



Next-Generation Sequencing – NGS core facility, principles of NGS platforms, applications, and experimental considerations

基因體定序 - NGS核心設施簡介、應用、及實驗設計之考量

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High Throughput Genomics Core
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LSL, 2017/4/25

Outlines

Part I: DNA Sequencing & NGS Core

- Evolution of sequencing technologies
- NGS platforms and comparisons

Part II: Principles of common NGS applications

- DNA
- RNA

Part III: Prep works

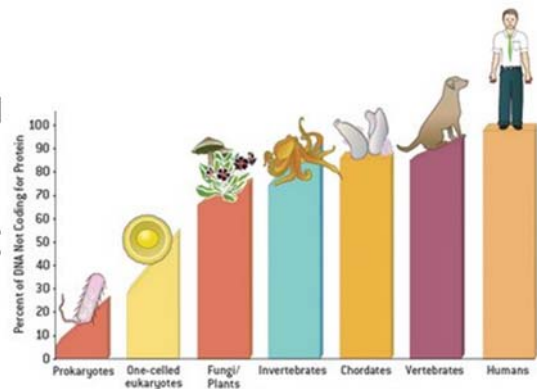
- Sample requirement and considerations
- Submission to NGS Core

Part IV: NGS Data & Resources

- Data types and QC
- Public resources

HT Genomics Core in Academia Sinica

- 2008: Established for biofuel pi
- 2013: Promoted as NGS servic
- Internal & collaborative projec
 - Pathogenic bacteria
 - Pathogenic/medicinal fungi
 - worms
 - Insects
 - Evo-devo: avian species
 - C3/C4 plants
 - Marine animals



*Need
Super
Sequencers!!*



http://www.ashg.org/education/everyone_1.shtml

NGS Service at BRC core

- Current NGS lineup:
 - 3 Illumina (HiSeq2500 *2, MiSeq)
 - Roche 454 GS+
 - PacBio Sequel
- **SOPs:** established for various NGS applications for both sequencing platforms
- Provides consultation on:
 - Project's need
 - suitable NGS experiment design
 - Sample preparation
 - Cost analysis



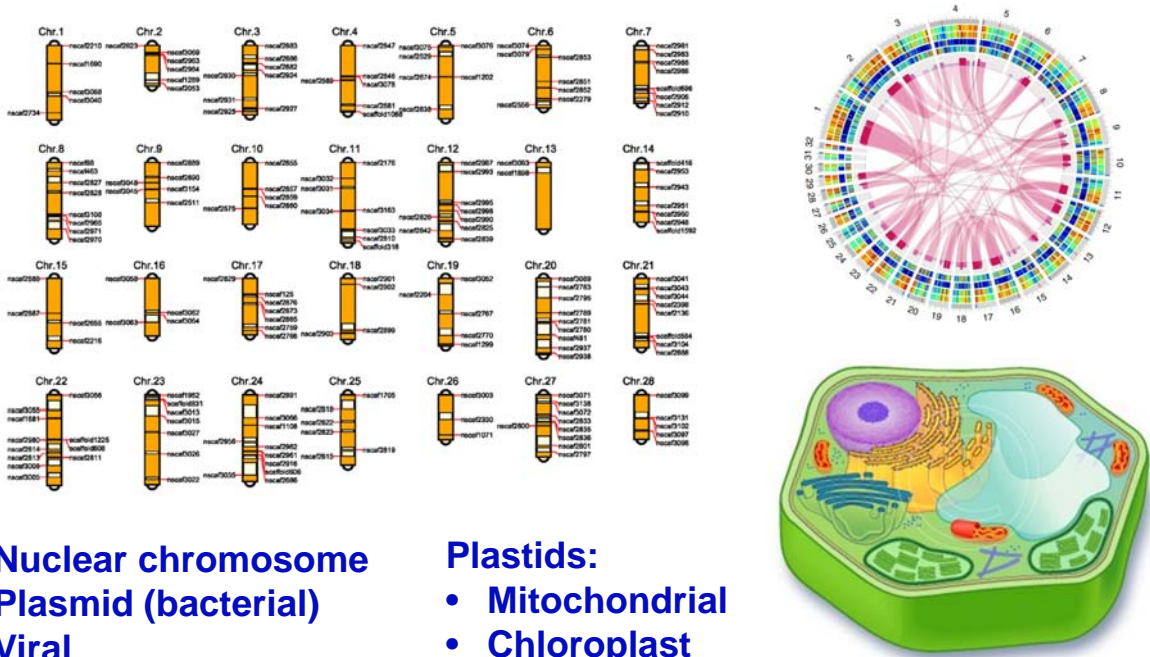
<http://ngs.biodiv.tw/NGSCore/>

I. DNA Sequencing

- Evolution of sequencing technologies
- NGS platforms and comparisons

Genome

- a full haploid set of chromosomes with all its gene; the total genetic constitution of a cell or organism.



- Nuclear chromosome
 - Plasmid (bacterial)
 - Viral
- Plastids:**
- Mitochondrial
 - Chloroplast

In general, genome size increases with organism's complexity.

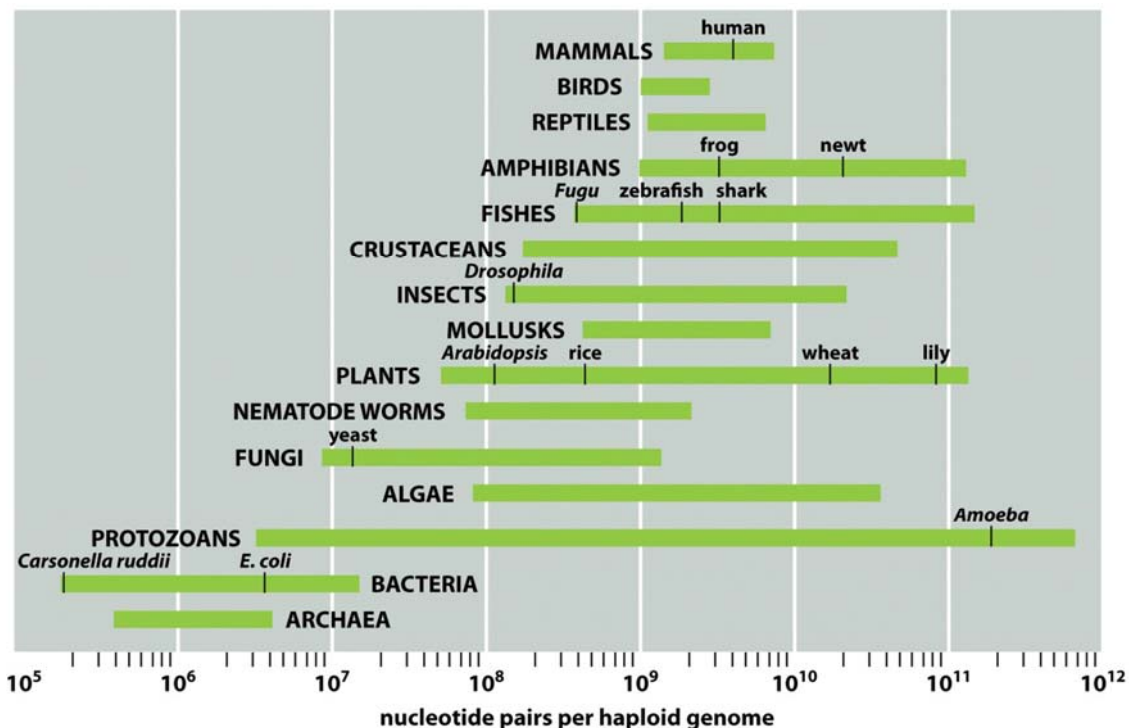
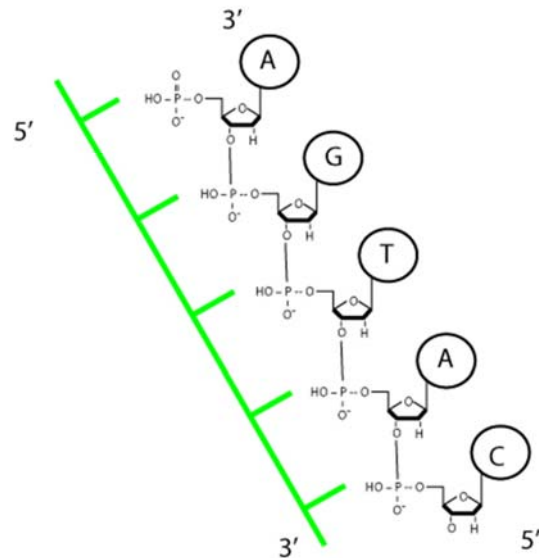
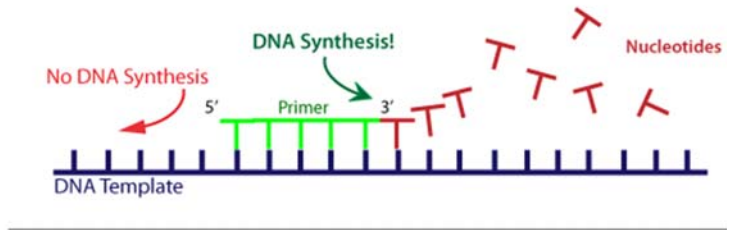
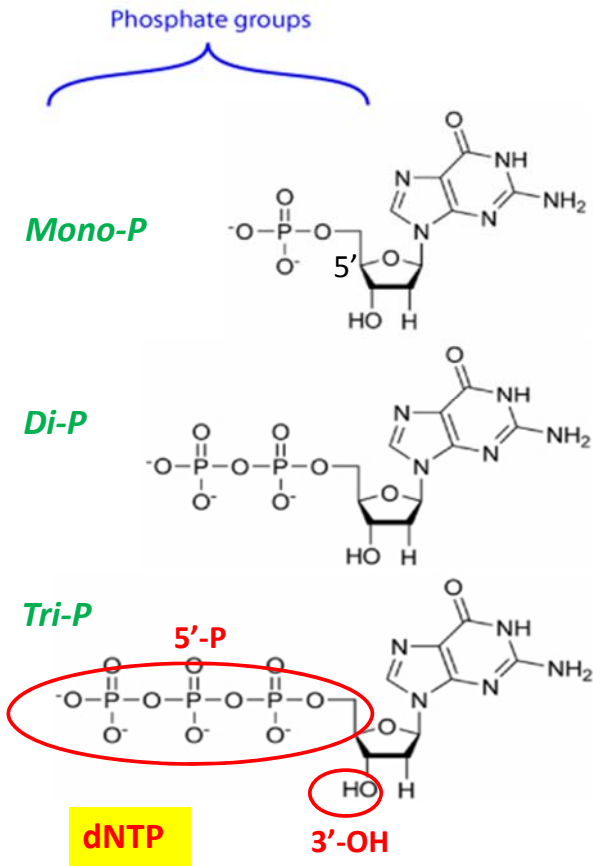


Figure 1-41 Essential Cell Biology 3/e (© Garland Science 2010)

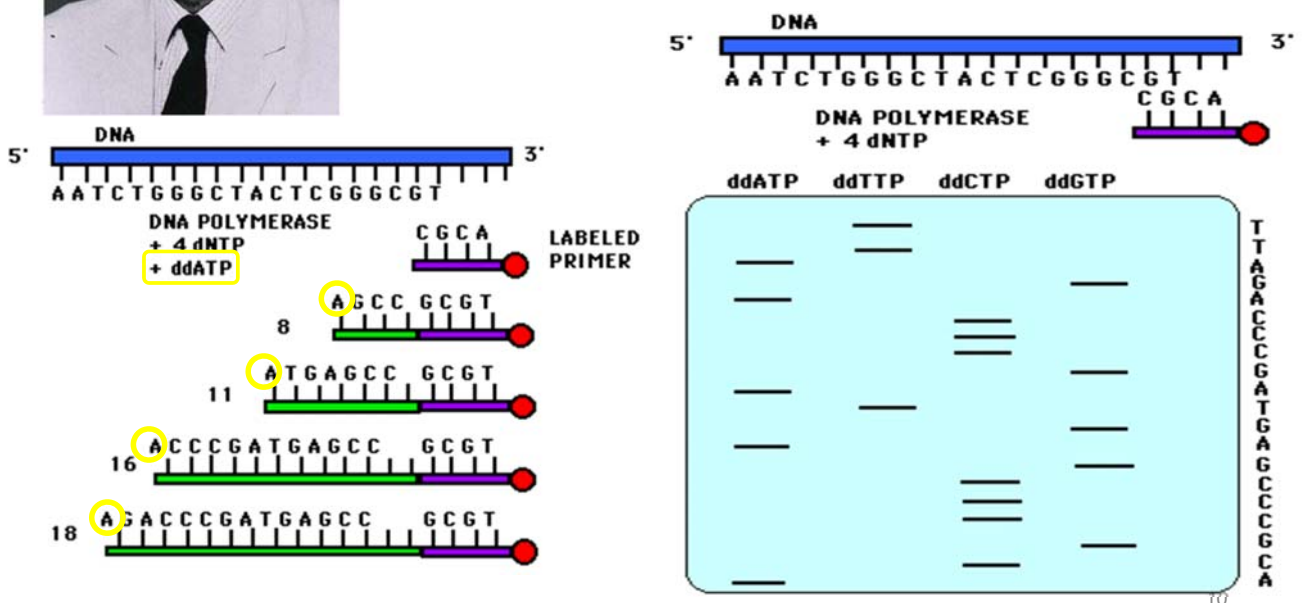
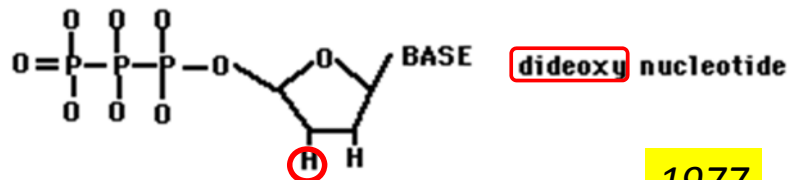
DNA synthesis - polymerization



<http://www.visionlearning.com/en/library/Biology/2/DNA-III/180>

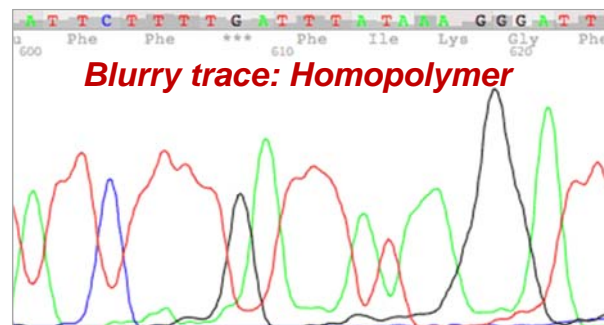
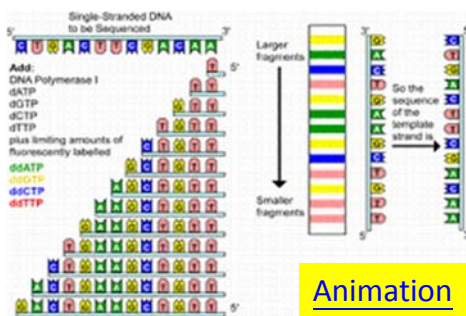
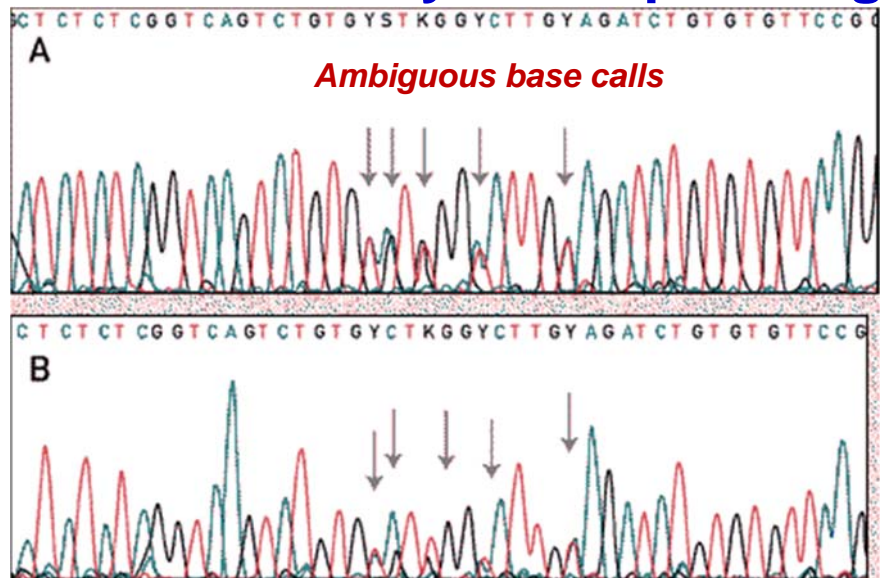
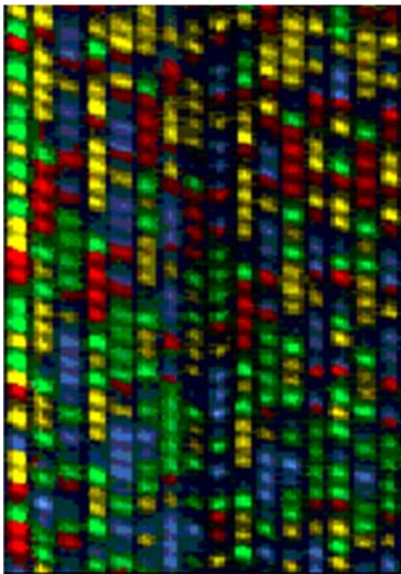
Sanger Seq.: chain termination w/ dideoxy nucleotide

