DNA Sequencing

차세대염기서열분석 (Next Generation Sequencing, NGS) 은 빠른 시간내에 대용량의 데이터를 생산하는 기술로, Human, Animal, Plant 및 Microorganism 등을 대상으로 다양한 연구목적에 활용할 수 있습니다.
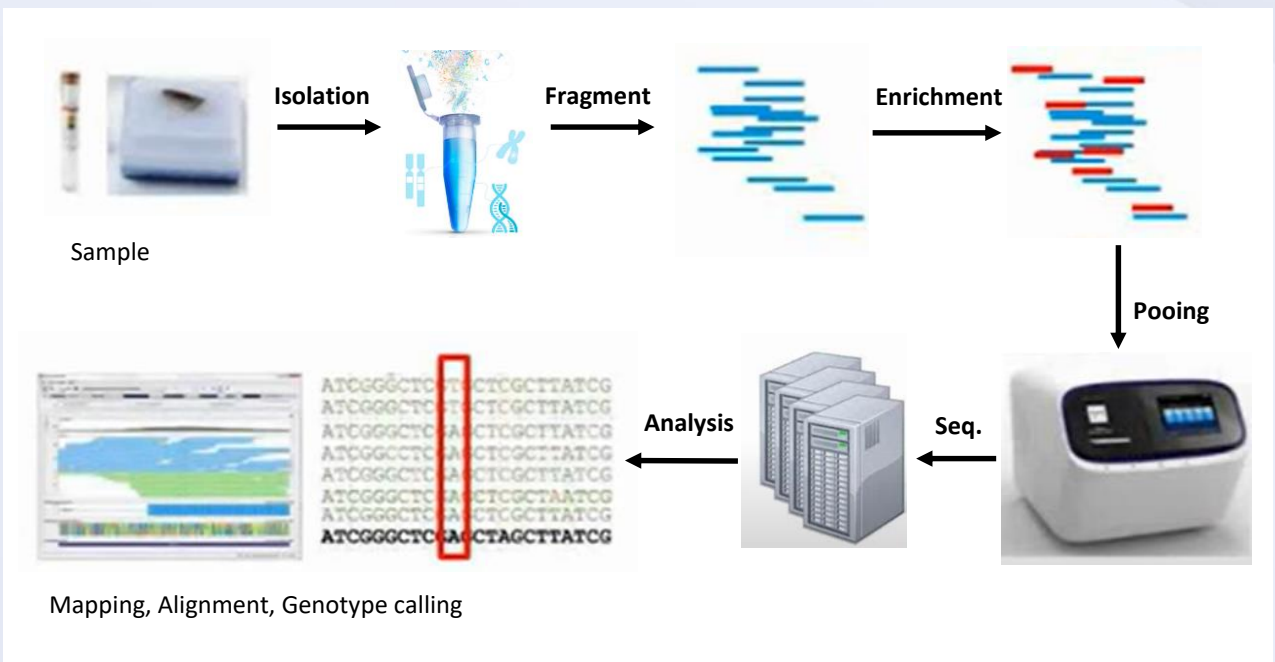
Human Exome Sequencing, Targeted Sequencing등 고객 여러분의 연구 목적에 맞춰 적절한 NGS application으로 실험 계획을 디자인해 드리며, 최적화된 분석 결과를 제공해 드립니다.
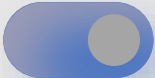
- Human Exome Sequencing
- Target DNA Sequencing

주요분석서비스

Application	Standard Analysis	Sample Requirements
Human Exome Sequencing	- Read Mapping - Variant calling (SNV, indels) - Variant annotation - Seq Depth > 50x	·DNA1μg (Conc. ≥ 50ng/$\mu\ell$) ·260/280 ration 1.8~2.0
Target DNA Sequencing	- Read Mapping - Variant calling (SNV, indels) - Variant annotation - Clinical annotation - Seq Depth > 100x	· 0.5~1μg (Conc. ≥ 10ng/$\mu\ell$) ·260/280 ration 1.8~2.0

