Sequencing Services

UNBIASED SHORT READ SEQUENCING PLATFORM

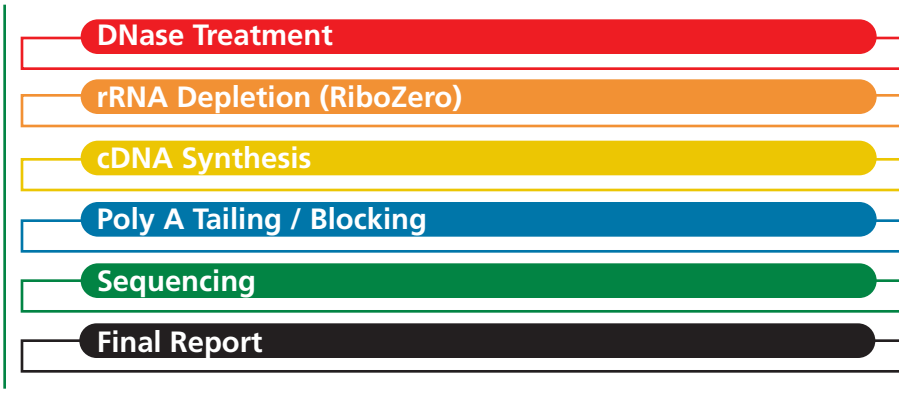
SEQ LL

SEQUENCE THE LOWER LIMIT

SeqLL brings a comprehensive range of services to flexibly implement custom solutions for NGS content. From project design to final report, we work with your team to define how your results are delivered. Sample preparation, sample sequencing, data depth and data interpretation are critical to your program success. Open communication ensures progress is managed to approved timelines.

true Single Molecule Sequencing (tSMS) simplifies sample preparation by eliminating library development, PCR amplification and reverse transcription. Accuracy is increased because we count individual molecules. Sensitivity is increased so rare transcripts are captured, small changes in transcription are above system noise. Accurate short reads (25-100bp) allow sequencing of degraded DNA and RNA samples like FFPE and ancient DNA more efficiently. Platform sensitivity means cfDNA can be confidently identified at lower sample concentrations, diseases potentially found earlier. Our team understands the need to quickly define the critical path of each drug discovery, drug development or assay panel development program. They come from industry, hear the important challenges and collaboratively build the project plans by bridging our experience with your expertise. tSMS provides:

- Flexible implementation
- True sample representation
- Low sample input requirements
- Rare transcript and expression change identification
- Genetic Biomarker panel development



RNA Sequencing

cDNA based RNA Seq - Transcription inititation studies, differentially expressed genes and pathway analysis related to oncololgy, cardiology, metabolic and infectious diseases have been investigated with tSMS. Programs rely on accurate primary data that is useful today as well as being useful for future investigations.

Typical solutions involve diverse techniques:
- Ribosomal RNA depletion
- Host DNA removal
- NSR primers for interference removal
- Direct RNA Sequencing
- Custom flow cell surface chemistry for novel investigations

RNA Seq
Differentially Epressed Genes
Transcription studies
Mutation and fusion detection
Spice Variant analysis
Differential Gene Expression

DRS - Direct RNA Sequencing - sequences RNA molecules without conversion to cDNA. The technology offers direct observation of RNA and allows you to understand the true nature of normal and disease transcriptomes and pathways without adding any sample preparation bias or sequencing artifacts.

