NEXT-GENERATION SEQUENCING SERVICE

Humanizing Genomics
macrogen

Humanizing Genomics macrogen | NGS NEXT GENERATION SEQUENCING

Whole Genome Sequencing

Introduction

De novo sequencing is typically performed without prior knowledge of the sequencing data. De novo sequencing has proven successful in confirming and expanding upon results from database searches, providing excellent resources for understanding a species. Some of the most crucial information, obtained by resequencing of an organism's genome DNA, are the individual variations in the genome, such as single nucleotide polymorphism (SNP), copy number variation (CNV), and structural variation (SV).

Sequencing Platforms

- HiSeq 2500/4000, HiSeq X, NovaSeq 6000
- NextSeq 500, MiSeq
- PacBio RS II, PacBio Sequel

Data Analysis*

De novo Sequencing

- Standard Data Analysis
  - Consensus sequence assembled into contigs
- Advanced Data Dnalysis
  - Gene prediction & annotation

Resequencing

- Standard Data Analysis
  - Mapping to the current reference genome
  - Analysis of mapping statistics
  - SNPs and InDels calling
  - Variant list in vcf or Excel formats
- Advanced Data Analysis

  A variety of options for mapping algorithms, variant detection algorithms, annotations, mapping to public databases

Exome / Targeted Sequencing

Introduction

Human exome sequencing is selective sequencing of coding regions of the human genome, after the exome has been efficiently captured.

- Three enrichment methods are available for specific applications:
  - Agilent SureSelect Human All Exome Kit
  - Illumina Nextera Exome Enrichment Kit
  - Twist BioScience Human Core Exome Kit
- Macrogen offers extensive data filtering and mapping for accurate SNP and InDel determinations.

Sequencing Platforms

- HiSeq 2500/4000, NovaSeq 6000
- NextSeq 500, MiSeq

Data Analysis*

Exome Sequencing

- Standard Data Analysis
  - Variant Calling (SNPs / InDels) & Annotation
- Advanced Data Analysis
  - CNV (Copy Number Variation)
  - Various Variant Calling Pipeline
  - Cancer Analysis / Family Analysis / Population Analysis

Customized Targeted Sequencing

- Standard Data Analysis
  - Variant Calling (SNPs / InDels) & Annotation
- Advanced Data Analysis
  - Various Variant Calling Pipeline
  - Population Analysis

Single Cell Sequencing

Introduction

Dissecting genomic, transcriptomic and epigenomic cellular heterogeneity using single cell Sequencing is essential for understanding biological system. With 10x chromium Gemcode technology system, Macrogen provides a high resolution of individual cell copy number variation, gene expression, cell surface protein expression, chromatin accessibility and immune diversity (TCR, BCR).

* Data Analysis may vary depending on the availability of reference and the type of platform.

Sequencing Platforms

- HiSeq 2500/4000, NovaSeq 6000

Data Analysis*

Single Cell RNA Sequencing

- Standard Data Analysis
  - Gene expression and cell clustering analysis
  - Simultaneous single cell analysis of gene expression, immune profiling and cell surface protein expression
- Advanced Data Analysis
  - Sample aggregation
  - Gene expression and cell clustering analysis (Seurat_2.3.4)
  - Cell identification
  - Differentially Expressed Genes (DEGs) analysis
  - Gene Ontology analysis
  - Trajectory analysis

Single Cell immune Profiling

- Standard Data Analysis
  - Paired full length V(D)J gene sequence identification
  - Clonality and diversity of TCR and/or BCR

Single Cell Surface protein Profiling

- Standard Data Analysis
  - Surface protein expression and cell clustering analysis

Single Cell ATAC Sequencing

- Standard Data Analysis
  - Chromatin accessibility profiles and cell clustering analysis

Single Cell CNV Profiling

- Standard Data Analysis
  - Chromatin accessibility profiles and cell clustering analysis

Exosome/Extracellular RNA Sequencing

Introduction

Exploration of extracellular vesicles (EVs) derived RNA from biological fluids and cell culture media have been in the center of spot-light for disease marker discovery and identification of RNA molecules in EV therapy. Macrogen provides exRNA sequencing service including high yield of exosome isolation, quality control of exRNA, high success rate of library preparation, sequencing, and expression profiles of diverse RNA types.

Sequencing Platforms

- HiSeq 2500

Data Analysis*

- Standard Data Analysis
  - RNA Composition Analysis
  - Expression Profiles of small RNA (miRNA, piRNA, snoRNA, snRNA, Y RNA, vault RNA) and mRNA
  - Novel miRNA prediction
- Advanced Data Analysis
  - Differentially expressed small RNAs
  - Customized Analysis

Transcriptome Sequencing

Introduction

Transcriptome sequencing using Next Generation Sequencing is a fast and reliable method for identifying genomic information. Macrogen provides whole mRNA transcript expression analysis, enabling novel gene discovery, identification of novel SNP and InDel, novel splice variant and chromosomal rearrangement discovery, and identifications of fusion genes.

Sequencing Platforms

- HiSeq 2500/4000, NovaSeq 6000

Data Analysis*

De Novo Sequencing

- Standard Data Analysis
  - *De novo* Assembly Statistics
  - Gene Expression Profile
- Advanced Data Analysis
  - Blastnt or Blastnr
  - Differentially Expressed Genes (DEGs)
  - Gene Annotations & Ontology Analysis

Resequencing

- Standard Data Analysis
  - Mapping Statistics
  - Gene Expression Profile
  - SNPs and InDels calling by mapping to reference genome (Human, Mouse, Rat)
  - Novel Transcripts
  - Alternative Spliced Transcripts
  - Fusion Gene (Human, Mouse, Rat)
- Advanced Data Analysis
  - Customized Analysis
  - Differentially Expressed Genes (DEGs)
  - Gene Ontology Analysis

* Data Analysis may vary depending on the availability of reference and the type of platform.

