

# Next Generation Sequencing

*From Basic Study to Clinical Research*

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**Oct. 2012**

## Company Introduction

威健創立於2003年

位於南港軟體園區二期



Quality is Assured



2006年通過ISO-17025認證

亞洲第一間Agilent認證晶片服務公司

提供專業高通量技術服務與試劑耗材銷售

2010年開始提供NGS服務

# WELGENE

## NGS Service



Sample QC  
樣本品管



GA IIx Sequencing  
定序服務



Data Analysis  
資料分析



Professional Report  
報告討論

It's the NGS Service in Taiwan

定序分析 臺灣完成 威健首選 完美把關

# Outline

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★ Introduction of NGS

★ Applications of NGS

DNA Level

RNA Level

Epigenetics

Metagenomics

# Outline

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## ★ Introduction of NGS

## ★ Applications of NGS

DNA Level

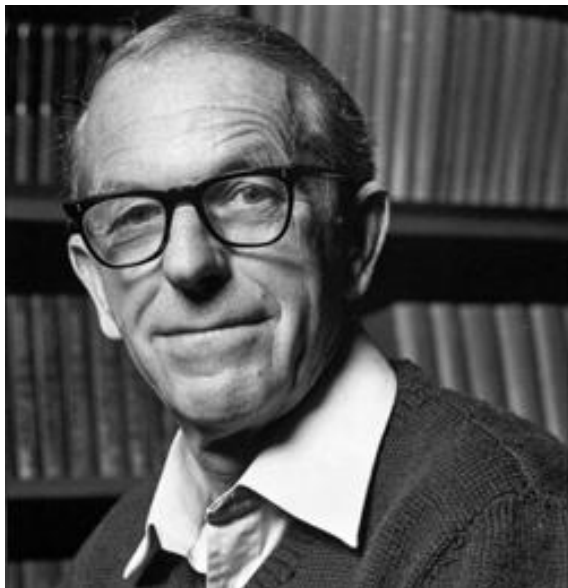
RNA Level

Epigenetics

Metagenomics

# Introduction

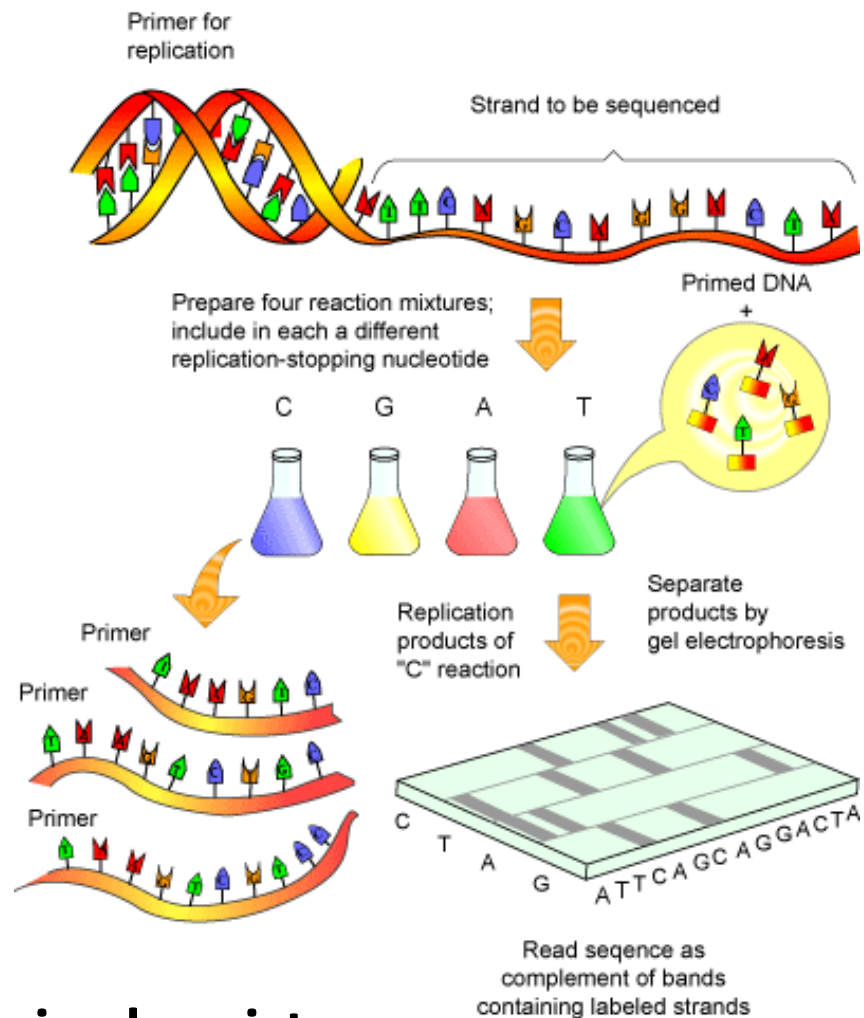
## Sanger Sequencing



**Frederick Sanger**  
born 13 August 1918

**Biochemist**

**two-times Nobel laureate in chemistry**



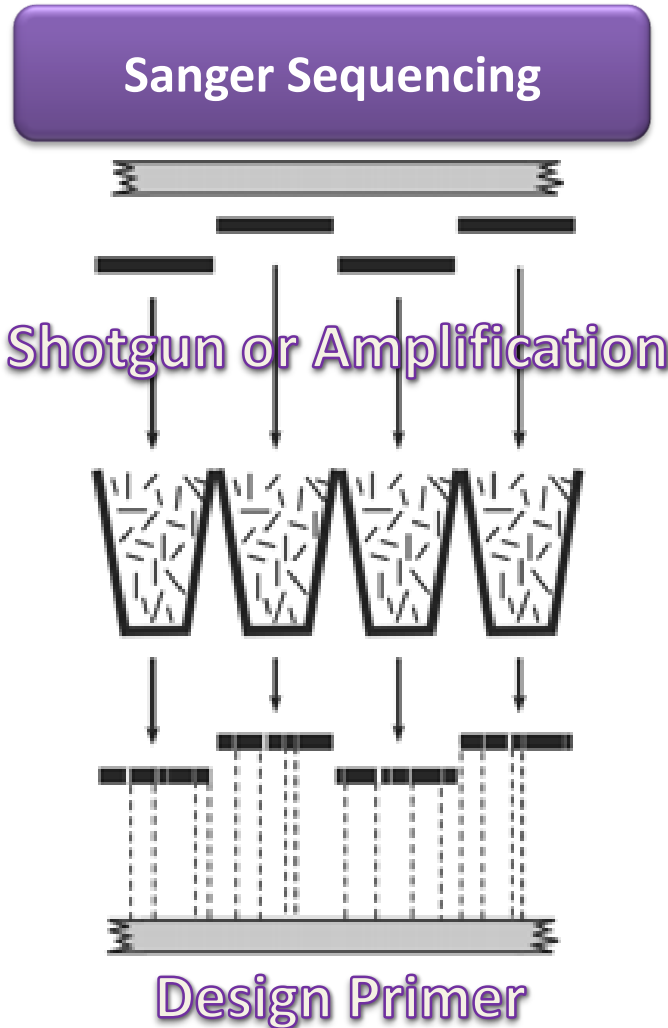
# Introduction

## Sequencing Technology Development



# Introduction

## Sanger vs NGS



# Introduction

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## Next Generation Sequencing

