Application of illumina iSeq System in Biomedical field

均泰生物科技有限公司
楊舜鈞 Ph.D.

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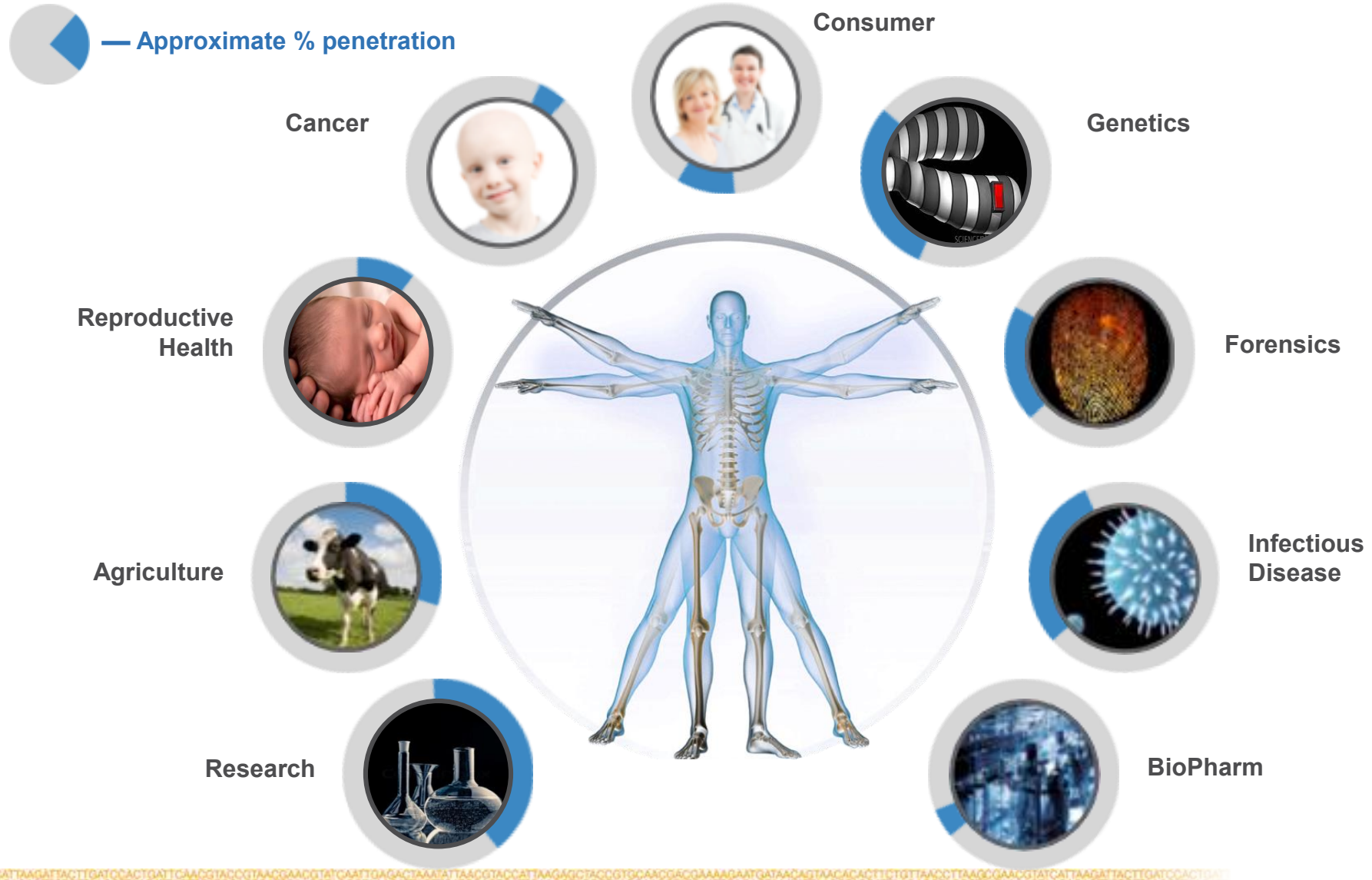
Application of Next Generation Sequencing



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Sequencing Power for Every Scale

HIGH THROUGHPUT
Whole Genome | Exome | Transcriptome

PERSONAL SCALE
FEW SAMPLES | RUN

PRODUCTION SCALE
MANY SAMPLES | RUN

NextSeq 550

HiSeq 4000

NovaSeq 6000

iSeq 100

MiniSeq

MiSeq

NextSeq 550

LOW THROUGHPUT
Targeted resequencing | Whole Genome (microbes)

For Research Use Only. Not for use in diagnostic procedures.

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iSeq™ 100

It's Time to Put Next Generation Sequencing Into Everyone's Hand

For Research Use Only. Not for use in diagnostic procedures.

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Convenient Single-use Cartridges

Eliminate run-to-run contamination

Self-contained reagents

Sequencing happens in cartridge

Reagents and samples never leave the cartridge

The iSeq™ 100 system has no on-instrument fluidics

No wash steps are required

For Research Use Only. Not for use in diagnostic procedures.

