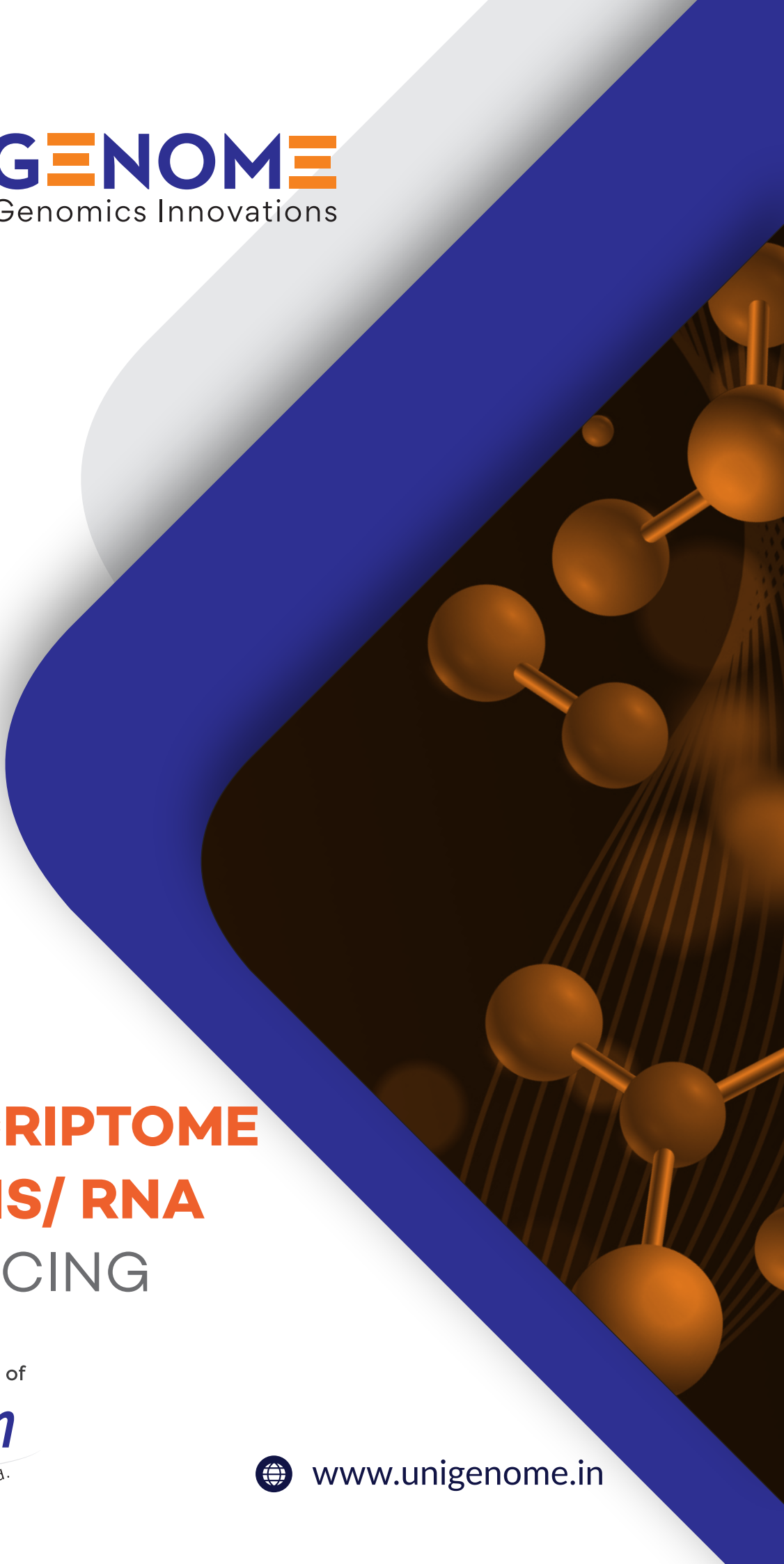
UNIGENOME
Leading Genomics Innovations



WHOLE TRANSCRIPTOME ANALYSIS/ RNA SEQUENCING

A Lifesciences Division of
Unipath
SPECIALTY LABORATORY Ltd.

www.unigenome.in

Whole transcriptome analysis/ RNA sequencing

RNA sequencing (RNA-seq) was developed more than a decade ago and since then has become a ubiquitous tool in molecular biology that is shaping nearly every aspect of our understanding of genomic function. RNA-seq is most often used for gene discovery and analysing differential gene expression (DGE). The essential stages of a DGE assay have not changed substantially from the earliest publications. The standard workflow begins in the laboratory, with RNA extraction, followed by mRNA enrichment or ribosomal RNA depletion, cDNA synthesis and preparation of an adaptor-ligated sequencing library. The final steps are computational: aligning and/or assembling the sequencing reads to a transcriptome, quantifying reads that overlap transcripts, filtering and normalizing between samples, and statistical modelling of significant changes in the expression levels of individual genes and/or transcripts between sample groups.

