform, this approach supports the demands of high throughput genomic newborn screening, where automation, reproducibility, and minimal sample consumption are critical.

Conclusion

This study demonstrates that high-quality genomic DNA, suitable for targeted NeoNGS workflows, can be reliably obtained from as little as two 3.2 mm DBS punches. Using the chemagic 360 automated extraction platform together with the DNA Blood Spot 3 Kit H96 kit, gDNA yields consistently exceeded 100 ng while maintaining the integrity required for downstream applications.

The extracted DNA supported robust and reproducible library preparation and sequencing performance, with high Q30 values and strong overall data quality. These results demonstrate that minimal DBS input does not compromise workflow performance, enabling efficient use of limited newborn samples.

Overall, this solution provides a scalable and automated approach for genomic newborn screening, combining low sample consumption with high reproducibility and throughput. This makes it well suited for routine screening environments where consistency, efficiency, and sample preservation are essential.

**Similar results have been obtained using the chemagic DNA Blood Spot 13 Kit H96 (CMG-779) kit*

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