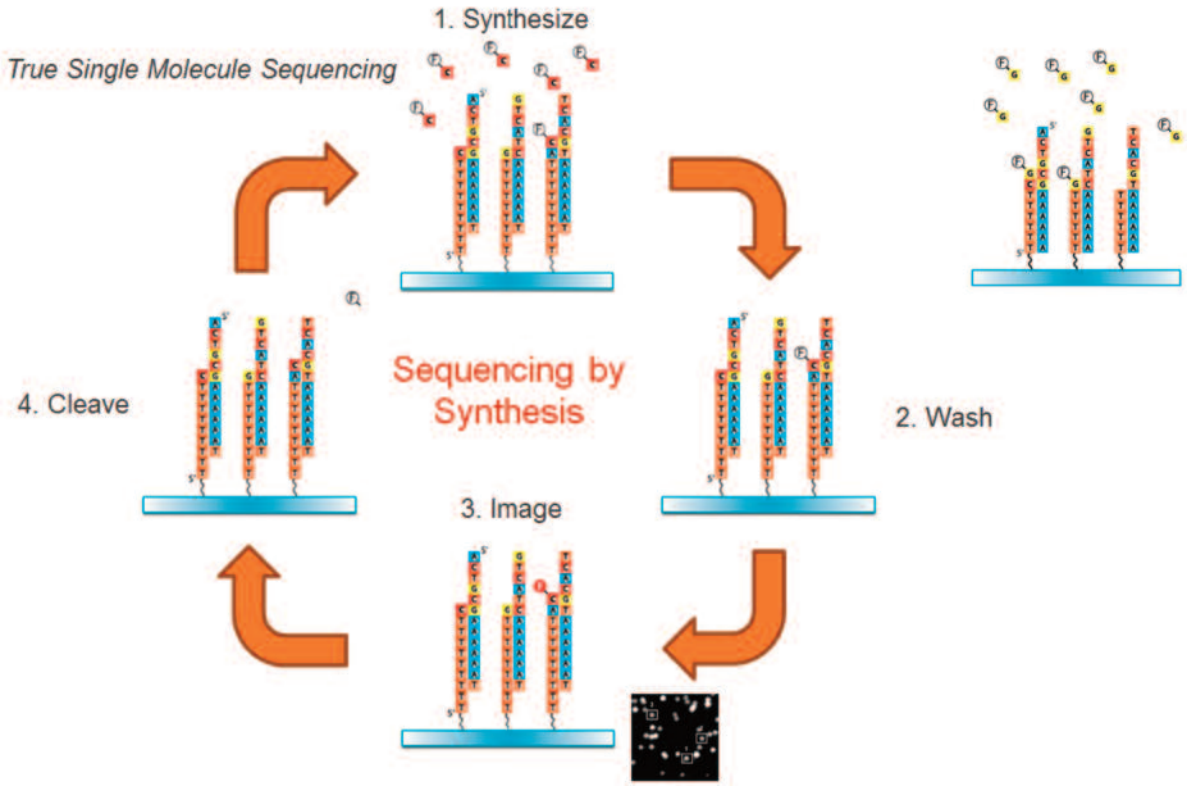
Library Interrogation ensures higher quality library content. Combining high sensitivity with amplification independent single molecule sequencing tSMS is able to confidently detect and identify Differentially Expressed Genes and rare transcription modification events. Interrogate your libraries to find those rare transcripts that have not been identified due to the introduction of amplification bias while creating your study libraries.

DRS
Simplified Sample Prep
Single molecule synthesis and reads
Authentic Transcriptome detail
Bias free data creation or generation
Evaluate 3' end formation for gene expression studies
Study transcription interference events
Direct transcriptome investigations and expression regulation studies

FFPE - Formalin Fixed Paraffin Embedded samples can degrade during processing or storage that can challenge other NGS systems to provide meaningful information and results. tSMS provides the coverage and sensitivity to accurately analyze and report content information from damaged and degraded FFPE samples.

Micro RNA Sequencing - allows any species of RNA to be profiled without the need for creation of specialized probes based on previous sequencing or external structural information. Accuracy is high over several orders of magnitude of transcript concentrations regardless of size or whether it is known or unknown.

Micro RNA Sequencing
Profile any Species of RNA
No need for custom probes
Sequence unknown
High Quantitation Accuracy
Sequence any size



True Single Molecule Sequencing (tSMS™)

DNA sequencing has undergone a revolution within the last fifty years. Beginning in the late 1970s, researchers have been improving the way we sequence DNA and trying to provide accurate results at a higher throughput.

