10x GENOMICS®

TECHNICAL NOTE

Sample Preparation Recommendations for the Chromium™ Genome Kit

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INTRODUCTION

The purpose of this document is to provide guidance for HMW gDNA extraction, including general recommendations, and links to protocols recommended by 10x Genomics (Fig 1). The Chromium™ Genome Protocols generate 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, such as increased haplotype phase block length and ability to call structural variants. For a detailed comparison of DNA Quality and application performance, please refer to our Technical Note CG00046 on our Technical Support Website. An excerpt from this Note is shown in Fig 2.

SUMMARY TABLE 1: DNA QUALITY LEVEL

DNA Quality	DNA Size (reported by 10x Software)	Comments
5	>80 kb	
4	60-80 kb	We prefer to run samples with DNA Quality Level >=4 for Supernova™ analysis
3	40-60 kb	We prefer to run samples with DNA Quality Level >=3 for Long Ranger™ analysis
2	20-40 kb	Possible to increase to DNA Quality Level 3 by using a >40 kb DNA Size Selection Protocol
1	<20 kb	Possible to increase to DNA Quality Level 2 by using a >20 kb Size Selection protocol

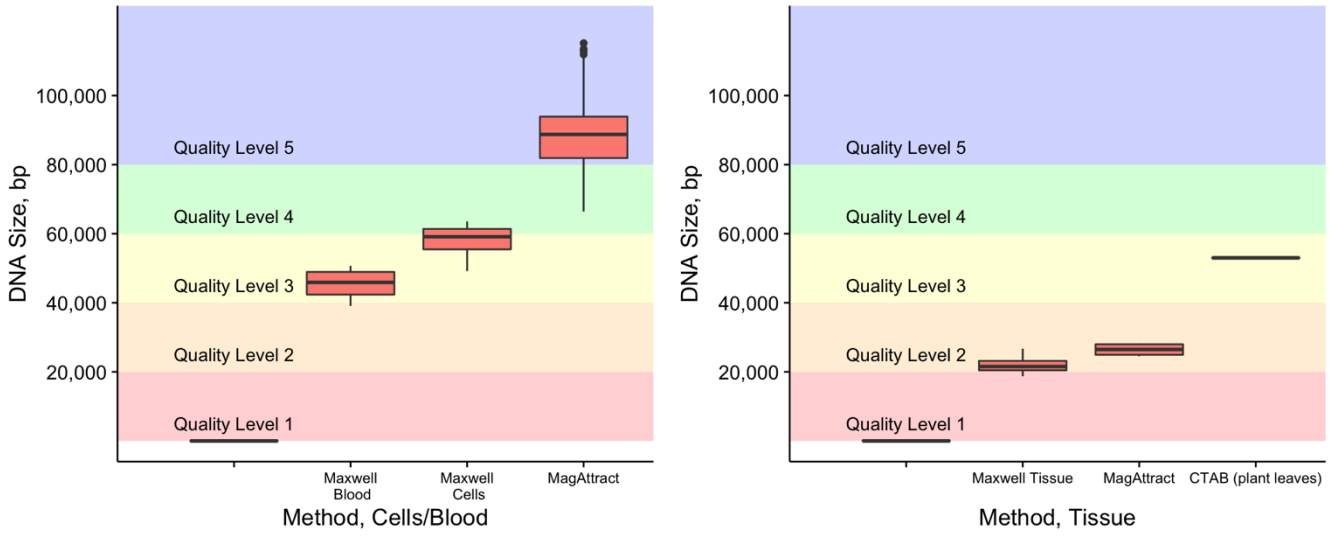

Fig 1. DNA Quality Levels obtained using 10x Genomics' Recommended Sample Prep Protocols.