1. High-throughput Sequencing (HTS or HT-Seq)
2. Massively Parallel Sequencing (MPS)
3. Deep Sequencing
4. Next-gen

# Outline

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★ Introduction of NGS

★ Applications of NGS

DNA Level

RNA Level

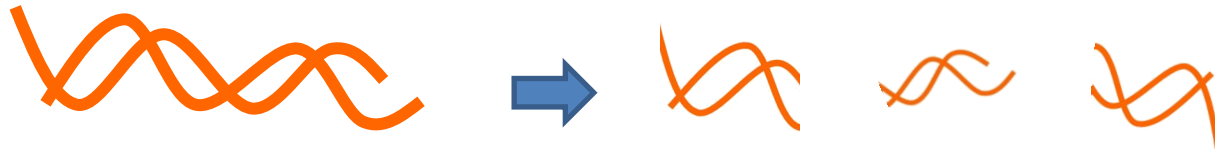
Epigenetics

Metagenomics

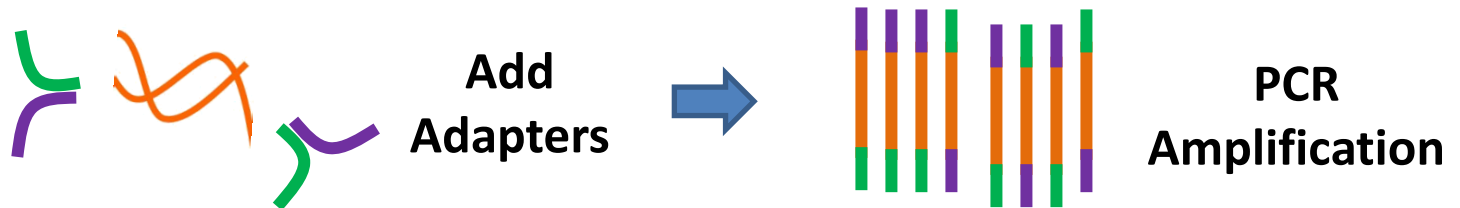
# Application of NGS

## Basic Workflow

### I. Sample Preparation & Fragmentation



### II. Library Construction



### III. Massively Parallel Sequencing & Analysis

# Application of NGS

## Sequencing Type

### I. Single Read (Single End)



### II. Paired-End



**Amount of Sequencing = Read length x Clustering No x Type of Seq**

# Application of NGS

## Basic Concepts

### I. To Find Known

- ▲ Mapping
- ▲ Counting / Similarity

### II. To Find Unknown

- ▲ Assembling
- ▲ Searching in Database / Prediction

# Application of NGS

## Powerful Methodology

De novo  
Whole genome sequencing  
Metagenomics  
Whole transcriptome  
Gene expression  
ChIP-seq  
Methylation  
Targeted resequencing

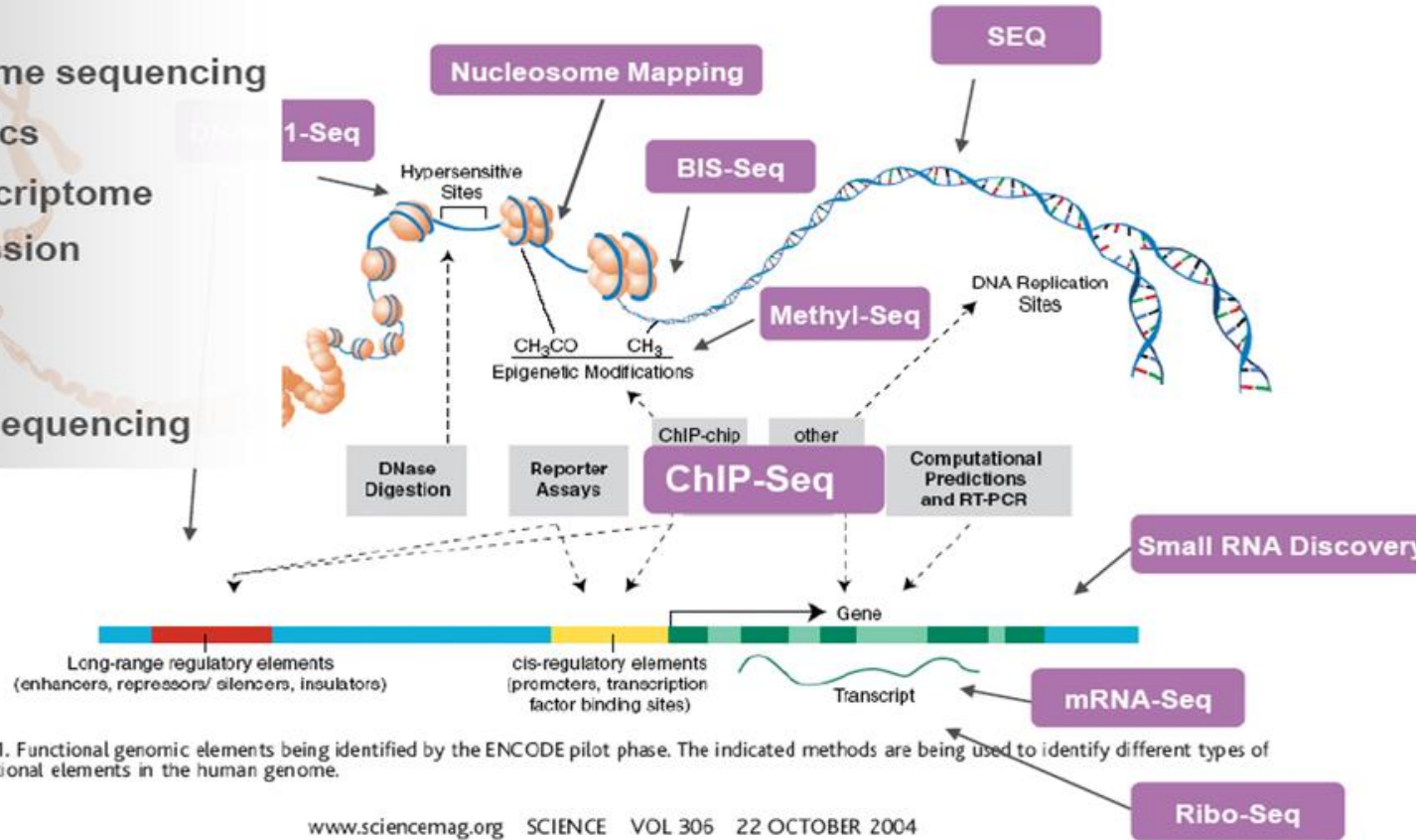


Fig. 1. Functional genomic elements being identified by the ENCODE pilot phase. The indicated methods are being used to identify different types of functional elements in the human genome.

www.sciencemag.org SCIENCE VOL 306 22 OCTOBER 2004

# Outline

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★ Introduction of NGS

★ Applications of NGS

**DNA Level**

RNA Level

Epigenetics

Metagenomics

# Application of NGS

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## DNA Level

- ▲ Genome *de novo* Sequencing
- ▲ Genome Resequencing
- ▲ Target (Exome) Sequencing