Unigenome helps the researchers with the best solutions for transcriptome sequencing using Illumina platforms. Our team of experienced scientists helps the customers from sample collection, data generation, analysis and provides support till the publication. Unigenome also helps scientists and researcher to get trained for the analysis of their data through step by step explanation of all the points via detailed report and practical exposure (If required).

Important factors affecting RNA sequencing study

A key consideration when designing an RNA-Seq study is the cost. The factors listed below can be used to calculate RNA-Seq study costs.

Study Size and Replicates

- Determine the total number of samples in your study and assess whether or not you will need to prepare and run replicates. Scientifically six replicates per sample are recommended but due to high cost factor, many researchers perform the RNA-seq studies in triplicates, duplicates or single.

Read Depth

- Usually RNA sequencing data is considered at a 100X depth of transcriptome size of given sample. For example, human genome size is 3.34 GB so its transcriptome size will be considered as 1% of genome size which is 33.4Mb. With this calculation, at 100X depth coverage the data should be 3.5 to 4 GB per sample. A sample for which genome size is unavailable, 5% of the nearest species reference genome size is considered as transcriptome size for data generation.

Sample Requirements

- We accept isolated RNA, microbial cultures, plant tissues, animal tissues, human tissues, blood samples, cell line samples etc

Isolated Total RNA

- 8-10µg of total RNA should be provided with RNA Integrity Number (RIN)> 6.
- RNA must not be degraded & should be free from DNA contamination.

Quality control of RNA SAMPLES

- Samples will be subjected to both qualification, quantification and those having RIN > 6 will be QC passed. However, inclusion of low RIN value of the samples will be processed upon customer's confirmation.
- Sample requirements for various sample types:

Human Samples

- **Cell Lines:** 1ml of 1OD culture or 106 cells should be pelleted, washed to remove media and can be sent in 1ml Trizol reagent with dry ice to Unigenome for further processing. Harvested cells can be stored at -20 degree or -80 degree (preferably -80 degree) until shipment.
- **Blood Samples:** 5-10ml of blood in Paxgene tubes. Blood samples collected in K2-EDTA, are prone to RNA degradation if stored longer than 24 hours. Paxgene tubes can maintain the RNA quality for longer period (at least 3-4 days).
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Microbial cultures

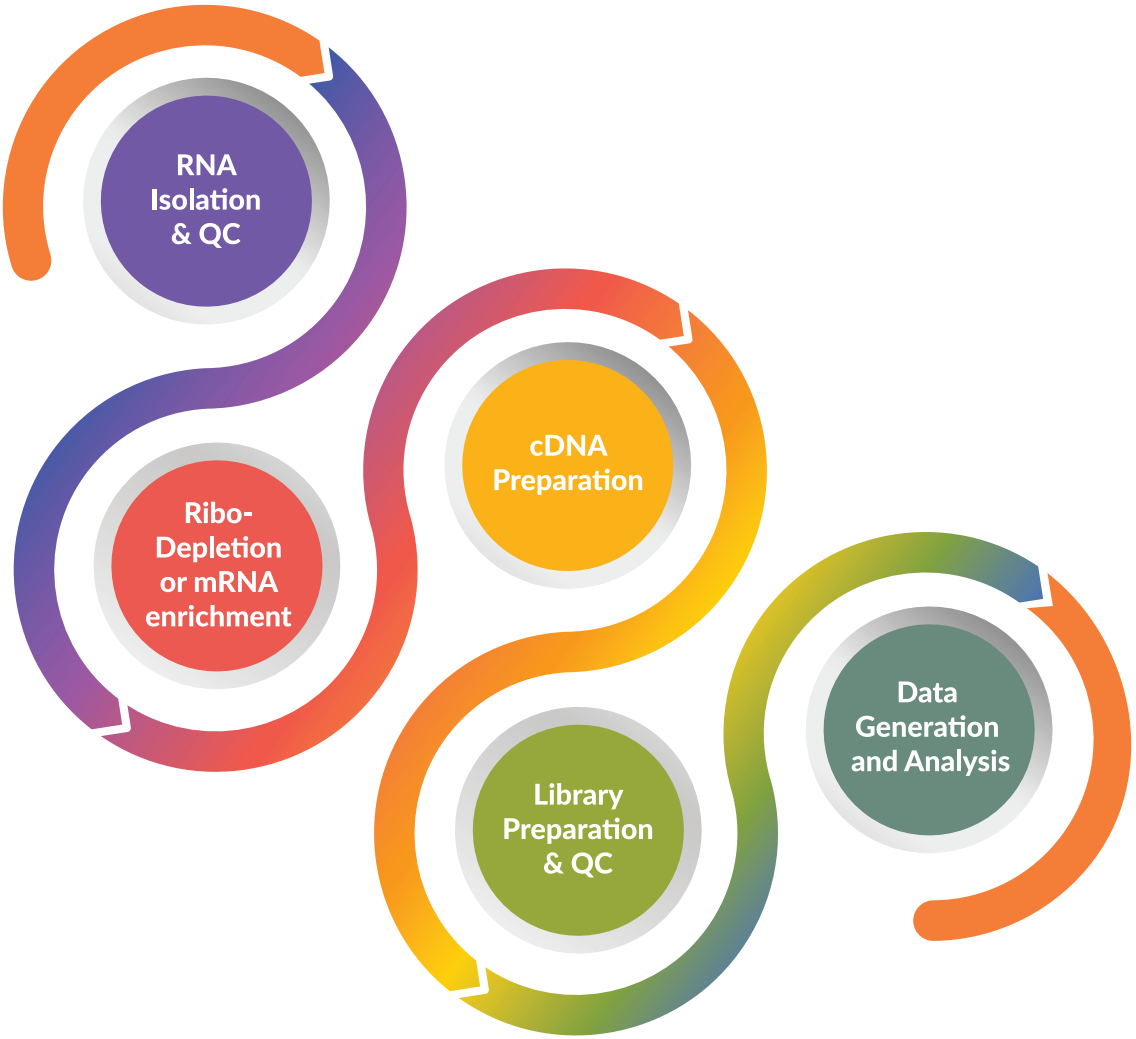
- 1ml of 1OD culture or 106 cells should be pelleted, washed to remove media and can be sent in 1ml Trizol reagent with dry ice to Unigenome for further processing. Harvested cells can be stored at -20 degree or -80 degree (preferably -80 degree) until shipment.

