10x GENOMICS®

TECHNICAL NOTE

Single-stranded DNA Damage and its Effects on Chromium™ Genome Application Performance

INTRODUCTION

The Chromium™ Genome Protocol generates long-range information across the length of individual DNA molecules. Starting the process with high molecular weight (HMW) genomic DNA (gDNA) will typically result in better application performance, including increased ability to call structural variants with Long Ranger™ and multi-megabase haplotype phasing with Supernova™. We often refer to the "DNA Quality Level" of a sample, which acts as a simple classification system to describe the average size of DNA molecules after running the Chromium Genome workflow (Table 1). For a thorough description of DNA Quality, see the Technical Note *Sample Preparation Recommendations for the Chromium Genome Kit* (CG00045).

DNA Quality Level	DNA size (reported by 10x Chromium Genome pipeline)	Applications
5	>80 kb	De novo assembly with Supernova and Long Ranger analysis
4	60-80 kb	De novo assembly with Supernova and Long Ranger analysis
3	40-60 kb	Long Ranger analysis
2	20-40 kb	Long Ranger analysis ‡
1	<20 kb	Long Ranger analysis possible, performance not thoroughly characterized ‡

Table 1. DNA Quality level as reported by running the Chromium Genome workflow and recommended applications. ‡ It is possible to increase the DNA Quality Level using the >40 kb or >20 kb DNA Size Selection protocol in *Sample Preparation Demonstrated Protocol – DNA Size Selection* (CG00018).

The DNA Quality Level is impacted by both the length of the HMW gDNA sample and the extent of damage to the DNA. The DNA length can be assessed by pulsed-field gel electrophoresis (see *Sample Preparation Demonstrated Protocol - High Molecular Weight DNA QC*, CG00019). However, single-stranded DNA (ssDNA) damage can be difficult to assess. While there are many mechanisms of DNA damage, in this Technical Note we demonstrate that single-stranded DNA damage caused by heat or enzymatic nicking can influence DNA quality and, subsequently, the performance of Chromium Genome Applications.

METHODS

Data presented in this Technical Note were generated using the human cell line GM12878 (Coriell). gDNA was extracted using a modified QIAGEN® MagAttract HMW protocol as outlined in the *Chromium™ Genome Reagents*

Kit v2 User Guide (CG00043). After extraction, the HMW gDNA was subjected to either heat or enzymatic nicking. Heat treatment was performed by incubating HMW gDNA at different temperatures (37°C, 56°C, or 65°C) in an Eppendorf® ThermoMixer® dry block for 15, 45, or 90 minutes. Enzymatic nicking was performed by incubating HMW gDNA at different temperatures (room temperature, 37°C, or 50°C), in different buffers (NEB Buffer 2.1 or Buffer 3.1), with varying units of BspQI (1, 1/5, 1/25, 1/125, 1/625, or 1/3125 dilution of 10 U/µl stock concentration) (NEB catalog no. R0712S) and different incubation times (45 or 60 min). The various conditions define mild, moderate, or severe treatment in this study.

RESULTS AND DISCUSSION

The heat- and enzyme-treated gDNA samples were analyzed via pulsed-field gel electrophoresis (Fig. 1a and 2a) prior to being processed using the Chromium™ Genome workflow and Illumina® sequencing. Samples treated with heat for 90 minutes were sequenced on an Illumina HiSeq 2500 and the resulting DNA Quality Levels are shown in Fig. 1b. Samples treated with BspQI under mild, moderate, or severe conditions were sequenced on an Illumina HiSeq 4000 and the resulting DNA Quality Levels are shown in Fig. 2b.

After running the Chromium Genome workflow, HMW gDNA samples treated with heat or enzymatic nicking had decreased DNA Quality Level and decreased size distribution of HMW gDNA compared to the untreated HMW gDNA controls (Fig. 1b and 2b). The magnitude of DNA damage was generally proportional to severity of the treatment - higher temperatures or higher concentrations of nicking enzyme led to larger decreases in DNA size distribution and DNA Quality Level. This DNA damage was not evident using pulsed-field gel electrophoresis (Fig. 1a and 1b).

