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Technical Note

DNA Prep

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Preparing DNA for PacBio® Whole Genome Sequencing for *de novo* Assembly: Quality Control and Shearing

Introduction

Single Molecule, Real-Time (SMRT®) Sequencing uses the natural process of DNA replication to sequence long fragments of native DNA. As such, starting with high-quality, high molecular weight (HMW) genomic DNA (gDNA) will result in longer libraries and better performance across difficult to sequence regions of the genome. This technical note is intended to give recommendations, tips and tricks for assessing and preserving the quality and size of your gDNA sample, shearing methods, and size selection procedures for samples intended to be used with whole genome sequencing for *de novo* assembly.

Topics Covered

DNA Quality Control (QC)

- Purity, quantification, and concentration
  - Qubit® fluorometer
  - NanoDrop® spectrophotometer
- Size
  - CHEF Mapper® System
  - Pippin Pulse™ System
  - FEMTO Pulse™ System

DNA Shearing

  - Megaruptor System
  - Needle shearing

DNA QC

We recommend using both fluorometric and spectrophotometric methods, for example using both the Qubit and NanoDrop instruments, when assessing your DNA sample. If the sample is pure gDNA, free of any RNA contaminants and other small molecules, the two methods should converge on similar amounts of gDNA.

Purity

Typically, we expect to see the NanoDrop A260/280 ratio between 1.8 and 2.0 and the A260/230 ratio ≥2.0 for ultrapure gDNA.

If the NanoDrop purity readings (A260/280 and A260/230) are out of the range specified above, we recommend performing an AMPure® purification step (see your selected library preparation protocol for AMPure instructions) followed by re-assessment of quantity and purity of the gDNA sample.

Quantification

If the measured NanoDrop concentration is significantly different (>50%) from the Qubit measurement, we recommend doing an AMPure purification step, as specified by your chosen library preparation protocol, followed by a re-measurement with both methods. Typically, a single AMPure purification step resolves the discrepancy.

- If the concentration measurement discrepancy after one AMPure purification step is ***reduced***, but not quite below the 50% difference threshold, we recommend proceeding with library preparation.
- If the concentration measurement discrepancy after one AMPure purification step is ***not reduced***, we recommend trying another cleanup approach such as a salt:chloroform wash protocol before a re-measurement with both methods.

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gDNA Sample	Qubit Conc. (ng/µl)	NanoDrop Conc. (ng/µl)	Concentration Difference	A260/280	A260/230
Example 1	49.0	34.5	34.7%	1.87	2.49
Example 2	72.2	19.4	52.8%	1.84	2.16

Table 1 - Example evaluation of gDNA samples. *Example 1 has a measured concentration difference of ≤50% with NanoDrop purity numbers in the expected range. Therefore, it is greenlighted to proceed to the next step. Example 2 has a measured concentration difference of >50% and therefore should go through an AMPure purification step before being re-evaluated.*

DNA Purity and Quantification – Alternative Methods

If you are unable to measure the quantity and/or purity with both the Qubit and NanoDrop instruments, it is still possible to generate a successful long-insert library following the guidance below.

- If you are using only a NanoDrop instrument and the purity numbers are in the appropriate range (A260/280 of 1.8-2.0 and A260/230 of ≥2.0), we recommend proceeding to library preparation.
- If you are using only a Qubit instrument, we recommend performing one AMPure purification step before a re-measurement of concentration and proceeding to library preparation.

Concentration

Additionally, we recommend having the gDNA at a concentration of ≥100 ng/µl prior to shearing and/or SMRTbell® library construction. This can be achieved by additional AMPure purification steps.