# Application of NGS

## DNA Level – Genome *de novo*

### ▲ Large Genome

Strategy:

Paired-end sequencing with gradient insert

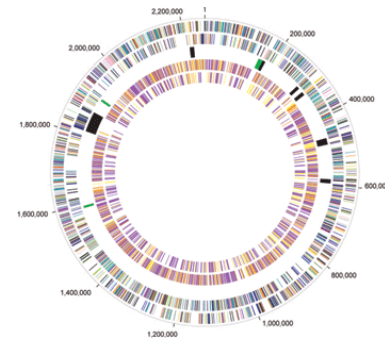
### ▲ Small Genome

Strategy:

Paired-end sequencing with high coverage

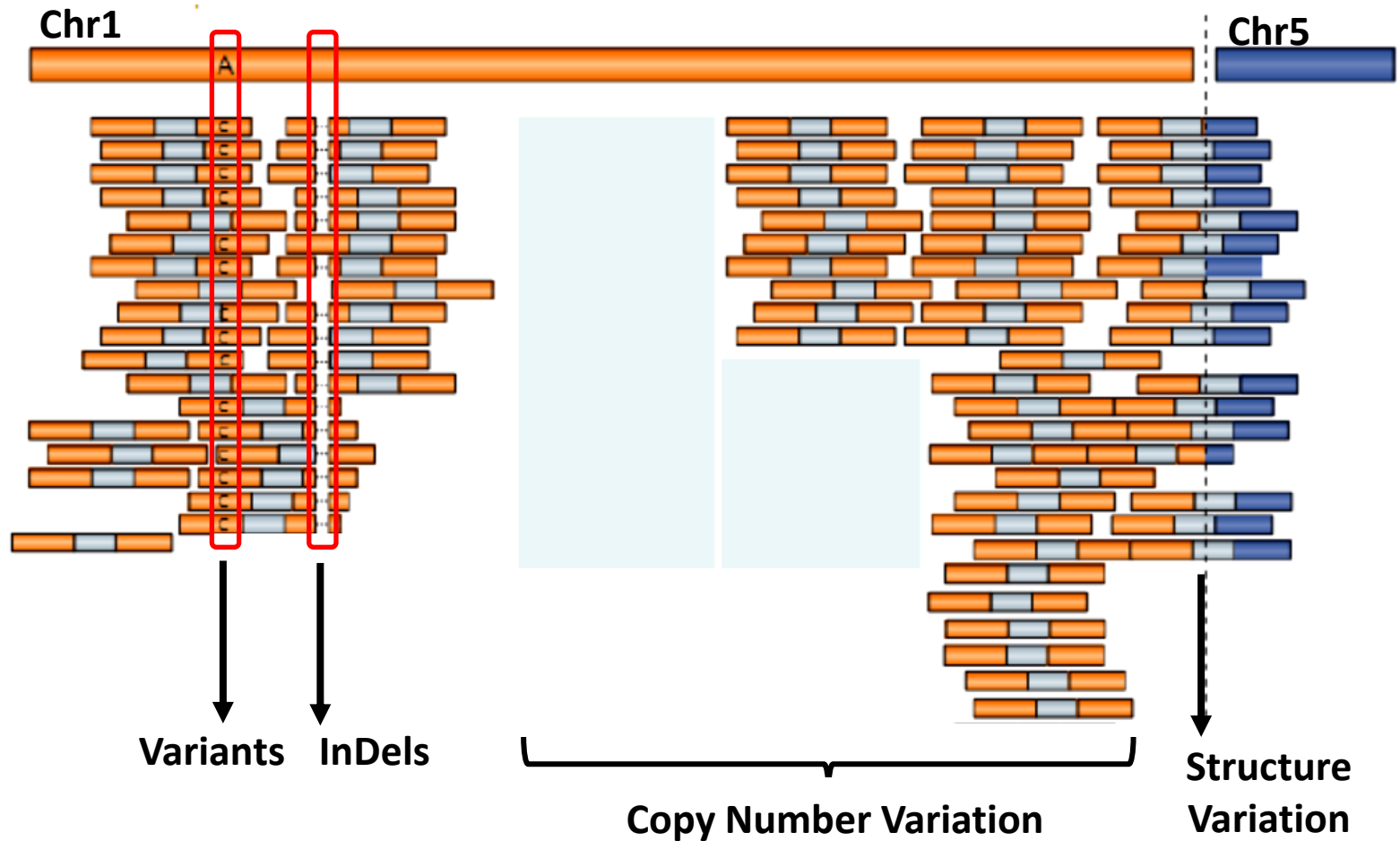


Ruiqiang Li, Wei Fan, et. al.  
Nature. 2009.



# Application of NGS

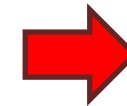
## DNA Level – Genome Resequencing



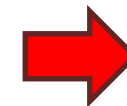
# Application of NGS

## DNA Level – Target / Exome Sequencing

Whole Genome



Sequencing



Sequencing

Target / Exome Region

Decreasing cost to add the sequencing depth

# Application of NGS

## DNA Level – Target / Exome Sequencing

1. Whole Exome Sequencing
2. Genome Wide Association Study (GWAS)
3. Clinical Research Panel



1kb ~ 5Mb



**Agilent Technologies**

**SureSelect<sup>XT</sup>**

500kb ~ 100Mb



**威健股份有限公司**  
Welgene Biotech Co., Ltd.

# Application of NGS

## DNA Level – Target / Exome Sequencing

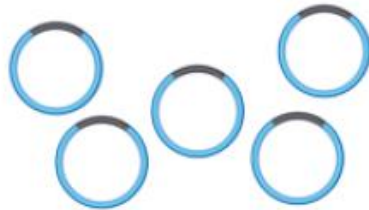
1) Digest genomic DNA.



2) Hybridize the HaloPlex probe library in presence of the Indexing Primer Cassette. Hybridization results in gDNA fragment circularization and incorporation of indexes and Illumina sequencing motifs.



3) Capture target DNA-probe hybrids. Biotinylation of probe DNA allows capture using streptavidin-coated magnetic beads.