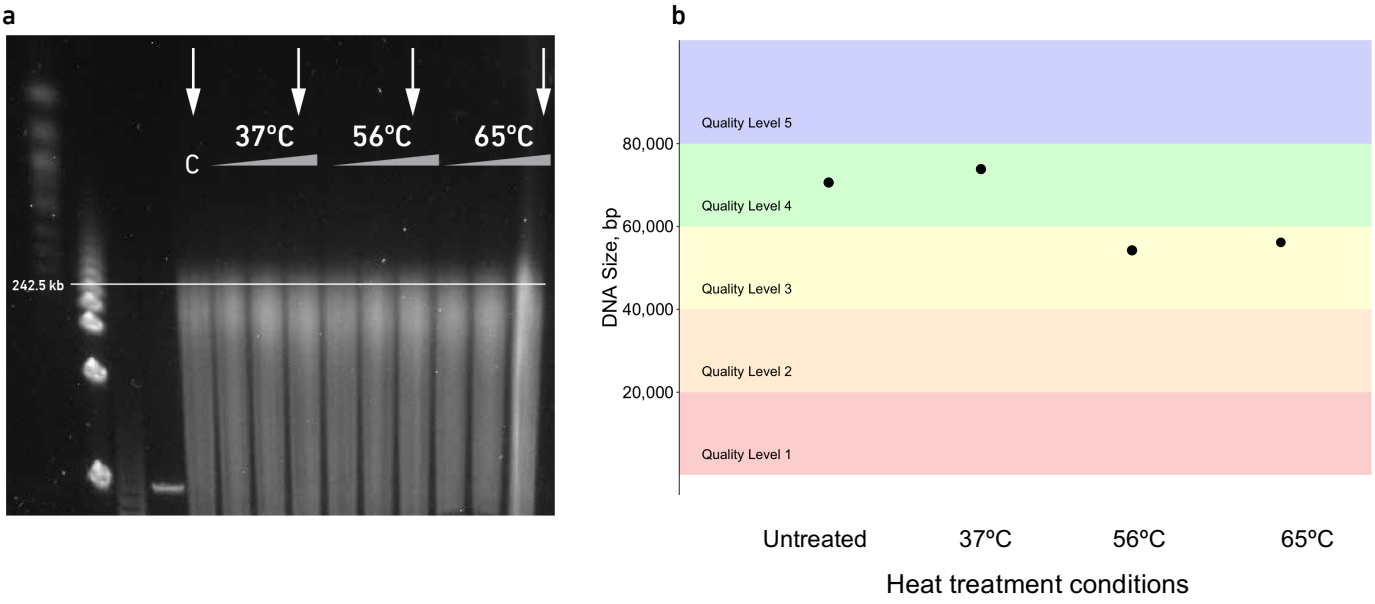

Fig. 1. (a) Pulsed-field gel electrophoresis and (b) DNA Quality Level of gDNA samples subjected to heat damage for increasing lengths of time (15, 45, or 90 min). Samples treated for 90 minutes at each temperature (white arrows, panel a) were sequenced and the resulting DNA fragment size calculated by the Long Ranger pipeline are presented in panel b. C: untreated control.