Downstream Step	Optimum Input Concentration
CHEF Mapper System	100 ng/lane
Pippin Pulse System	100 ng/lane
FEMTO Pulse	5-500 pg/µl
Needle Shearing	250 ng/µl
Megaruptor	25-50 ng/µl

Table 2 - Optimal input concentration for various DNA sizing and shearing methods. *DNA sizing and shearing methods are listed in order of preference for HMW gDNA preparation.*

Size

*To determine whether you need to shear the gDNA, we recommend determining the gDNA size distribution by **one** of the following DNA sizing methods:*

1. CHEF Mapper System (BioRad)
2. Pippin Pulse System (Sage Science)
3. FEMTO Pulse System (Advanced Analytical)

- If the gDNA mean fragment size is in the range of 10 kb to 100 kb, we recommend proceeding directly to SMRTbell library preparation without shearing of the DNA. This will ensure obtaining the very longest possible sequencing reads and highest yield from the runs performed on PacBio Systems.
- If the gDNA is well above 100 kb, typically identified by being beyond the resolution of the sizing instrumentation used, we recommend shearing the gDNA.

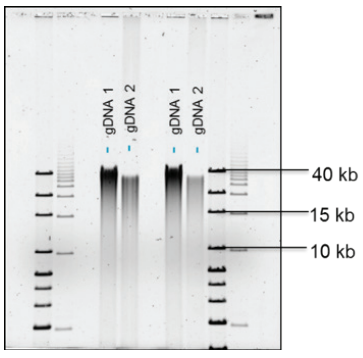

Figure 1. DNA QC example using Pippin Pulse. *The size distribution of gDNA1 is 20 kb to 40 kb. It is possible to construct a library with gDNA 1 without shearing the sample and achieve long sequencing reads. However, gDNA 2 is degraded with the mean fragment size <40 kb. This gDNA is still viable for library preparation but will likely not produce the optimal read lengths or yield on PacBio Systems.*

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DNA Shearing

The two recommended methods for shearing gDNA to the desired size distribution are needle shearing and the Megaruptor System (Diagenode). To ensure sufficient yields of final >30 kb libraries, input gDNA must be sheared carefully so that the average size of fragmented DNA remains well above the desired size selection cut-off. The response of individual gDNA samples to recommended shearing parameters may differ and must be determined empirically and evaluated by pulsed-field gel electrophoresis (PFGE). Test shears are highly recommended.

- For needle shearing, we recommend starting with a gDNA concentration of 250 ng/µl and performing the shearing as recommended in our official guideline protocols (see pages 4-5 in our SMRTbell Express Template Preparation Protocol).
- For shearing with the Megaruptor System, we recommend starting with a gDNA concentration of 25-50 ng/µl and shearing according to the manufacturer's recommendations at either 50-60 kb setting range for >30 kb libraries or 75 kb setting for >40 kb libraries. For further recommendations on using the Megaruptor System for shearing, please see pages 4-5 in our SMRTbell Express Template Preparation Protocol.

AMPure Purification and SMRTbell Library Prep

After shearing the gDNA to the desired mean fragment size, we recommend performing an AMPure purification step and resuspending the gDNA in Elution Buffer (included with PacBio Template Prep Kits) at a concentration of ~140 ng/µl prior to SMRTbell library preparation. The gDNA is then converted to SMRTbell library molecules according to our recommended library preparation protocols, which are posted on the PacBio Documentation webpage. Specific protocols for constructing long-insert libraries for *de novo* assembly applications are listed below.

Recommended Protocols

Procedure & Checklist – Preparing Greater Than 30 kb Libraries Using SMRTbell Express Template Preparation Kit

Procedure & Checklist – Preparing Greater Than 30 kb SMRTbell Libraries Using Needle Shearing and BluePippin Size-Selection on PacBio RS II and Sequel Systems

Procedure & Checklist – Preparing Greater Than 30 kb SMRTbell Libraries Using Megaruptor Shearing and BluePippin Size-Selection on PacBio RS II and Sequel Systems

Additional Protocols

Procedure & Checklist – Preparing Greater Than 15 kb Libraries Using SMRTbell Express Template Preparation Kit

Procedure & Checklist – >20 kb Template Preparation Using BluePippin Size-Selection System (15 – 20 kb Cutoff) for Sequel Systems

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