NGS Workflow

Sample → Isolation → Fragment → Enrichment → Pooing → Seq. → Analysis → Mapping, Alignment, Genotype calling

Transcriptome Sequencing

Human, mouse 및 동식물 등 다양한 시료로부터 정상군과 대조군의 유전자 발현량의 차이, novel gene, fusion gene 분석이 가능합니다. 약물 treat 후 유전자 발현 차이를 보거나, 질환에 연관된 유전자의 기능 연구에 활용할 수 있습니다. mRNA, small RNA 등 다양한 종류의 전사체 분석이 가능합니다.
시료양이 작은 경우 (FFPE 시료포함) Ion AmpliSeq transcriptome library 제작 kit을 이용하여 유전자 발현 분석이 가능 합니다. AmpliSeq Transcriptome 서비스는 2만여 개의 유전자의 발현 차이를 빠르고 정확하게 분석한 데이터를 제공합니다. (human, mouse 가능)
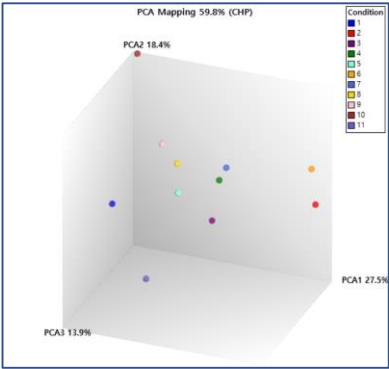
- RNA Sequencing
- Small RNA Sequencing
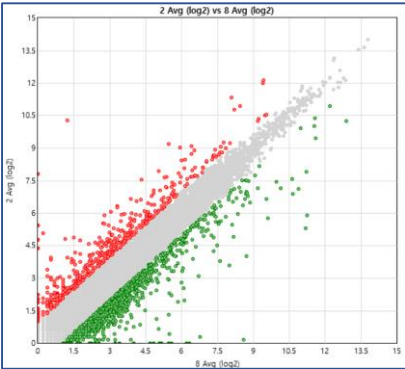- AmpliSeq RNA Sequencing

주요분석서비스

| Application	Standard Analysis	Sample Requirements
RNA Transcriptome Sequencing*	- Reference alignment - Abundance Estimation - Differential expressed genes (DEGs) - Novel gene, fusion genes - Seq Depth 15-20M Reads	·Total RNA 1μg (Conc. ≥ 100ng/μℓ) ·RIN ≥ 7 ·DNA-free
Small RNA Sequencing	- Reference alignment - Abundance Estimation - Discovery of novel/known miRNA - data size 5M Reads	·Total RNA 1μg (Conc. ≥ 100ng/μℓ) ·RIN ≥ 7 ·DNA-free
Ampliseq Transcriptome Sequencing –**Human, mouse**	- Diffential expressed genes - data size 7-10M Reads	·Total RNA 0.1μg (Conc. ≥ 10ng/μℓ) ·FFPE> 10ng ·RIN ≥ 7 ·DNA-free

분석 예시

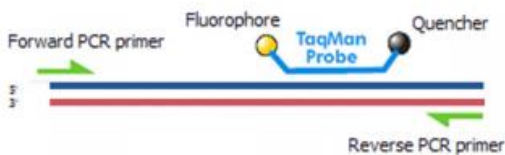| PCA plot	Scatter plot	Hierarchical Clustering

Transcriptome Sequencing
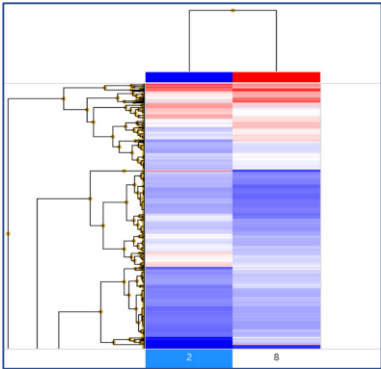
❖ **Ion AmpliSeq™ Transcriptome Gene Expression Kit**

(Easy gene-level transcriptome sequencing)

✓ 약물의 기전 및 처리 물질의 효과를 빠르게 확인 가능
✓ RNA Expression 스크리닝, 저렴한 비용으로 빠른 결과 제공
✓ Mouse, Human AmpliSeq RNA panel

Gene Expression 연구 방법

▪ Highly correlated results : Panel 의 design이 MicroArray Quality Control (MAQC), qPCR data 와 최적의 correlation 도출!