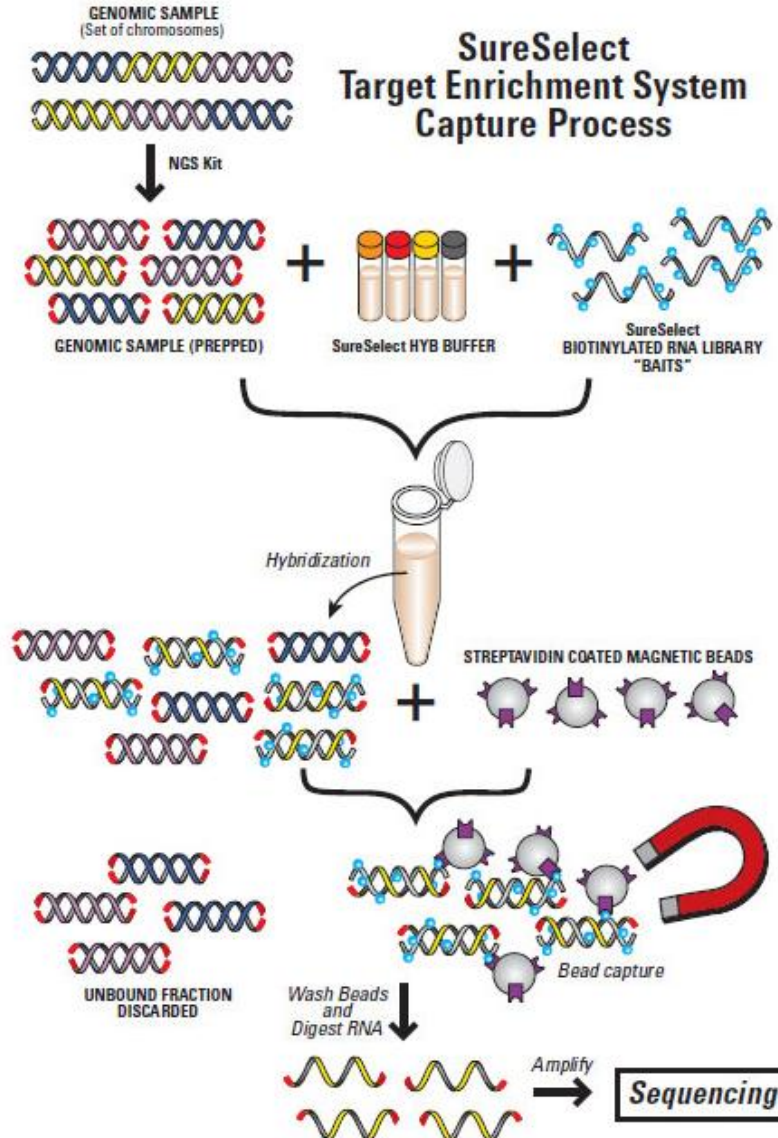


4) PCR amplify targeted fragments to produce a sequencing-ready, target-enriched sample.



# Application of NGS

## DNA Level – Target / Exome Sequencing



# Outline

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★ Introduction of NGS

★ Applications of NGS

DNA Level

**RNA Level**

Epigenetics

Metagenomics

# Application of NGS

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## RNA Level

- ▲ Transcriptome *de novo* Sequencing
- ▲ RNA Sequencing
- ▲ Small RNA Sequencing

# Application of NGS

## RNA Level – Transcriptome *de novo*

(without reference genome)



Assembling



Unigene



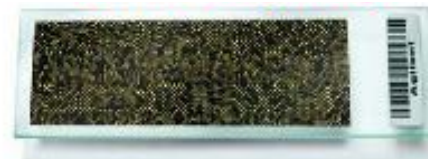
Expression



Annotation



SSRs Markers



Custom Array

# Application of NGS

## RNA Level – RNA Sequencing



**Mapping**

**Gene Expression (RPKM/FPKM)**

**Alternative Splicing**

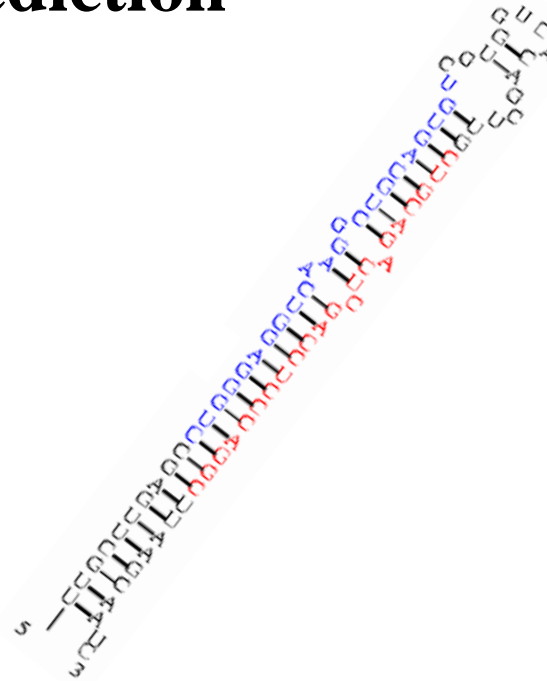
**SNPs**

**Novel Genes & Transcripts Prediction**

# Application of NGS

## RNA Level – Small RNA Sequencing

- analysis of known miRNA (miRBase) 18-40nt
- analysis of miRNA differential expression
- novel miRNA prediction
- target prediction



# Application of NGS

## RNA Level – Array vs NGS

	Array	NGS
Gene Expression	O	O
Alternative Splicing	X	O
<i>de novo</i>	X	O
Small RNA expression	O	O
Novel miRNA prediction	X	O

**Next  
Generation  
Sequencing**

**Microarray**

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  - RNA Level
  - Epigenetics**
  - Metagenomics