MEASURING DNA SIZE

At 10x Genomics, and throughout this Note, we report DNA Size obtained from by analyzing the Linked-Read sequencing data with 10x Genomics software. Our software accurately calculates the length of each individual DNA molecule after the dilution, denaturation, and partitioning steps of the product workflow. Therefore, there will always be a discrepancy in comparing QC results from a starting sample of HMW DNA (e.g. pulsed-field gel) with the DNA Size measured after the Chromium™ Genome workflow for that same sample. Because we measure the length of every gDNA molecule separately, we are able to provide the distribution of DNA Size (histogram) as well as a single summary metric (length-weighted mean) for each sample in both Long Ranger™ (reference-based pipeline) and Supernova™ (*de novo* assembly pipeline).

At 10x Genomics, we are constantly testing and optimizing 3rd party kits in order to provide preferred protocols for HMW gDNA preparation that are user-friendly, robust, and will yield excellent application results with the Chromium™ Genome Workflow. On the 10x Genomics Support Website, we currently provide protocols for HMW gDNA preparation from cell suspensions, whole blood, and fresh-frozen tissue. We will periodically update the Technical Support website as we continue to expand our collection of sample prep protocols.

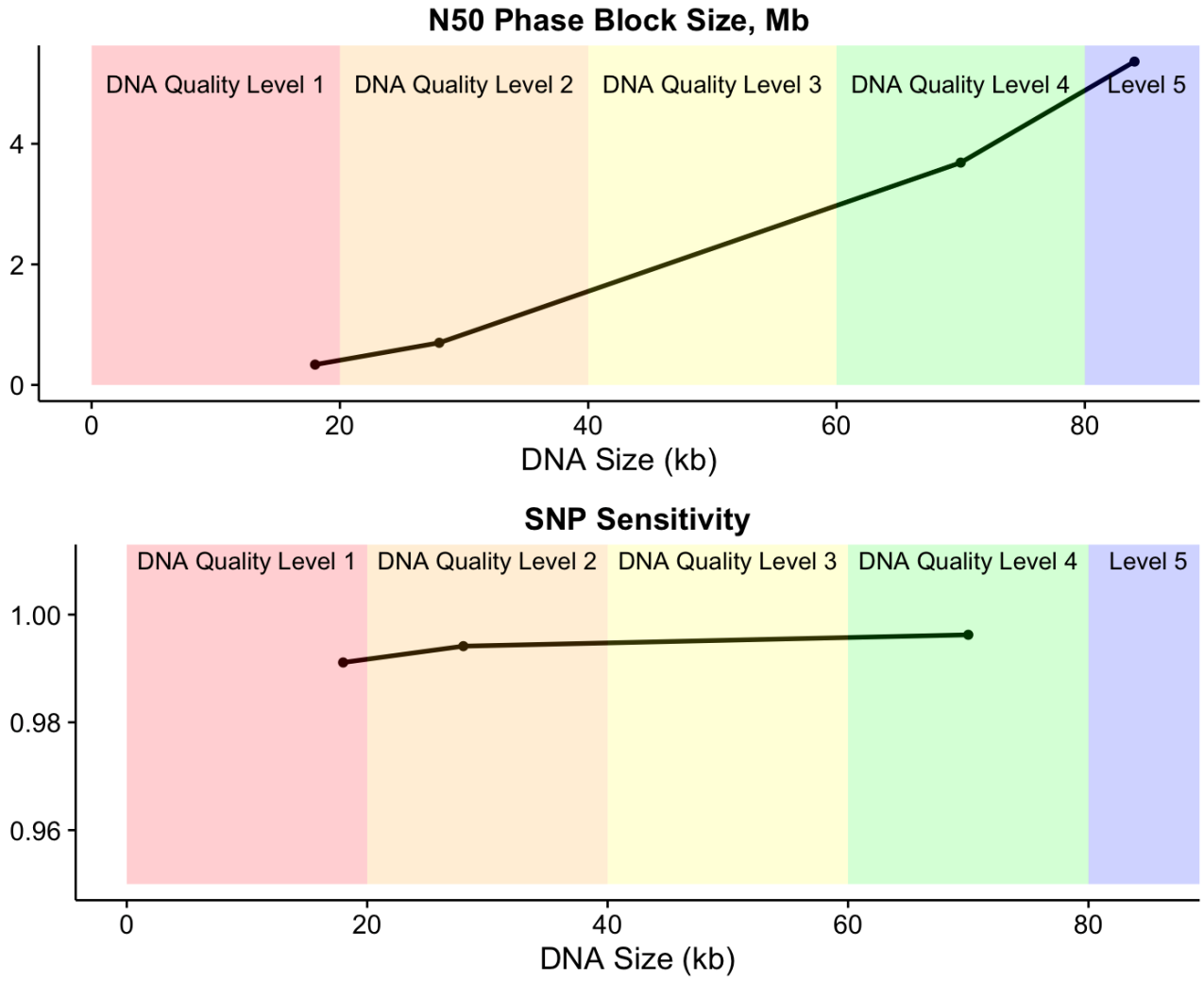

Fig 2. DNA Quality Level: Application performance examples. N50 Phase Block size is significantly dependent on DNA Size (above), whereas SNP sensitivity does not show a significant dependence on DNA Size (below). Data was generated using NA12878 DNA processed with Chromium™ Genome Protocol. Additional application examples can be found in Technical Note CG00046 on our Technical Support Website.

FACTORS TO CONSIDER WHILE EXTRACTING HMW gDNA FROM YOUR SAMPLES

1. **Quality level of DNA based on application goals.** Your application goal will determine the quality level of DNA required. Please refer to our Technical Note CG00046 on our Technical Support Website for demonstrations of application performance versus DNA Quality. This Note covers a comprehensive set of application metrics, ranging from SNP sensitivity (reference-based applications) to scaffold size (*de novo* assembly applications). In general, *de novo* assembly requires a higher DNA Quality Level than reference-based analysis. Two examples from this Note are presented in Fig 2.

