Effective Date: 11/17/2023 Previously Updated: 5/23/2022

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General

Please thoroughly review these policies prior to submitting samples. If you have any questions regarding these policies or any other aspect of next generation sequencing, please contact us at nextgen-seq@biotech.wisc.edu.

Unless otherwise specified, all data and reagents distributed by the University of Wisconsin Biotechnology Center DNA Sequencing Facility are intended for research purposes only. They are not intended nor certified for diagnostic or clinical use. Clinical services may be available through Wisconsin State Lab of Hygiene Clinical Testing.

We reserve the right to change the policies listed herein at any time. The effective date and date of previous update are noted in the header of this document. A notification of major policy changes will be given within the UWBC Requester Portal submission wizard. It is otherwise the responsibility of the submitter to review policies prior to submission to verify that they comply with the most current polices.

Submission Queue

Submissions must be approved by NGS staff prior to samples being dropped off or shipped to UWBC NGS. A request is entered into our queue upon receipt of samples. The official record is the information provided in the submission record seen in the UWBC Requester Portal. Any additional requests must be fully documented and approved.

Standard Turnaround Times*

QC only, gDNA shearing, etc. – 48 hours
DNA extraction, library preparation, and sequencing – 6-8 weeks
Library preparation and sequencing – 4-6 weeks
Sequencing only – 1-2 weeks

* Turnaround times are not guaranteed. We make every effort to meet or beat these turnaround times. For instrument issues beyond our control or other unforeseen issues, we try to inform clients of significant delays. Additionally, holidays and times when the University is closed may affect turnaround time.

Minimum and Optimal Sample Requirements

Submitted Biological Samples (e.g., tissues, blood)

– If samples are not provided as directed by NGS guidelines, we cannot guarantee that DNA extraction will be successful. While we make every effort to meet DNA input requirements for downstream use, we do not guarantee a specific mass, size, or quality of DNA. Many factors such as species, tissue type, sample age, and previous storage conditions are factors that impact the success of DNA extraction.

Submitted Input (Standard Molecular Weight) DNA – Quantification and/or size and quality analysis by the UWBC DNA Sequencing Facility is the standard by which input requirements are made. If input mass is not met, as specified by NGS guidelines, you will be informed via email. You may elect to either resubmit samples of sufficient quality and quantity or proceed with the existing samples. Should you choose to proceed with samples that do not meet our requirements, the samples will be run AS IS, and we make no assurances on the quality of downstream data.

Submitted Input (High Molecular Weight) DNA – The quantity and quality of high molecular weight (HMW) DNA intended for library preparation and/or sequencing on Oxford Nanopore or PacBio sequencing instruments is assessed via best practices using spectrophotometry, fluorometric quantification, and pulsed field gel electrophoresis. While we apply best practices, we do not guarantee downstream outcomes following our assessment of HMW DNA. There are factors beyond our control affecting library preparation and sequencing outcomes. These include, but aren't limited to, species, tissue type, or DNA context-specific issues. Where there is precedent, we make every effort to inform you ahead of time of any issues we expect.

While spectrophotometry allows for identification of some organic molecules that absorb in the 220nm to 340nm range we monitor, it can't detect all molecules that may be present and cause inhibition of downstream library preparation and/or sequencing. Pulsed field gel electrophoresis (PFGE) is a powerful tool to assess the size of HMW DNA and it also allows for assessment of bound, charged molecules. We do our best to interpret the results of PFGE, but certain profiles may mask underlying issues or have alternative interpretations. Fluorometric quantification of HMW DNA can be difficult in situations where DNA is heterogenous, which is common with HMW DNA extractions. DNA heterogeneity leads to highly variable quantification, inaccurate masses of DNA being input into library preparation, loss of higher weight DNA during library preparation, under-representation of longer reads during sequencing, and possibly over-representation of shorter reads during sequencing.

Submitted Illumina Library – Quantification and size/quality analysis by the UWBC DNA Sequencing Facility is the standard by which input requirements are made. If input mass is not met or libraries fail to meet requirements for sequencing, as specified by NGS guidelines, you will be informed via email. You may elect to either resubmit samples of sufficient quality and quantity or proceed with the existing samples. Should you choose to proceed with samples that do not meet our requirements, the samples will be run AS IS, and we make no assurances on the quality of downstream data.