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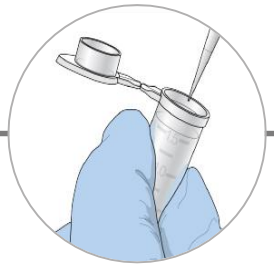
Setting Up a iSeq™ 100 Run

Thaw

Thaw at 2°C to 8°C for 24 hours or in room temperature bath for 6 hours

Note: Keep package intact for water bath thaw

6–24 hrs



Dilute

Dilute libraries to 60–120 pM

Load

Load libraries in the sample reservoir

<5 mins

Sequence

Initiate

On-instrument run setup using guided, LRM software

Set-up

Select from pre-configured runs or enter a run manually

BaseSpace SEQUENCE HUB

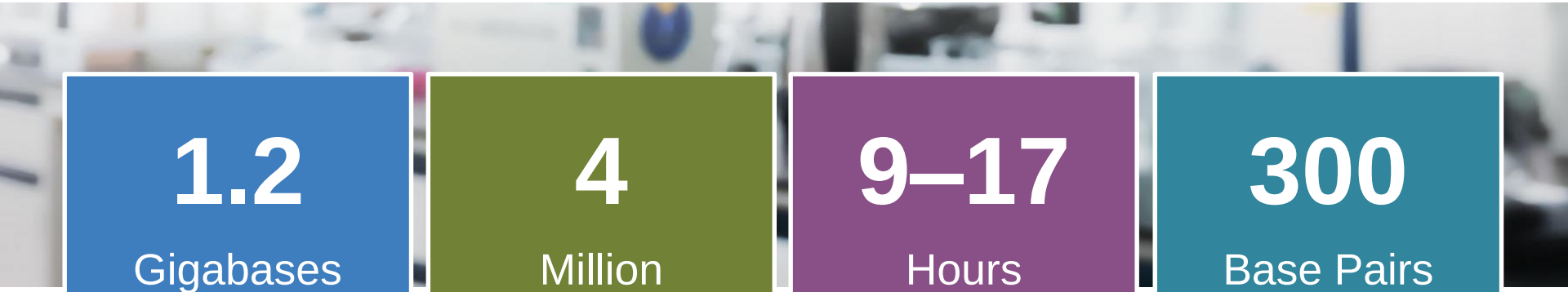
Monitor

Monitor runs remotely on BaseSpace™ or on-instrument

<5 mins

iSeq™ 100 Specifications

2x250 available in 2019



1.2	4	9–17	300
Gigabases	Million	Hours	Base Pairs
1.2 billion nucleotides sequenced per run	4 million fragments sequenced per run	Total time sequencing	Up to 300 bp fragments sequenced per read

Run Configuration	Reads (M)	Output	Run Time
1x36 bp	4	144 Mb	9 hrs
1x50 bp	4	200 Mb	9 hrs
1x75 bp	4	300 Mb	10 hrs
2x75 bp	4	600 Mb	13 hrs
2x150 bp	4	1.2 Gb	17 hrs

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Illumina Sequencing Workflow

Comprehensive Workflow

Sample Preparation | **Library Preparation** | **Sequencing** | **Analysis**

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Whole Genome Sequencing

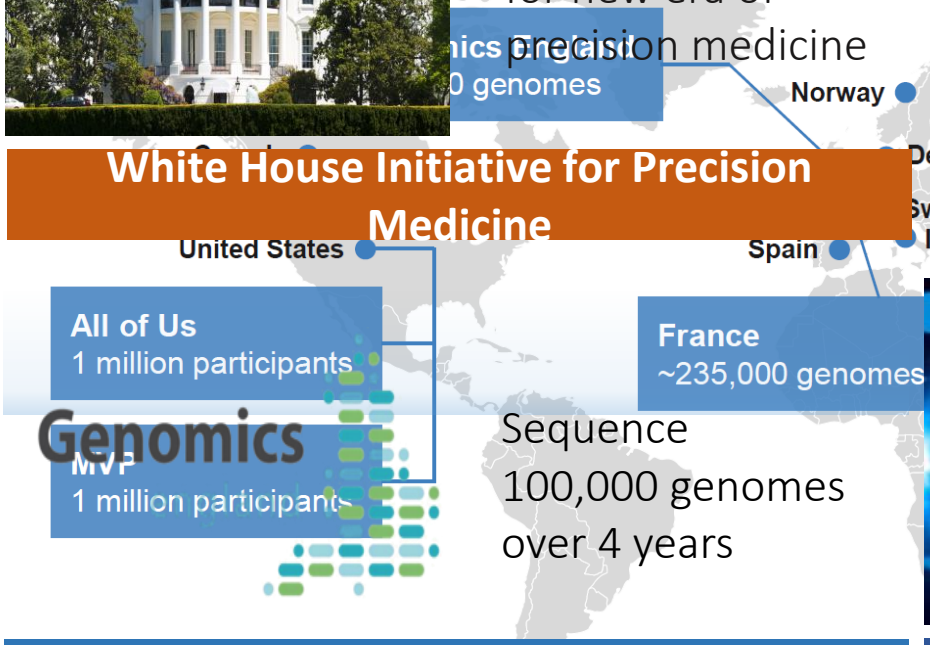
The complete, accurate genetic make-up of an individual

$215M investment; Calls for new era of precision medicine

White House Initiative for Precision Medicine

$10B over 15 years; 2 million people

China's Precision Medicine Initiative



Sequence 100,000 genomes over 4 years

Genomics England

$745M to build 235,000 genomes-per-year sequencing operation

France Investment in Genome Sequencing

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Whole genome

1. $3x10^9$ bps
2. Containing exon, intron, regulatory element, short tendon repeat…..