2. **HMW gDNA extraction protocol.** We have found that the method used for gDNA preparation strongly correlates with DNA Quality after running Chromium™ Genome. Mild extraction protocols (no heat or harsh buffers) produce gDNA molecules that are longer and less damaged (fewer nicks and abasic sites). We strongly encourage following our gDNA extraction protocols, as they are thoroughly tested in the Chromium™ Genome Workflow. Fig 1 demonstrates typical results from for cell suspensions, whole blood, and fresh-frozen tissue using 10x Genomics' Demonstrated Protocols (or in the case of CTAB extraction, a protocol kindly provided by our collaborators Allen van Deynze and Kevan Stoffel at UC Davis).

3. **QC of gDNA (optional).** HMW DNA QC is challenging. We provide guidance for HMW gDNA QC in our support site (HMW gDNA QC). We have found that both DNA length (which can be measured on a gel) and DNA damage (such as nicking, which cannot be measured on a gel) are important for predicting the resulting DNA Quality Level after running Chromium™ Genome. We have observed DNA damage with protocols that use heat or harsh chemicals such as phenol and also with archival samples that are many years old. Therefore, at 10x Genomics we don't rely on HMW DNA QC when deciding whether or not to run a particular sample. Rather, we use the gDNA extraction method itself as the main factor in setting expectations for application performance. We run pulsed-field gels to confirm our expectations and help set up a DNA Size Selection.

4. **DNA Size Selection (optional).** DNA Size Selection can be used to remove DNA molecules below a certain size cutoff. We provide protocols for >20 kb and >40 kb. It is important to note that some types of damage can cause a larger molecule to break down a bit during the Chromium™ Genome Workflow. DNA Size Selections are very predictable and reproducible on gDNA samples with minimal damage. Variability has been observed when performing a Size Selection on archival/damaged DNA samples. In these cases there is always an improvement in DNA Quality, but the degree of improvement is difficult to predict.