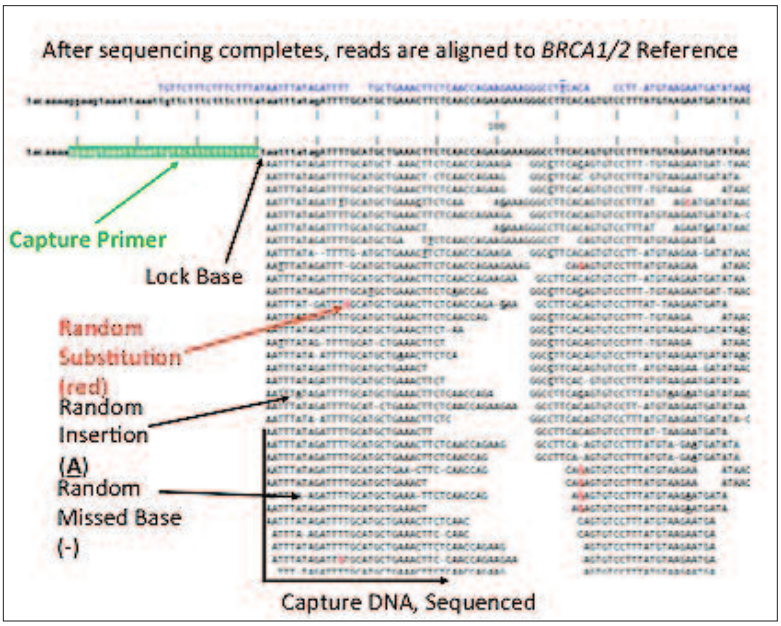
The first generation of DNA sequencing technology, Sanger Sequencing, involved adhering nucleotides (dNTPs) and modified nucleotides to a template strand of DNA that was later run through gel electrophoresis to determine the exact order of the sequence. While this method of sequencing is accurate, it can be slow and extremely costly, keeping many smaller research labs from being able to run tests that could further their studies. Though this technique is still a valid way to sequence, scientists quickly began looking for technology that would allow for achieving faster and less expensive results.

To accomplish this, numerous approaches were developed resulting in second and third generation methods of sequencing. True Single Molecule Sequencing (tSMS™) improves upon the methods developed for first-generation sequencing. The tSMS™ technology, differs from previous methods in that it sequences strands in a massively parallel manner without any amplification steps, meaning that many strands can be sequenced at once and also provide unique information.

Flow cells are packed very densely with genetic material, allowing for higher throughput. The tSMS method typically utilizes a dT50 primer to initiate sequencing from a 3′ poly-A tail, though other capture primers may be used to increase the specificity of sample hybridization. When the strand is adhered to the flow cell, a laser illuminates the field, causing the incorporated "Virtual Terminators" to fluoresce. A CCD camera then takes pictures of the flow cell, marking the location of each strand. When the incorporation chemistry is complete, scientists and informatics experts work to align the sequence information prior to distributing these alignments to researchers and customers. The tSMS™ method is advantageous as it allows researchers to directly sequence the DNA without sample preparation steps like ligation or PCR amplification. Furthermore, the technology allows for massively parallel sequencing, increasing the throughput tremendously without requiring phased incorporations.

High Accuracy Sequencing

Effective error rates are very low as errors are random and can be easily identified and eliminated as they are not repeated in amplification cycles.



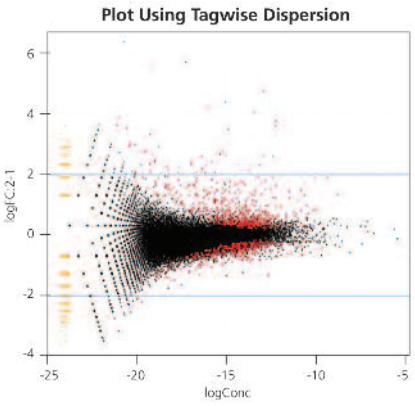
Bioinformatic Services

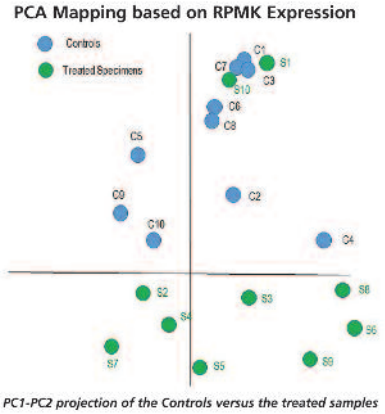
As we push the limits of NGS methods and protocols we are able to expand into broader and more sophisticated applications that address complex biological questions. The corresponding data set generated becomes larger and more complex. The old standard of applying a basic data set review may not adequately address subtle differences that your research requires. Maximizing the value of your work begins with creating a protocol that specifies all areas from specimen collection, sample processing and finally optimizing the data set review.