# Application of NGS

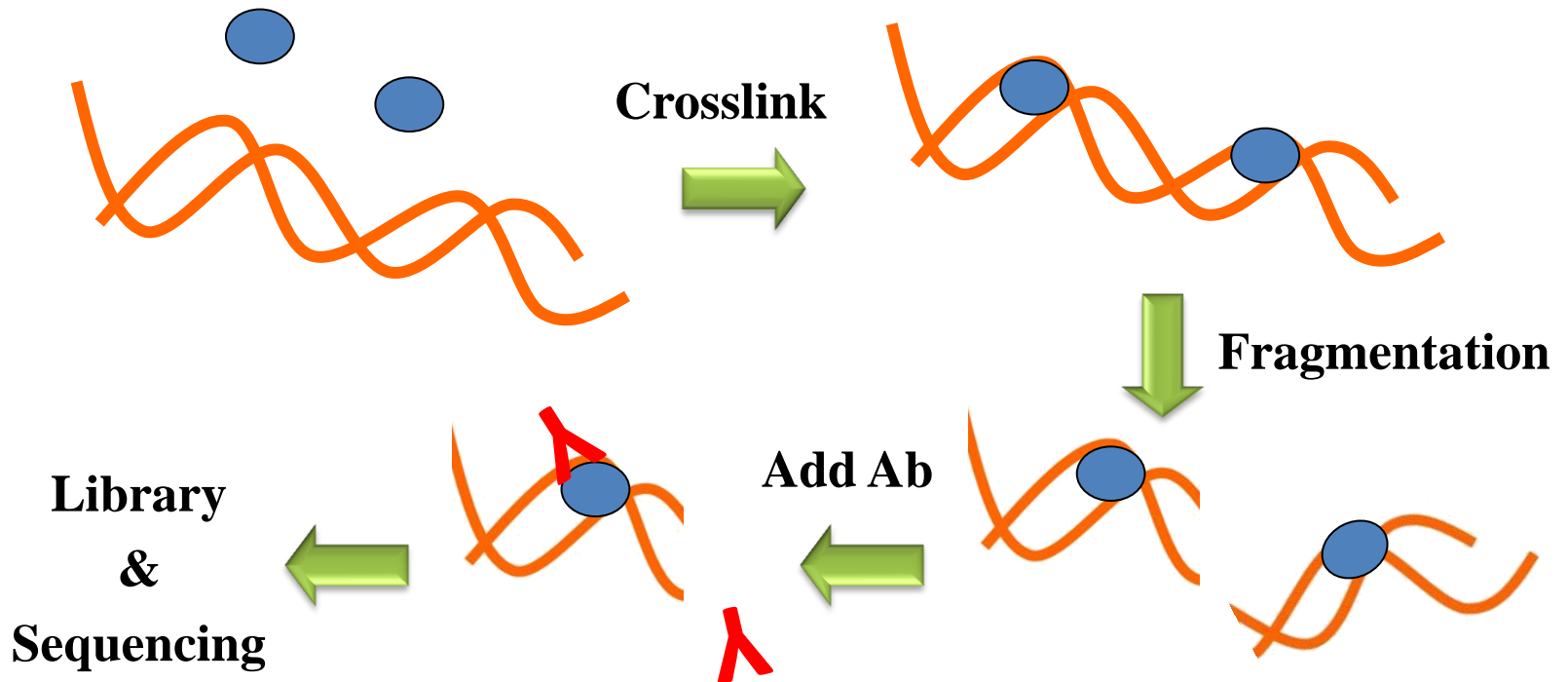
## Epigenetics

- ▲ ChIP-seq
- ▲ Bisulfite Sequencing
- ▲ MeDIP-seq

# Application of NGS

## Epigenetics – ChIP-seq

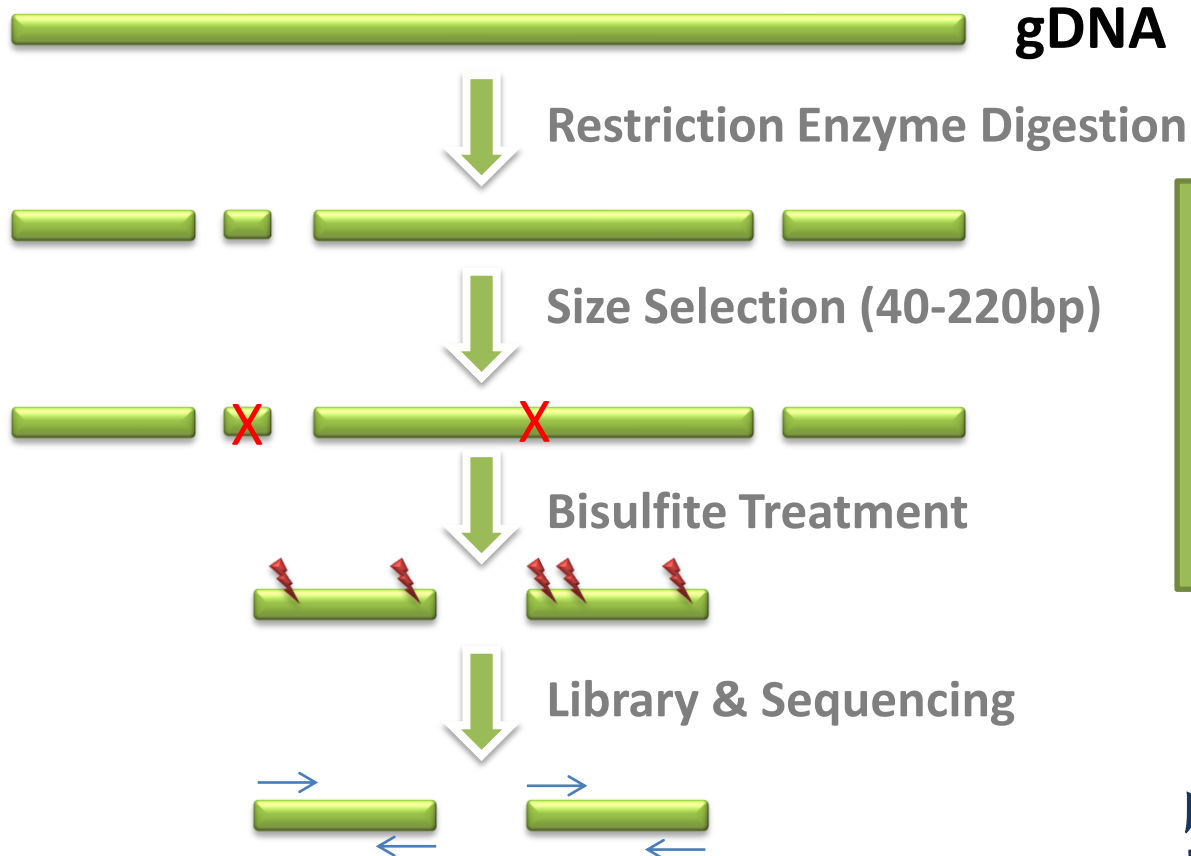
### Chromatin immunoprecipitation (Transcription Factor / Histone Modification)



# Application of NGS

## Epigenetics – Bisulfite Sequencing

WGBS (Whole Genome Bisulfite Sequencing)  
RRBS (Reduced Representation BS)