Kit	on Ampliseq Transciptome **Mouse** Gene Expression Kit	Ion Ampliseq Transciptome Human Gene Expression Kit
Target	>90% representation of mouse RefSeq genes	>95% representation of human RefSeq genes
No. of gene designed	20,767 (well-annotated RefSeq genes) 3,163 (XM and XR genes)	20,802
Recommended sample multiplexing	8 samples per Ion 540™ Chip	8 samples per Ion 540™ Chip

Metagenome Sequencing

Metagenomic sequencing을 통해서 다양한 샘플의 박테리아, 균류 등을 확인 및 비교할 수 있습니다. NGS 분석을 통해서 배양이 어려운 균의 마이크로바이옴 분석이 가능하여, 군집다양성의 계통 분류학적 비교가 가능합니다. 연구자가 데이터를 이용할 수 있도록 통계 분석 결과와 시각적 데이터를 제공합니다.

- 16S rRNA taxanomic Sequencing
- Amplicon Metagenome Sequencing
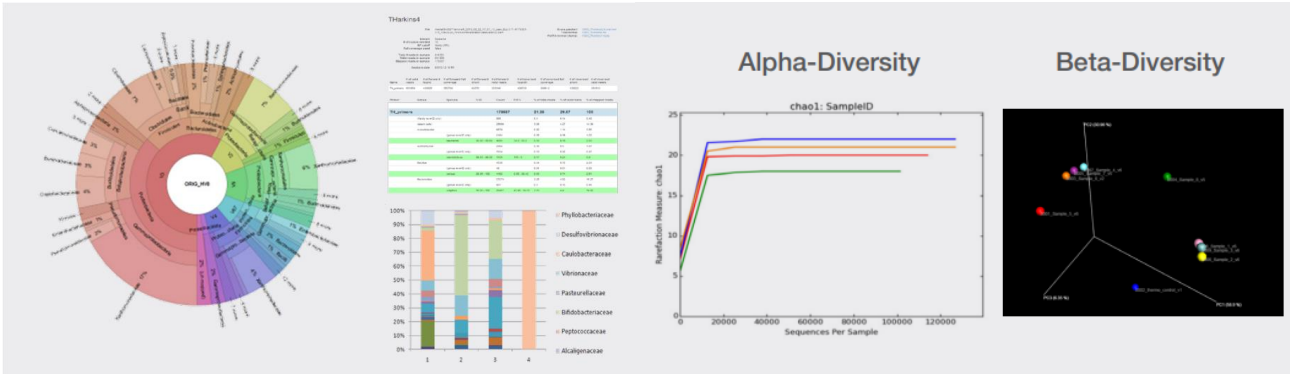- Shotgun Metagenome Sequencing

주요분석서비스

Application	Standard Analysis	Sample Requirements
Full –length 16S rRNA Sequencing Amplicon Metagenome Sequencing	- Taxonomic profiling - Comparison analysis of microbial community - Diversity statistics (alpha-, beta- diversity) - Seq Depth > 50,000 reads	·gDNA>100ng (Conc. 20ng/$\mu\ell$) ·260/280 ration 1.8~2.0
Shotgun Metagenome Sequencing	- Remove host sequences - Metagenome assembled genomes - Prediction genomic region - Gene annotation - Taxonomy assign	·High quality gDNA ·260/280 ration 1.8~2.0