SeqLL offers Basic, Advanced and Custom Bioinformatics packages to serve as a partner in your research at the levels required to meet the outcomes of even the most challenging projects.

Typical Plots from a standard data review include

- Digital Gene Expression tables (DGE) can be created
- Differentially expressed genes can be located and reported
- MW and MA plots vs Controls provide insight into the effect
- Principal Component Analyses
- The biological function of differentially expressed





PC1-PC2 projection of the Controls versus the treated samples

Custom Sequencing - projects and protocols can be used to optimize the data set being generated during sequencing. The customization is not limited to programmable features of a system.

Direct Capture - utilizes custom flow cells which are created allowing only specific sequences to be captured minimizing costly time consuming sample preparation and not introducing bias due to amplification.

Not So Random Primer Sequencing - Transcriptome studies based on quantitative sequencing can estimate levels of gene expression by measuring target RNA abundance in sequencing libraries. Sequencing costs are proportional to the total number of sequenced reads, and in order to cover rare RNAs, considerable quantities of abundant and identical reads are needed. This major limitation can be addressed by depleting a proportion of the most abundant sequences from the library. However, such depletion strategies involve either extra handling of the input RNA sample or use of a large number of reverse transcription primers, termed not-so-random (NSR) primers, which are costly to synthesize. Taking advantage of the high tolerance of reverse transcriptase to mis-prime, it is possible to use as few as 40 pseudo-random (PS) reverse transcription primers to decrease the rate of undesirable abundant sequences within a library without affecting the overall transcriptome diversity. PS primers are simple to design and can be used to deplete several undesirable RNAs simultaneously, thus creating a flexible tool for enriching transcriptome libraries for rare transcript sequences.[1]

Sample Preparation Services

Talk to our expert staff about their experience in sample preparation about developing protocols that meet your exact needs including cDNA synthesis; Ribosomal RNA depletion; Poly A tailing and blocking and gene enrichment.

