

4. DNA Extraction and Quantification

4.1. Information Regarding DNA Extraction and Quantification

Importance of DNA Quantity

It is critical to load the correct mass of DNA into the GemCode Platform. Operating outside the recommended input amount can result in a reduction in application performance. Underloading the mass of genomic input material into the system will typically result in a higher PCR duplication rate, and a reduction in variant-calling performance. Overloading the genomic input material will reduce the number of Linked-Reads per Molecule (LPM). This causes a reduction in the density of long-range information used for haplotype phasing and structural variant calling.

Importance of DNA Length

The GemCode Platform produces long-range information over the scale of the length of input DNA. Loading longer DNA into the GemCode Platform will typically result in better application performance on both haplotype phasing and structural variant calling. The GemCode Platform delivers optimal performance on input DNA with a length-weighted mean of 50–70 kb.

Handling of High Molecular Weight DNA

Best practices for handling high molecular weight (HMW) DNA include using nuclease-free reagents and consumables, minimizing pipetting steps, and using wide-bore pipette tips where possible. Pipette HMW DNA slowly to avoid shearing the DNA.

4.2. DNA Extraction

4.2.1. DNA Extraction Overview

The GemCode platform supports the extraction of DNA from cultured cells using the Qiagen MagAttract HMW Kit (catalog no. 67653), with minor modifications. Please refer to the manufacturer's brochure (Qiagen MagAttract HMW DNA Kit Handbook 08/2013) for details regarding reagent preparation, storage, and troubleshooting.

The following DNA extraction protocol is a combination of two Qiagen protocols for Disruption/Lysis of Tissue (Qiagen MagAttract HMW DNA Kit Handbook 08/2013, page 18) and Manual Purification of High-Molecular-Weight Genomic DNA from Fresh or Frozen Tissue (Qiagen MagAttract HMW DNA Kit Handbook 08/2013 pages 19-21). For a single DNA extraction, use 1×10^6 cultured cells.

4.2.2. DNA Extraction Protocol

- a. Count the cells and use 1×10^6 cells per extraction. Place the cells/media in a **2 ml** microcentrifuge tube. Centrifuge the tube for **5 seconds** at **15000 x g** to pellet cells.
- b. Aspirate and discard the media, leaving only the cell pellet behind.
- c. Using a P1000 pipette, add **220 μ l** of Buffer ATL to the sample. Pipette mix **10 times** using a P1000 pipette with a wide bore tip.
- d. Using a P20 pipette, add **20 μ l** of Proteinase K. Mix by inverting the tube **10 times**.
- e. Incubate for **12–16 hours** in a Thermomixer set to **56 °C** and mixing at **900 rpm**.
- f. **STOPPING POINT:** Samples can be incubated overnight before proceeding to the next step.
- g. Quick-spin samples for **2 seconds** at **maximum speed** in a benchtop mini-centrifuge to remove condensation that may have formed on the lid of the tube overnight
- h. Using a P20 pipette, add **4 μ l** of RNase A to the sample. Pipette mix **10 times** using a P200 pipette with a wide bore tip. Incubate for **3 min.** in a Thermomixer set to **25 °C**. Mix at **1400 rpm**.
- i. Using a P200 pipette, add **150 μ l** of Buffer AL to the sample. Pipette mix **5 times** using a P200 pipette with a wide bore tip.