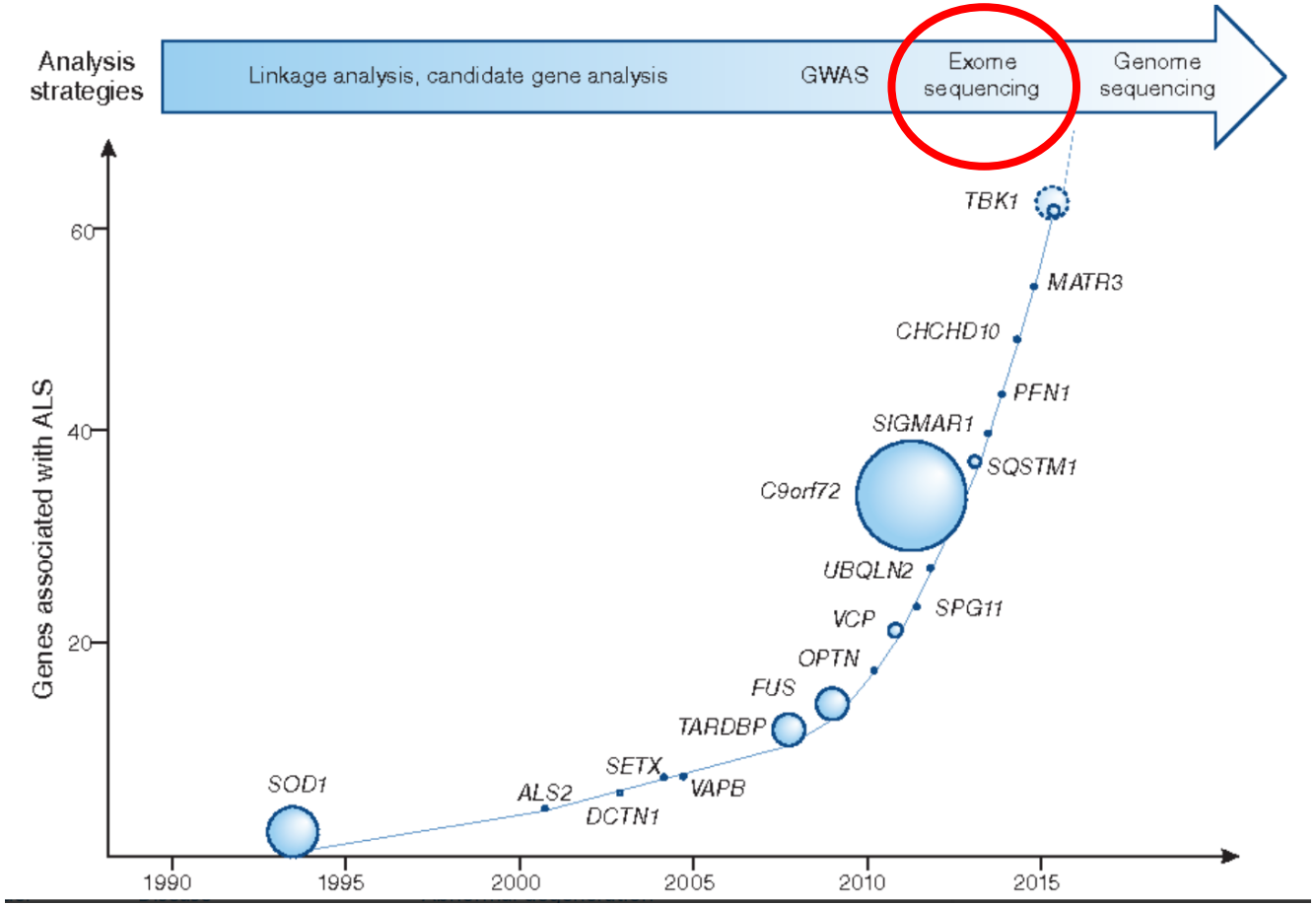
Whole exome

1. 30-40M bps (<1.5% of whole genome)
2. Containing exon only (CDS+UTR)
3. mutations in the exome are thought to harbor 85% of mutations that may cause disease

Whole Exome Sequencing

The efficient way to identify the genetic variants in all of an individual's genes

Amyotrophic Lateral Sclerosis as a example



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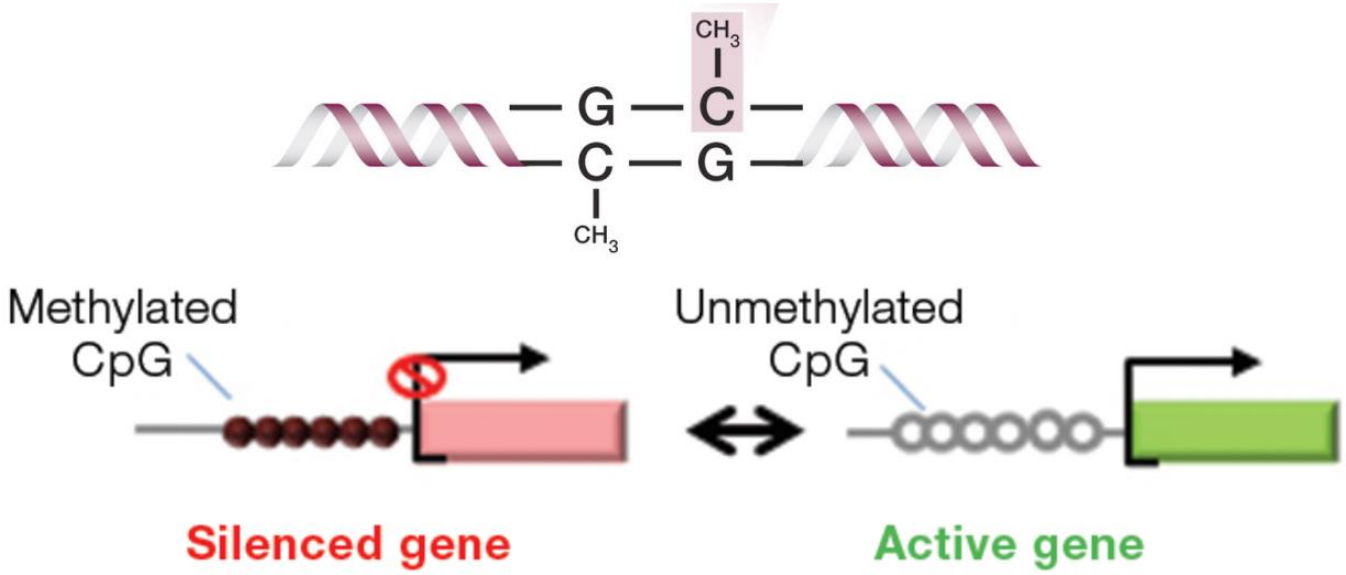
Common Studies Using DNA Methylation