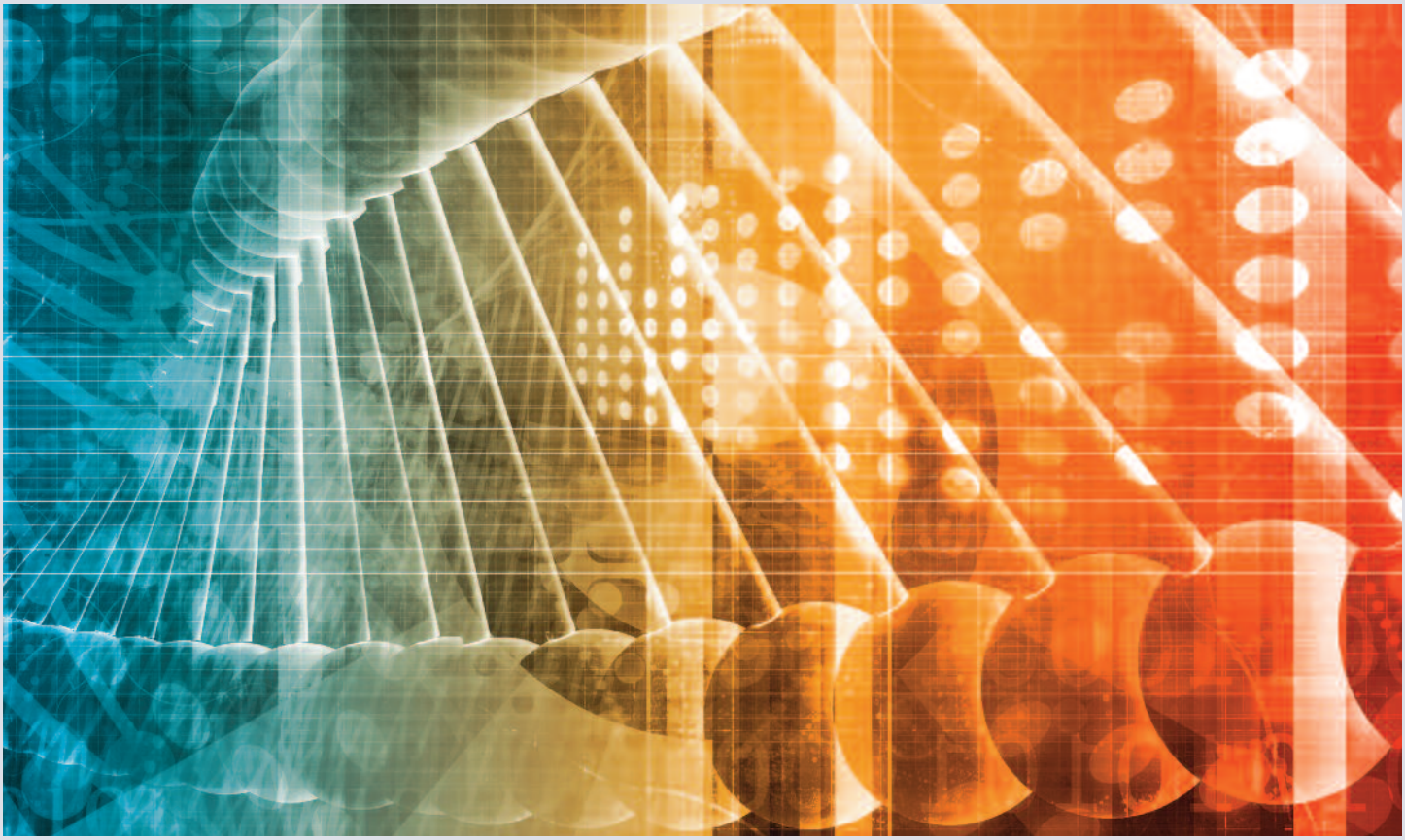
Research Studies

SeqLL's staff is happy to discuss specific requirements for custom projects such as:

- Tumor Profiling
- Rare Cell Populations
- Exosomal RNA or DNA investigations
- Disease Pathway Modulation
- Genetic Biomarker Panel Identification

[1] Courtesy of Dr. Charles Plessy, RIKEN JAPAN
http://www.biotechniques.com/BiotechniquesJournal/2016/April/Targeted-reduction-of-highly-abundant-transcripts-using-pseudo-random-primers/biotechniques-364067.html

DNA Sequencing



Short Read DNA Sequencing - is a highly accurate, cost effective technology. The tSMS technology is a platform that has been optimized for short read RNA and DNA sequencing. This has resulted in benefits such as:

- Low sample inputs and amounts required due to the system's high sensitivity allow accurate results for applications such as single circulating tumor cells.
- No bias added due to external amplification steps
- Flexibility as sequencing of enriched materials from solution capture can be accomplished using a wide variety of external kits and sample preparation protocols.

Digital Gene Expression - can provide the information needed with a much lower of required reads reducing the cost per sample without sacrificing the information required.

ChIP Seq - can provide valuable information in epigenetic research looking across the genome for DNA-protein interactions.

Exome Capture - protocols extract and sequence the exome (s) in a genome and compare variations against known references.

Mate - Pair & Paired End Sequencing allows the researcher to effectively generate information regardless of the limitation on long reads allowing de novo sequencing to assemble an entire genome.

tSMS Flexibility allows wide applicability of the technology to areas such as microbial detection and infection time course assessment.

Typical Performance Specifications

Strand Output	12-20M usable strands / channel 600M - 1B usable strands / run
Total Output	420 - 600 Megabases per hour
Throughput	105 - 180 Megabases / hour
Read Length	25 - 55 bases in length 33 - 36 average length
Run Time Accuracy	30 quads > 99.995% consensus accuracy @ >20X coverage
Raw Error Rate	~2% substitutions <1.5% insertions <3.0% deletions Consistent from 20-80% GC content of the target DNA (independent of read length)
Standard Template Size	25 - 5,000 bases
MicroRNA Template Size	For < 20 bases

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