Submitted PacBio Library – Submitted PacBio libraries will be assessed by Qubit and either Agilent Femto Pulse or TapeStation, depending on library size. As of the publication of these policies, our experience with customer prepared PacBio libraries is slim. Please consult with staff on expectations and guidelines.

Submitted Oxford Nanopore Library – As of the publication of these policies, our experience with customer prepared Oxford Nanopore libraries is slim. Please consult with staff on expectations and guidelines.

Libraries Prepared by UWBC

Illumina Library Preparation

See the appropriate section under Minimum and Optimal Sample Requirements above. All library preparation-specific guidelines must be followed. By submitting DNA that fails to meet the specified guidelines, you accept responsibility for library preparation costs regardless of success. Illumina library preparations don't include spectrophotometric analysis (e.g., NanoDrop), so it is the responsibility of the submitter to determine quality prior to submitting DNA. NanoDrop service may be requested for an additional cost. Except where noted, Illumina library preparations don't include DNA size/integrity analysis (e.g., TapeStation) of input DNA, so it is the submitter's responsibility to determine that the size/integrity of DNA is appropriate. Highly fragmented DNA (e.g., FFPE, eDNA) or DNA that has been extracted, stored, or handled improperly may lead to poor quality libraries or failure to produce a library. TapeStation or other comparable analysis service may be requested for an additional cost.

GBS – DNA intended for GBS library prep can vary across species and extraction. For samples that meet the specified guidelines, we consider library preparation to be successful if ~95% of samples generate a successful library.

In-House Amplicon (16s/18s/ITS) – Success of library preparation highly depends on quality of the input DNA and enrichment of target DNA. For many of our targeted assays, the input DNA being supplied is derived from complex substrates containing organic molecules/compounds that may carry forward during DNA extraction and inhibit amplification and/or sequencing. In addition, many of these complex substrates contain multiple organisms from which DNA is being isolated and where the biomass of your target(s) may be scarce. Since only fluorometric quantification is performed prior to library preparation, we have no means to ascertain inhibitor content in DNA nor exact mass of DNA being targeted. Thus, we make no guarantees of successful library preparation and will only consider repreparation if assay positive controls, where applicable, fail.

Custom Amplicon – For targeted amplicon library preparation, the statements above for In-House Amplicon (16s/18s/ITS) apply. The custom amplicon service has two stages: Evaluation/Optimization and Production.

> *Evaluation/Optimization*: Prior to approval of any custom amplicon service, it is the responsibility of the submitter to provide at least two peer-reviewed publications containing full methods for amplification of the target of interest. Those methods must include full primer sequences; reaction conditions detailing polymerase, primer, and other reaction volumes and/or concentration; and cycling conditions. In the absence of peer-reviewed articles, a lab protocol or similar may be accepted at our discretion. For each custom amplicon assay, an evaluation/optimization is performed with the guidance of the provided methods and is performed on a time and materials basis. If a quote hasn't been provided, you may request one for an estimate of these charges. For the purposes of the evaluation, we prefer to use an appropriate control DNA or mix of DNAs. In the absence of an appropriate control sample, we will source an appropriate control DNA from a vendor or from an internal source. If a control DNA can't be procured, the experimental/submitted DNA will be substituted for the evaluation. If we are unable to replicate the assay, either with or without control DNA, we will still charge for the cost of time and materials. The evaluation is limited to the assessment of the size and

quantity of the amplified product(s). Compared to the expected size from provided methods, the evaluation is deemed successful if comparable. The evaluation is limited to initial amplification – no complete library is prepared, and no sequencing is performed during this stage. If optimization is required, we may be able to work with you to provide feedback on changes or options we can try to improve outcomes. However, optimization will be performed at our discretion, as we are a service provider and not a research lab. Optimization is charged on a time and materials basis and only performed upon approval by the submitter.

Production: After successful evaluation of the assay, we begin library preparation from submitted samples. The same DNA used for evaluation will be used as a control during production. If library preparation succeeds for the control DNA and fails for any submitted DNA, the submitter is still responsible for the cost of all failed library preparations. We otherwise make no guarantees of successful library preparation, which extends to sequencing performance and quality metrics (e.g., Q score, on-target/off-target) as these can't be determined *a priori*.