FACTORS TO CONSIDER WHILE USING EXISITING gDNA SAMPLES

In many cases, customers wish to process existing gDNA samples with the Chromium™ Genome Assay, as opposed to preparing fresh HMW gDNA from a biological sample. Each case will vary, but the following outlines a generalized decision process:

1. What type of application performance are you looking for? Please refer to our Technical Note CG00046 on our Technical Support Website, or contact 10x Genomics Technical Support for guidance.

2. What was the method used to extract the gDNA? This can help set expectations for DNA Quality (see tables 2 and 3 below). If you have any questions, contact 10x Genomics Technical Support for guidance.
3. If you don't know the extraction method, do you have any QC data for your existing gDNA sample?
4. If your sample likely does not meet the required quality, is it possible to re-extract gDNA from your biological specimen? This may not be possible but should be considered for best results.
5. Alternatively, do you have enough gDNA (quantity and quality) for a DNA Size Selection? As noted in the table above, performing a DNA Size Selection (removing gDNA molecules <20 kb or <40 kb) can significantly increase the DNA Quality Level as described above.

GENERAL BEST PRACTICES FOR OBTAINING HIGH QUALITY HMW gDNA

There are a few general principles to follow for high-quality HMW gDNA isolation. We provide several gDNA preparation protocols, but for alternative protocols, please consider the following advice:

DO'S

• Use a wide-bore pipet tip and pipet HMW gDNA slowly to prevent DNA from shearing

• Elute and store HMW gDNA in TE or a similar buffer (not water)

• Collect and store biological specimens correctly to ensure optimal DNA quality yield.

• DNA size selection is a great option for improving data quality if DNA quality is not optimal

• Use physical grinding methods while extracting HMW gDNA from tissue samples, as chemical lysis buffers can shear/damage the DNA

DON'TS

• Avoid steps that can denature, nick, or damage the DNA (heat incubation, extreme pH buffers, chemical dissociation buffers for tissue samples)

• Avoid vortexing to mix samples as it can shear the DNA, and use brief "pulse vortexing" only if absolutely necessary

OTHER METHODS REQUIRING HMW gDNA

HMW gDNA >100 kb may not be a common sample prep requirement, but it is an important sample type for different projects, including:

- **Traditional genome mapping:** Most high-quality genomes, from fruitfly to apple to human, were assembled using a physical scaffold generated from a large-insert BAC library (for example, barley), which requires DNA 100's kb in size. *Large-insert BAC library protocols are an excellent starting point for running Chromium™ Genome.*
- **Next-generation genome mapping:** New technologies (e.g. optical mapping) also use HMW gDNA >100 kb in size to generate physical scaffolds for genome sequencing and assembly. *Optical mapping sample prep protocols are an excellent starting point for running Chromium™ Genome.*

SUMMARY TABLE 2: gDNA EXTRACTION METHODS AND SAMPLE TYPES

Sample Type	10x Recomm-ended	Kit	DNA Quality Level	Why recommended by 10x?	Requirements	Source
Cells	Yes	QIAGEN® MagAttract®	5	Good quality, easy protocol	1e6 cells	10x Validated Protocol for Cells
Cells	Yes	Promega Maxwell®	3-4	Automated	1e6 cells	Write-up in progress. Contact support@10xgenomics.com for a pre-print protocol
Blood	Yes	QIAGEN® MagAttract®	5	Good quality, easy protocol	200 ul whole blood	10x Demonstrated Protocol for Whole Blood
Cells	Yes	Promega Maxwell®	3	Automated	300 ul whole blood	Write-up in progress. Contact support@10xgenomics.com for a pre-print protocol
Fresh-Frozen Tissue (Mammalian)	Yes	QIAGEN® MagAttract®	2	Easy protocol, but quality is not optimal for all applications	25 mg tissue	10x Demonstrated Protocol for Fresh-Frozen Tissue
Fresh-Frozen Tissue (Mammalian)	Yes	Promega Maxwell®	2	Automated protocol, but quality is not optimal for all applications	25 mg tissue	Write-up in progress. Contact support@10xgenomics.com for a pre-print protocol
Fresh-Frozen Tissue (Single insect)	Yes	QIAGEN® MagAttract®	2-3	Easy protocol	1 insect, preferably larval	See notes below
Tissue (Plant)	Yes	CTAB Extraction	3	High quality DNA	2g leaves	UC Davis Genome Center Website
Tissue (General)	Yes	Gel Plug	3-5	Universal protocol but will require sample-specific optimization. This technique is used for preparing samples for optical mapping	Varies	Zhang *et al*, 2012. DNA must be released from gel plug prior to running Chromium™ Genome, see notes below for a protocol