Frederick Sanger



<http://www.bio.davidson.edu/Bio111/sea.html>

Fluorescent Dye: Terminator Cycle Sequencing

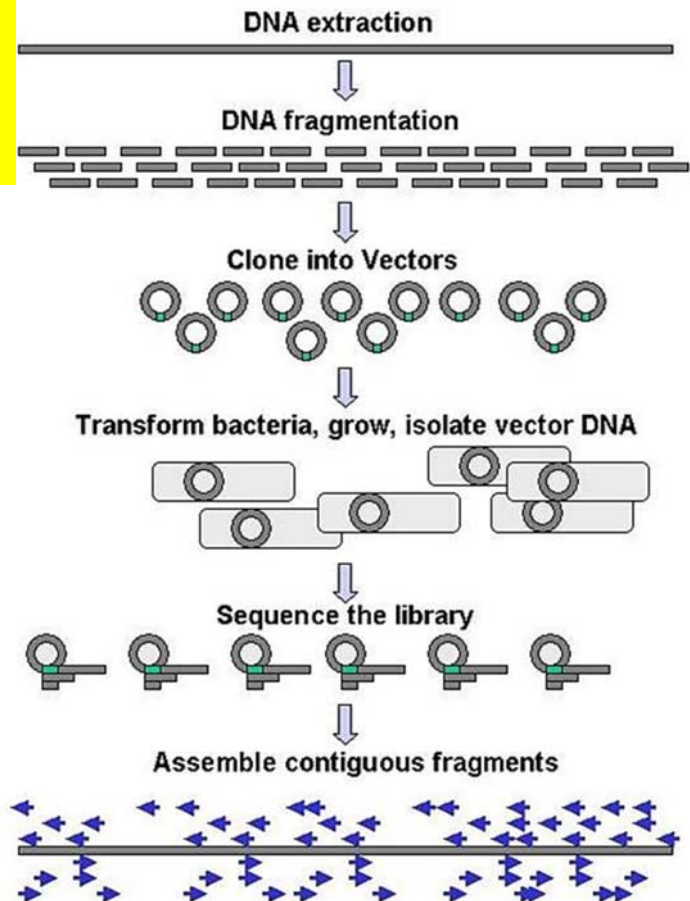


Genome Sequencing: Hierarchical cloning

BAC: 100-200kb

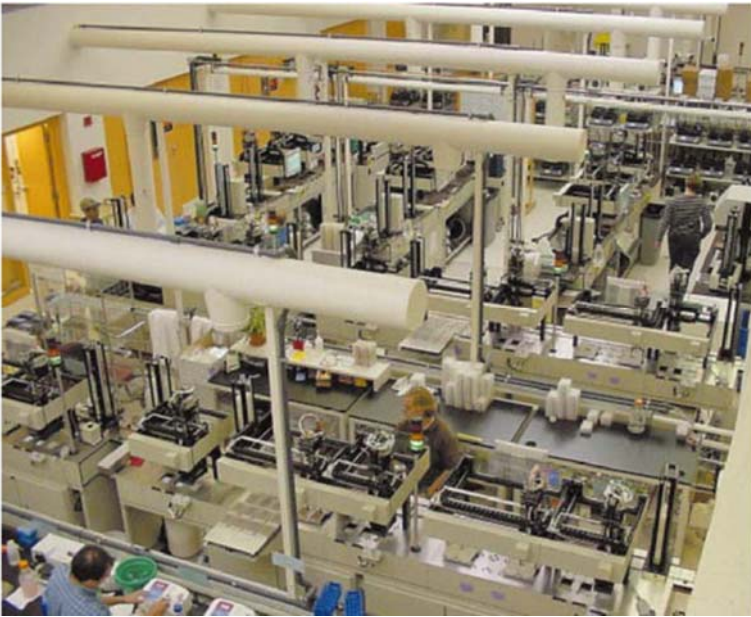
Fosmid: 30-40 kb

Plasmid: 1-10kb

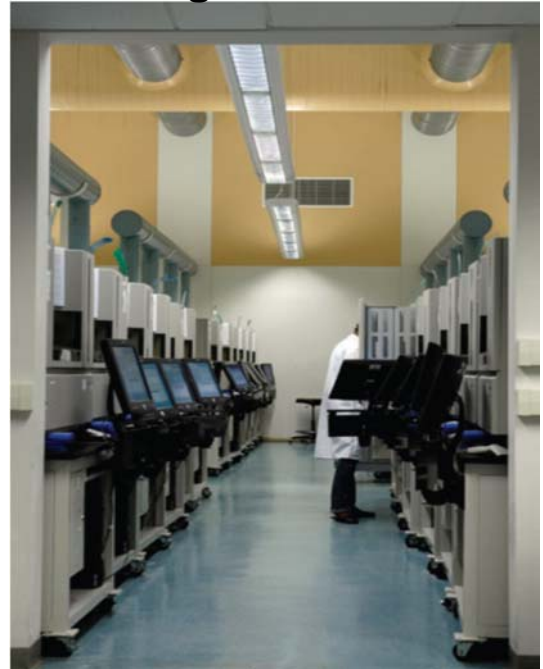


Large scale Cappillary Sequencing

Library factory - Whitehead Institute



Sequencing factory - Sanger Institute



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Nature 409, 860-921(15 February 2001)

Next generation Sequencing

- Improvements in:
 - Enzymes
 - Chemistry
 - Image analysis
 - increased sequencing capabilities.
- Potentials to dramatically accelerate biological and biomedical research
 - *by enabling the comprehensive analysis of genomes, transcriptomes and interactomes,*
 - *by tending to become inexpensive, routine and widespread, rather than requiring very costly production-scale efforts.*

[From: Introduction to NGS.](#)

NGS – massive parallel sequencing

Current Popular platforms:

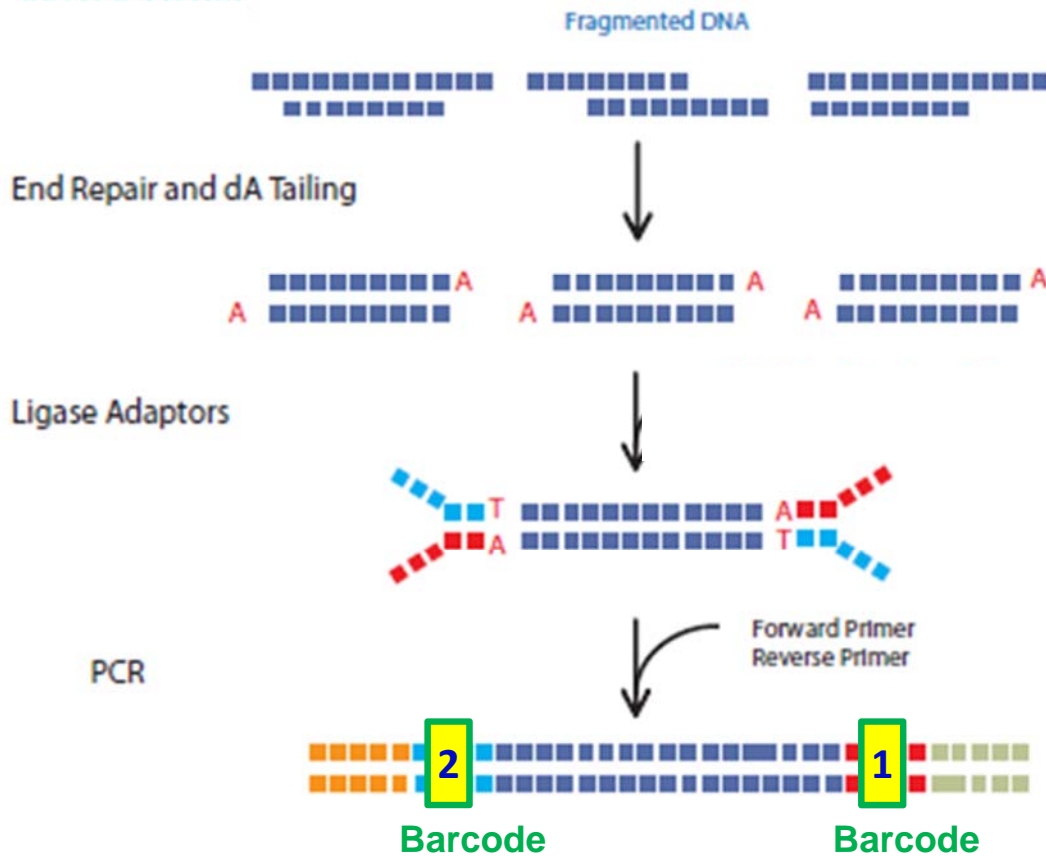
- **2nd-Gen: clonal amplification**
 - Roche 454: GS FLX, , 454 Jr., 454 XL+, 454 Jr.
 - Illumina: GA, Miseq, HiSeq, NovaSeq
 - Life Technologies: SOLiD, Ion Torrent, Ion Proton
- **3rd-Gen: single molecule sequencing**
 - Pacific Biosciences: PacBio RS II, Sequel
 - Oxford Nanopore Technologies

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Advances in Sequencing Technologies

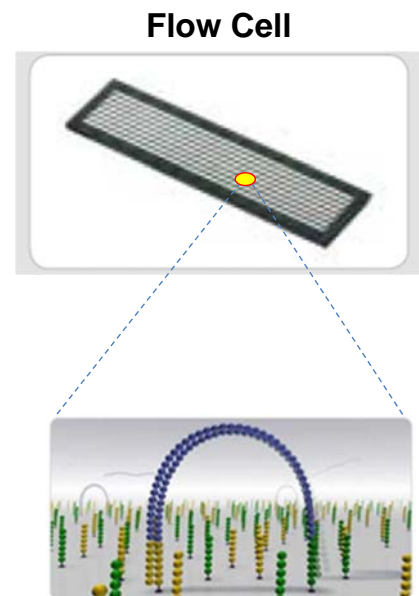
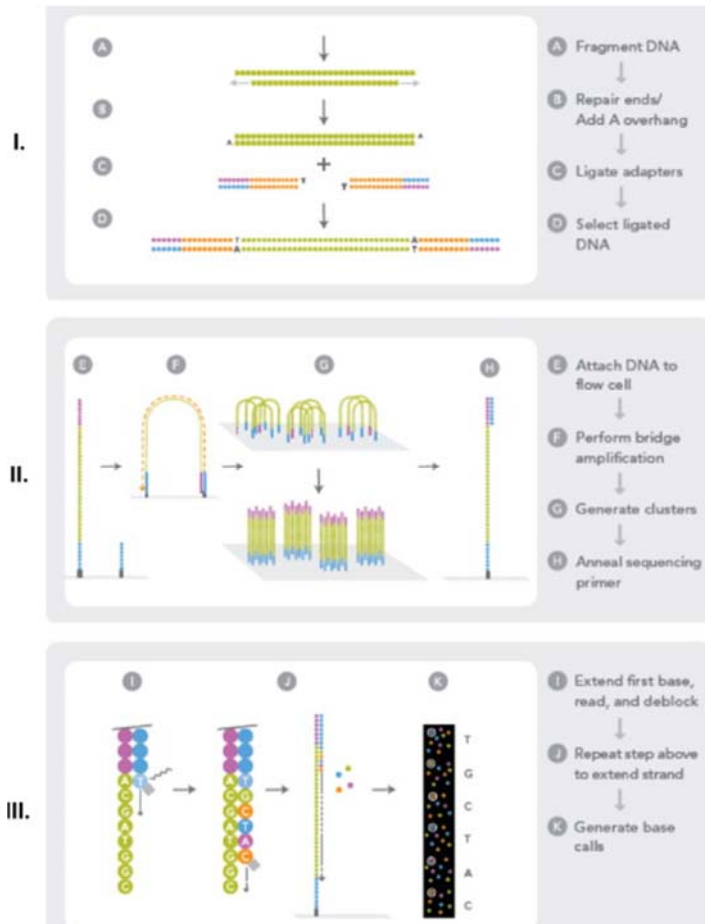


NGS Library Preparation Workflow



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Illumina/Solexa: Cyclic Reversible Terminator



Illumina – Flow cell imaging



GA IIx



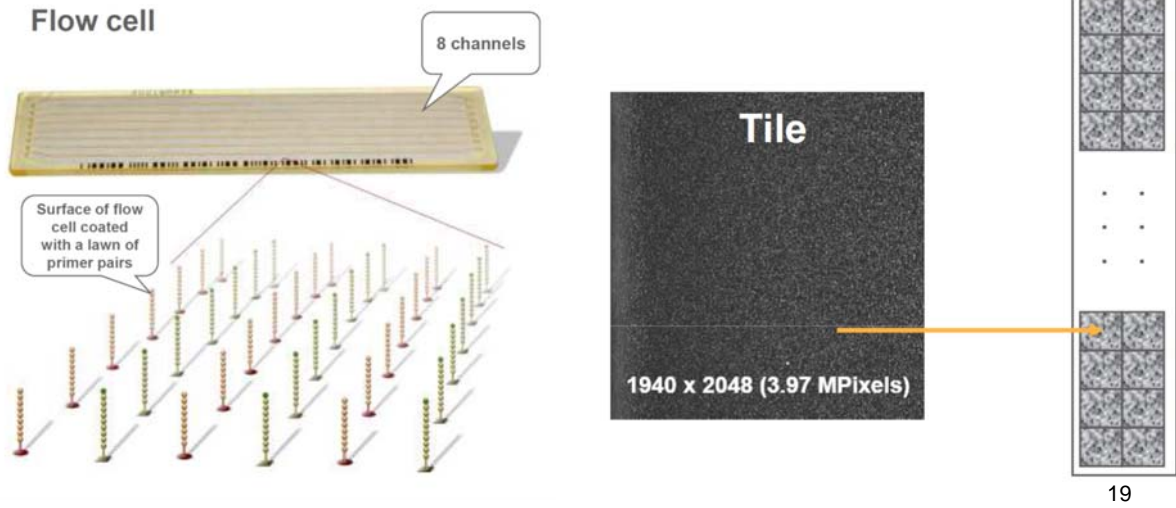
HiSeq 2500
(HT*8 / Rapid*2)



NextSeq 500

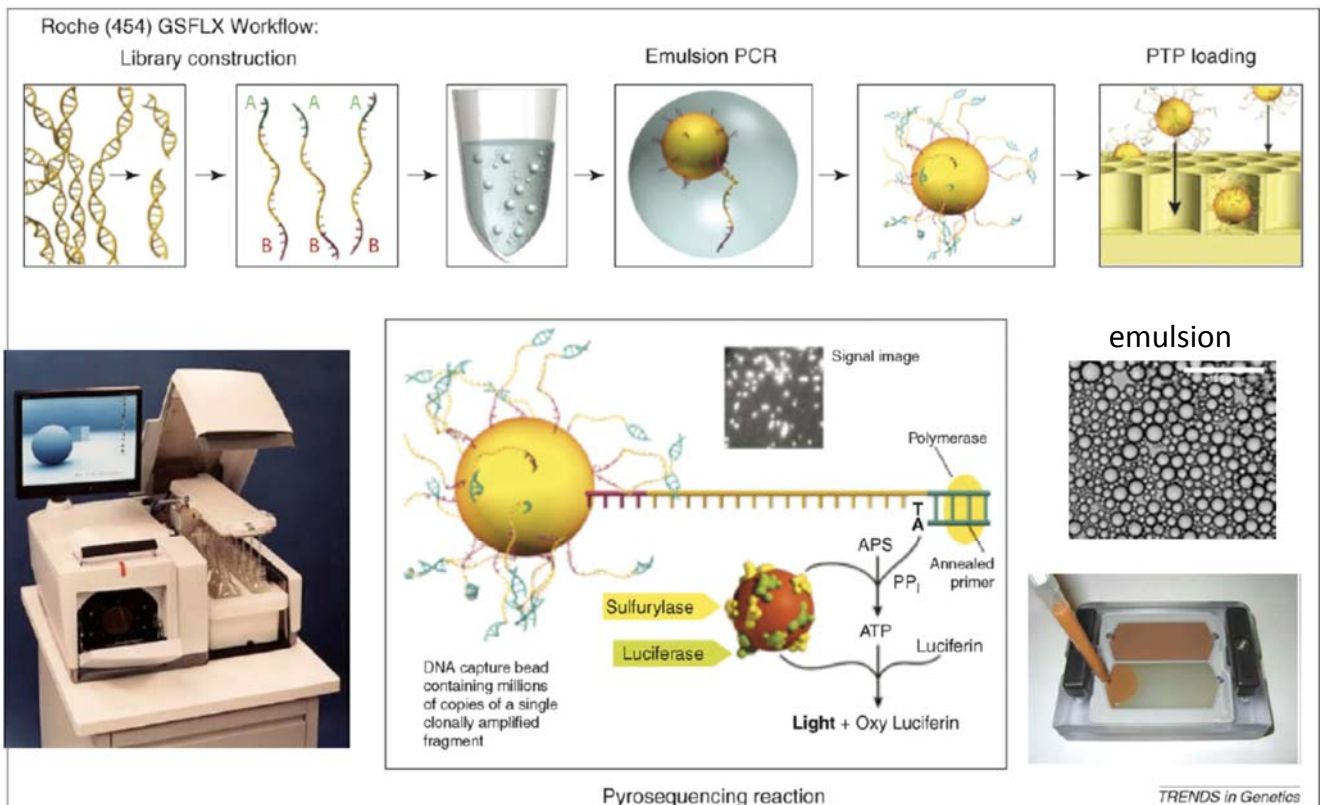


MiSeq v2



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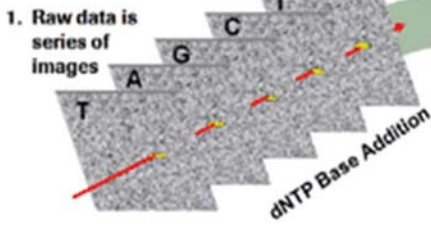
454: emPCR & pyrosequencing



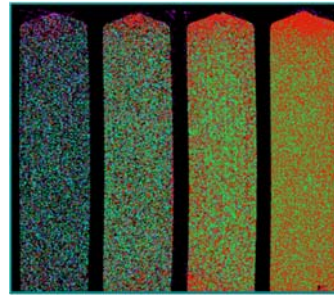
454 flowgram and read length profile

GS FLX Data

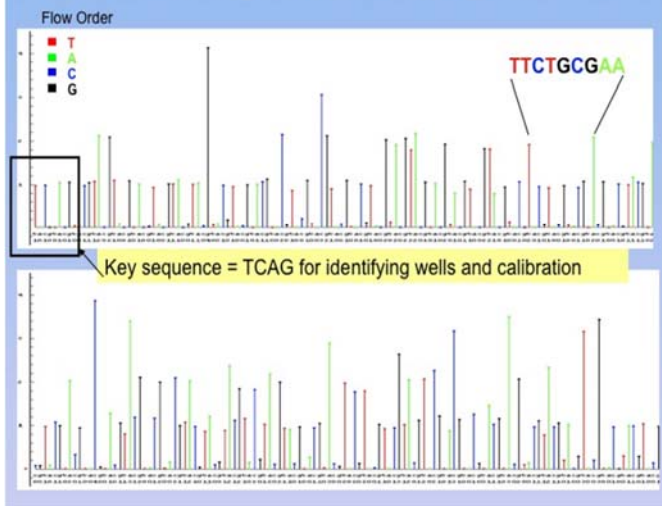
Image Processing Overview



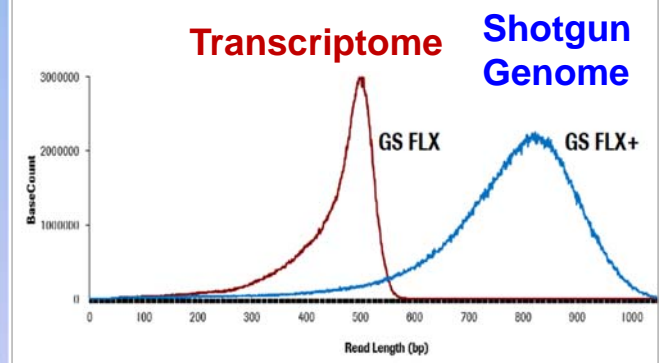
2. Each well's data extracted, quantified and normalized