Small RNA Sequencing

Particularly, small RNA molecules encoded from genomes are responsible for regulating the gene expression.
Macrogen's NGS technology is able to sequence and quantify all the small RNA families in a sample and profile the miRNA, siRNA, piRNA and other non-coding RNAs.

Data Analysis*

Small RNA Sequencing

- Standard Data Analysis
  - Expression Profiles of small RNA (piRNA, snoRNA, snRNA, Y RNA, vault RNA)
  - Novel miRNA
- Advanced Data Analysis
  - Target Prediction of Known and Novel miRNA
  - Gene Set Analysis
  - Differentially expressed miRNA

Epigenome Sequencing

WGBS (Whole Genome Bisulfite Sequencing) Methylation Sequencing

Introduction

Methylation is one of the major pathways of gene expression regulation in chromosomal DNA. Therefore, to characterize methylation of bases is to understand regulation of genes.
Comparison of the sequence obtained from the bisulfite-treated library to the published sequence enables the identification of differential methylations. The methylation state on the target regions, e.g. CpG islands) can be quantitatively haracterized.

Sequencing Platforms

- HiSeq X
- HiSeq 2500/4000, NovaSeq 6000
- MiSeq System

Data Analysis*

- Standard Data Analysis
  - Global Methylation Profiles
  - Specific Methylation Profiles [CG Islands / Differentially Methylation Regions (DMRs)]
- Advanced Data Analysis
  - DMR-Associated Genes
  - Gene Set Analysis
  - Comparative Analysis

Comparison of Epigenomic Application

Application	Restriction Enzyme	Bisulfite Conversion	Advantages
WGBS	-	Yes	High resolution
RRBS	Yes	Yes	Each sequencing-read contains at least one CpG site

Long Read Sequencing

Introduction

Researchers continue to face challenges in finishing genomes, characterizing variations, and understanding the function of key biological markers although NGS technology has dramatically developed. Achieving accurate read lengths of 1,000 base pairs (bp) or longer in a single sequencing reaction is valuable for investigators in many areas of research.
Macrogen introduces PacBio RS II / Sequel sequencing technology to resolve the long reads (up to 10kb) of DNA strand, allowing observation of structural and cell type variation not clarified by the other sequencing technologies. The flexibility of the sequencing systems of long reads in Macrogen allows you to switch easily between platforms and applications as investigational needs change in the dynamic research environment.

Sequencing Platforms

- PacBio RS II / PacBio Sequel

Applications

Genome Finishing & *De novo* Assembly

- Support of multiple applications: genome finishing, metagenomics, *De novo* assembly, meta-assembly with long & short reads.
- High-quality reads of up to 20Kb

Whole Human Genome Phasing

- Examines the unique haplotype content of two homologous chromosome
- One assay to phase over 94% of heterozygous SNPs and InDels
- Simple push-button analysis within a day

* Data Analysis may vary depending on the availability of reference and the type of platform.

Metagenome Sequencing

Introduction

NGS is emerging as a powerful tool for profiling complex microbial communities. This new technology dramatically reduces both the time and cost of DNA sequencing, making it possible for a small laboratory to completely sequence the genome of their favorite bacterium.

For many bacterial species of interest, various strains have been sequenced. These reference strains are attractive subjects in genetic engineering, as derived strains can easily be compared at the genomic level against the parental strain using Macrogen's NGS techniques.

Amplicon Metagenomic Sequencing

Metagenomic sequencing enables efficient identification of microorganism diversity in a specific environment.

Sequencing Types & Platforms

- 16S rDNA sequencing using MiSeq system
  - V3–V4 Regions
  - Customized Regions
- Full-length 16s rRNA sequencing on PacBio RS II
- 18S rDNA/ ITS sequencing

Shotgun Metagenomic Sequencing

Shotgun metagenomic sequencing is a comprehensive sampling method of all the genes in all the organisms present in a given mixed sample. The method enables to evaluate the microbial diversity and detect the abundance of species under different conditions. For shotgun sequencing, DNA is purified and randomly sheared into smaller fragments before sequencing. Shotgun metagenomic sequencing provides information both about which organisms are present and what metabolic processes are possible in the community.

Data Analysis*

Amplicon Metagenome Sequencing

- Standard Data Analysis
  - Community Diversity Analysis (OTU)
  - Probiotics Analysis
- Advanced Data Analysis
  - Phylogenetic Tree
  - Hierarchical Taxonomy Graph
  - Heatmap
  - PCA biplot

Shotgun Metagenome Sequencing

- Standard Data Analysis
  - Assembly
  - Gene Prediction & Annotation
  - Taxonomy Analysis

* Data Analysis may vary depending on the availability of reference and the type of platform.

Technologies

HiSeq X ten
HiSeq 2500/4000
NovaSeq 6000
MiSeq / NextSeq 500
PacBio RS II / Sequel
Ion PGM / Proton
10X Genomics

Bioinformatics

De novo Assembly
Reference Mapping
Variant Calling (SNP / InDel)
CNV & SV
Gene Expression
Functional Annotations

Applications

Whole Genome Sequencing (*De novo* / Re-seq)
Exome / Targeted Sequencing
Single Cell Sequencing
Exosome/Extracellular RNA Sequencing
Transcriptome (mRNA / small RNA) Sequencing
Epigenome Sequencing
Metagenome Sequencing
Long Read Sequencing

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Overview of Macrogen's NGS Research Application and Sequencing Platform

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