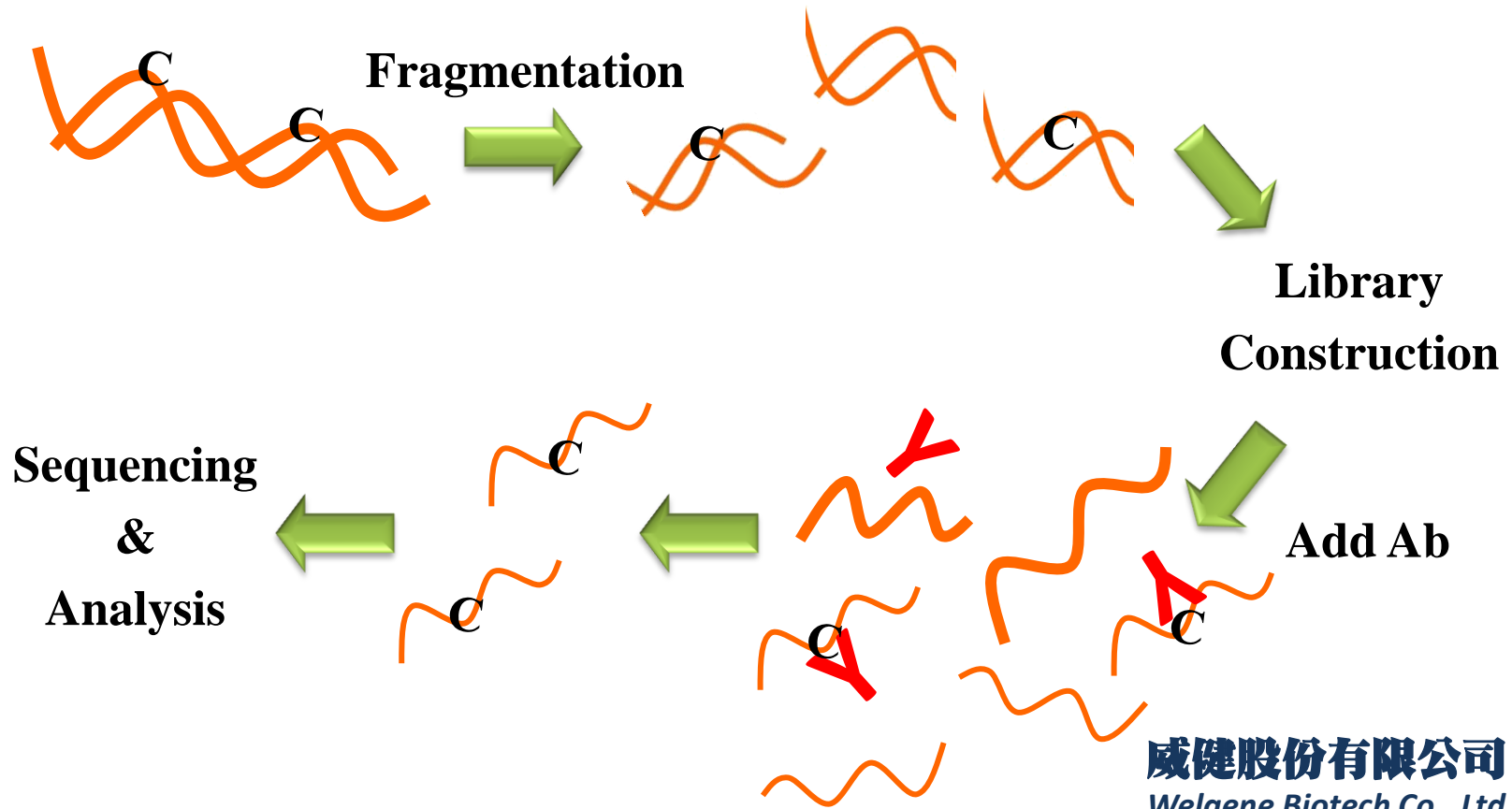
**MSP I**  
Recognition Site

5'... CCGG... 3'  
3'... GGCC... 5'

# Application of NGS

## Epigenetics – MeDIP-seq

**Methylation DNA Immunoprecipitation**  
(5-mc / 5-hmc)



# Application of NGS

## Epigenetics – NGS for Methylation

Methods	Resolution	Coverage	Cost	5-hmc
WGBS	Single Base	Genome	High	No
RRBS	Single Base	Subset of Genome	Cost-effective	No
MeDIP-seq	50~100bp	Genome	Cost-effective	Yes

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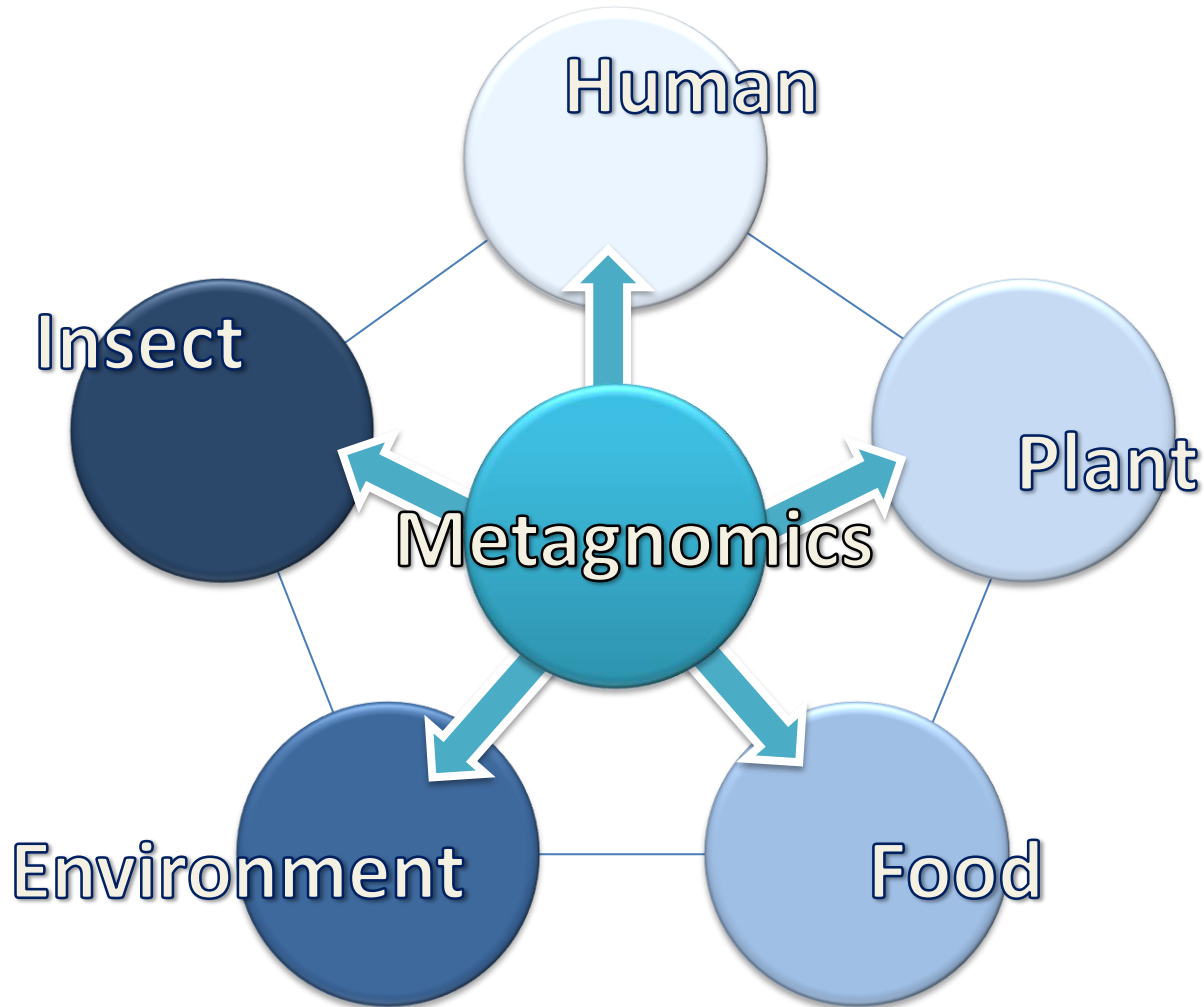
# Application of NGS