Plant TISSUES

- Seedling, leaf, stem, flower, fruit, grain etc any plant tissue can be used for RNA sequencing as per experimental requirements.
- Minimum 3-5 gm of tissue sample should be transported in RNA later solution.
- The volume of RNA later should be at least ten times the volume of tissue.
- Plant tissue samples should be harvested and immediately immersed in RNA later solution.
- Ideally, if sampling has to be done from the field, 50ml falcon along with RNA later solution should be ready in dry ice box, so that immediately after harvesting sample can be immersed in the RNA later solution.

Note: All types of samples should be transported in dry ice (-20°C) containing cool packs to Unigenome, Ahmedabad, Gujarat, India.

Workflow of RNA sequencing



Bio-informatics Pipeline of RNA sequencing

There are two approaches for analysis of transcriptome data:

- **Denovo analysis:** This is basically reference genome free analysis. Thus this approach is mostly used for species for which no prior reference genome or transcriptome is available.
- **Reference based analysis:** This approach is used when there exist proper annotated genome for particular species.

Denovo based transcriptome analysis/Deliverables:

- Clean reads after filtration of adapter and low quality bases
- Denovo assembly of reads to generate transcripts
- Clustering of transcripts to generate unigenes
- Assembly statistics and validation

- Coding DNA sequences (CDS) prediction
- Annotation of the predicted CDS against NCBI's NR database, uniprot, Pfam and KOG database
- GO functional classification of CDS in biological processes, cellular components and molecular function
- Expression profiling of CDS
- Differential expression analysis of CDS (if more than 1 samples)
- Significantly Up-regulated and down-regulated (log2fold>2 and P-value <0.05)
- Pathway analysis using KEGG
- Identification of transcription factor (in case of plant)
- Simple sequence repeats (SSRs) discovery
- Graphical presentation of differentially expressed genes in terms of heatmap, volcano plot and scatter plot
- Comprehensive compiled report and data deliverables

Denovo based transcriptome analysis/Deliverables:

- Clean reads after filtration of adapter and low quality bases
- Mapping of transcriptome data on reference genome
- Differential gene expression (DEG) analysis
- Functional annotation of expressed genes in samples
- Significantly Up-regulated and down-regulated (log2fold>2 and P-value <0.05)
- Heatmap and volcano plot for DEG
- Pathway analysis using KEGG for DEG
- Comprehensive compiled report and data deliverables

Optional

- Submission of data to NCBI (raw data and assembled contigs)
- Further analysis, figures, and tables required by specific journals are provided as per customer's requirement

Timeline: It depends upon genome size, technology selected, number of samples, complexity and coverage required. The generalized time line is six to ten weeks

Note: Additional/customized Analysis is also provided upon request with extra charges.If you have data generated, our team of expert bioinformaticians can help you analyze your data and translate it into meaning full output.

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