Cancer, Aging, Obesity, Diabetes, Alzheimer's, Development, and many more…

Epigenome Wide Association Studies (EWAS)	Gene Expression & Genetics Studies
Discover / Screen for Biomarkers	Expand Understanding

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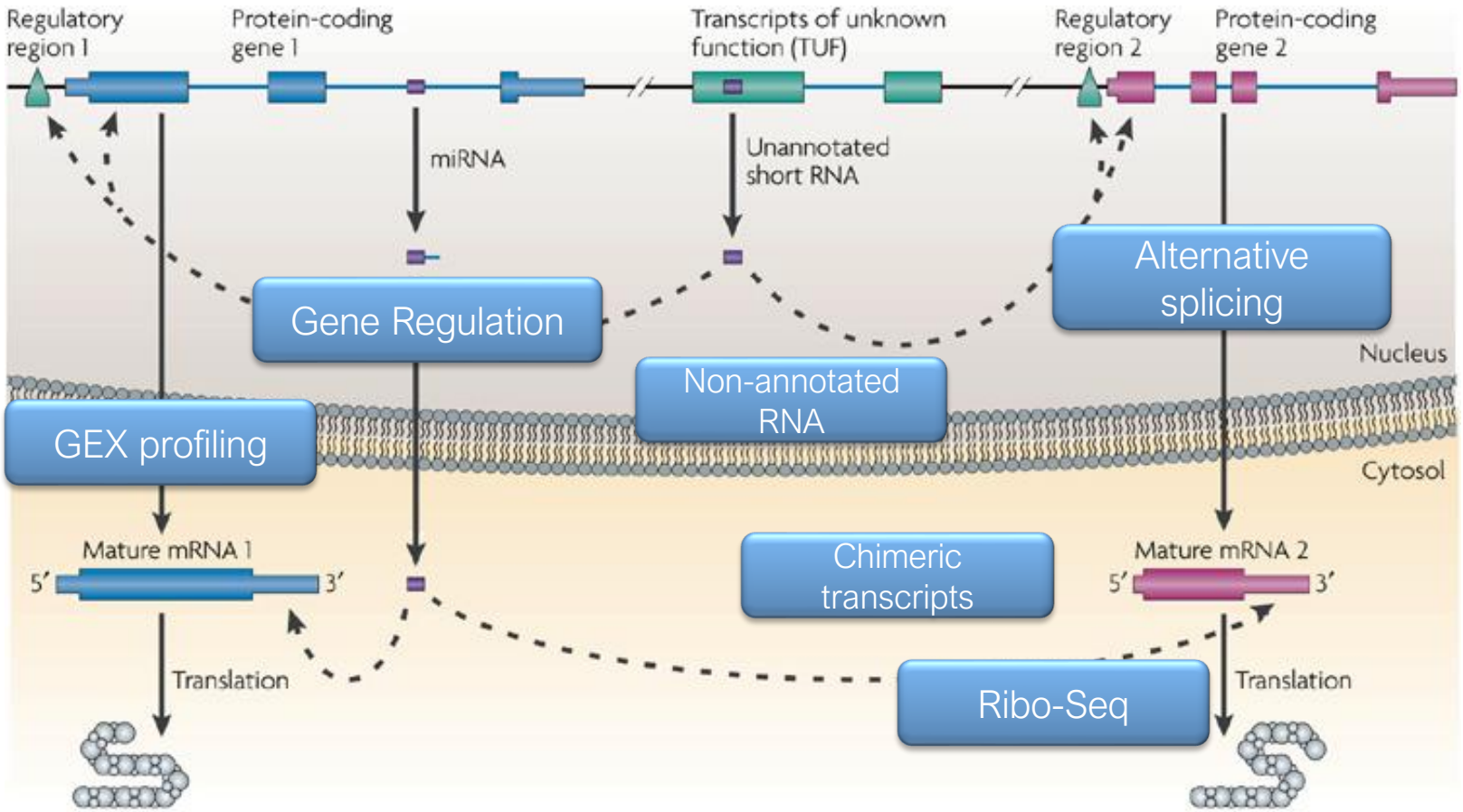
Understanding the Transcriptome: RNA Sequencing



Nature Reviews Genetics 8, 413-423 (June 2007) - Modified

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Solutions for Target Resequencing with iSeq

WGS,

Target Resequencing

Amplicon Based

Capture Based

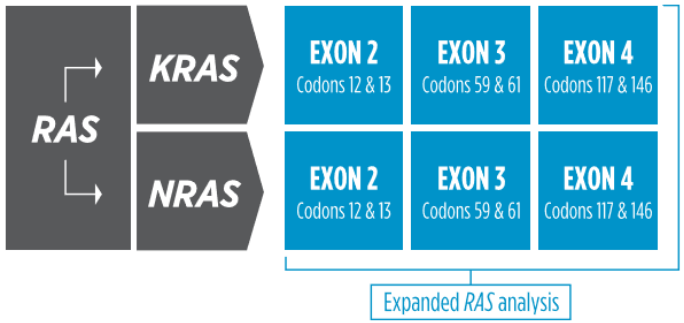
Vectibix:

is an epidermal growth factor receptor (EGFR) antagonist indicated for the treatment of wild-type RAS (defined as wild-type in both KRAS and NRAS as determined by an FDA-approved test for this use) metastatic colorectal cancer (mCRC)

***KRAS* and *NRAS* are part of the *RAS* family of oncogenes**[1,2] (56 variants)

RAS status definitions[2]:

- **Nonmutated or wild type (WT) *RAS*** means the *RAS* genes (*KRAS* and *NRAS*) do not have mutations
- **Mutated or mutant type (MT) *RAS*** means that at least one of the *RAS* genes (*KRAS* or *NRAS*) contains a mutation that affects its function



mCRC = metastatic colorectal cancer; MT = mutant type; WT = wild type.