DNA Library Prep – Libraries being prepared from gDNA are prepared via enzymatic fragmentation of the DNA while libraries being prepared from short DNA <~600bp (e.g., fragmented, digested, or amplified) are prepared without fragmentation. The process is robust but may be inhibited in certain contexts. DNA derived from organisms in complex substrates containing organic molecules/compounds and/or heavy metals or organisms containing cell walls with high levels of organic molecules/compounds that carry forward during DNA extraction may inhibit library preparation and/or sequencing. DNA with high GC- or AT-content may also present issues in preparation and/or sequencing. It is the responsibility of the submitter to inform us if any of the above conditions exist. You are responsible for the cost of any library preparations that are unsuccessful due to failure to inform us of any of the above conditions.

Index PCR – It is the responsibility of the submitter to ensure that the amplified products being provided to the UWBC DNA Sequencing Facility are optimized, specific to the target of interest, contain the required primer sequences for additional amplification, and that there is sufficient enrichment so that subsequent amplification is effective. We run an in-house positive control to ensure our process is effective and we will reattempt amplification if there is failure to amplify our positive control. We make no guarantees of successful library preparation.

ChIP, Cut&Run, Cut&Tag – Successful library preparation depends on multiple factors outside of our immediate control. For ChIP, proper fragmentation of DNA is critical. For all methods, the number of input cells and choice of antibody can greatly affect outcomes. It is highly recommended that the proper controls be used for each method. A proper control for ChIP is the "input control". A proper control for CUT&Run and CUT&Tag requires an antibody to an abundantly expressed target or an "IgG control". In any situation, the control must meet input mass requirements. Additionally, for ChIP, the input control must be in an acceptable size range. ChIP library preparation includes size analysis prior to prep while CUT&Run and CUT&Tag don't include size analysis. Where controls that meet specifications have been provided, we will re-prepare failed libraries at no additional cost or not charge for failed library preparations, depending on availability of DNA. Where libraries are successfully prepared from the aforementioned controls, and library preparation for any other sample fails, the submitter is still responsible for the cost of all failed library preparations. We otherwise make no guarantees of successful library preparation.

Exome – A combination of DNA Library Prep above and hybridization/capture is used to prepare these libraries. The limitations stated in DNA Library Prep apply, although the input expectations are limited to human cells or tissues. Other model organisms for which capture baits are available may apply if previously discussed and approved by UWBC DNA Sequencing. It is worth noting again that DNA inputs derived from FFPE or other fixed tissues may lead to poor quality libraries or failure to produce a library. Successful library preparation is determined by library size and mass metrics, not by sequencing performance and quality metrics. Therefore, we make no guarantees for sequencing metrics (e.g., on-target, off-target, coverage uniformity).

Targeted Panels – Accepted inputs for panels include DNA from FFPE or fixed tissues as well as fresh/frozen cells and tissues. It is worth noting again that DNA inputs derived from FFPE or other fixed tissues may lead to poor quality libraries or failure to produce a library. The panels are designed to detect SNPs, indels, and SV breakpoints. There are limitations in our ability to detect these variants when DNA is highly fragmented due to the design of the panels. As such, we make no guarantees of successful library preparation from DNA derived from FFPE or fixed tissues. By extension, there is no guarantee of sequencing performance and quality metrics (e.g., Q score, on-target/off-target). For DNA derived from fresh/frozen cells and tissues, successful library preparation is determined by library size and mass metrics, not by sequencing performance and quality metrics.

Oxford Nanopore Library Preparation

See the appropriate section under Minimum and Optimal Sample Requirements above. All library preparation-specific guidelines must be followed. By submitting DNA that fails to meet the specified guidelines, you accept responsibility for library preparation costs regardless of success. Oxford Nanopore library preparations always include HMW QC which includes spectrophotometric analysis (e.g., NanoDrop), Agilent Femtopulse, and Qubit. NGS staff will note if values are lower than required and will provide you the option to continue or provide new or more materials. Highly fragmented DNA (e.g., FFPE, eDNA) or DNA that has been extracted, stored, or handled improperly may lead to poor quality libraries or failure to produce a library.