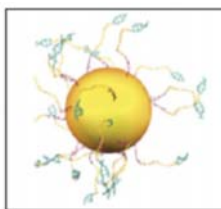
Example of a Flowgram



Significantly more bases from Sanger-like reads



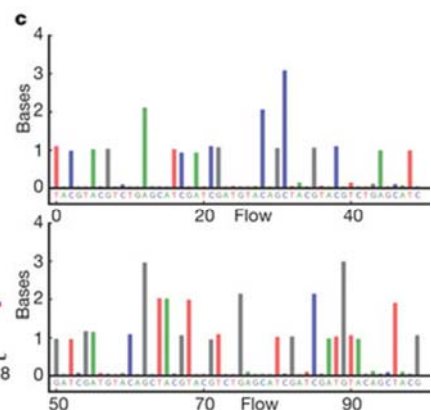
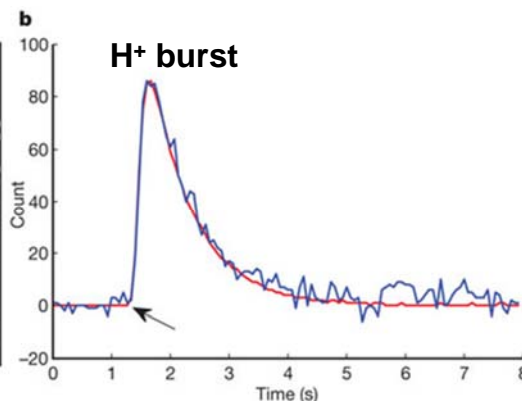
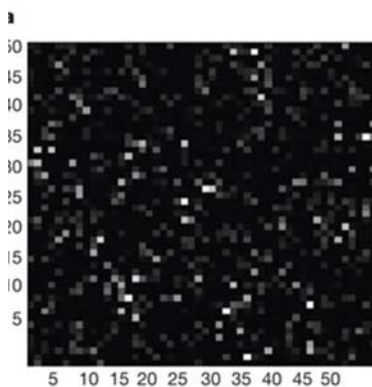
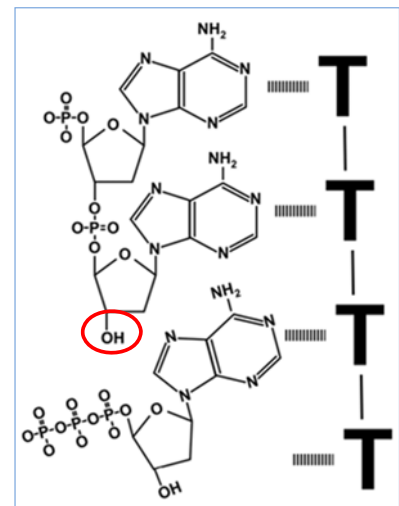
Ion Torrent/Proton: Sensing bulk release of H⁺



emPCR



Semiconductor



Life Technologies – proton sensing

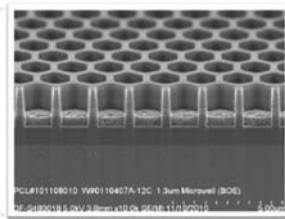
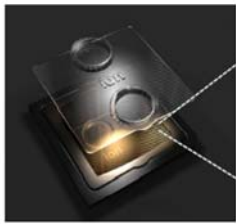
PGM™ for genes.
Proton™ for genomes.
Sequencing for all.



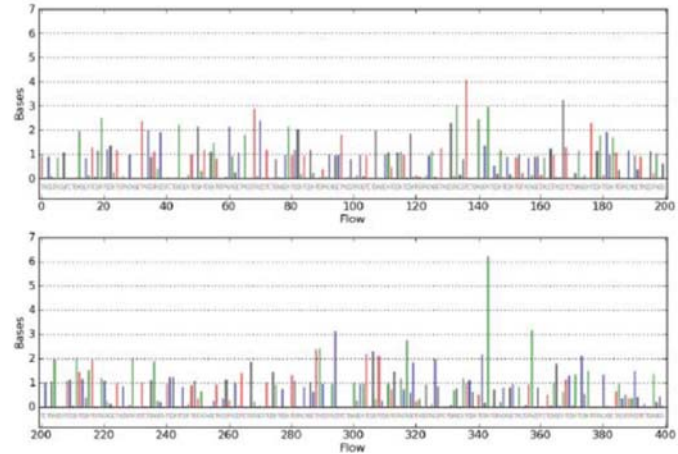
Ion PGM™ Sequencer



Ion Proton™ Sequencer

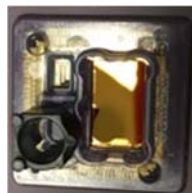
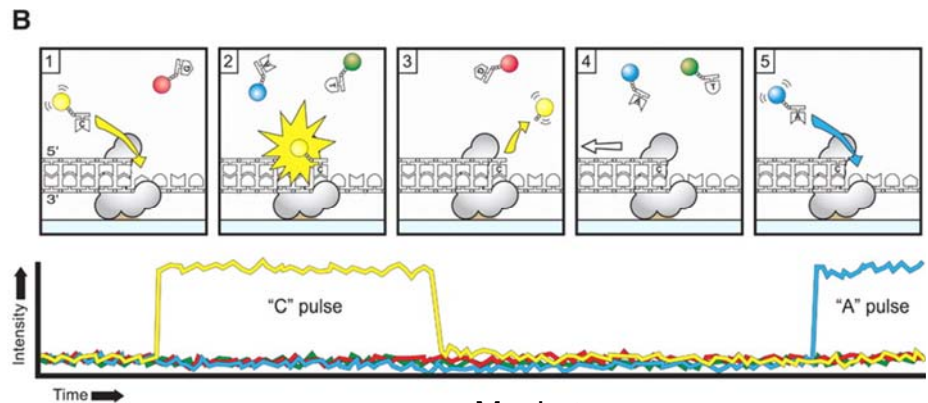
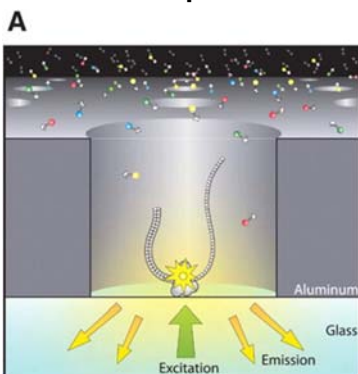


Chip: 台積電



PacBio: 3rd-Gen SMRT Sequencing

- Single Molecular Real Time (SMRT) real-time technology
- ZMW (zero-mode waveguides), a 100-nm hole with DNA/Polymerase complex immobilized at the bottom; recording fluorescence released from P-dNTP upon incorporation

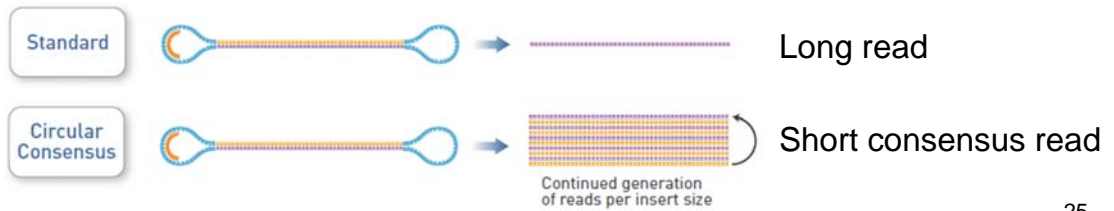
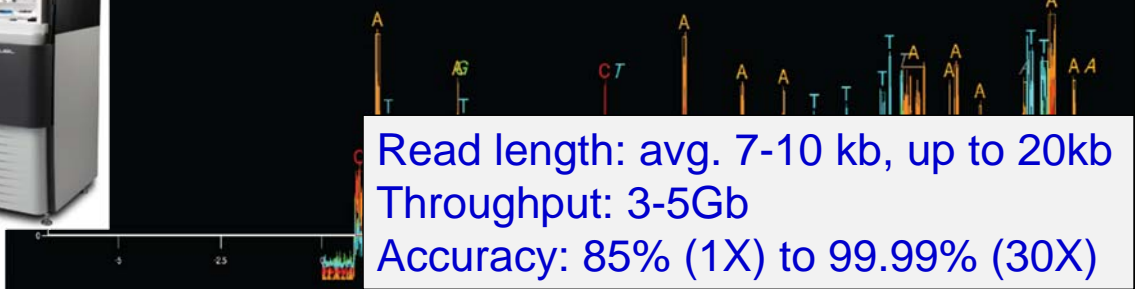


Movie trace

Signal Processing and Base Calling

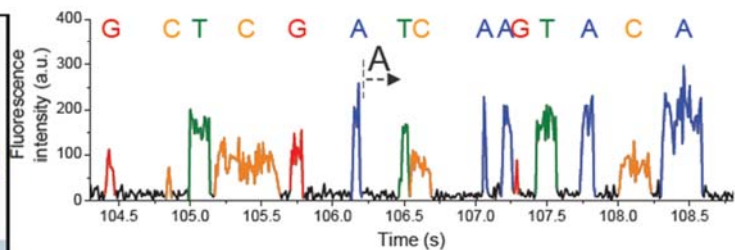
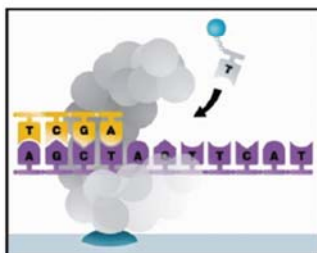
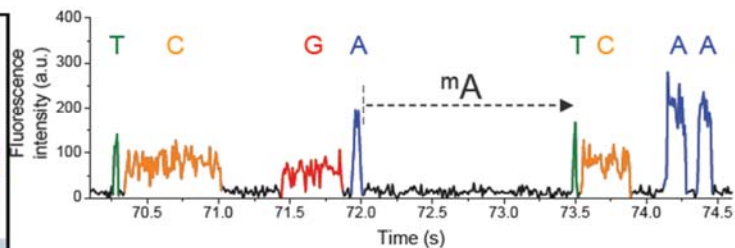
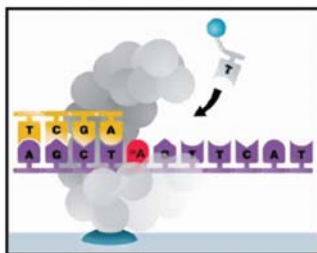


Converting pulses of light into DNA bases and kinetic measures



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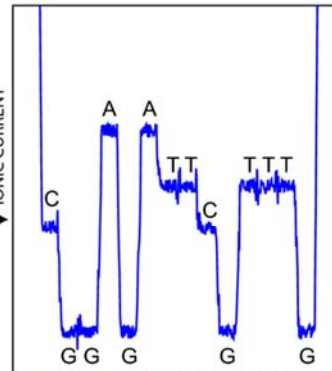
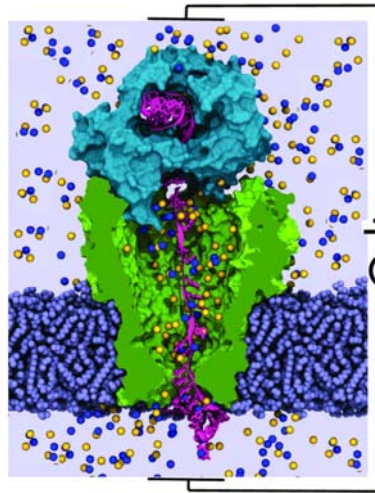
Key Feature: Kinetic Information



- Differentiation between modified and non-modified bases
 - Epigenetics, DNA damage, New, novel modifications
- Direct observation (e.g. no bisulfite)

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NanoPore Sequencing Technology

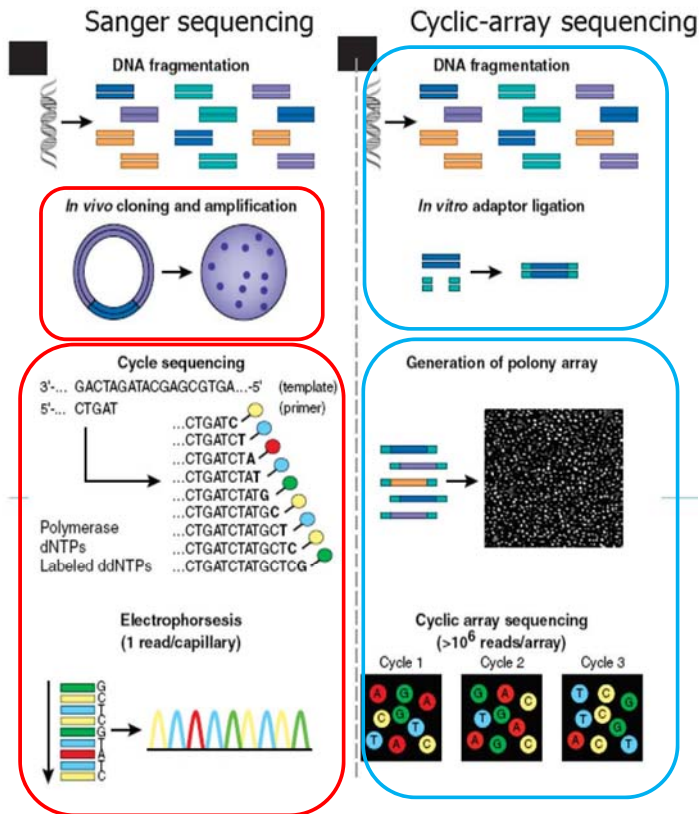


NGS Platforms & Features



	Roche 454 GS +	Illumina HiSeq 2500 (*2)	Illumina MiSeq	PacBio Sequel
Chemistry	Pyrosequencing	Cyclic reversible terminator		Real-time single molecule polymerization
Chip format				
Output/run	500-850 Mb	HT mode: 1 Tb Rapid mode: 150 Gb	up to 15 Gb	Current: 3-5 Gb Future: 7-10 Gb
Read length	400-1000 nt	PE 50-250 nt	PE 50-300 nt	1-15 kb
# Fragments /lane	1 M reads	150-180 M (Rapid) 200-250M (HT)	12-15 M (v2) 20-25M (v3)	350-500 K / SMRT cell
Data quality	> 99%; homopolymeric errors; tolerate high GC%	> 99.9%; Tolerate homopolymer; sensitive to high GC	> 99.9%; Tolerate homopolymer; sensitive to high GC	Raw ~ 85%; CCS ~ 99.9%; homopolymeric errors; tolerate high GC%
Application	De novo genome & transcriptome; Long amplicons	Highest throughput De novo assembly; Re-sequencing	De novo assembly; Re-sequencing; amplicon	Genome assembly; structural variation; phasing; Iso-Seq

Next-generation DNA sequencing



Advantages:

- adaptor-mediated library construction
- Clonal amplification to enhance signal intensity
- No bacterial cloning, colony picking, chr. Walking
- Array-based sequencing
- Massive parallel sequencing
- Much cheaper per *output unit*

<http://ueb.ir.vhebron.net/NGS>

II. Principles of common NGS applications

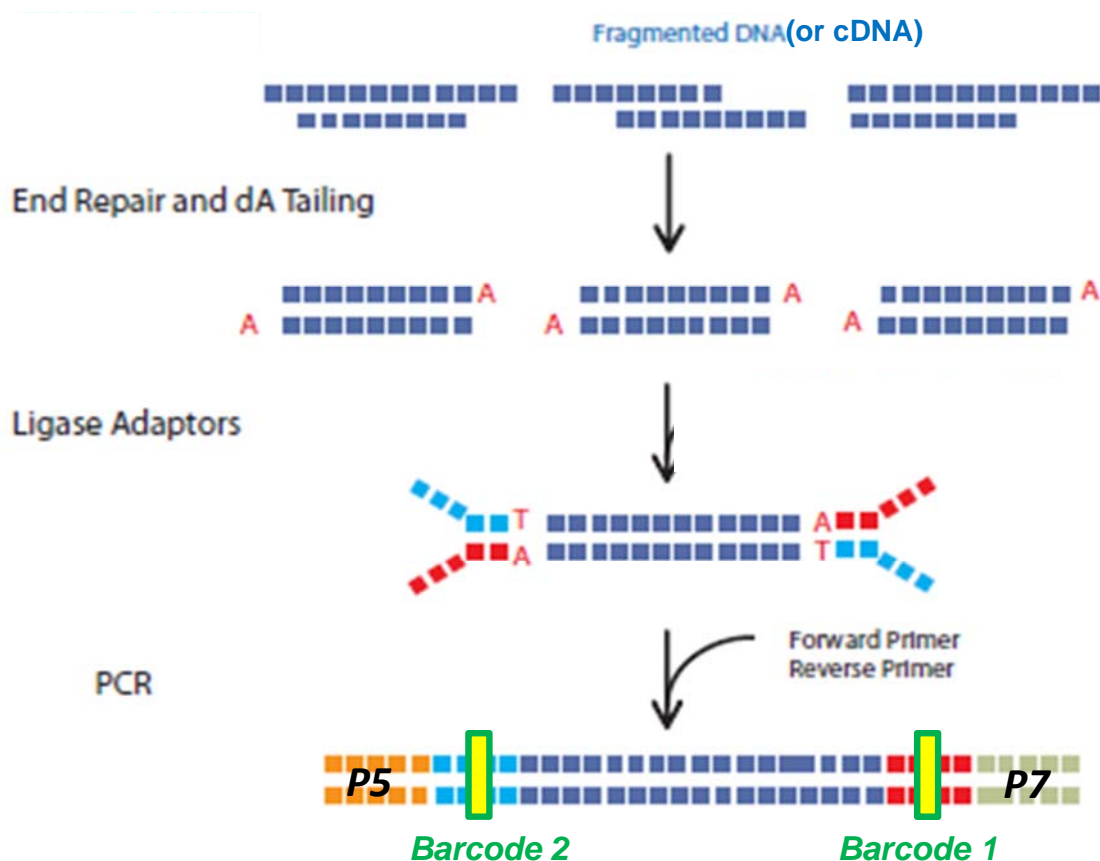
- DNA applications
- RNA applications

Genome Sequencing

- *De novo*
- *Re-sequencing*

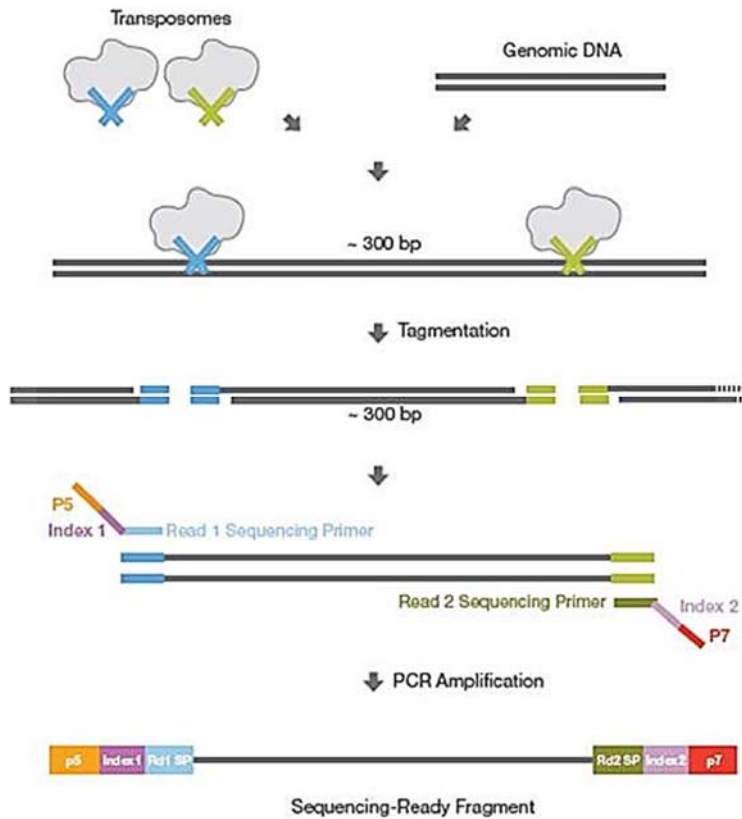
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1. Shotgun gDNA Library Preparation