SUMMARY TABLE 3: GENERAL METHODS

General Method	Typical DNA Quality	Notes
Column	2	
Mag beads	3-5	Varies by vendor. Our recommendations are above
Gel Plug	3-5	
Phenol Prep	2-3	
Archival samples	1-3	Mixed/unknown methods. Damage (nicking) suspected

DESCRIPTION OF METHODS FOR HMW gDNA SAMPLE PREPARTION

MAGNETIC BEADS

Magnetic bead-based purification is one of the most commonly used methods to isolate gDNA from biological samples. Kits are available in many different formats and from many different vendors. The overall workflow for magnetic bead-based gDNA preparation consists of 5 basic steps:

1. **Pre-Processing** (optional) – For example, homogenization of solid tissue
2. **Lysis** – Lyse cells and nuclei, liberating gDNA into solution. Lysis buffers often contain ProteinaseK and RNase
3. **Binding** – Bind DNA to silica-coated magnetic beads by adding a buffer containing a chaotropic salt and isopropanol
4. **Washing** – Wash magnetic beads to remove cellular debris including proteins, lipids, and sugars
5. **Elution** – Elute gDNA from the magnetic beads by the addition of a slightly basic, low molarity buffer (e.g. 10 mM Tris, pH 8.5)

There are several challenges in using magnetic bead-based methods for preparing HMW gDNA >100 kb. Many protocols contain vigorous mixing and high-heat incubation steps, which can shear the DNA. We optimized the QIAGEN® MagAttract® HMW (PN 67563) kit to produce HMW gDNA >200 kb (DNA Quality Level 5 after Chromium™ Genome) in ~70 minutes, with only 15 minutes hands-on time. We offer HMW gDNA extraction protocols for cell suspensions, whole blood, and fresh-frozen tissue, and single insects (described below). For best results, we recommend using the QIAGEN® MagAttract® HMW (PN 67563) kit, along with our optimized protocols.

For automated HMW gDNA extraction, we recommend the Promega Maxwell® Instrument. We have tested cell suspensions, blood, and tissue. However, the resulting DNA Quality Level is lower than our QIAGEN® MagAttract® protocols as presented above. Contact 10x Genomics Technical Support for a pre-print version of these protocols.

CTAB/PRECIPITATION

HMW gDNA extraction from plants is particularly difficult, due to the presence of many different types of molecules that are absent in animal cells, including polyphenols and polysaccharides. This wondrous diversity of molecules across different plant species results in fragrant roses, spicy serrano peppers, and starchy potatoes, but it also makes sample prep very challenging! Our collaborators at UC Davis (Kevan Stoffel, Allen van Deynze) have developed protocols for the preparation of HMW gDNA >100 kb from different plant species. These published methods involve grinding plant tissue in liquid nitrogen, followed by CTAB and chloroform clean-ups. Their general method is published and available for download: Stoffel *et al.*, 2012. A specific implementation of this method for chili pepper (which was sequenced at 10x using Chromium™ Genome and assembled with Supernova™ software) can be downloaded from the UC Davis Genome Center Website.