DNA Library Prep – Adapters are ligated onto native DNA, with or without fragmentation. DNA derived from organisms in complex substrates containing organic molecules/compounds and/or heavy metals or organisms containing cell walls with high levels of organic molecules/compounds that carry forward during DNA extraction may inhibit library preparation and/or sequencing. It is the responsibility of the submitter to inform us if any of the above conditions exist. You are responsible for the cost of any library preparations that are unsuccessful due to failure to inform us of any of the above conditions.

PacBio Library Preparation

See the appropriate section under Minimum and Optimal Sample Requirements above. All library preparation-specific guidelines must be followed. By submitting DNA that fails to meet the specified guidelines, you accept responsibility for library preparation costs regardless of success. PacBio library preparations always include HMW QC which includes spectrophotometric analysis (e.g., NanoDrop),

Agilent Femtopulse, and Qubit. NGS staff will note if values are lower than required and will provide you the option to continue or provide new or more materials. Highly fragmented DNA (e.g., FFPE, eDNA) or DNA that has been extracted, stored, or handled improperly may lead to poor quality libraries or failure to produce a library.

DNA Library Prep – Adapters are ligated onto native DNA, with or without fragmentation. DNA derived from organisms in complex substrates containing organic molecules/compounds and/or heavy metals or organisms containing cell walls with high levels of organic molecules/compounds that carry forward during DNA extraction may inhibit library preparation and/or sequencing. It is the responsibility of the submitter to inform us if any of the above conditions exist. You are responsible for the cost of any library preparations that are unsuccessful due to failure to inform us of any of the above conditions.

DNA Extraction Policies

These policies apply to high molecular weight (HMW) and standard (non-HMW) DNA extractions. Given the wide variety of species, tissue sources, preservation methods, and context-specific variations, we make no general guarantees of success. For "standard" species (e.g., human, mouse), "standard" input types (e.g., blood, cell lines), and where we have precedent (e.g., manufacturer supported protocol or UWBC previously performed), we will provide a reasonable assurance of successful DNA extraction. Success is generally defined as sufficient quantity and quality for downstream use but doesn't imply a guarantee of specific metrics (e.g., concentration, 260/280, 260/230). For "non-standard" species and inputs and where we have no precedent, we offer no assurances of success. You will be responsible for the cost of all failed DNA extractions unless failure resulted from UWBC staff errors or equipment malfunctions. In these cases, UWBC will either attempt re-extraction if sufficient material exists and only after issues have been resolved or not charge for the failed extractions. Please also see DNA Extraction Deliverables.

Deliverables

Illumina Sequencing

- See Sequencing Deliverable Exceptions below.
- See Data Storage below.
- Data is delivered in FASTQ file format, along with a MultiQC report, within 2 business days of run completion. Further analysis may be contracted separately with the Bioinformatics Resource Center (BRC) at brc@biotech.wisc.edu.
- We do not perform read trimming for the purposes of removing adapter, low-quality, or any other sequence. There are cases where we mask out cycles of a read for NovaSeq X Plus lanes or flow cells, but this must be explicitly requested - this isn't trimming.
- A request for 2x150 10B Half Lane is considered satisfied when ≥360M **aggregate** passing filter reads are provided. **This isn't a guarantee of reads per sample.**
- A request for 2x150 Shared (10M read increments) is considered satisfied when **aggregate** passing filter reads are ≥80% of the request. For example, ≥8M reads on a 10M read request. **This isn't a guarantee of reads per sample.**

- We strive to deliver data that meets or exceeds the metrics indicated in the table below. **There are many different factors that can affect these metrics such as species, library prep kit, GC content, nucleotide diversity, % PhiX used, etc.**

Instrument	Reads Passing Filter (PhiX Control DNA)	Reads Passing Filter (mixed species)	% of bases at Q30 or greater
MiSeq v2	12-15M		75%
MiSeq v3	22-25M		70%
MiSeq nano	1M		75%
MiSeq micro	4M		75%
NovaSeq X Plus 1.5B lane	200M		85%
NovaSeq X Plus 1.5B flowcell	1.6B		85%
NovaSeq X Plus 10B lane	1.25B	1.125B	85%
NovaSeq X Plus 10B flowcell	10B	9B	85%
NovaSeq X Plus 25B lane	3.25B	2.9B	85%
NovaSeq X Plus 25B flowcell	26B	23B	85%
NextSeq2000 P1	100M	60M	85%
NextSeq2000 P2	400M^	240M	85%^
NextSeq2000 P3	1.2B		85%

M = Million, B = Billion
^300M and 80% for 2x300

Oxford Nanopore Sequencing

- See Sequencing Deliverable Exceptions below.
- See Data Storage below.
- Data is delivered in FASTQ file format, along with a run report, within 2 business days of run completion. Raw data files (FAST5 or POD5) are available upon request and are subject to the terms in the Oxford Nanopore Sequencing Services Contract.
- We use default software/run settings and parameters that are indicated by Oxford Nanopore Technologies. Requests for changes to these parameters must be approved by us. Parameters are as follows:
    - Basecalling models and model classes – The “High Accuracy” (HAC) model is the default model. We reserve the right to choose the basecalling model that is best suited, as these models evolve/improve.
    - Q-score filtering – The Q-score filtering threshold is set by the basecalling model and a “pass” or “fail” status to all reads. The default Q-score threshold is set at 9 (~87%). Pass and Fail reads are delivered.

  - Barcode demultiplexing/trimming - All barcoded libraries are demultiplexed. The barcode is part of the read and barcode trimming (beginning, middle, and both ends) is turned off. Any resulting data will contain barcode sequence, so care should be taken in downstream data processing.
    - The quality score threshold used for determining barcode pass/fail and subsequent assignment is the default value.
- We strive to deliver data that meets or exceeds the metrics indicated in the table below. There are no guarantees of output, read length, or read counts. Output on a PromethION flow cell is inversely proportional to DNA input length. **There are many different factors that can affect these metrics such as species, DNA length, DNA quality, library prep type, GC content, etc.**

Flow Cell	Estimated Total Output Passing Filter	Estimated Total Reads Passing Filter
PromethION 72 hour	~40-100+Gb	~10-100+M
PromethION 12 hour	~5-20Gb	~1-20M

PacBio Sequencing

- See Sequencing Deliverable Exceptions below.
- See Data Storage below.
- Data is delivered in BAM file format within 2 business days of run completion. Both CCS (circular consensus sequence or HiFi) and subreads are delivered for Sequel II data. Only CCS data is delivered for Revio data.
- We use default run settings and parameters that are indicated by PacBio for the library type being sequenced. Requests for changes to these parameters must be approved by us.
  - Barcode demultiplexing/trimming - All barcoded libraries are demultiplexed. The barcode is part of the read and barcode trimming (beginning, middle, and both ends) is turned off. Any resulting data will contain barcode sequence, so care should be taken in downstream data processing.
    - The quality score threshold used for determining barcode pass/fail and subsequent assignment is the default value.
- We strive to deliver data that meets or exceeds the metrics indicated in the table below. There are no guarantees of output, read length, or read counts. Output on a SMRT cell is dependent on the quality of the native DNA. **There are many different factors that can affect these metrics such as species, DNA length, DNA quality, library prep type, GC content, etc.**

Flow Cell	Estimated Total Output Passing Filter
8M SMRT Cell (Sequel II)	20-25 Gb
25M SMRT Cell (Revio)	90 Gb

Sequencing Deliverable Exceptions

- While we will make every effort to create successful sequencing runs with high quality data output, we cannot be responsible for improperly prepared samples. Therefore, we make no guarantees on the quantity and quality of data generated from Illumina/PacBio/Oxford Nanopore sequencing if:
    - Libraries are prepared by the user, where we have minimal control over quality.
    - Custom primers are supplied with incorrect sequences or inappropriate melt temperatures.
    - Samples are submitted that do not meet UWBC minimum submission requirements (stated on guidelines located on UWBC website).
    - Non-Illumina barcode/index methods are used. Barcodes placed at the 5'end of the sequence and those with no redundancy are especially prone to read errors.
- Libraries prepared by UWBC – We strive to ensure all libraries we prepare meet these specifications. As noted, run success is DNA/RNA dependent to a certain degree so variation occurs, and some libraries will not perform as well as expected. Facility prepared libraries that grossly underperform due to Facility or instrument errors will be re-run as soon as possible; however, there may be a wait for a run that will meet or exceed the requested parameters.
- Libraries prepared by customer – Customer prepared libraries that do not meet these specifications can be re-run at the submitting lab's expense.
- If your sequencing libraries grossly underperform, or if you have other questions/concerns regarding your data, please contact us immediately at nextgen-seq@biotech.wisc.edu to address your concerns.

QC Deliverables

- For QC of Illumina libraries and input DNA for ChIP, we will provide:
    - Concentration of each sample/library or pooled libraries.
    - A PDF report from the Agilent TapeStation including one defined region for each sample/library. **The raw data file from the TapeStation is not delivered.**
- For QC of input DNA for Oxford Nanopore or PacBio library preparation/sequencing, DNA that is explicitly submitted for high molecular weight HMW DNA QC, or that which is included with our HMW DNA extraction service, we will provide:
    - A NanoDrop trace report
    - Concentration of each sample
    - A PDF report from Agilent Femto Pulse (HMW DNA only) including at least one defined region for each sample or a PDF report from Agilent TapeStation including one defined region for each sample. **The raw data from the Femto Pulse or TapeStation is not delivered.**
- Does not pertain to libraries prepared by the facility, DNA submitted for Illumina library preparation/sequencing (excludes ChIP), or DNA extracted by UWBC for Illumina library prep.
- Requests made for additional information not included in the reports (e.g. additional regions) and raw data files may be requested, but they will be at our discretion at an additional cost.

DNA Extraction Deliverables

- See Shipping below.
- DNA is eluted with the elution buffer appropriate for the kit/method utilized, as determined by the manufacturer or the UWBC. DNA is provided to you in the assay vessel into which it was eluted. Request for transfer to a specific vessel will incur a transfer/consumable fee.
- Where the DNA is to be used in downstream library preparation and sequencing, residual DNA (if applicable) will be maintained for 6 months after extraction after which it will be properly disposed.
- For extraction of "standard" (not high molecular weight) DNA, **QC is not included** unless the DNA is being used for subsequent library preparation. You don't need to request QC unless this is a "DNA Extraction Only" request and you require that information.
- For extraction of high molecular weight DNA, **QC is included**. Refer to QC Deliverables.

Data Storage

- Deliverable sequence data will be stored in your download directory. These files will be kept for 60 days from the date they were created by the DNA Sequencing Facility, but the PI is ultimately responsible for long term storage.
- For internal users, we highly recommend signing up for ResearchDrive, where 25Tb of storage is provided to each PI at no cost. We sync our data storage nightly to UW Madison DoIT's ResearchDrive. For external users, we highly recommend and support the use of Globus.
- **We are not responsible for failure to download data within 60 days. If there are circumstances preventing access to your data, please contact UWBC and we will work with you to get you your data.**

Sample Storage and Disposal

- All input DNA submitted after 9/12/2019, along with any intermediate and/or derived products (to include undiluted stock libraries), will be stored for 6 months from the sample receipt date on the request record and then disposed of.
- All customer prepared libraries submitted after 4/23/2018 will be stored for 6 months from the sample receipt date on the request record and then disposed of.
- All libraries that have been diluted to 2nM by the facility to be used for sequencing will be stored for 4 years from the sample receipt date on the request record and then disposed of.
- All biological samples (e.g., tissues, blood, cells) provided for DNA extraction that aren't completely depleted during extraction will be kept for 6 months from the sample receipt date on the request record and then disposed of following our biosafety protocol.
- **You are responsible for arranging the shipment or pickup of any samples prior to disposal. Residual biological samples can only be picked up. See Shipping below.**

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Shipping

- We can only ship DNA. **Any biological samples sent to us will not be shipped back.** You may arrange to pick them up or we will properly dispose of them 6 months from submission.
- You are responsible for all handling and delivery charges incurred through the process of shipping DNA. We do our best to properly package samples for shipment, but we aren't liable for damage/loss of samples through shipping.
- **We do not ship internationally.** If you wish to arrange for international shipping, a courier must be contracted, all paperwork must be completed by you or them, and courier pickup must be done at our building. You are responsible for all handling charges incurred through the packaging of DNA.