분석 예시

Krona Chart, 퍼센트 ID, 매핑

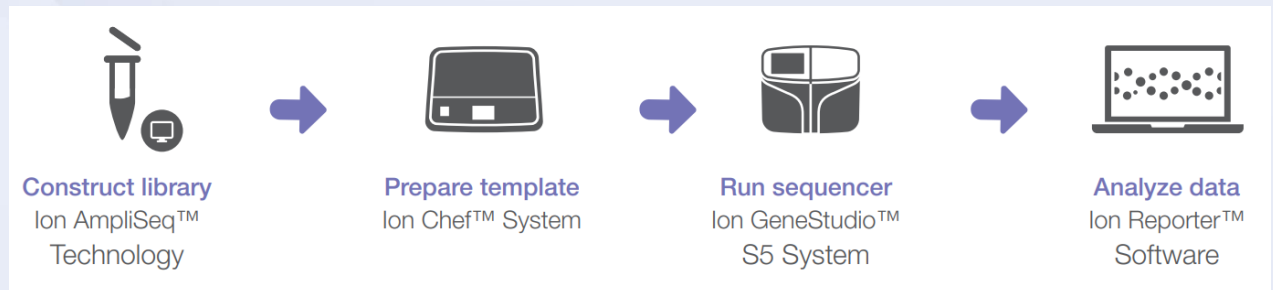alpha-· beta-diversity (QIIME software)

Metagenome Sequencing

❖ AmpliSeq Microbiome Health Research Kit

Panel	Target	Amplicons	Detail
Target Species Pool	Species-specific identification	230	73 targets associated with cancer immunological and gastrointestinal disorders for Research
16S rRNA Gene pool	Microbiome profiling	12	8 of 9 V regions

✓ Cancer, immunological, and gastrointestinal (GI)
   관련의73개 bacteria 종에 specific 한 pool

✓ 16S rRNA hypervariable region 중 8곳을 cover 하는 pool

Workflow using the Ion AmpliSeq Microbiome Health Research

Construct library
Ion AmpliSeq™
Technology

Prepare template
Ion Chef™ System

Run sequencer
Ion GeneStudio™
S5 System

Analyze data
Ion Reporter™
Software

List of Species (암, 면역, 장 관련 73ea bacteria)

Species			
Akkermansia muciniphila	Campylobacter rectus	Gardnerella vaginalis P	arvimonas micra
Anaerococcus vaginalis	Chlamydia pneumoniae	Gemmiger formicilis	Peptostreptococcus anaerobius
Atopobium parvulum	Chlamydia trachomatis	Helicobacter bilis	Peptostreptococcus stomatis
Bacteroides fragilis	Citrobacter rodentium	Helicobacter bizzozeronii	Phascolarctobacterium faecium
Bacteroides nordii	Cloacibacillus porcorum	Helicobacter hepaticus	Porphyromonas gingivalis
Bacteroides thetaiotaomicron	Clostridium difficile	Helicobacter pylori	Prevotella copri
Bacteroides vulgatus	Collinsella aerofaciens	Holdemania filiformis	Prevotella histicola
Barnesiella intestinihominis	Collinsella stercoris	Klebsiella pneumoniae	Propionibacterium acnes (Cutibacterium acnes)
Bifidobacterium adolescentis	Desulfovibrio alaskensis	Lactobacillus acidophilus	
Bifidobacterium animalis	Dorea formicigenerans	Lactobacillus delbrueckii	Proteus mirabilis
Bifidobacterium bifidum	Enterococcus faecalis	Lactobacillus johnsonii	Roseburia intestinalis
Bifidobacterium longum	Enterococcus faecium	Lactobacillus murinus	Ruminococcus bromii
Blautia obeum	Enterococcus gallinarum	Lactobacillus reuteri	Ruminococcus gnavus
Borrelia burgdorferi	Enterococcus hirae	Lactobacillus rhamnosus	Slackia exigua
Campylobacter concisus	Escherichia coli	Lactococcus lactis	Streptococcus gallolyticus
Campylobacter curvus	Eubacterium limosum	Mycoplasma fermentans	Streptococcus infantarius
Campylobacter gracilis	Eubacterium rectale	Mycoplasma penetrans	Veillonella parvula
Campylobacter hominis	Faecalibacterium prausnitzii	Parabacteroides distasonis	
Campylobacter jejuni	Fusobacterium nucleatum	Parabacteroides merdae	