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2. Tn tagging - Nextera Library prep



1. TemplateDNA + transposome complex (contain adaptor)

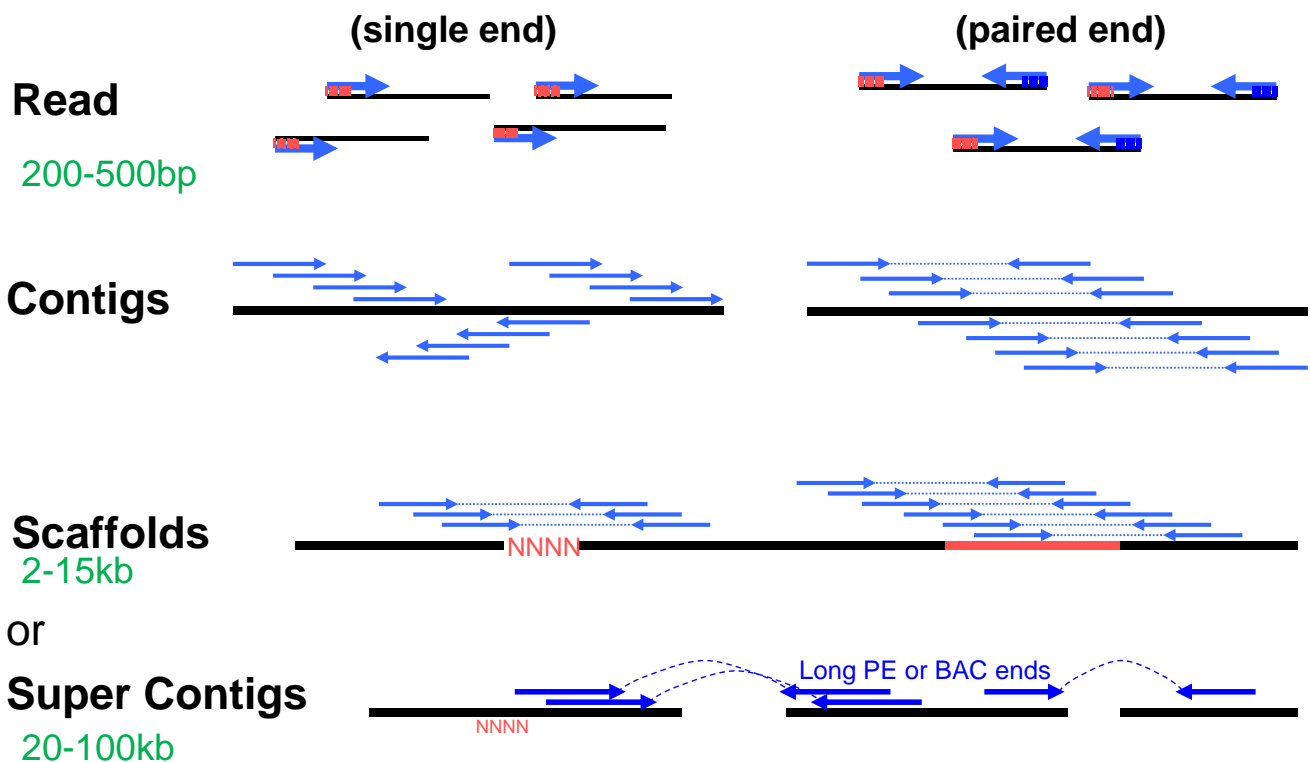
2. Tagmentation breaks DNA and add adaptor to ends

3. PCR amplification to engineer barcode and sequencing primers

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http://www.gtbiotech.com.tw/products/Nextera_XT_DNA.asp

Hierarchical Genome Assembly



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De novo Genome Sequencing

- **Local assembly: with size selection**
 - shotgun PE: *end-overlapping*
 - 200~600bp insert; PE2*150~300bp, 50-200X coverage
- **Scaffolding:**
 - Mate-pair: 2~15kb (size selected), 20-50X
 - 454 long PE: 20~30kb jump
- **Bridging over small gaps: LIPE**
 - Shotgun 1~1.6kb insert
- **Gap filling: PacBio, NanoPore long Reads**
 - 1~30 kb

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Re-sequencing: Variant detection

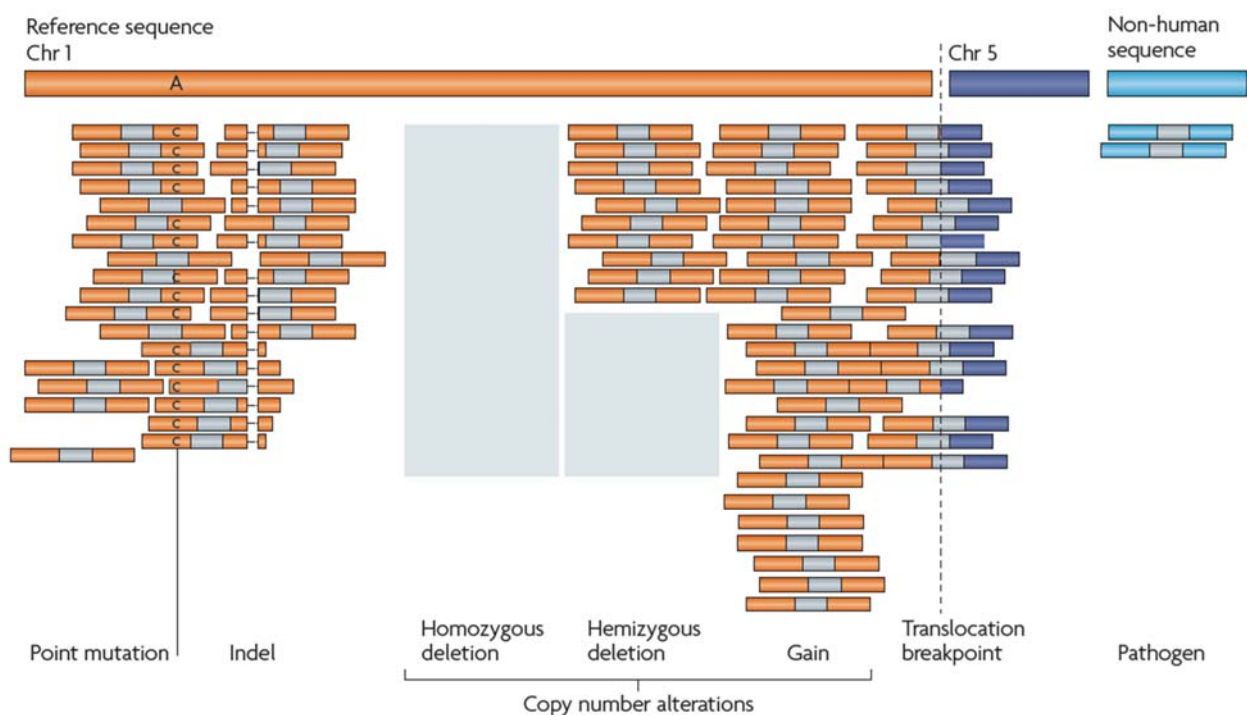


Figure 3 | Types of genome alterations that can be detected by second-generation sequencing. Sequenced

Whole Genome Re-Sequencing

- **Variant calling: SNPs & short INDELS**
 - shotgun PE: SR or PE
 - Read length: 100~150bp
 - >30X coverage per haploid genome
- **Genome re-arrangement: longer INDELS and break point**
 - Gel-size selection
 - PE2*150~300; 30-50X coverage
- **Copy number variation:**
 - PE2*100-150; >30X coverage

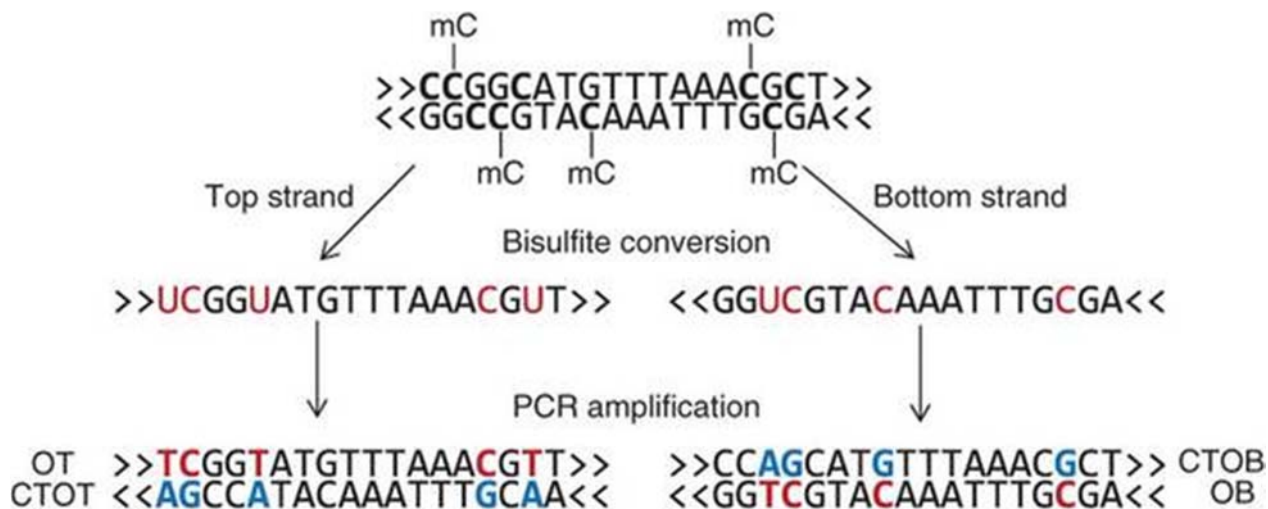
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Epigenetic sequencing

- *Bisulfite-seq*
- *ChIP-seq*
- *Histone-IP*

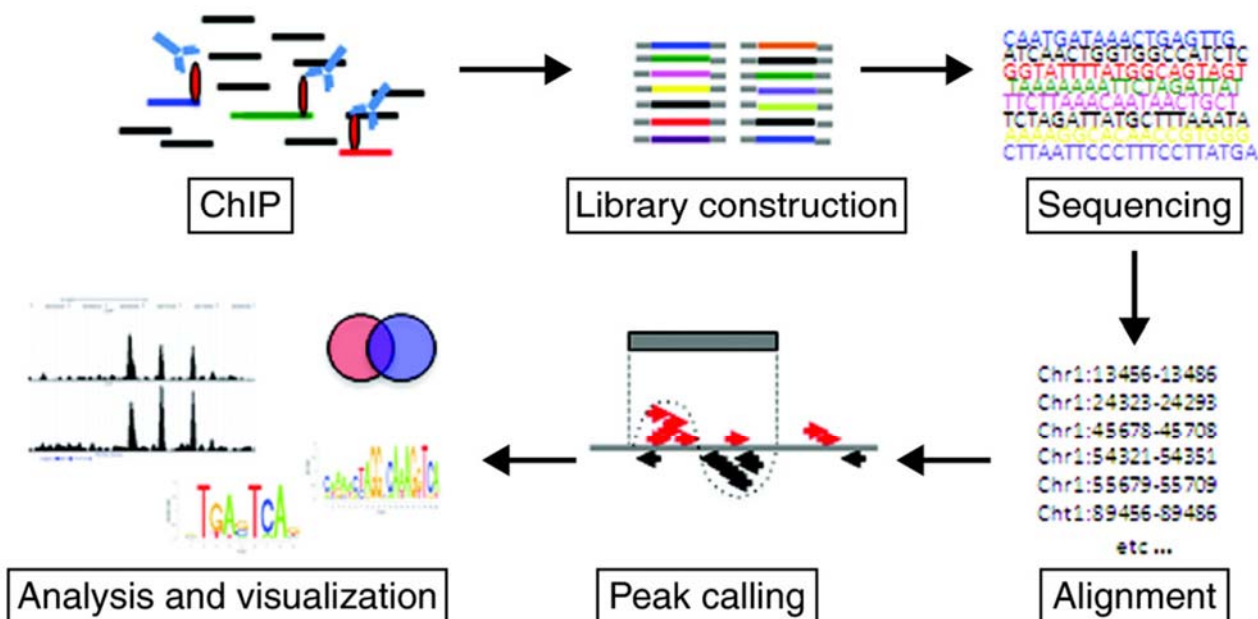
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Effect of bisulfite treatment of DNA



<http://www.nature.com/nmeth/journal/v9/n2/full/nmeth.1828.html>

ChIP-seq procedure



ChIP-Seq considerations

1. Sample requirement:

- Qubit assay: 10-100 ng (eg. 15 ng in 40 ul)
- Purity: OD 260/280 ratio at 1.7-1.9
- Smear pattern: BioAnalyzer HS DNA, distribution at 0.2-0.8kb, major~250bp
- Optional: qPCR confirmation of target genes

2. Controls:

- Negative: Mock (no primary Ab), Total INP, un-induced
- Positive: [primary Ab \(ChIP-grade\)](#) from independent sources

3. Sequencing format:

- SE or PE; read length 50-100nt
- barcode 12-48-plex

Sequencing and data QC

• Sequencing Depth and Barcoding:

- 10-20 million reads can reveal can provide reasonable for a ChIP-Seq sample
- May require more reads to identify rare binding events
- SR: normal ChIP-Seq; with unique mapping provides quantitative detection
- PE or long read: to increase mapping precision, or for Allele-specific ChIP-seq

• Data Quality Control:

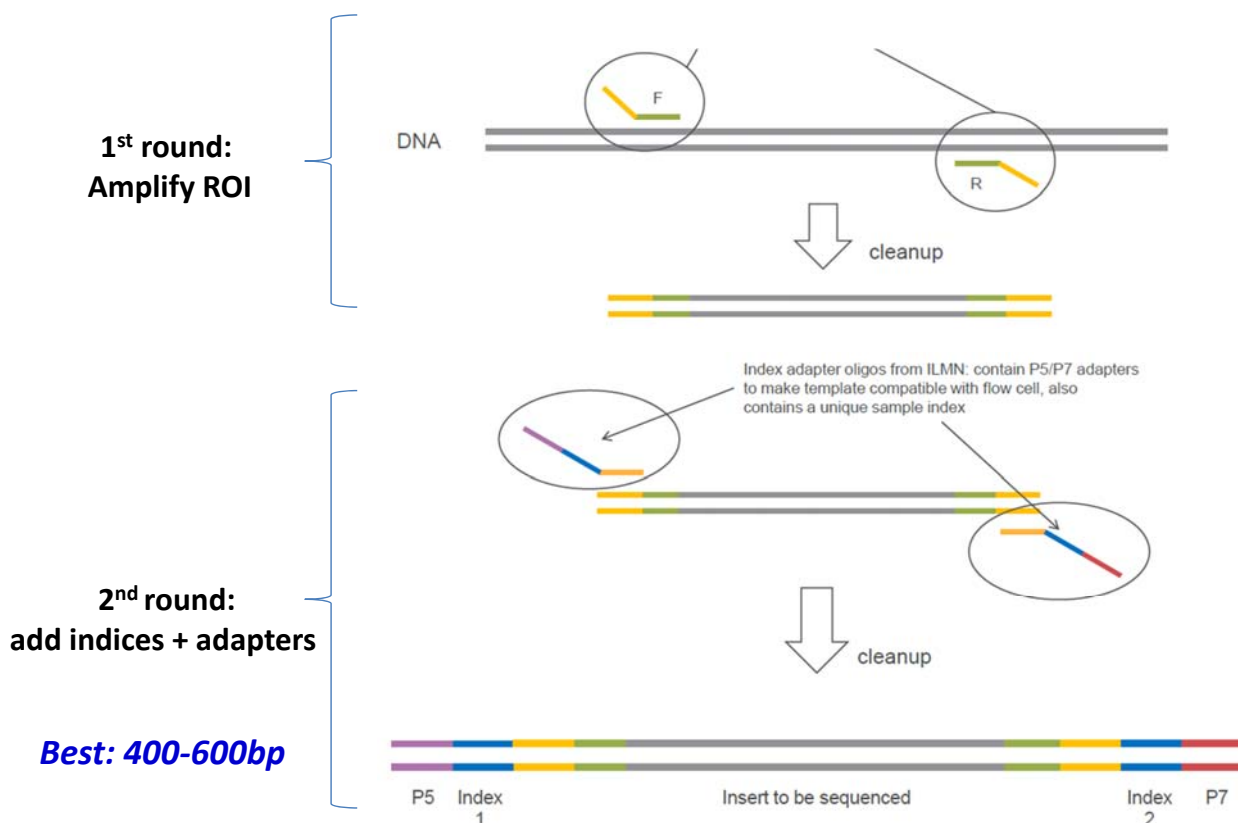
- low dupreads
- mapping to both strands
- similar GC% distribution of whole genome (low GC bias)
- data visualization in Genome Browser or IGV