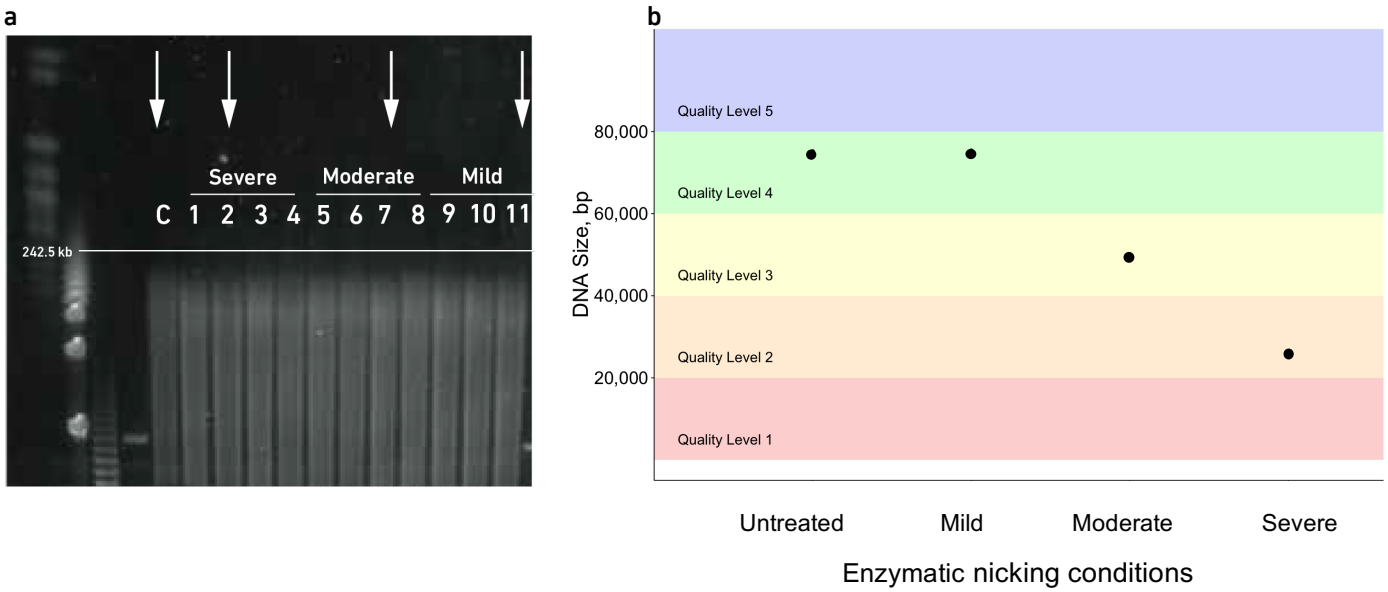

Fig. 2. (a) Pulsed-field gel electrophoresis and (b) DNA Quality Level for gDNA samples subjected to severe (samples 1-4), moderate (samples 5-8), or mild (samples 9-11) enzymatic nicking based on temperature, buffer, units of BspQI, and incubation time. One sample from each enzymatic treatment (white arrows, panel a) was sequenced and the resulting DNA fragment size calculated by the Long Ranger pipeline are presented in panel b. Treatment conditions for sequenced samples: Sample 2 (severe treatment): 10 units BspQI, NEB Buffer 3.1, 50°C, 60 min; Sample 7 (moderate treatment): 2 units BspQI, NEB Buffer 2.1, 37°C, 45 min; Sample 11 (mild treatment): 0.0032 units BspQI, NEB Buffer 2.1, 37°C, 45 min. C: untreated control.

CONCLUSION

We demonstrate that exposure of HMW gDNA to heat or enzymatic nicking affects downstream DNA Quality Levels. This damage cannot be assessed by pulsed-field gel electrophoresis and therefore should be minimized when extracting and storing gDNA. Be aware that other sources of ssDNA damage, such as oxidative compounds, while not explicitly demonstrated in this Note, would also be expected to reduce DNA quality. The following HMW gDNA extraction protocols were developed and tested by 10x Genomics to deliver high-quality gDNA with minimal ssDNA damage:

- *Chromium Genome Reagent Kits v2 User Guide* (GC00043)
- *Sample Preparation Demonstrated Protocol – HMW DNA Extraction from Whole Blood* (CG00015)
- *Sample Preparation Demonstrated Protocol – DNA Extraction from Fresh Frozen Tissue* (CG00072)
- *Sample Preparation Demonstrated Protocol – Salting Out Method for DNA Extraction from Cells* (CG00116)

When optimizing your own HMW gDNA extraction protocol, or when modifying a 10x Genomics Demonstrated Protocol for your particular sample type, we recommend the following best practices to minimize ssDNA damage:

- Avoid and/or reduce heat steps in gDNA extraction protocols, **particularly during the final steps of the protocol such as eluting from beads or resuspending a precipitated pellet**
- Use nuclease-free buffers to store gDNA (do not use water)
- Use gDNA preparations within 6 months (when stored at -20°C) or 2 weeks (when stored at 4°C)
- Incorporate chelating compounds such as EDTA throughout the extraction protocol

REFERENCES

- *Chromium Genome Reagent Kits v2 User Guide* (GC00043)
- *Sample Preparation Demonstrated Protocol – High Molecular Weight DNA QC* (CG00019)
- *Sample Preparation Demonstrated Protocol – DNA Size Selection* (CG00018)
- *Sample Preparation Recommendations for the Chromium Genome Kit* (CG00045)
- *Technical Note - Chromium Genome Application Performance as a Result of DNA Quality and Sequencing Depth* (CG00046)

Notices

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