## Metagenomics

- ▲ Bacteria 16S rDNA
- ▲ Archaea 16S rDNA
- ▲ Fungi 18S rDNA
- ▲ Fungi ITS

# Application of NGS

## Metagenomics

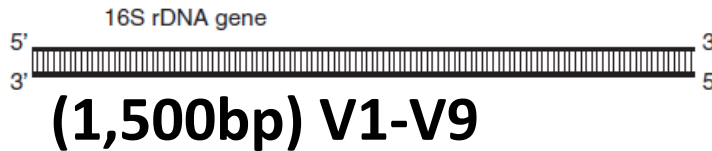


# Application of NGS

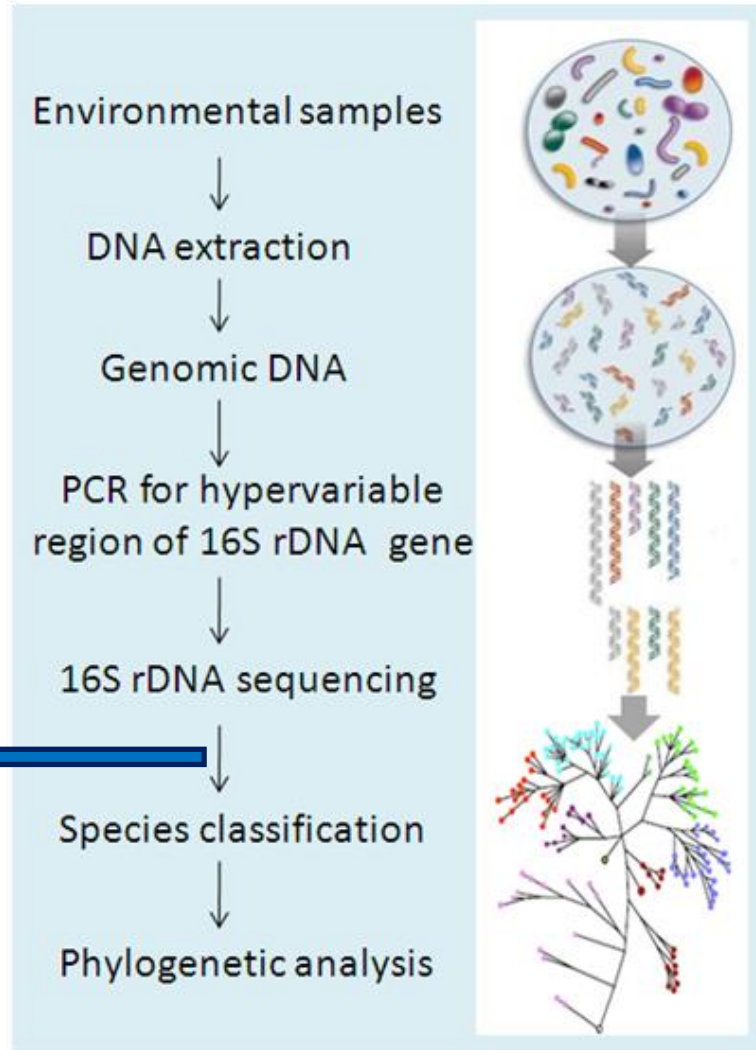
## Metagenomics



Schematic of rDNA gene with the 16s, 5.8s and 23s regions. 10 – 100 copies are in the bacterial genome. ITS = Internal Transcribed Spacers.



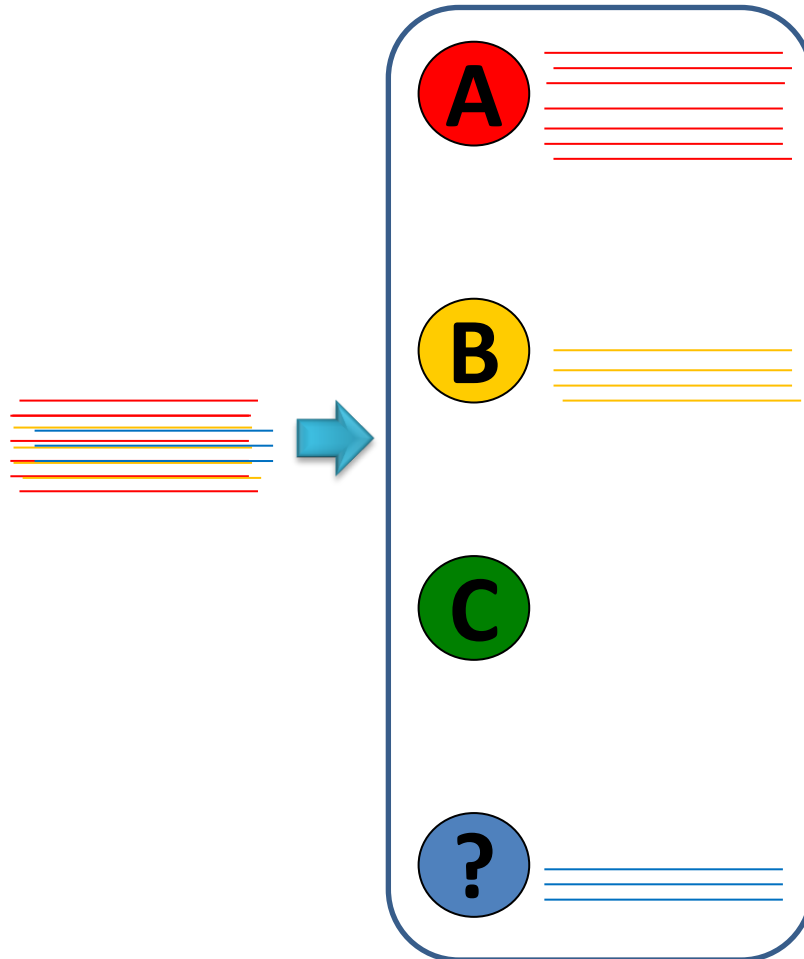
OTU(Operational Taxonomic Unit) analysis