Amplicon sequencing

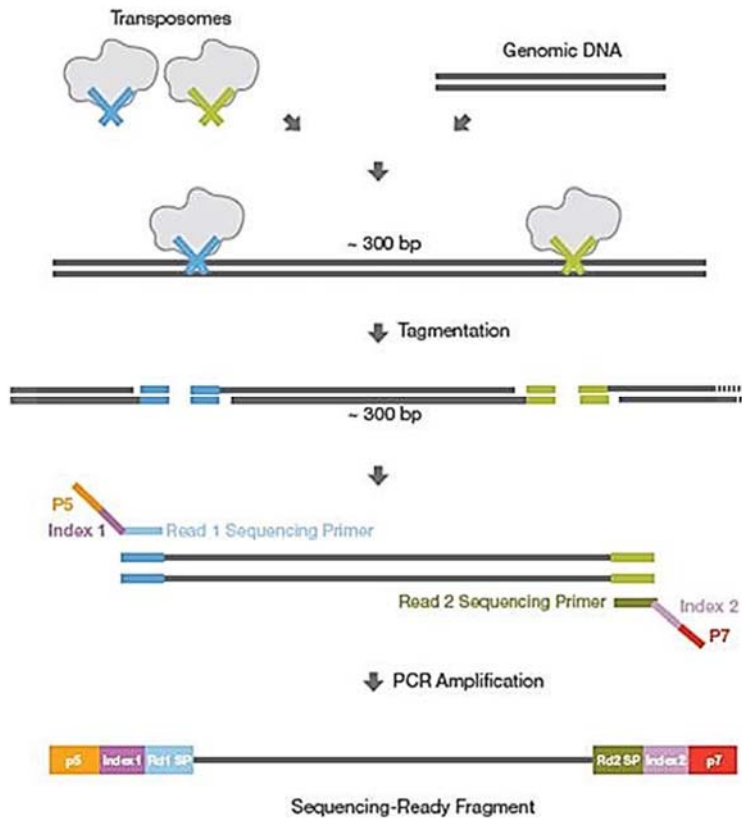
- 1. Two-step PCR**
- 2. Nextera XT tagmentation**

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Amplicon library: 2-step PCR



Nextera Library prep - Tn tagging



1. TemplateDNA + transposome complex (contain adaptor)

2. Tagmentation breaks DNA and add adaptor to ends

3. PCR amplification to engineer barcode and sequencing primers

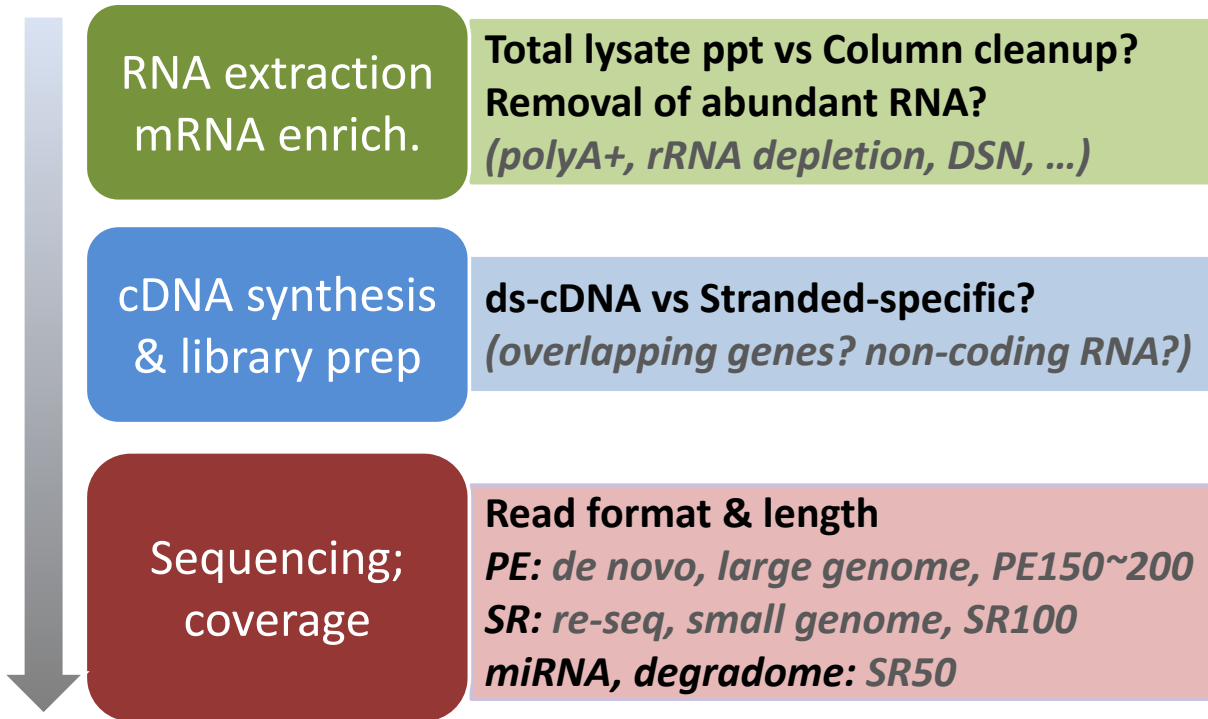
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http://www.gtbiotech.com.tw/products/Nextera_XT_DNA.asp

Transcriptome sequencing

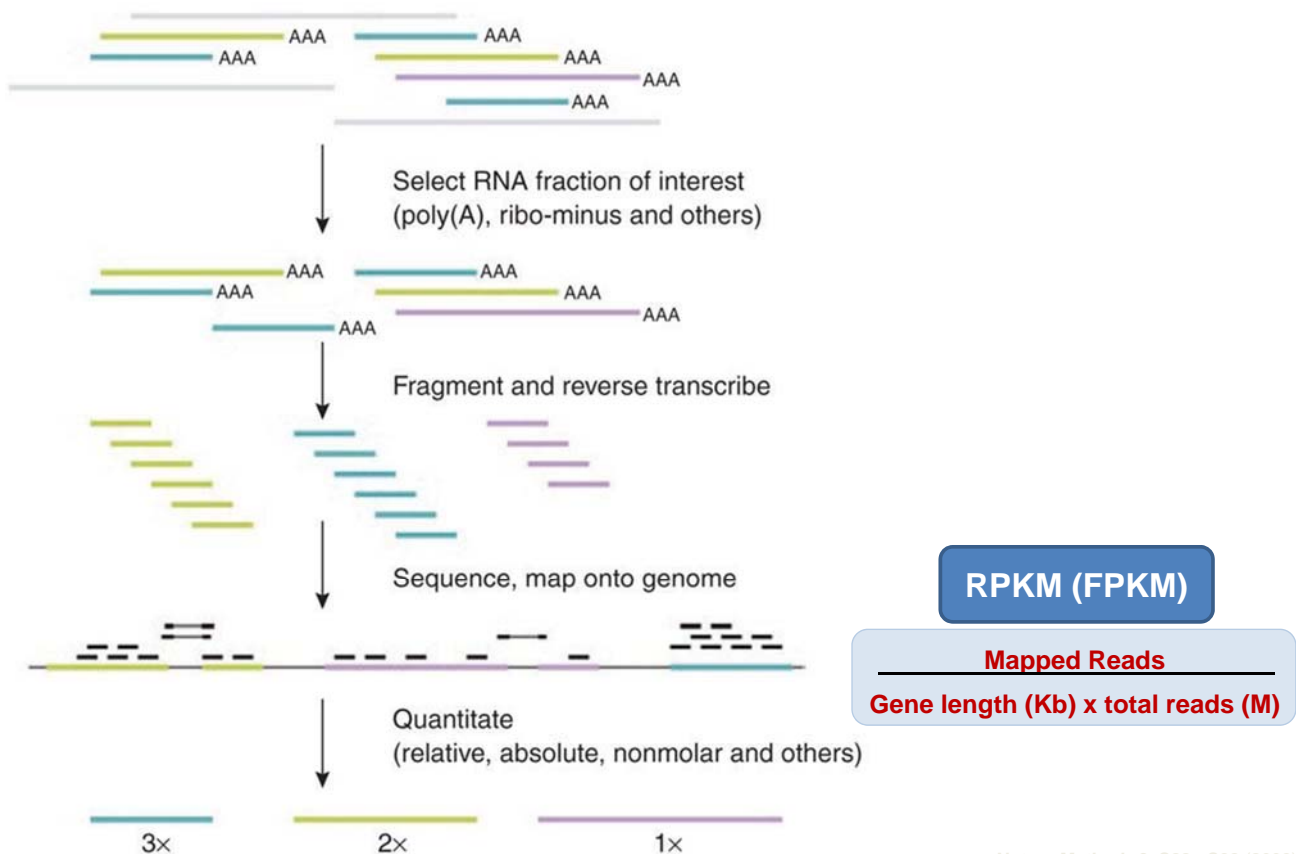
- *mRNA vs stranded*
- *smRNA, non-coding RNA*

RNA-seq: considerations



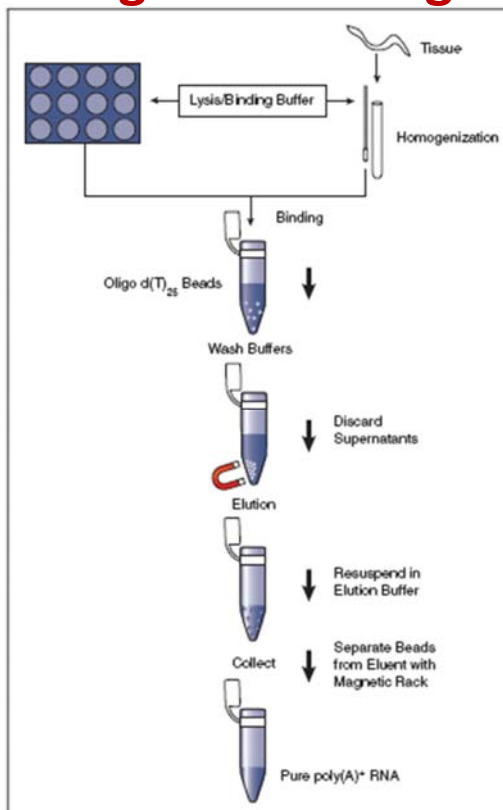
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Transcriptome profiling: RNA-seq

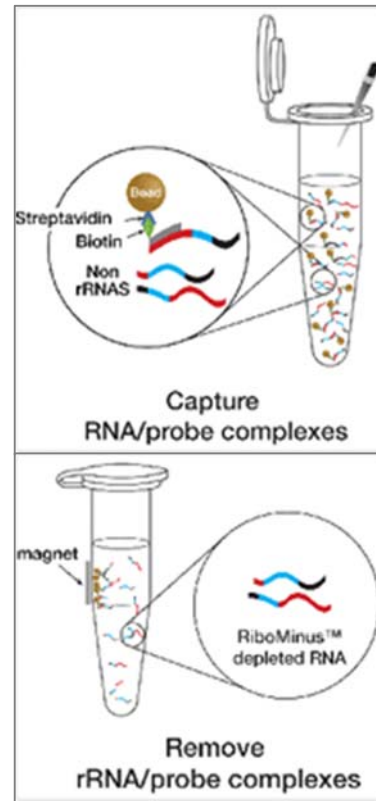


mRNA enrichment

Oligo-dT binding



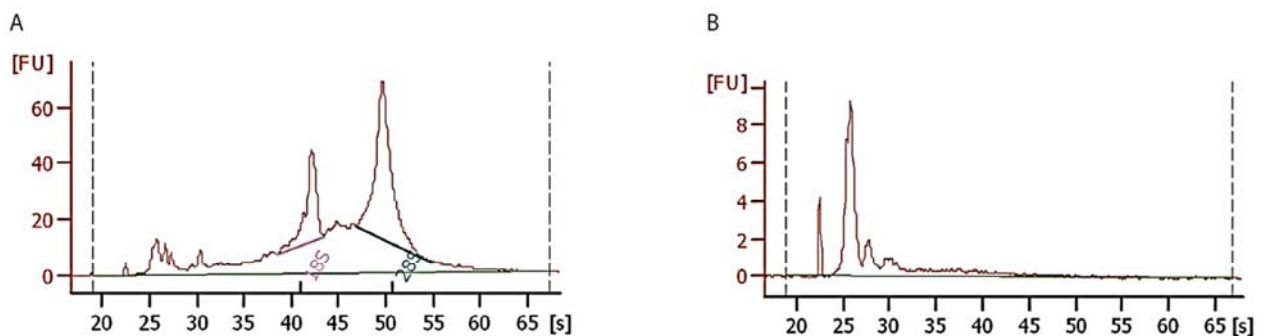
rRNA removal



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rRNA Removal from Eukaryotic RNA

Figure 2. Removal of rRNAs from total RNA.

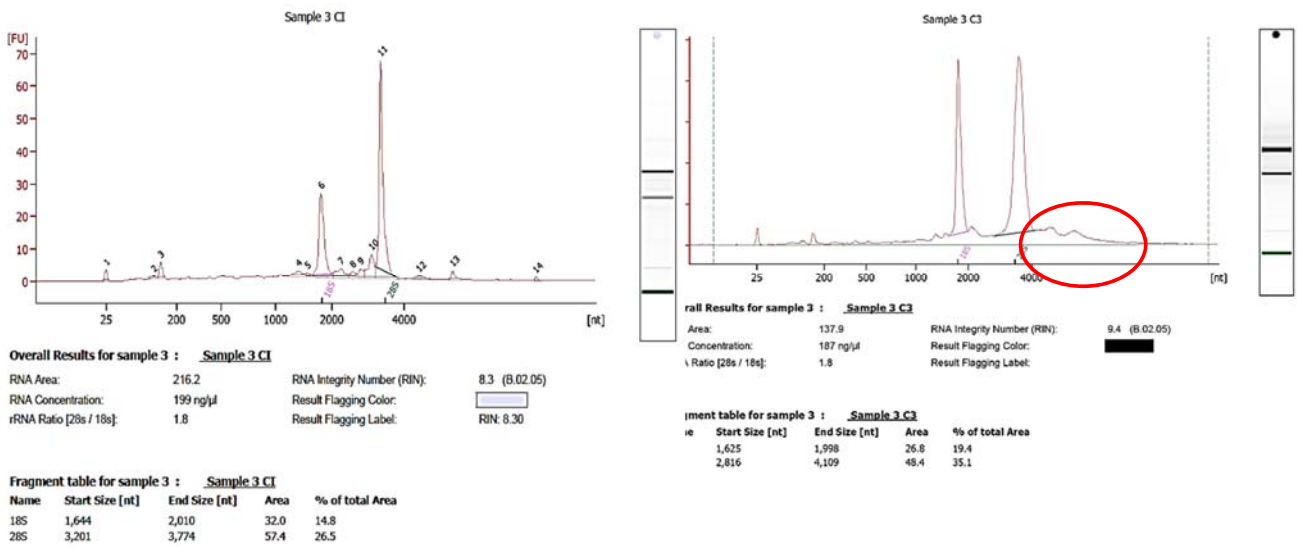


Removal of ribosomal RNAs from total RNA. Total RNA from MCF-7 cells was examined using a nanochip on a Bioanalyzer before (A) and after (B) treatment with the Ribo-Zero Gold Kit.

rRNA depletion works for degraded RNAs, but require deeper sequencing.

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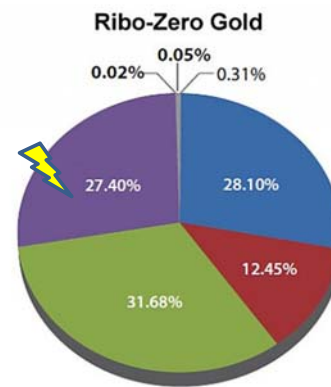
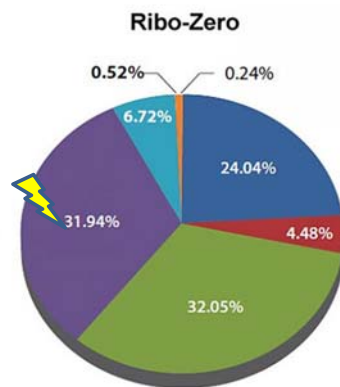
DNA contamination in RNA sample



Have seen contamination up to 30%!!

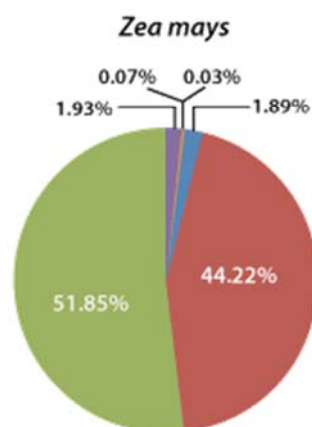
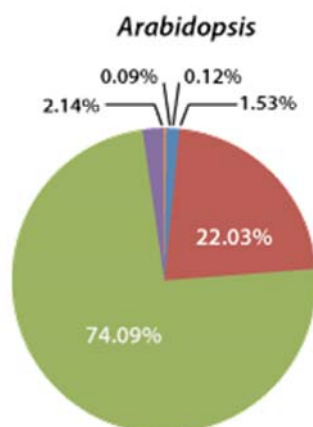
rRNA removal by RiboZero depletion

Oligo version



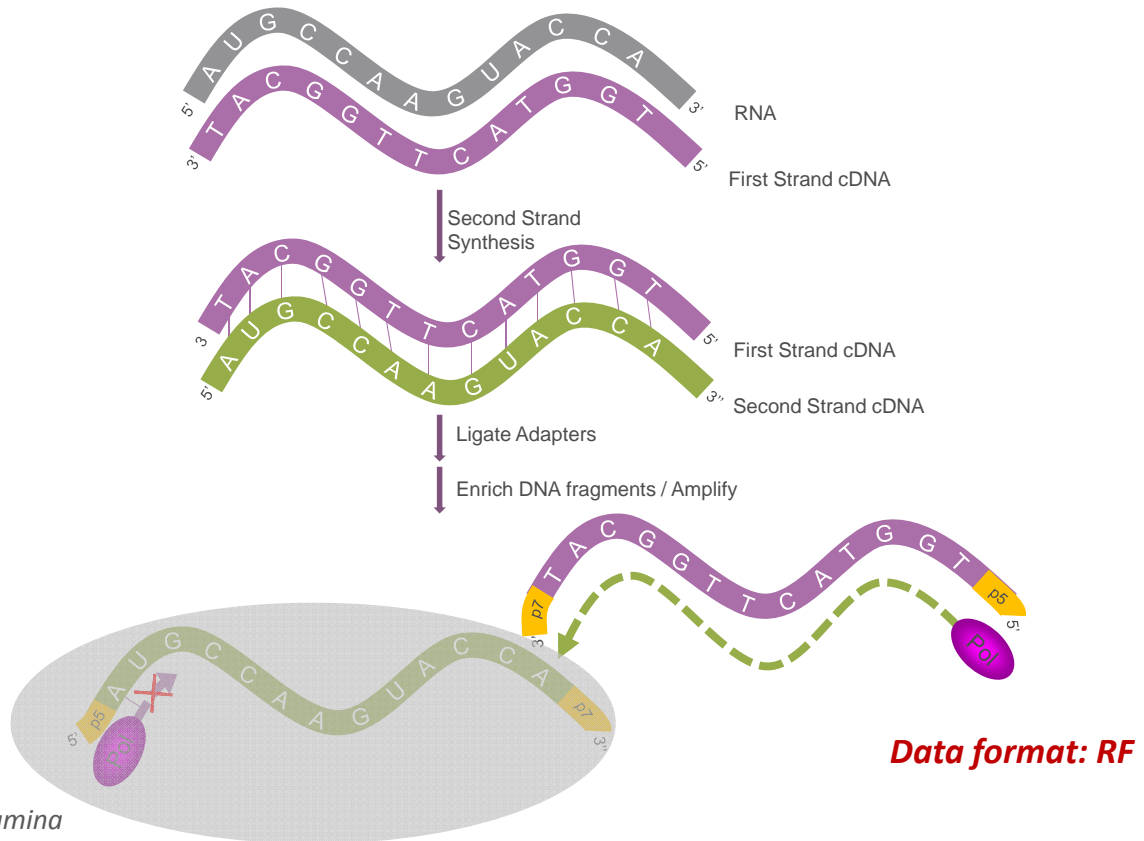
- Aligning exomic
- Partial exomic
- Intronic
- Genomic
- 16S mtrRNA
- 12S mtrRNA
- 28S mtrRNA