FDA–cleared companion diagnostic kit with illumina MiSeqDx at 2017.07

TruSight One

Genes Targeted **(4813 genes)**

TruSight One
Targeting > 4,800 genes; enabling labs to expand and streamline their assay portfolio and sequencing portfolio

TruSight HLA
Accurate, unambiguous, phase-resolved HLA typing in a single assay

TruSight RNA Pan-Cancer
Targeting 1385 oncology genes for gene expression, variant and fusion detection in all RNA sample types including FFPE

TruSight Myeloid
Uses expert-defined content to identify somatic mutations in myeloid malignancies

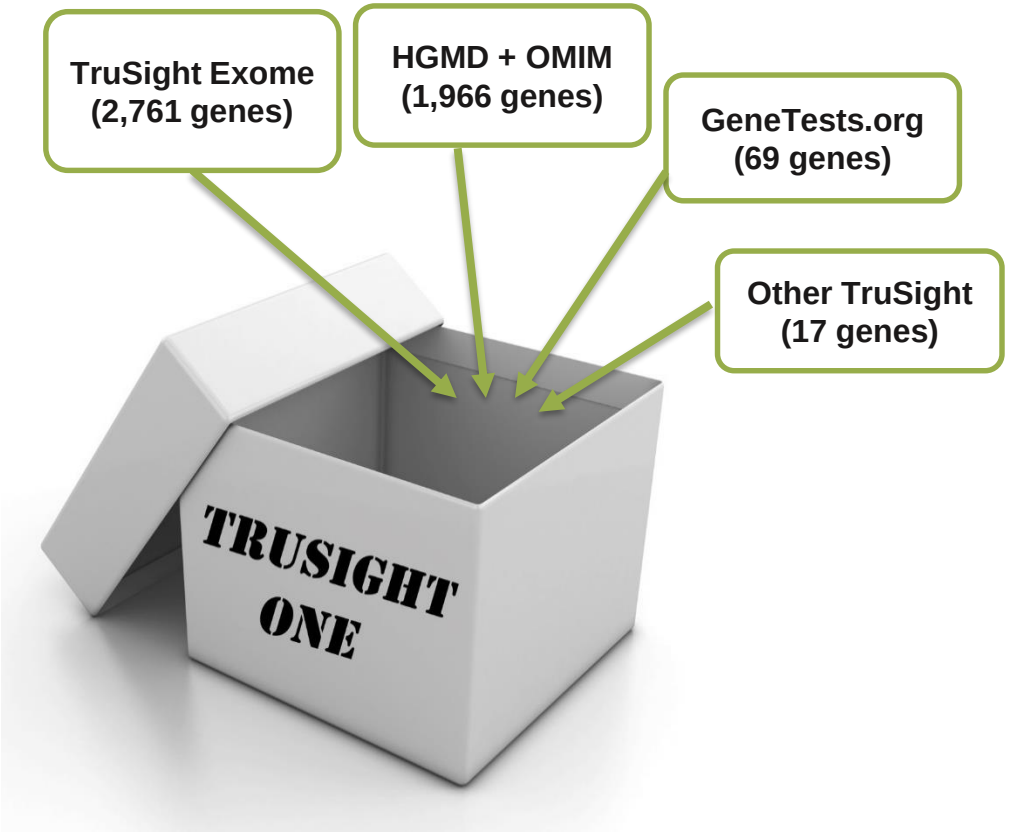
TruSight Cancer
Targeting genes previously linked to a predisposition towards cancer

TruSight Tumor 15
Focused panel assesses common somatic variants in solid tumors

TruSight Cardio (174 genes)
Focusing on identifying inherited cardiac conditions

TruSight Inherited Disease (552 genes)
Focusing on severe, recessive pediatric onset diseases

TruSeq Neurodegeneration kit (118 genes)



(8N)

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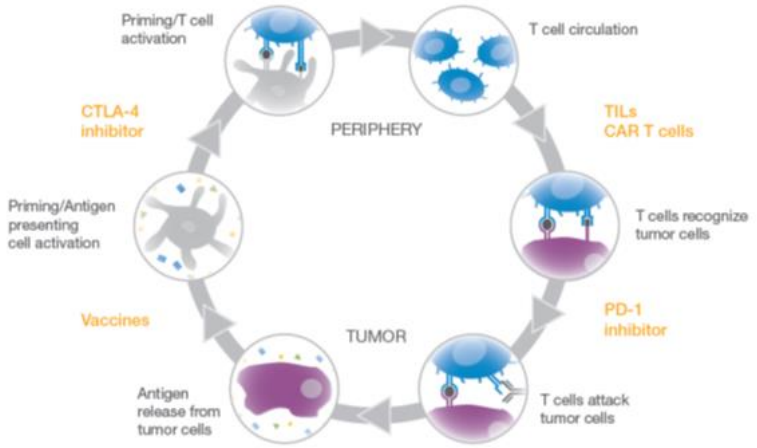
Companion Diagnostic Development

Partnering to Power Oncology Precision Medicine

(not suitable for iSeq)



Companion Diagnostic product (under development) based on TruSight™ Tumor 170 content

Loxo to expand oncology menu for NextSeqDx

Companion Diagnostic product (under development) based on TruSight™ Oncology 500 content