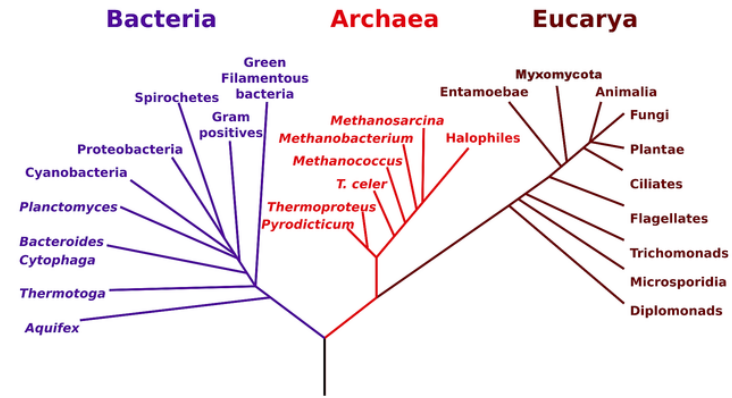
# Application of NGS

## Metagenomics

### OTU(Operational Taxonomic Unit) Analysis

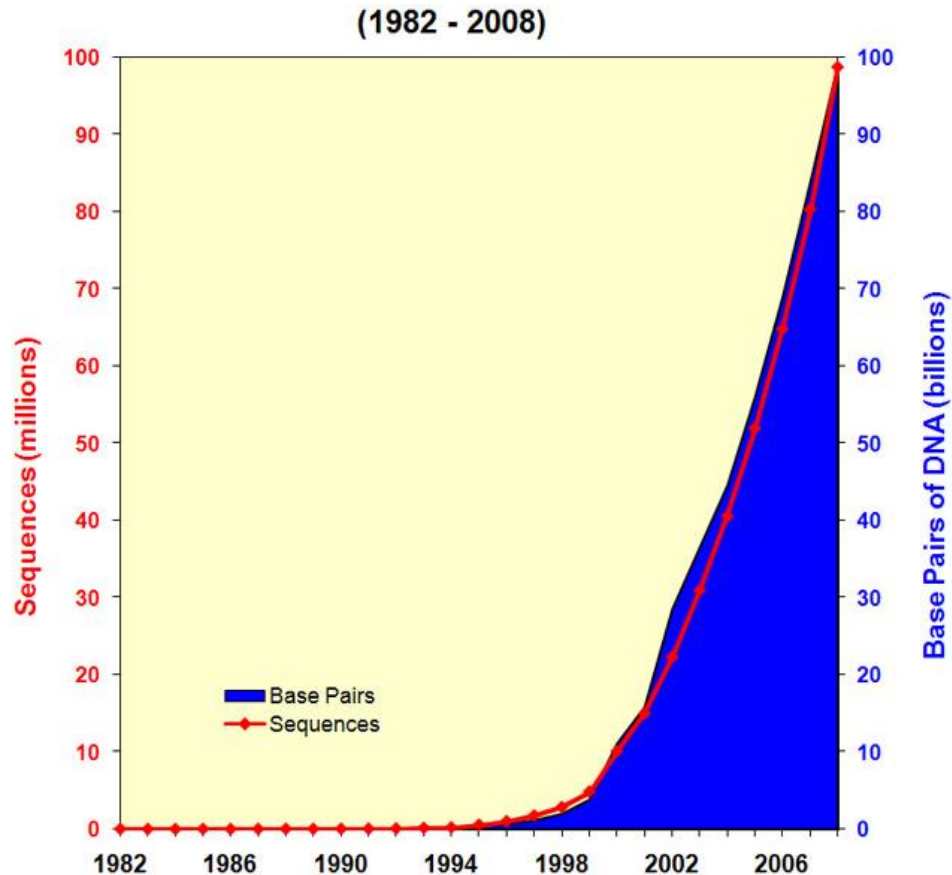


### Phylogenetic Tree of Life



# It's the NGS time

## Growth of GeneBank



1. Data generated by 1000 Genome Project had surpassed total data deposited in NCBI

2. For the sequencing **cost dramatically decreased** sequencing technology is widely used in research

3. Current Challenge : **Data mining** and disclose biological issue

# Difficult Points

## Face the Problem

# Analysis !?

## Hardware

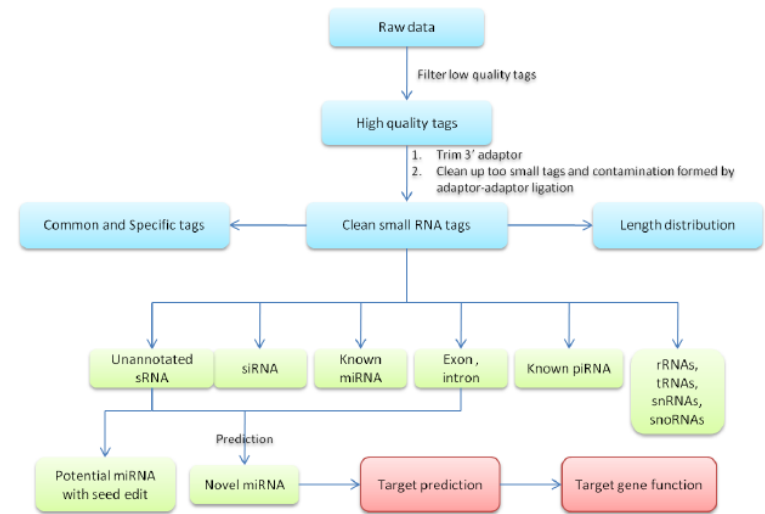


Server



PC

## Software



Pipeline

# NGS Data Mining

## To Solve this Problem

1. Bio IT
2. Commercial Software (GeneSpring NGS/CLC...)
3. Cloud (DNAnexus)
4. Professional Biotech Co.



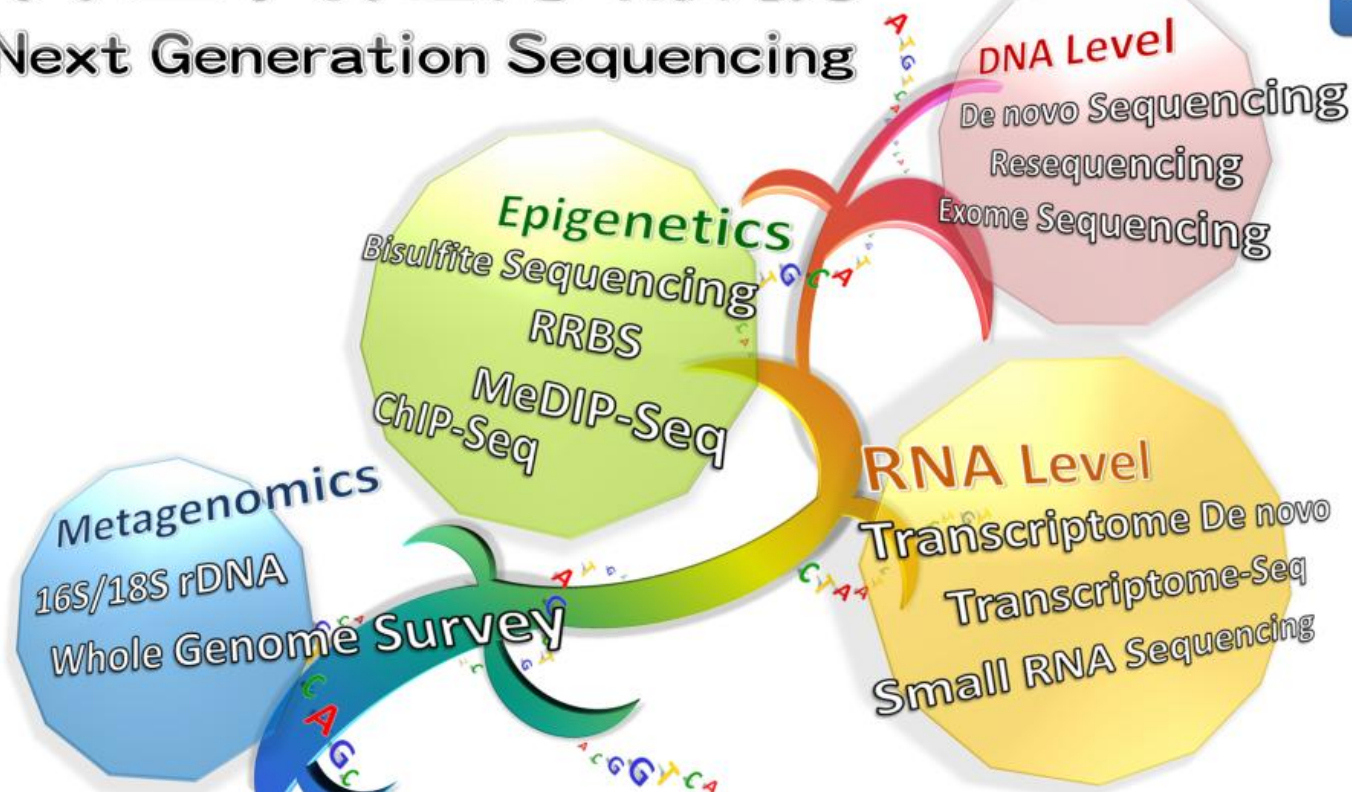
**WELGENE**

**DNAnexus**  
<https://dnanexus.com/>



# 次世代定序服務

Next Generation Sequencing



# Welgene NGS Service

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## Advantages

- ▲ Professional & Flexible
- ▲ Multiple Check Points
- ▲ Highest Sequencing Quality
- ▲ Latest & Most Often Analysis Pipeline
- ▲ Clear & Easy to Understand Report (E-Report)

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*Thanks for your Attention*

*& Discussion Time*