Species divergence

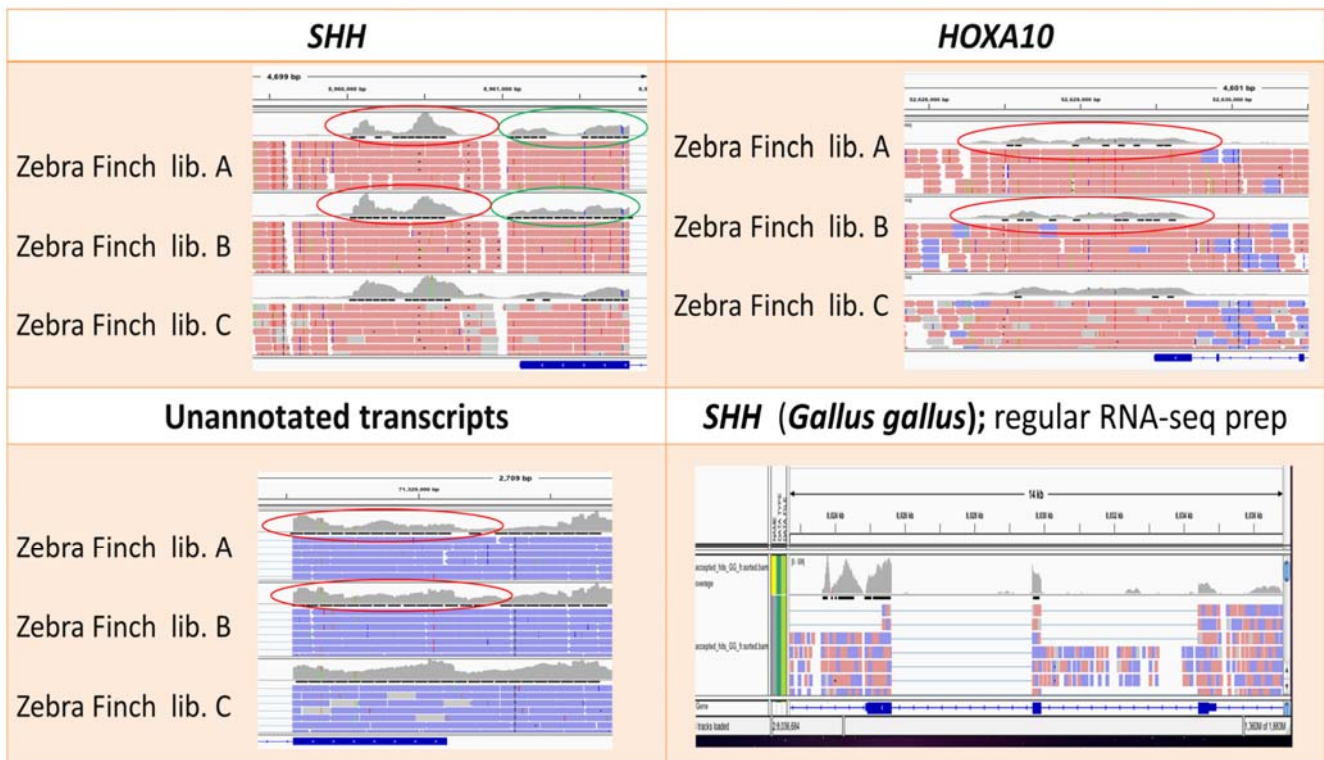


- Intronic
- Intergenic
- mRNA
- Cytoplasmic rRNA
- Chloroplast rRNA
- Mitochondrial rRNA

Strand-specific RNA-seq prep



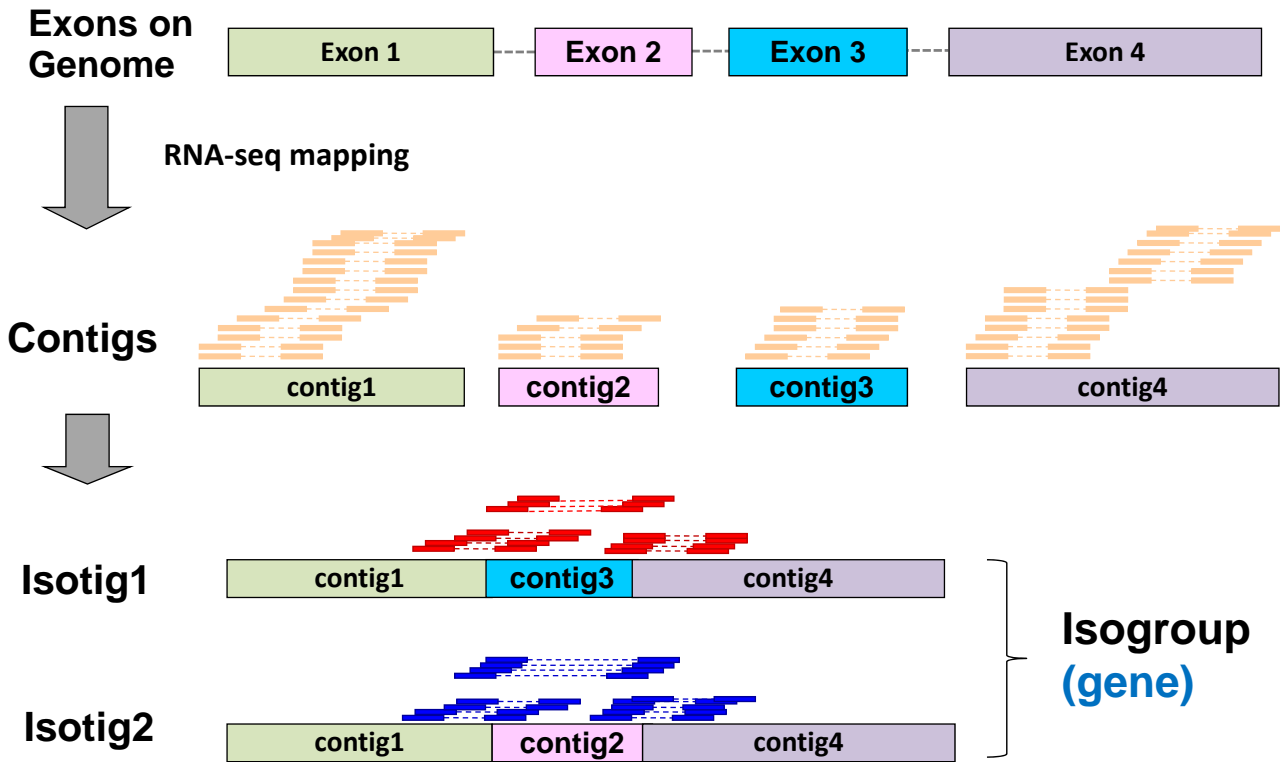
Stranded RNA-seq: >90% strand-specificity



*Genome viewer: IGV genome viewer (version 2.2), plus strand(Blue), minus strand(Red), no paired end info (could be plus or minus strand, Gr non-stranded protocol the amount of blue alignments are almost equal to red alignment

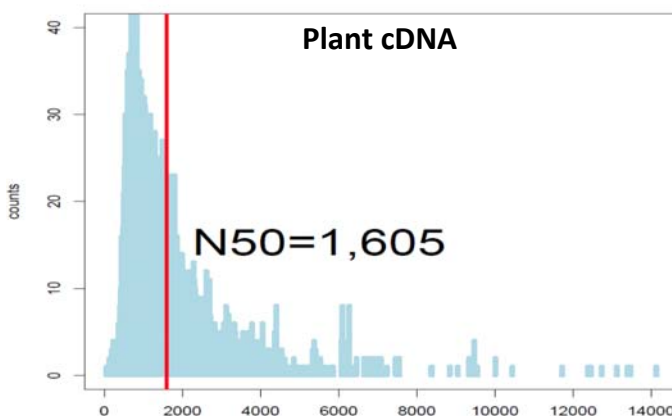
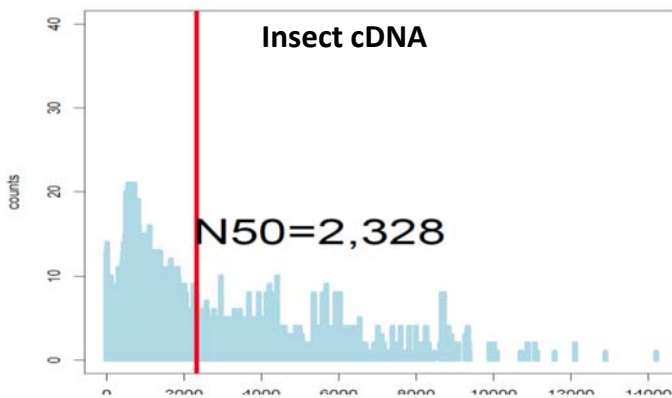
Courtesy of Chih-kuan Chen

Transcriptome assembly



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454 Isotig histogram and assembly stats



Newbler 3.0 assembly	Insect cDNA	Plant cDNA
total # of Reads	994,094	1,704,310
total # of Bases	428,812,259	726,697,350

isotig Metrics (Transcript)

# Isotigs	16,983	31,349
# Bases	26,766,462	43,294,186
Avg IsotigSize	1,576	1,381
N50 IsotigSize	2,328	1,605
Largest IsotigSize	14,210	14,135

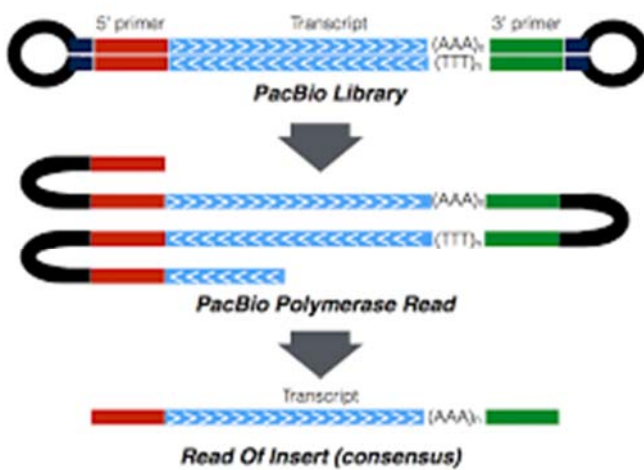
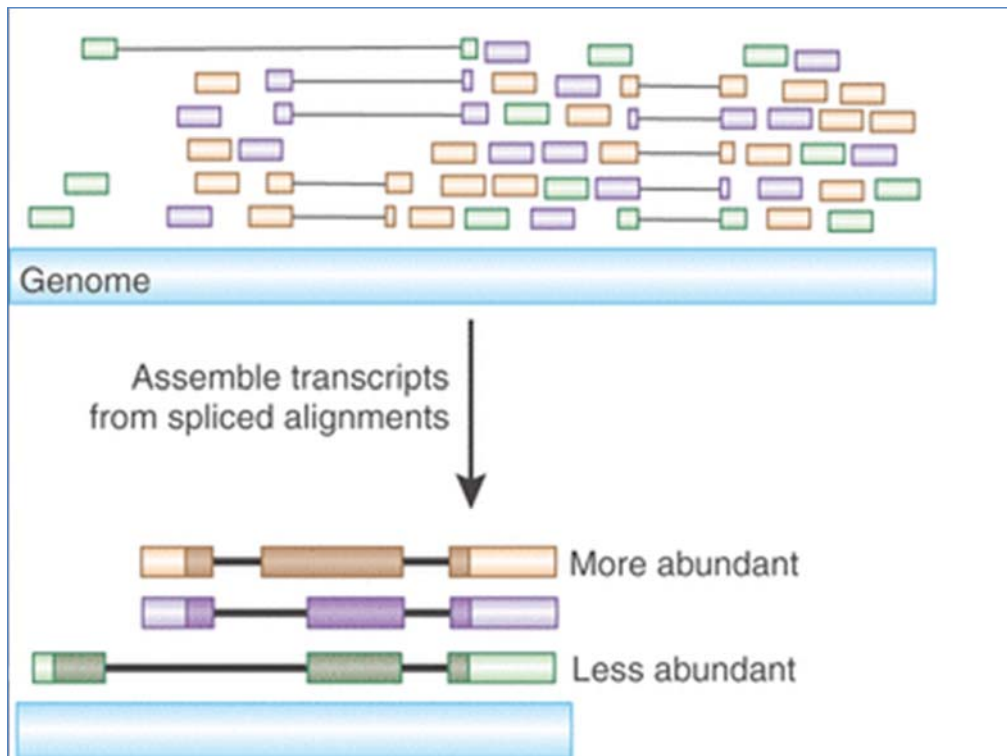
isogroup Metrics (Gene)

# Isogroups	12,843	20,939
avgContigCnt	1.6	1.9
largestContigCnt	250	464
avgIsotigCnt	1.3	1.5
largestIsotigCnt	72	64
# with OneIsotig	10,903	14,657

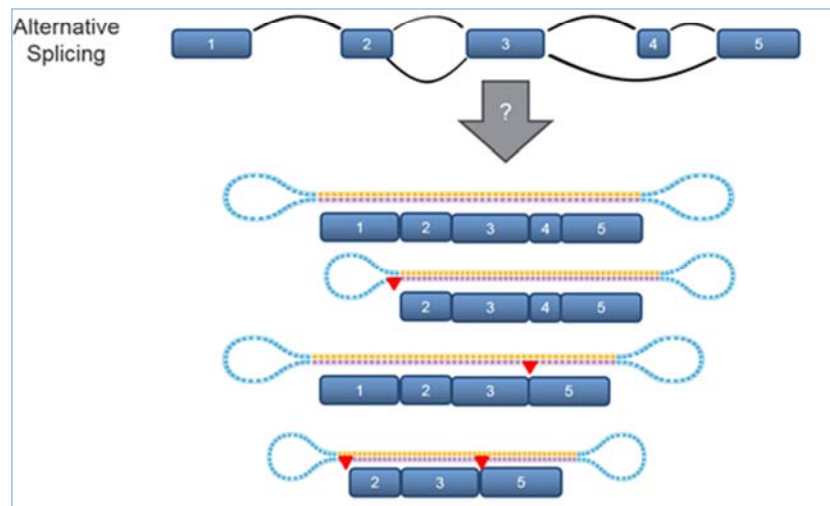
By: Jin Tang, Yuchuan Teng

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TopHat and Cufflinks



PacBio: Iso-Seq

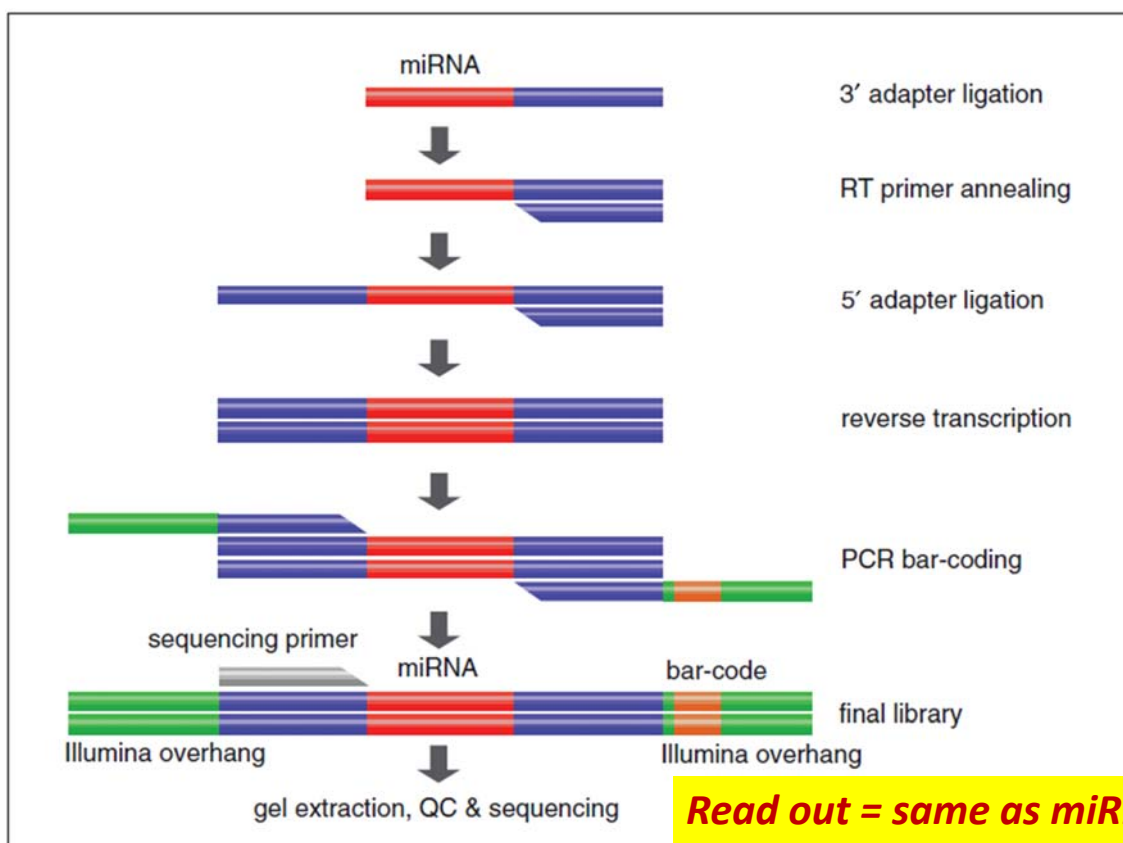


Transcriptome Sequencing

- **Sample:**
 - mRNA to pair with miRNA or lincRNA study?
- **mRNA enrichment method**
 - Oligo-dT vs Ribo-depletion
- **Controls and Biological replicates**
- **Time course?**
- **Re-sequencing (mapping) vs de novo assembly**
- **NGS:**
 - Shotgun: SR or PE
 - PE2*100~200bp
 - Coverage depends on need for detection sensitivity
- **Splicing variants? Fusion junction?**
 - Gel-size selection
 - PE2*150~300