Bristol-Myers Squibb to expand oncology menu for NextSeqDx

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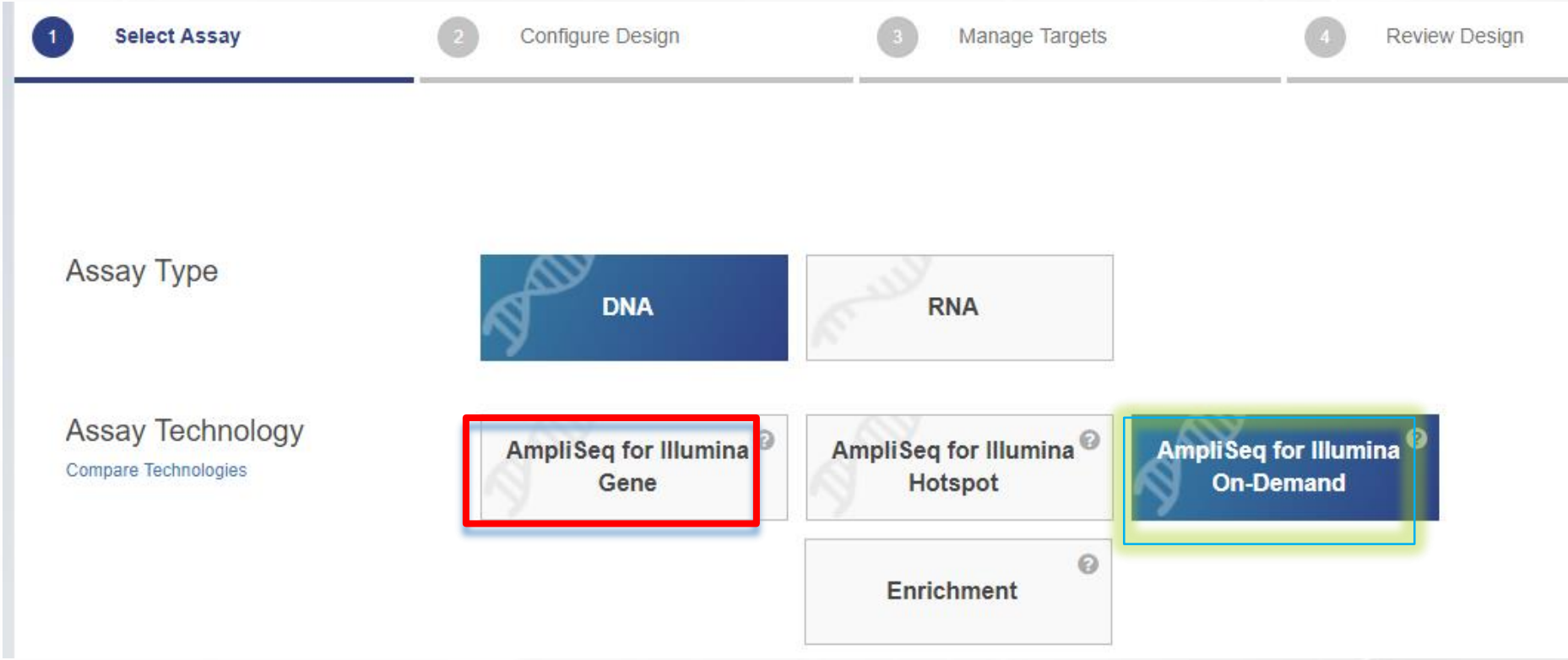
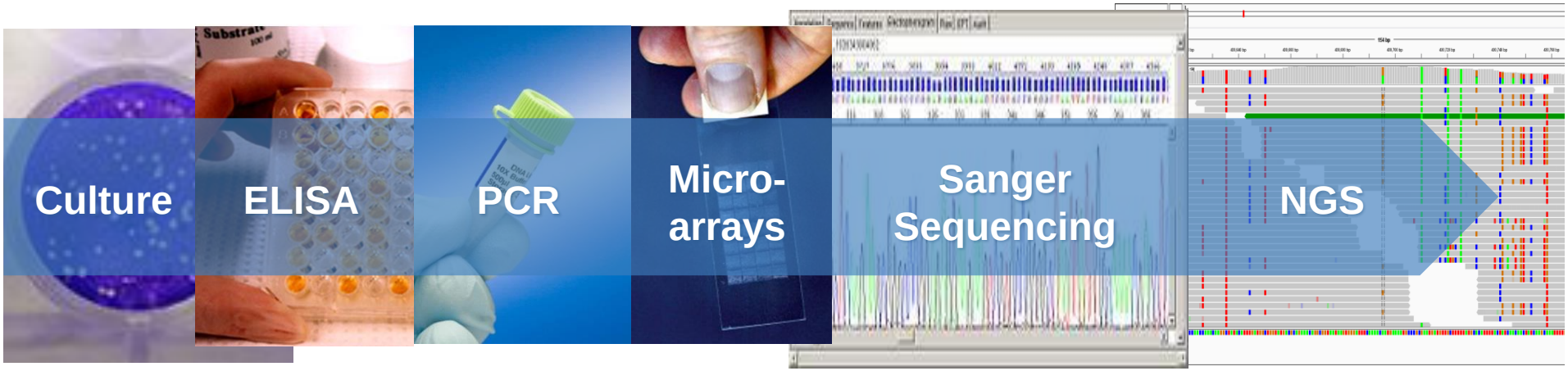
Custom Design:

BaseSpace DesignStudio sequencing

Start Design



NGS for microbes detection



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Rapid Whole-Genome Sequencing for Investigation of a Neonatal MRSA Outbreak

Published at June 14, 2012

MRSA outbreak and origin investigation in an UK hospital

Public Health Research Update

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Genetic Epidemiology with MiSeq®: Tracking Influenza H7N9 in China

Emergence of a Respiratory Pathogen

- In winter of 2013, patients in eastern China were hospitalized with severe lower respiratory tract infections associated with an avian strain of influenza A virus (H7N9).
- By mid-April, 60 cases were confirmed in five Chinese provinces, rising to 130 in May. The viral transmission route was not clear, but the strain was found in chickens, implicating spread through live animal markets.
- June reports show infections continue in ten provinces, with a mortality rate of ~20%, no cases outside of China, and no confirmed human-to-human events.
- Simultaneous infection with several influenza strains raises concerns about the rapid emergence of genetic adaptations that could lead to a human pandemic.