GEL PLUG

This is the most common method for preparation of HMW gDNA up to and above 1 Mb in size, and has been widely used since the 1980's (Schwartz and Cantor). The basic principle of DNA extraction using gel plugs:

1. **Prepare the biological sample:** Requires single cell or nuclei suspensions. Most commonly, samples are ground in liquid nitrogen and filtered to remove remaining tissue, then washed extensively via centrifugation.
2. **Embed the sample in agarose:** The cell/nuclei suspension is combined with molten agarose, and cast into a mold. The solidified agarose "plugs" can then be handled easily. Agarose provides several advantages for isolating and processing HMW gDNA. First, the solid matrix formed by agarose protects the fragile chromosomal DNA from shearing (in fact, the entire chromosomes remain completely intact). Second, the pore sizes in an agarose matrix (~200 nM) allow for rapid diffusion of proteolytic digestion enzymes into the sample to digest cellular debris. Third, since it is easy to perform buffer exchanges, the gel plugs can be stored in a preservative solution (4°C without damage for many years) and then dialyzed into TE buffer/water prior to use.
3. **Lyse and digest the cells:** Depending on sample types, the content of lysis buffer will vary, but should particularly contain EDTA (to chelate nucleases), detergent (to disrupt cell membranes), and Proteinase K (to digest histone proteins).
4. **Wash and store the gel plugs:** At this stage, most cellular components are degraded, but the chromosomal DNA remains intact. The gel plugs are thoroughly washed to remove proteins, cells, and other debris.

The general gel plug method was first published in 1984 (Schwartz and Cantor), and a very good expansion of this method for a variety of plant species was subsequently published by Hong-Bin Zhang and Rod Wing at Texas A&M University (Zhang *et al.*, 1995). However, our preferred source for gel plug protocols is a 2012 Nature Methods paper from Meiping Zhang and Hong-Bin Zhang, which includes specific protocols for plants, insects, and other animal tissue sources (Zhang *et al*, 2012).

There are challenges when preparing HMW gDNA using gel plugs. The workflow involves unfamiliar reagents and techniques, making it very different from traditional DNA extractions. Additionally, the protocol itself takes several days from start to finish.

This is the general prep method used for optical mapping (BioNano Genomics, OpGen), so we recommend checking to see if your sample type has been analyzed with these methods and if so, using those specific protocols as a starting point.

Releasing HMW gDNA from gel plugs: In order to use the HMW gDNA for downstream applications including Chromium™ Genome, the agarose matrix must be enzymatically degraded. Steps for digesting agarose plugs:

1. **Equilibrate the gel plug in TE buffer.** Equilibrate a single gel plug in TE. Place one gel plug in a 2 ml tube filled with TE and replace the buffer several times over the course of 24 hours.
2. **Melt the gel plug.** Remove the liquid buffer, leaving only the gel plug behind. Melt the plug in a 72°C water bath for 1-2 minutes. Take care to work quickly and do not leave the gel plug at 72°C longer than absolutely necessary to melt it.
3. **Add the enzyme.** Add 100 ul TE buffer and 2 ul of beta-agarase (New England Biolabs, PN M0392) to the tube. Mix by slowly pipetting the contents of the tube 3X with a wide-bore pipet tip.
4. **Incubate the tube at 42°C for 2 hours.** The HMW gDNA solution can then be stored at 4C and used directly in the Chromium™ Genome Workflow.

RECOMMENDATIONS FOR DIFFERENT SAMPLE TYPES

CELL SUSPENSIONS

At 10x Genomics, we routinely use cultured cells to test the performance of our sample prep protocols. Cultured cells are easy to grow, and isolating HMW gDNA is straightforward and easy with our protocols. The following are examples obtained with the recommended protocol in our User Guide (MagAttract).

Live cells: Typical DNA Quality Level: 5

Frozen cell pellets (shipped on dry ice): We have received frozen cell pellets from both collaborators and vendors. The viability of cells in a frozen pellet is very low (0% to 10% viability measured by Trypan Blue staining), but typically, samples are DNA Quality Level 4.