- j. Using a P1000 pipette, add **280 µl** of Buffer MB to the sample. Mix by inverting **10 times**.
- k. Vortex the MagAttract® Suspension G for **60 seconds**. Using a P200 pipette, add **40 µL** to the sample.
- l. **NOTE:** *If this is the first time using a particular vial of MagAttract Suspension G, increase the vortex mixing step to **3 minutes**.*
- m. Incubate the sample for **3 min.** in a Thermomixer set to **25 °C**. Mix at **1400 rpm**.
- n. Quick-spin the sample tube in a benchtop microcentrifuge for **2 seconds** and place on a DynaMag™-2 Magnetic Rack for **1 min.** to allow bead capture. Using a disposable transfer pipette or a P1000 pipette, remove and discard the supernatant. Take care not to disturb the bead pellet.
- o. Remove the sample from the magnetic rack. Using a P1000 pipette, add **700 µl** of Buffer MW1 directly to the bead pellet. Incubate the sample in a Thermomixer set to **room temperature (15–25 °C)** for **2 min.** Mix at **1400 rpm**.
- p. Repeat **Steps n. and o.**
- q. Quick-spin the sample tube in a benchtop microcentrifuge for **2 seconds** and place on a DynaMag™-2 Magnetic Rack for **1 min.** to allow bead capture. Using a disposable transfer pipette or a P1000 pipette, remove and discard the supernatant. Take care not to disturb the bead pellet.
- r. Remove the sample from the magnetic rack. Using a P1000 pipette, add **700 µl** of Buffer PE directly to the bead pellet. Incubate in a Thermomixer set to **room temperature (15–25 °C)** for **2 min.** Mix at **1400 rpm**.
- s. Repeat **Steps q. and r.**
- t. Quick-spin the sample tube in a benchtop microcentrifuge for **2 seconds** and place on a DynaMag™-2 Magnetic Rack for **1 min.** to allow bead capture. Using a disposable transfer pipette or a P1000 pipette, remove and discard the supernatant. Take care not to disturb the bead pellet.
- u. Using a P1000 multichannel pipette, carefully rinse the beads by adding **700 µl** of nuclease-free water.
CRITICAL 1: Leave the sample tubes in the magnetic rack for this step.
CRITICAL 2: Do not pipette water directly onto the beads. Slowly pipette the water down the side of the tube opposite the magnetic pellet.
- v. Incubate for **exactly 60 seconds** at room temperature. Using a P1000 multichannel pipette, remove and discard the supernatant.
CRITICAL 1: Leave the sample tubes in the magnetic rack for this step.
CRITICAL 2: The timing of this step is extremely important. If a multichannel pipette is not available, take care to ensure that each tube has the exact same incubation time. Do not exceed 60 seconds.
- w. Repeat **Steps v. and w.**
- x. Remove the sample tubes from the magnetic rack. Using a P200 Pipette, add **150 µl** of Buffer AE directly to the bead pellet. Incubate for **3 min.** in a Thermomixer set to **room temperature (15–25 °C)**. Mix at **1400 rpm**.
- y. Quick-spin the sample tubes for **2 seconds** in a benchtop mini-centrifuge and place on a DynaMag Magnet Rack for **1 min.** to allow bead capture. Using a P200 pipette, transfer the supernatant containing purified high molecular weight DNA to a new sample tube with a wide-bore tip.
- z. Store the HMW DNA sample at **4 °C** or proceed directly to **Step 4.3, DNA Quantification**.

4.3. DNA Quantification

4.3.1. DNA Quantification Overview

The Qubit® Fluorometer system is used for quantification of the HMW DNA extracted and purified in **Step 4.2, DNA Extraction**. Both the Qubit™ dsDNA BR Assay Kit and the Qubit™ dsDNA HS Assay Kit will be used to achieve accurate DNA quantification. Refer to the manufacturer's handbook for details and advice regarding operation of the Qubit Fluorometer, as well as both of the assay kits mentioned above. Accurate quantification is critical for successful sequencing when using the GemCode platform. Care must be taken during this process to pipette very accurately.

4.3.2. DNA Quantification Workflow

- a. Quantify the HMW DNA extracted in Section 4.2.
- b. Calibrate the Qubit Fluorometer for the dsDNA BR Assay per the manufacturer's instructions. Be sure to accurately pipette the standard solutions. Once the calibration is successful, proceed directly to next steps.
- c. Measure the concentration of each DNA sample and record the concentration in ng/μl. Using a P2 pipette, **take at least 2 concentration measurements per sample tube**. If the replicate results are inconsistent, mix the DNA sample by slowly pipetting the entire volume of the sample **2 times** using a P200 pipette with a wide-bore tip. Repeat the DNA concentration measurements. Once the tube yields a consistent concentration reading, proceed to the next step.
- d. Prepare a working stock for each DNA sample at **20 ng/μl** in nuclease-free water. Use wide-bore pipette tips to add the correct volume of DNA to nuclease-free water. Using a P200 pipette with a wide-bore tip, mix the DNA sample by slowly pipetting the entire volume of the sample **2 times**.

4.3.3. Quantification of DNA Working Stocks

- a. Calibrate the Qubit Fluorometer for the dsDNA HS Assay per the manufacturer's instructions. Be sure to accurately pipette the standard solutions. Once the calibration is successful, proceed to the next step.
- b. Measure the concentration of each DNA working stock, and record the concentration in ng/μl. Using a P2 pipette, **take at least 2 concentration measurements per sample tube**. If the replicate results are inconsistent, mix the DNA sample by slowly pipetting the entire volume of the sample **2 times** using a P200 pipette with a wide-bore tip. Repeat the DNA concentration measurements. Once all working stocks yield a consistent concentration reading, proceed to the next step.

STOPPING POINT: Samples can be stored at **-20 °C** for up to 6 months.