A CDC scientist harvests H7N9 for research purposes. www.cdc.gov

Identification of influenza H7N9 in China, 2013

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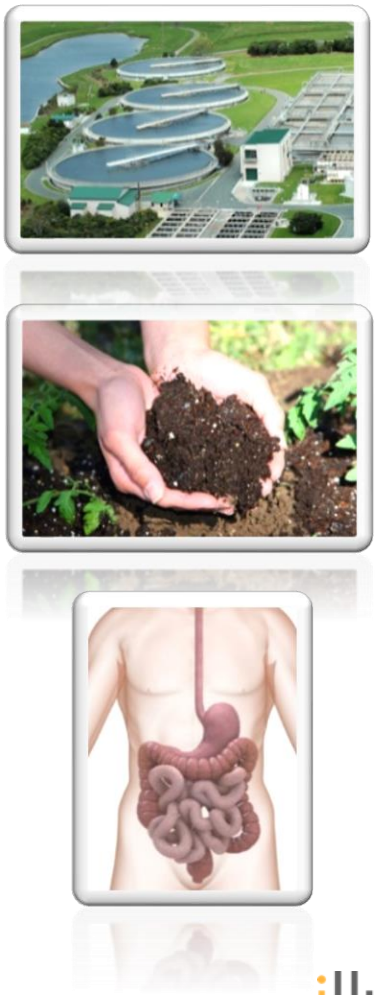
Metagenomics and Microbial Diversity

Diverse Applications

Metagenomic studies can give insight into:

- Microbial diversity in an environmental habitat
- Abundance of microbial species
- Gene content in a sample and the discovery of novel genes
- Meta-transcriptomics: analysis of the expressed genome in a sample
- Pathogen detection
- Signature profile of an environment or disease state



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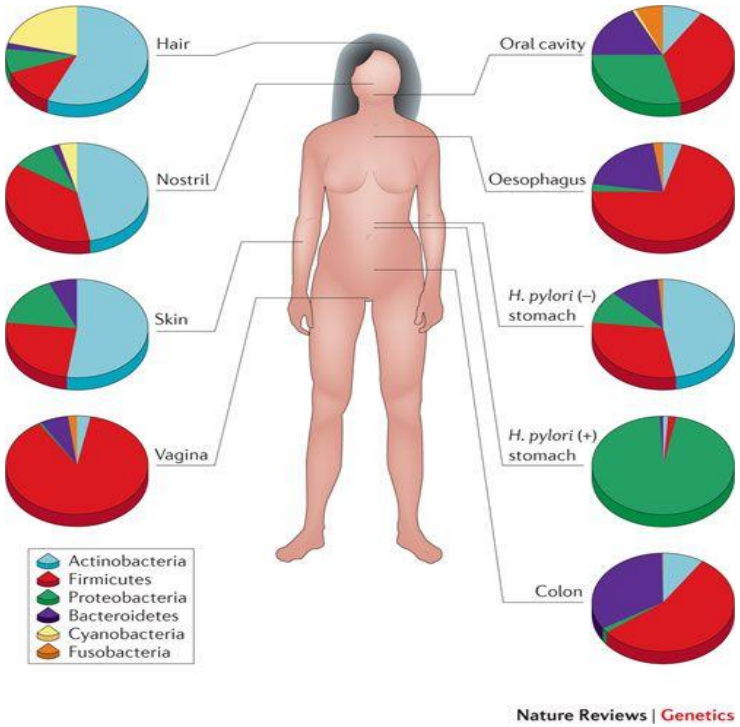
Microbiome and human body environment



- Microbial community vary at different body sites on the same person (*different ecosystem*)
- Bacteria of a specific body site from different persons have more common characteristic (*common ecosystem*).
- The microbiome of a healthy and diseased person looks very different (*rearranged ecosystem*)

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Throughput to Match Microbiology Applications

High Throughput

Low Throughput

Shotgun metagenomics

- **Microbial diversity**
- **Gene content and discovery**

16S rRNA Metagenomics

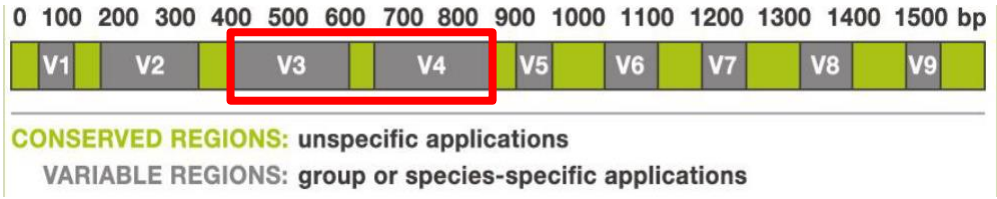
- **Relative abundance of microbial diversity**
  - 16S for bacteria and archaea
  - ITS for Yeast
  - 100K reads/sample

Microbial genomics

- **Detection**
- **Identification**
- **Antibiotic sensitivity testing**
- **Molecular epidemiology**