Cryopreserved vials of cells (slow-frozen in media containing DMSO using standard protocols for cryopreservation): We have extensively tested cryopreserved cells and found that the HMW gDNA quality obtained from our QIAGEN® MagAttract® protocol is equivalent to live cultured cells. Typical DNA Quality Level: 5.

WHOLE BLOOD

We routinely extract HMW gDNA from whole blood. The following are examples obtained with the recommended protocol in our Demonstrated Protocol (MagAttract).

Tube type: We strongly recommend collecting blood in an EDTA vacutainer (purple cap). We have tested different EDTA tube types (vendors, volumes), as well as different organisms, and this this is a robust collection mechanism. We have tested coagulated blood (extracted into a non-EDTA tube) and found that the prepared HMW gDNA was only 30 kb on a pulsed-field gel. In this case, a blood sample was redrawn into an EDTA tube, resulting in a DNA Quality Level 5 sample.

Buffy coat (instead of whole blood): We have limited experience with buffy coat samples. However, inputting 200 ul of buffy coat into our recommended blood extraction protocol resulted in a DNA Quality Level 5 sample.

Storage conditions

- **Fresh:** Typically, whole blood is shipped to our facility at room temperature or at 4°C (not frozen). We prepare HMW gDNA from blood samples within 36 hours of extraction for best quality results. We have not tested significantly extending the interval between blood extraction and HMW gDNA preparation.
- **Frozen, -20°C:** We have tested short-term (~1 month) storage at -20°C with no degradation in sample quality.
- **Frozen, -130°C (LN2 vapor phase):** We have tested a blood sample stored at -130°C for >1 year. DNA Quality Level 5.

FRESH (OR FRSH-FROZEN) TISSUE

The quality of HMW gDNA extracted from tissue depends on biological sample quality (how the sample was harvested and preserved) and dissociation method.

Chemical versus physical dissociation of solid tissue: The QIAGEN® MagAttract® protocol recommended above uses a chemical tissue dissociation buffer, whereas the gel plug protocol recommended above uses a physical tissue dissociation process to release cells and DNA from the samples. In our experience, chemical dissociation buffers cause significant DNA damage, partly due to the ingredients in the buffer itself and partly due to the experimental conditions for using the buffer (high heat for >12 hours). Using a chemical tissue dissociation buffer is easier than grinding, but the DNA quality is noticeably reduced (DNA Quality Level 2). The gel plug method referenced above uses physical dissociation (rather than chemical) and can produce DNA Quality Level 5 samples.

SINGLE INSECTS

A main advantage of the Chromium™ Genome Workflow is that it requires a very small amount of input HMW gDNA (~1 ng), thus enabling genome assembly from very small biological samples, including single insects. We have prepared DNA from single insects using a modification of our QIAGEN® MagAttract® protocol for cultured cells (located in the Chromium™ Genome Reagent Kit User Guide Man.No.CG00022 on our support website). In our hands, we observed a high degree of sample-to-sample variability, so we suggest repeating this HMW gDNA prep protocol on several different replicates and choosing the sample with the highest starting DNA quality. Steps for Single Insect HMW gDNA sample preparation (QIAGEN® MagAttract®):

1. Prepare a chilled work surface by placing a metal block on ice (we use an aluminum tube rack), covering with Parafilm, and wiping the Parafilm with 70% Ethanol.
2. Alternatively, place a sterile Petri dish on ice.
3. Quickly place a single insect (we used snap-frozen mosquito pupae) on top of the Parafilm, and mince the insect into a fine paste using a sterile razor blade.
4. Quickly transfer the tissue paste into 200 ul of PBS buffer and proceed with step (d) of the "gDNA Extraction" protocol on page 8 of the Chromium™ Genome Reagent Kit User Guide (Man.No.CG00022).

Notices

Document Number

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For technical information or advice, please contact our Customer Technical Support Division online at any time.

Email: support@10xgenomics.com

10x Genomics 7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA