New to NGS? Let us help!

This introductory guide to Next Generation Sequencing covers:

- Selecting the right Next Generation Sequencing service
- Read length and coverage
- Sample preparation guidelines
- Sequencing data output and bioinformatics analysis
- Consultation and experimental design

and more...

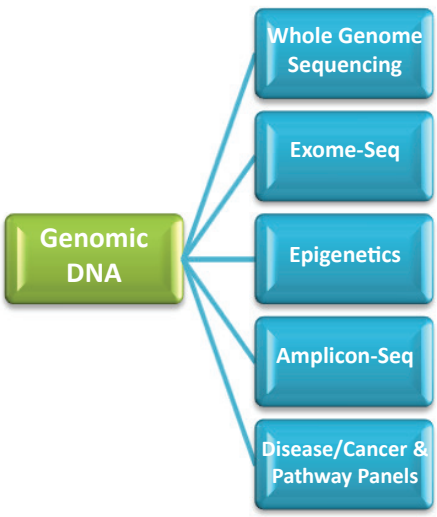
Advantages of using abm's Next Generation Sequencing services

- Cost-effective services with quick turnaround time
- Certified Illumina® CSPro Partner for providing high quality NGS services
- Strict quality control at multiple steps and industry standard NGS platforms from Illumina®
- Dedicated NGS specialists to help with experimental design and data analysis
- RNA-Seq, miRNA-Seq, WGS, metagenomics, epigenetics, Amplicon-Seq, and more!
- Cancer, disease, and pathway gene panels available
- Customizable bioinformatics analysis to suit your project needs
- Confidentiality of samples and sequencing results

Take your research to the next level with NGS

How do I get started?

abm's Next Generation Sequencing services are split into several categories depending on the starting material, required sample preparation and library construction, and the desired data output. In general, the common NGS workflow begins with experimental design and sample quality control, followed by library construction and sequencing. The data is analyzed with specialized bioinformatics software and a summary report is provided along with the results.



Genomic DNA

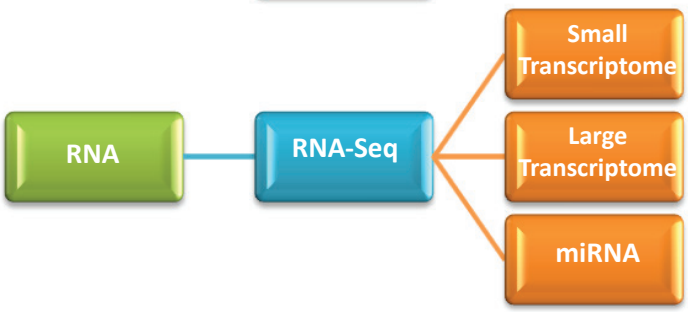
Whole Genome Sequencing (WGS): Characterization of a full genome. Determine the full DNA sequence of an organism's genome, including identifying gene variants and single nucleotide polymorphisms (SNPs).

Exome Sequencing: A cost-effective alternative to WGS to selectively sequence the coding regions of the genome while maintaining high coverage in sequencing depth.

Epigenetic Profiling: Identify differentially methylated DNA regions associated with cancer, other diseases, and regulation of growth and development.

Amplicon Sequencing: Ultra deep sequencing of PCR products for analyzing genetic variations, ideally suited for samples such as the hyper-variable regions of antibodies as well as population samples for SNP detection.

Panel Sequencing: Pre-designed panels providing insights into "if and how" the genetic material impacts and/or triggers various pathological conditions.



Total RNA

Small Transcriptome Sequencing: Transcript profiling of organisms with small transcriptomes (i.e. bacteria and viruses).

Large Transcriptome Sequencing: Transcript profiling of organisms with large transcriptomes (i.e. human and mouse).

miRNA Sequencing: Specifically focusing on quantification and discovery of microRNAs. RNA isolation method must be capable of retaining miRNAs.

Single End or Paired End Reads?

For single end reads, each fragment is only read from one end, generating the sequence of base pairs. For paired end reads, each fragment is read from both ends, allowing for sequencing of longer fragments with higher accuracy. This improves the ability to identify the relative positions of various reads in the genome, making it more effective than single end reads in resolving structural rearrangements such as gene insertions, deletions, or inversions. It can also improve the assembly of repetitive regions. This degree of accuracy may not be required for all experiments and are slightly more expensive, but are generally recommended as they improve the genome alignment and any further downstream analysis as well.

What read length do I need?

Read length refers to the number of base pairs that are read at a time. For example, one read might consist of 50 base pairs, 100 base pairs, or more. For a read length of 50 base pairs, single end reads would read 50 base pairs from each fragment, while paired end reads would consist of 2 x 50bp reads, covering up to 100 base pairs on the same fragment. Longer reads can provide more reliable information about the relative locations of specific base pairs, particularly if the same read sequences can appear in multiple places within a genome. However, it is usually more expensive to generate longer reads. Longer reads are generally not required or recommended for cases where the precise sequence is not important (i.e. RNA-Seq for gene expression levels), as this unnecessarily increases the cost and time for sequencing.

How much depth of coverage will I require?

The depth of coverage is a measure of the number of times that a specific genomic site is sequenced during a sequencing run. In exome sequencing, for example, the target might be 60X coverage, meaning that — on average — each targeted base is sequenced 60 times. This does not mean that every targeted base is sequenced every time; some segments may be read 100 or more times, while others might only be read once or twice, or not at all. The higher the number of times that a base is sequenced, the better the quality of the data. If you want to have 10X coverage of a genome that is 300 million bases (0.3 gigabases or 0.3G) in size, you would need 3 billion base pairs (3G output). If your read length is 100 base pairs, then you would require a minimum of 30 million reads to get 10X coverage of the genome.

For RNA-Seq, we generally recommend a minimum of 20 million reads per sample. For organisms will small transcriptomes, such as bacteria and viruses, we recommend 8 to 10 million reads. For more detailed analyses to determine, for example, allele-specific expression or expression of low-abundant transcripts, 40 million to 80 million reads may be required. For sequencing projects that require higher accuracy — such as studies of alternate splicing — 20 to 40 million paired end reads will provide better results.

How do I prepare and ship my sample?

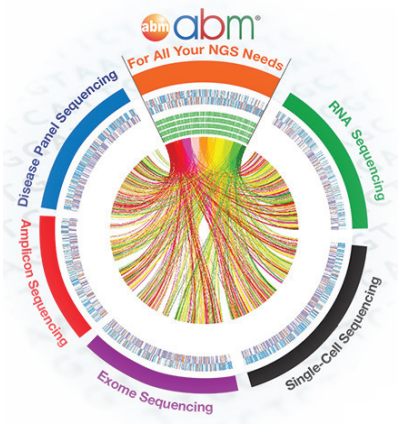
Each NGS service has specific sample preparation guidelines and minimum requirements for both amount and quality of the samples. It is essential that there is enough starting material at or higher than the recommended working concentration for best results. Quality assessment, such as running the samples on a gel or bioanalyzer, must be performed on the samples prior to shipment to abm, as degraded samples with poor quality that do not pass abm's internal quality control will not be processed. All samples are required to be shipped on dry ice and need to be accompanied with an order confirmation number, which can be acquired by sending a completed Order Form to technical@abmgood.com.

Please refer to our NGS Sample Guideline Requirements, Waybill Instructions, Commercial Invoice for Template Samples, and Dry Ice Label Template for more details on sample preparation and shipping.

What types of bioinformatics analysis will be done?

If the reference genome is available (i.e. human or mouse), read mapping and alignment to the reference genome is included in the service (additional fees may apply if the reference must be imported into our alignment database).

- Data is available in the industry standard format: FASTQ (default).
- Other formats available upon request (additional fees may apply).
- RNA-Seq includes: Read mapping (Tophat), gene expression quantification (Cufflinks/FPKM), and QC (RseQC/FastQC).
- WGS and Exome-Seq include: Read mapping (BWA), deduplication (Picard), and local realignment, base quality recalibration, and variation calling (GATK).
- All NGS services come with 3 months FREE storage of raw data.



Can I request additional bioinformatics analysis to be done with my data?

Our trained specialists can help you tailor the bioinformatics analysis to your research needs. For example, a comparison of two RNA-Seq samples can be performed to find differentially expressed genes using Cuffdiff. Variant calling can be carried out to identify variants, indels, and even single nucleotide mutations or polymorphisms in gene sequences. Specialized bioinformatics analysis will require more processing time and may incur additional fees. For more details or a custom quotation for your sequencing project, please contact NGS@abmgood.com.

How can I place an order?

If you are ready to place an order, please send a completed Order Form to technical@abmgood.com. You will receive an order confirmation shortly thereafter and can proceed with shipping your samples to abm. Our strength is in our ability to customize any service to meet your research needs. Please do not hesitate to contact us about our services:

- Custom bioinformatics analysis
- Request for sequencing services not listed on our website
- Bulk order discounts for large sample numbers
- and more...

For help with project design, NGS consultation, or custom quotations, email us at NGS@abmGood.com

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