16S rRNA Metagenomics: Microbiota

Using tailed PCR primers



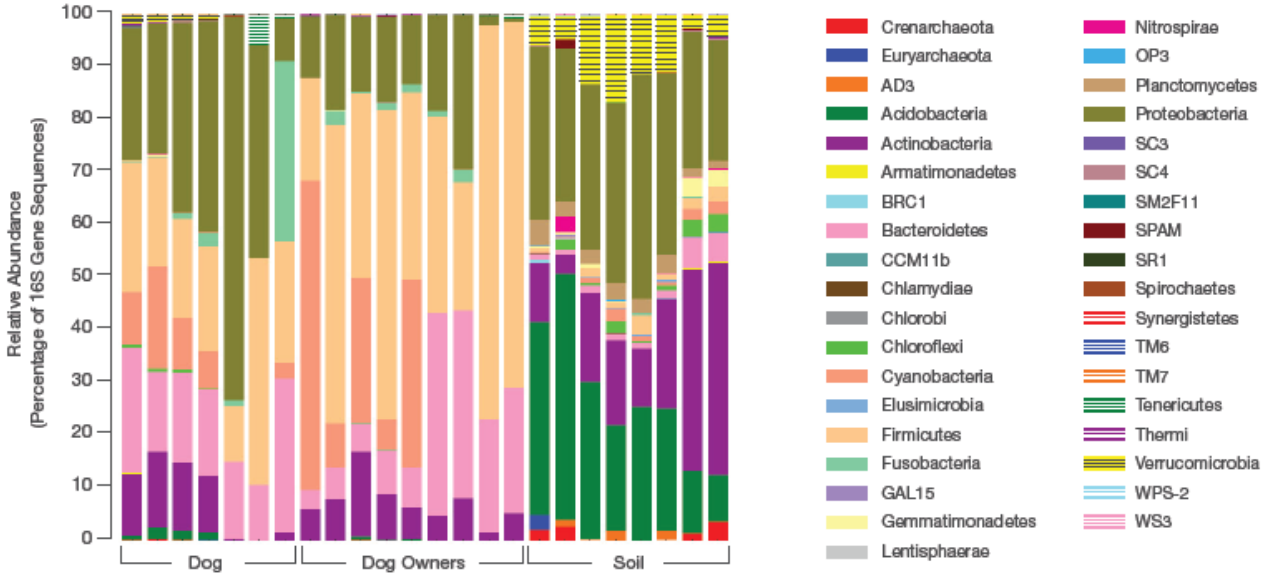
Primers targeting 16S hyper-variable region
- design amplicon (V3, V4), 460 bp

Tailed PCR
- Incorporates adapters and barcodes

Pool
- multiple samples

Sequencer
- **2x150** or **2x300**

QIIME taxon assignment at phylum level



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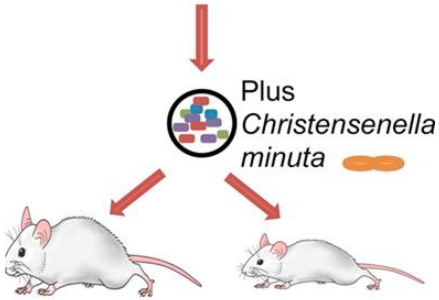
Gut Microbiomes vs Personal Health

Metabolism

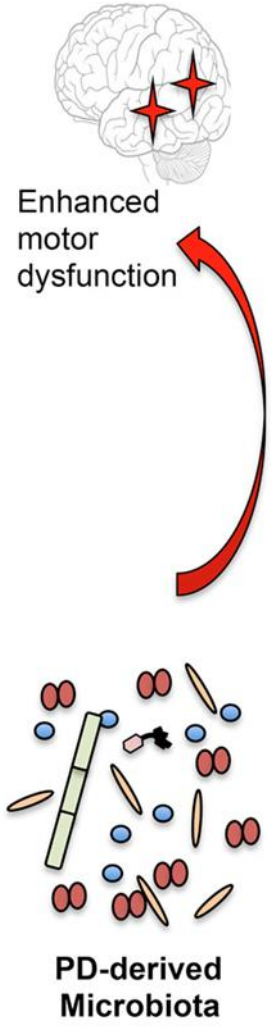
Lean individuals have higher levels of the highly heritable taxon Christensenellaceae



Germfree mice receiving C. minuta amended stool gain less weight

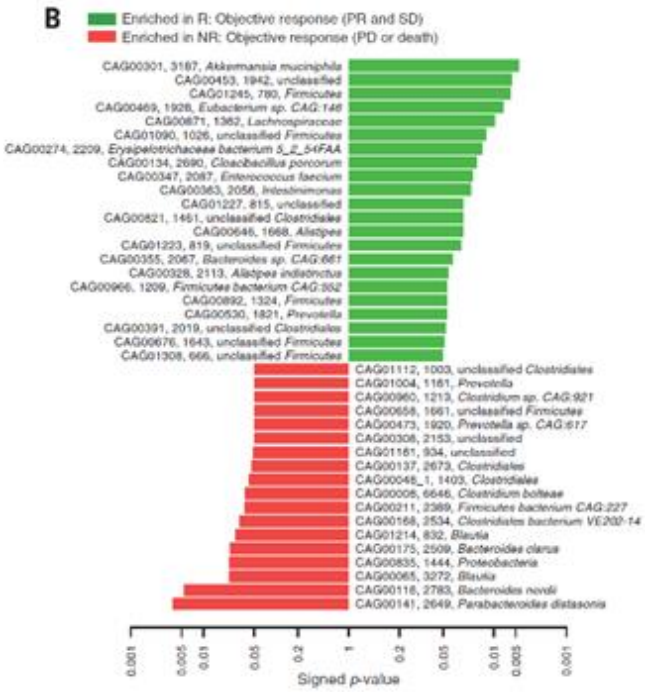
Cell 159, 789-799, 2014

Neuron Degeneration



Cell 167, 1469-1480, 2016

ImmunoOncology



Science 359: 91-97, 2018

Thank you!

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