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smRNA library prep - Directional



Summary of NGS Project Design

1. **Application type:**
 - NGS platform, sequencing format
 - data scale/coverage depth
 - Sequencing bias?
2. **Sample nature:**
 - Ploidity, plastids, pure/meta sample
 - Genome size, GC%, repeats
3. **Experimental Design:**
 - Biological replicate (n=3?)
 - Control set?
4. **NGS bioinformatic algorithms**
 - For long or short reads? SR vs PE?
 - Computational demand

III. NGS Core & Prep works

- Sample requirement
- Considerations
- Submission to NGS Core



Documentation

Home / Documentation

<http://ngs.biodiv.tw/NGSCore/documentation/>

Forms

Sequencing Application Form for Illumina	Download
Sequencing Application Form for PacBio Sequel	Download
Sequencing Application Form for Roche 454	Download
Sample Submission Form	Download

Guides

NGS Sample Preparation Guide	Download
Guidelines for Chloroform Purification (PacBio)	Download
16S Metagenomic Library Prep Guide (Illumina)	Download
Info for mRNA-Seq and smRNA-Seq	Download
Chip-Seq Q&A	Download

Core News

- ⊙ Launching of the 3rd-Gen sequencing service of PacBio Sequel system
- ⊙ Promotional Discount on HiSeq
- ⊙ 本核心實驗室之儀器使用效益獲得肯定
- ⊙ Web server (Pydio) has been disabled

Lecture & Seminar

- ⊙ 2016-03-24

Related Web Links

Application Type

(S-B)	TruSeq Methylation DNA (Bisulfite Seq)
(S-Ca)	TruSeq Paired-End DNA, Gel free
(S-Cb)	TruSeq Paired-End DNA, Gel plus
(S-D)	TruSeq Long Insert Paired-End DNA (1-2Kb)
(S-E)	TruSeq Small RNA (miRNA)
(S-G)	Degradome
(S-I)	TruSeq CHIP DNA (ChIP Seq)
(S-Ma)	Nextera Mate-Pair DNA, Gel free (no size selection)
(S-Mb)	Nextera Mate-Pair DNA, Gel plus (4 sizes, 1-15Kb)
(S-Mc)	Nextera Mate-Pair DNA, Gel plus (6 sizes, 1-40Kb)
(S-N)	TruSeq Synthetic Long-Read DNA
(S-P)	Indexing PCR (2nd Step PCR)
(S-Q)	Nextera DNA
(S-R)	Nextera XT DNA
(S-S)	TruSeq Stranded RNA, Poly-A
(S-T)	TruSeq Stranded RNA, Ribo-zero
(S-Wa)	Low-Input Stranded RNA, Poly-A (Clontech)
(S-Wb)	Low-Input Stranded RNA, Ribo-zero (Clontech)
(S-Wc)	Low-Input Stranded RNA, aRNA (Epicentre)
(S-Xa)	Human Exome Capture (Agilent)
(S-Xb)	Human Exome Capture (SeqCap)

Illumina HiSeq 2500 Sequencing Services

HiSeq High-Throughput Mode (8 lanes):

	Mode Type (200-250M clusters per lane)
(HTSR100)	HiSeq HT v4, Single Read 100
(HTPE125)	HiSeq HT v4, Paired End 2*125

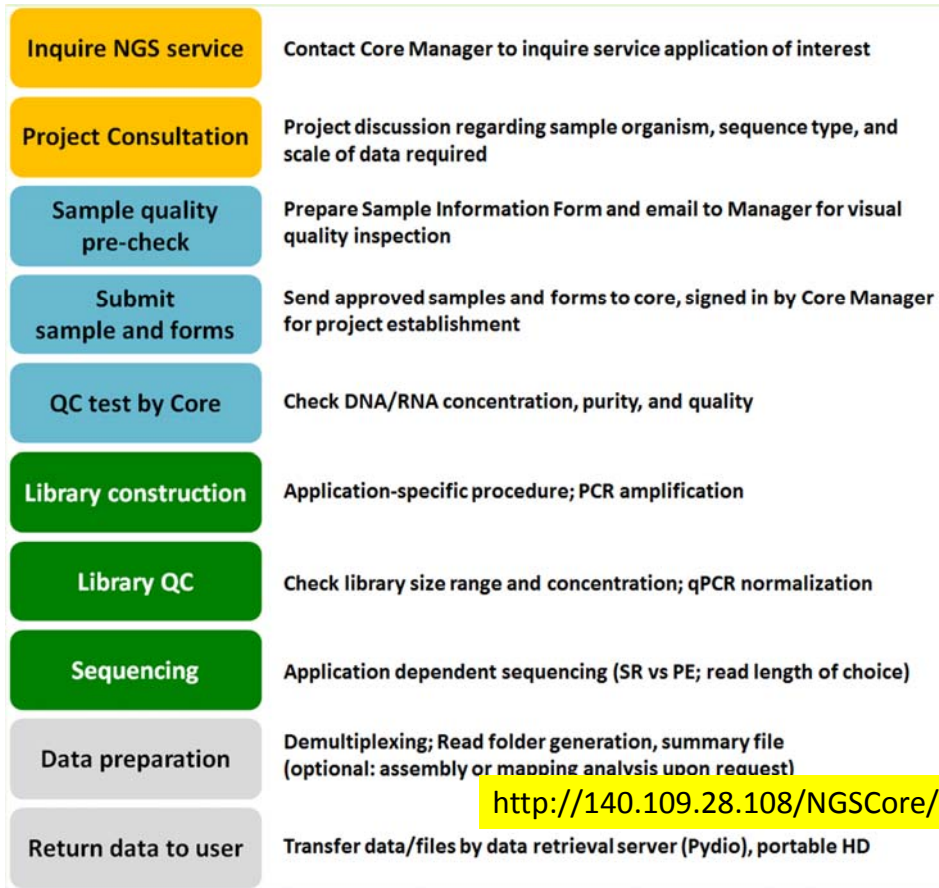
HiSeq Rapid Mode (2 lanes per chip)

	Mode Type (150-180M clusters per lane)
(RpSR50)	HiSeq Rapid v2, Single Read 50
(RpSR100)	HiSeq Rapid v2, Single Read 100
(RpPE100)	HiSeq Rapid v2, Paired End 2*100
(RpPE150)	HiSeq Rapid v2, Paired End 2*150
(RpPE200)	HiSeq Rapid v2, Paired End 2*200
(RpPE250)	HiSeq Rapid v2, Paired End 2*250

Illumina MiSeq Sequencing Services

	Mode Type (10-15M clusters in v2; 20-25M clusters in v3)
(MS50)	MiSeq 50 cycles v2, PE2*25 or SR50
(MS150)	MiSeq 150 cycles v3, PE2*75 or SR150
(MS300)	MiSeq 300 cycles v2, PE2*150 or SR300
(MS500)	MiSeq 500 cycles v2, PE2*250
(MS600)	MiSeq 600 cycles v3, PE2*300

NGS Service Workflow



http://140.109.28.108/NGSCore/?page_id=40

中央研究院 新世代基因體定序核心實驗室
Illumina Sequencing Application Form 定序服務申請表

Dataset ID <small>(user only)</small>	Case ID <small>(user only)</small>
--	---------------------------------------

Please Read NGS Sequencing Service Policy Carefully Before Submitting Your Requisition
請詳細定序服務條款 (見第二頁)

User Information					
Principal Investigator	Name (中):		Name (En):		
	Institute:		Phone:	E-mail:	
Applicant <small>(contact person)</small>	Name (中):		Name (En):		
	Institute:		Phone:	E-mail:	
Project Name <small>(brief description)</small>					Funding Sources
Library Construction Services					Note
# of Sample Submitted	Quality & Quantity Assessment				
	<input type="checkbox"/> Qubit DNA <input type="checkbox"/> BioAHS DNA <input type="checkbox"/> Qubit RNA <input type="checkbox"/> BioRNA Nano <input type="checkbox"/> qPCR (for Ready-to-seq Library) <input type="checkbox"/> BioRNA Pico				
# of Library Construction	Application Type (see table below)				
	<input type="checkbox"/> Gel-free <input type="checkbox"/> Gel-plus <input type="checkbox"/> Size Selection (at extra cost)				
Sequencing Requisition					
Sequencing Mode (select ONE only)			Format	Read Length	Multiplexing
HiSeq HT	HiSeq Rapid	MiSeq	SR / PE		# of Lib per Lane
					Total Lane(s)
Note (user only)					
Signature of Core Manager Date (YYYY/MM/DD)			Signature of PI Date (YYYY/MM/DD)		
請款日期 <small>(user only)</small>			收款日期 <small>(user only)</small>		
Application Type	Sample Requirement	Application Type	Sample Requirement		
(S-B) Methylation DNA	1-2 ug in 25 ul	(S-P) Indexing PCR (2 nd Step PCR)	>300 ng in 20 ul		
(S-C) Paired-end DNA	2-3 ug in 50 ul	(S-Q) Nextera DNA	75-150 ng in 25 ul		
(S-D) Long Insert Paired-end DNA	2-3 ug in 50 ul	(S-R) Nextera XT DNA	2-5 ug in 10 ul		
(S-E) Small RNA (miRNA)	8-10 ug in 10 ul	(S-S) Stranded mRNA (Poly-A)	4-6 ug in 50 ul		
(S-G) Degradome	100-150 ug in 100 ul	(S-T) Stranded mRNA (Ribo-Zero)	4-6 ug in 50 ul		
(S-I) CHIP DNA	5-10 ng in 25 ul	(S-W) Low-input Stranded mRNA	5-100 ng in 25 ul		
(S-L) Ready-to-seq Library	>10 nM in 20 ul	(S-X) Human Exome Capture	2-3 ug in 50 ul		
(S-M) Mate-Pair DNA	10-20 ug in 100 ul	(S-Z) Customized Prep			
(S-N) Synthetic Long-Read DNA	1-2 ug in 50 ul	(QC) QC-only			

中央研究院 新世代基因體定序核心實驗室
NGS Sequencing Service Policy 定序服務條款

A. Sample Requirement

- Please check on NGS Sample Preparation Guide ([download](#)) to ensure that your samples meet our requirement and be well-packaged. NGS core facility reserves the right to determine whether the unqualified sample can proceed to the library preparation.
- Additional fees would be charged if there are any needs to re-prepare library or do extra experiments requested by the user due to sample issues. The final service charge will be re-calculated depending on real experiment cost.

B. Sample Submission

- As a part of our standard service procedure (see [Service Flow](#)), please email to Dr. Mei-Yeh Lu the NGS Application Form (Illumina or PacBio) and the Sample Submission Form prior to the actual submission for visual quality inspection. Please kindly include PI in all communicating emails, allowing PI to be updated timely on the project status and information.
- All sample drop-offs should accompany appropriate forms signed by PI for project approval, and please contact Dr. Lu to make an appointment in advanced before you bring your samples to the NGS core facility. For contact information, please visit our website [Contact & Location](#).

C. Acknowledgement & Authorship

- We appreciate your support if you mention NGS core facility in the acknowledgement section when the data using our sequencing services is published. You may add a sentence like this:

We thank the High Throughput Sequencing Core hosted in the Biodiversity Research Center at Academia Sinica for performing the NGS experiments.

- If there are substantial contributions from NGS core facility in your research, including intellectual input, data analysis and interpretation, or special experiment design, it might be appropriate to involve core members in the list of authorship.
- Please kindly share your publications on [here](#) in case we miss it. Thank you.

中央研究院 新世代基因體定序核心實驗室
NGS Sample Submission Form 樣品明細表

Submission Date		Case ID (use only)																			
Application Type		1. Please refer to NGS Sample Preparation Guide for the labeling and packing, and ensure the quantity and quality of your samples fulfill all the requirements. 2. Please attach Gel Image (required) or BioAnalyzer Traces (optional) at the end of the NGS Sample Submission Form. 3. Sample Name must be the same as the labeling on the tube cap.																			
Organism or Species		Sample Type: <input type="checkbox"/> genomic DNA <input type="checkbox"/> amplicon <input type="checkbox"/> total RNA <input type="checkbox"/> ChIP DNA <input type="checkbox"/> cDNA <input type="checkbox"/> Ready-to-seq Library <input type="checkbox"/> plasmid DNA <input type="checkbox"/> mRNA <input type="checkbox"/>																			
Genome Size or DNA Length		Enzyme Treatment & Usage: Dissolved in: <input type="checkbox"/> DNase <input type="checkbox"/> RNase <input type="checkbox"/> RNase Inhibitor <input type="checkbox"/> H ₂ O <input type="checkbox"/> EB <input type="checkbox"/>																			
Purification Method																					
Sample Name (tube labeling)	optional Qubit (ng/ul)	Nano Drop (ng/ul)	Vol. (ul)	Amt. (ug)	OD 260/280	OD 260/230	optional rRNA Ratio	optional RIN	Notes	Sample ID (use only)											
1																					
2																					
3																					
4																					
5																					
6																					
7																					
8																					
9																					
10																					
11																					
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中研院生物多樣性中心 新世代基因體定序核心實驗室 NGS Core Lab 陽明城大樓 A603 負責人 呂國雄 博士 (02)2787-1199 or 1198 ext. 9
Update: 2017/4/25

中央研究院 新世代基因體定序核心實驗室
Supplemental Information of NGS Samples

Gel Image (required)

1. Post-run staining only.
2. Please indicate sample no. & major marker sizes (ladder should cover at least 0.1-10kb).

a. Gel percentage: ___% of TAE or TBE agarose gel
b. Run condition: ___voltage for ___min
c. Loading amount: ___ng of sample per lane; ___ng of ladder per lane

中央研究院 新世代基因體定序核心實驗室
Supplemental Information of NGS Samples

BioAnalyzer Electropherogram (optional)

1. Please arrange images of BioA traces according to the sample number on the sample submission form.
2. BioA DNA High Sensitive 1000 7500 12000; or BioA RNA 6000 Nano Pico

Sample # 1	Sample # 2
Sample # 3	Sample # 4

高通量定序核心實驗室樣品送件流程

1. 填妥樣品送件單，E-mail至NGS Core負責人信箱，樣品確認沒問題即可送件
2. 參考NGS Core樣品送件須知，將樣品備妥
3. 預約定序樣品送件時間，即可進行送件
4. QC通過後將開始進行收費
5. 定序完成後寄送通知信件，即可上網下載定序資料

Sample Submission

1. Prior project communication; email files for preview
2. Submission by appointment:
 - a. Samples: Provide samples in low-bind tubes
 - b. transferred on ice/dry ice/liquid N₂
 - c. Submit along with NGS application form & Sample form
 - d. Attach gel images and/or BioAnalyzer traces for quality check
 - e. Make appointment in advance

General Sample Requirements

DNA:

- RNase-treated and purified
- Submission amount > 3X of library input
- High purity (NanoDrop ratios, BioA, gel)
- Long integrity (>23~48kb)
- Low in inhibitors and contaminants (eg. EDTA, CTAB)
- Concentration: 200-800 ng/ul

RNA:

- DNase-treated and purified
- Submission amount >2X of library input
- High purity (NanoDrop ratios, BioA, gel)
- High in rRNA ratio, RIN
- Low in inhibitors and contaminants
- Concentration:
 - mRNA 200-1000 ng/ul
 - smRNA: 500-2000 ng/ul

Auxiliary equipments



**Qubit
Fluorometer**



**BioAnalyzer
(up to 11 samples)**



**Fragment Analyzer
(up to 96well plates*3)**

**Covaris
(DNA shearing)
0.2~10kb**

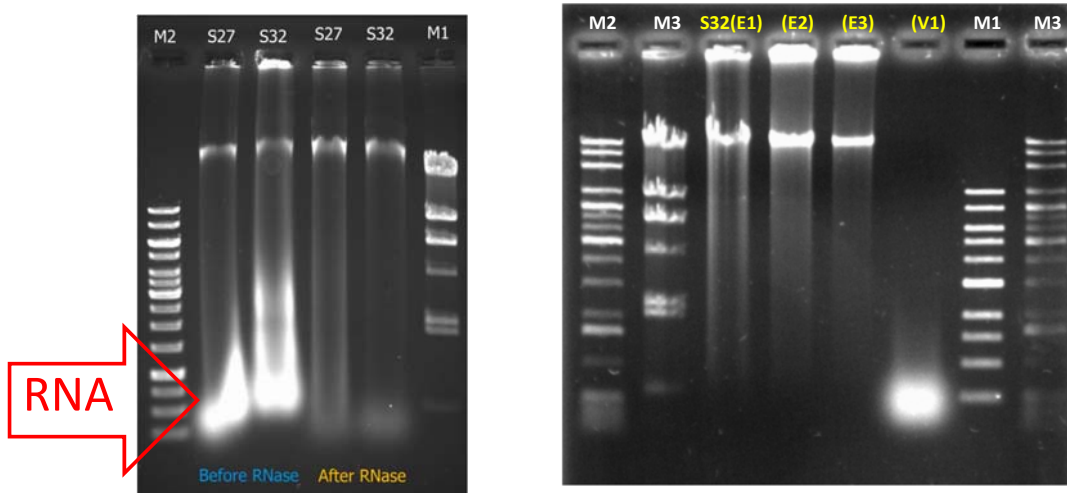


**BluePippin
(gel size selection)**



LC480 qPCR

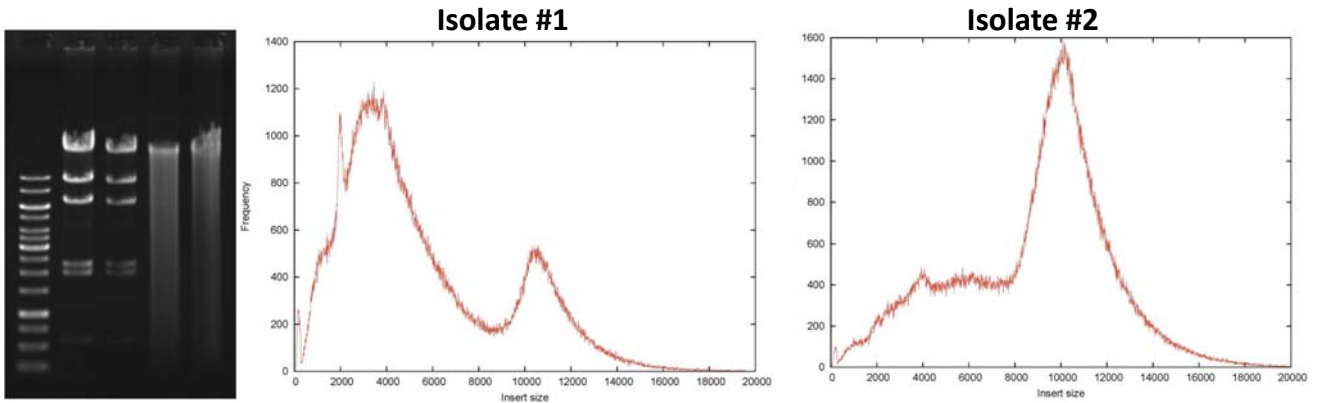
Genomic DNA QC



	OD 260/280	OD 260/230	NanoDrop (ng/uL)	Qubit DNA (ng/uL)	RNA Carry over (NanoDrop/Qubit)
S32-original	2.04	2.05	2250.8	56.0	40.19 X
S32_V1	2.16	2.52	1207.40	7.29	165.62 X
S32_E1	1.77	0.94	394.50	131.00	3.01 X
S32_E2	1.67	0.82	45.06	14.10	3.20 X
S32_E3	1.75	0.75	11.48	4.49	2.56 X

Fungal genome MP – 1~20kb

Assembly version	Assembly size (bp)	num. scaffolds	average (kb)	largest scaff (kb)	N50 (kb)	N50 (n)	N90 (kb)	N90 (n)	valid nuc	Ns
Hong Kong version	31,259,288	1,434	22	407	79	122	12	484	31,259,288	0
NTU version	~30Mb	NA	NA	NA	~50kb	NA	NA	NA	NA	NA
BRC v1 (PE+3kb ; sequenced in Japan)	33,490,075	2,555	13	2,449	421	18	48	94	33,052,597	437,478
BRC v2 isolate 1 (PE + 5 MP libraries up to 20kb)	31,254,640	97	322	3,610	2,385	6	650	16	30,893,103	361,537
BRC v2 isolate 2 (PE + 5 MP libraries up to 20kb)	31,591,454	107	295	5,891	3,404	4	1,061	10	31,091,726	499,728



RNA QC

BioAnalyzer RNA ladder

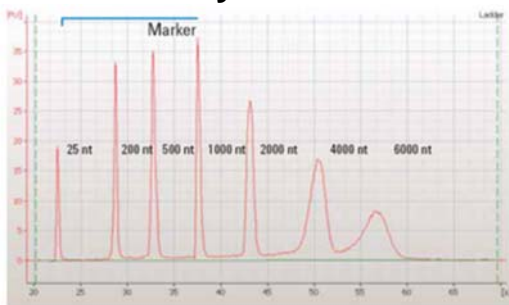
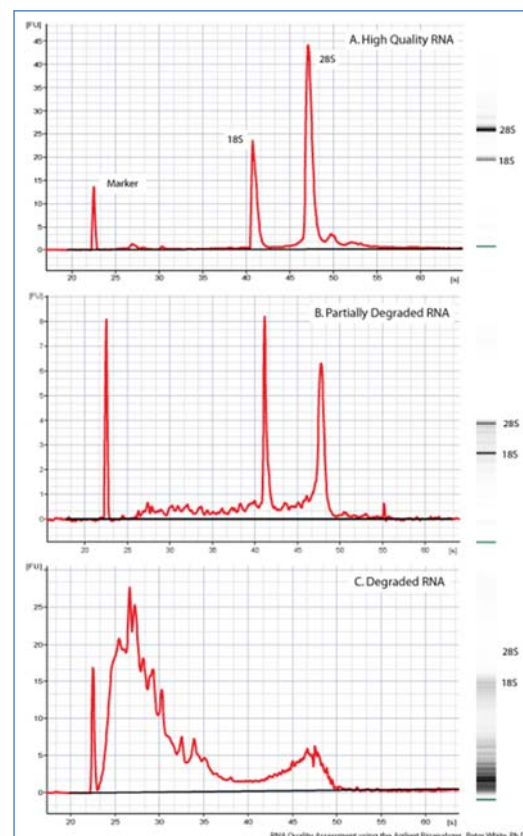
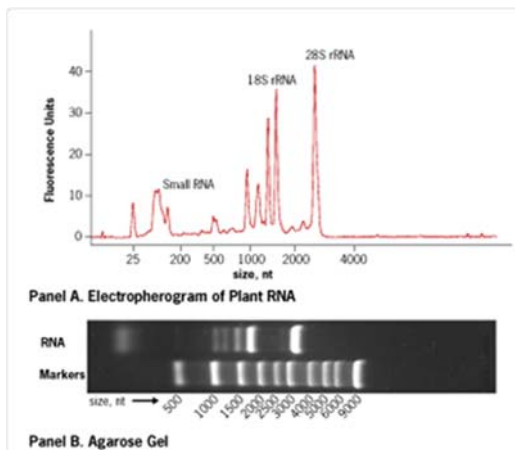


Figure 1 RNA 6000 Nano ladder

Human RNA – various degradation

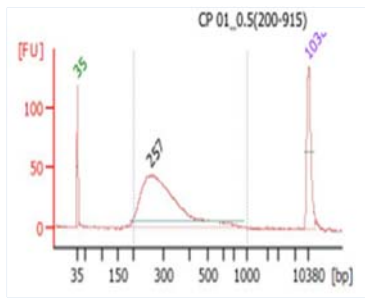


Plant total RNA

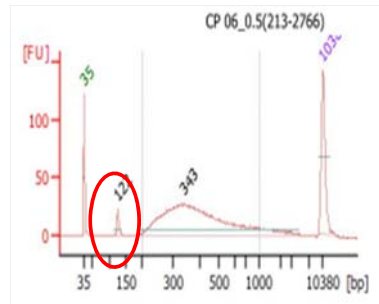


Low Input Sample – biased sequencing

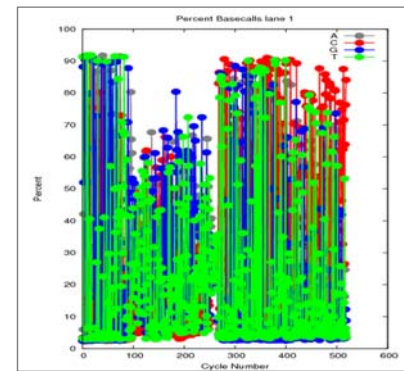
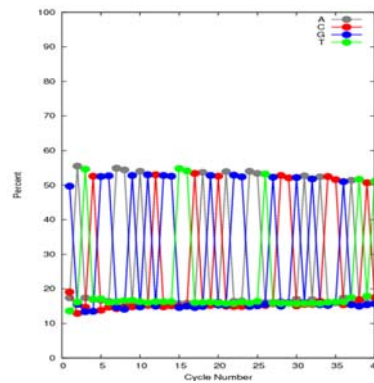
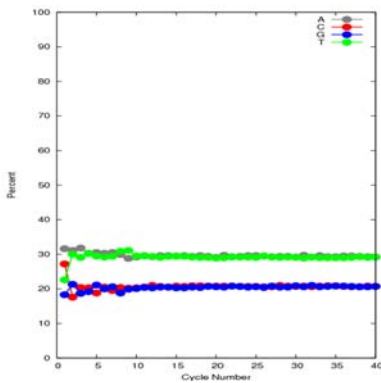
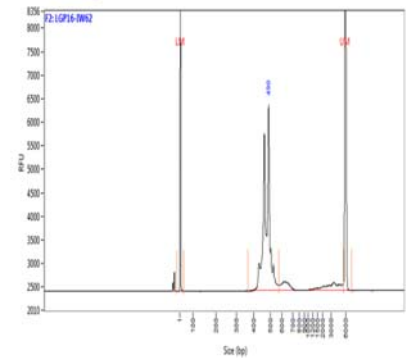
Normal input- Little bias



Low input - adaptor bias

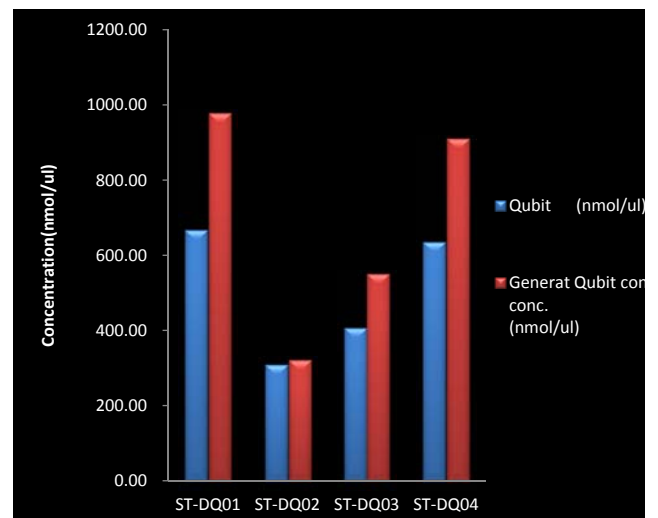
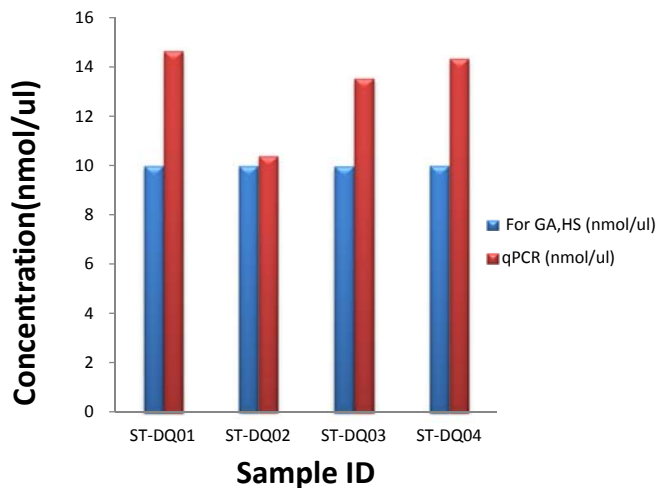


Amcplion – extreme bias



Library molar normalization – NGS qPCR

Sample	bp	Qubit (ng/ul)	Qubit (nmol/ul)	For GA,HS (nmol/ul)	qPCR (nmol/ul)	Ratio of qPCR/Qubit	Generat Qubit conc.to qPCR conc. (nmol/ul)
ST-DQ01	321	141.5	667.90	10	14.66	1.47	979.14
ST-DQ02	359	73	308.10	10	10.40	1.04	320.42
ST-DQ03	376	101	407.00	10	13.55	1.36	551.49
ST-DQ04	361	151.5	635.90	10	14.33	1.43	911.24



IV. NGS Data & Resources

- Data types and QC
- Public resources

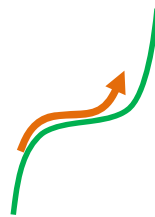
79

Types and Characteristics of NGS Reads

- Read length:

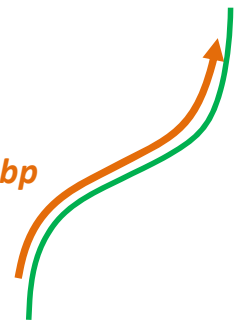
Short

50-300bp



Long

500-15,000bp



- Read types:

SR



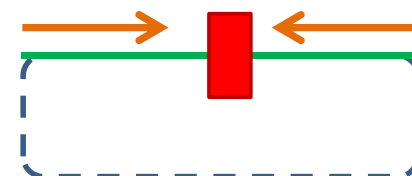
50bp-20kb

PE



50-300 bp;
1~1.5 kb jump

MP

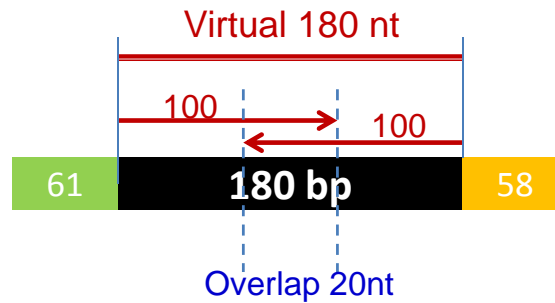


50-300bp;
2~15kb jump

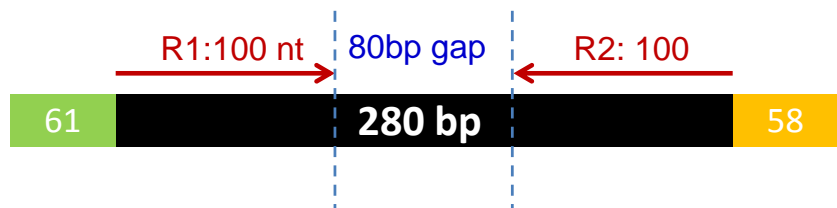
80

Insert size vs Library Fragment Size

300-bp fragment:
Ends overlapped 20 bp



400-bp fragment:
Ends gapped by 80 bp



Illumina Read – fastQ file

```

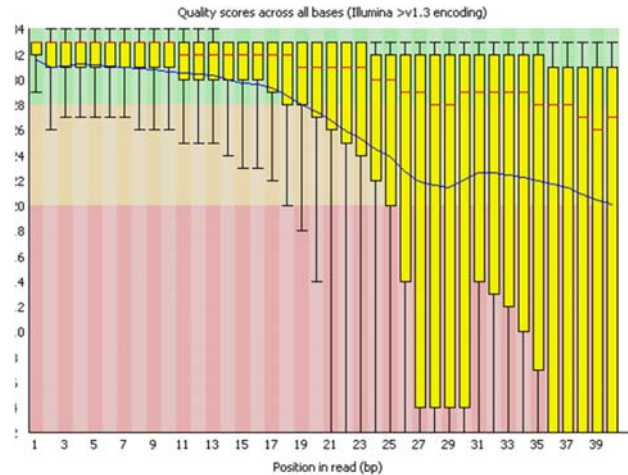
Sequence header Machine ID, FC ID Lane ID Index sequence
no control
Y/N: failing PF or not
Read1 or Read2
@HWI-D00368:32:H8R31ADXX:2:1101:2034:2140 1:N:0:CAGATC
TTTGNCGAGAACTGGAATTGAACCAATATTTAAGTCTTACAAGGAATTCGTTTTAAC
+
@@@F#2ADFDHHHJJJJGHHIIJIIJJJIJGGJHEIIJIIJIIJIIJJJIJJJIGI
Q-score header
    
```

Seq. performance assessment – Base Q

Phred quality scores Q: logarithmically related to error probabilities

$$P \text{ by } Q = [- 10 * \log_{10}(P)]$$

Phred Score Q	Error probability	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%



FastQC Report

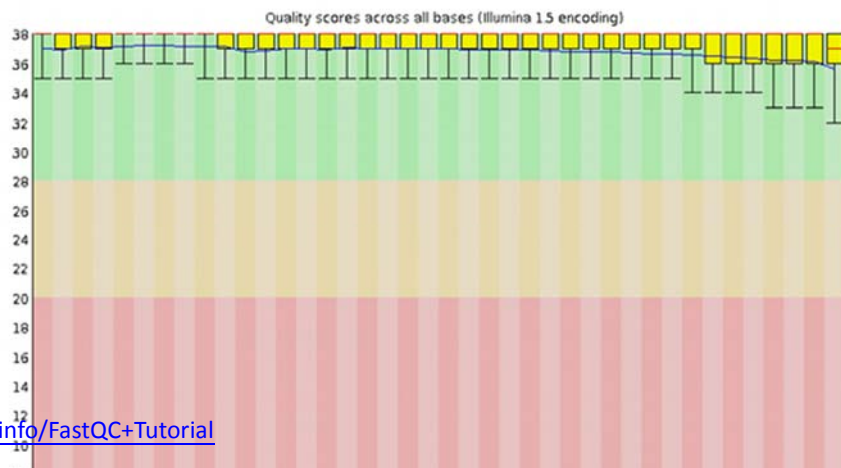
Summary

- ✓ Basic Statistics
- ✓ Per base sequence quality
- ✓ Per sequence quality scores
- ⚠ Per base sequence content
- ✓ Per base GC content
- ✓ Per sequence GC content
- ✓ Per base N content
- ✓ Sequence Length Distribution
- ✓ Sequence Duplication Levels
- ✓ Overrepresented sequences
- ⚠ Kmer Content

Basic Statistics

Measure	Value
Filename	good_sequence_short.fastq
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	250000
Filtered Sequences	0
Sequence length	40
%GC	45

Per base sequence quality



Data Trimming - Trimmomatic

Overview | Group | Publications | Supporting Info | Teaching | Software | Internal

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News

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Apr 4, 2013
Sequencer
Apr 4, 2013
Mozod
Feb 28, 2012
Fascination of Plant Days May 18
Jan 7, 2012
Perrinelli NSQ >500k
Jan 6, 2012
Sequencers Wild is wki of the month
Dec 24, 2011
Forschungszentrum Jülich
Nov 15, 2011
Sequencers Wild published
Sep 2, 2011
New Location

Contact Info

Bjorn Usadel,
Worringer Weg 1
52002 Aachen
Germany

You are here: [Supporting Info](#) » Trimmomatic

Trimmomatic: A flexible read trimming tool for Illumina NGS data

Citations

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*, *btu170*.

Description

Trimmomatic performs a variety of useful trimming tasks for Illumina paired-end and single ended data. The selection of trimming steps and their associated parameters are supplied on the command line. The current trimming steps are:

- ILLUMINACLIP: Cut adapter and other Illumina-specific sequences from the read.
- SLIDINGWINDOW: Perform a sliding window trimming, cutting once the average quality within the window falls below a threshold.
- LEADING: Cut bases off the start of a read, if below a threshold quality
- TRAILING: Cut bases off the end of a read, if below a threshold quality
- CROP: Cut the read to a specified length
- HEADCROP: Cut the specified number of bases from the start of the read
- MINLEN: Drop the read if it is below a specified length
- TOPHRED33: Convert quality scores to Phred-33
- TOPHRED84: Convert quality scores to Phred-64


It works with FASTQ (using phred + 33 or phred + 64 quality scores, depending on the Illumina pipeline used), either uncompressed or gzipped FASTQ. Use of gzip format is determined based on the .gz extension. For single-ended data, one input and one output file are specified, plus the processing steps. For paired-end data, two input files are specified, and 4 output files, 2 for the 'paired' output where both reads survived the processing, and 2 for corresponding 'unpaired' output where a read survived, but the partner read did not.

<http://www.usadellab.org/cms/?page=trimmomatic>

Data pre-processing: eg. FastX-toolkit

FASTX-Toolkit

FASTQ/A short-reads pre-processing tools



Home | Download & Installation | Galaxy Usage | Command-line Usage | License | Useful Links | Contact

Introduction

The FASTX-Toolkit is a collection of command line tools for Short-Reads FASTA/FASTQ files preprocessing.

Next-Generation sequencing machines usually produce FASTA or FASTQ files, containing multiple short-reads sequences (possibly with quality information).

The main processing of such FASTA/FASTQ files is mapping (aka aligning) the sequences to reference genomes or other databases using specialized programs. Example of such mapping programs are: [Blat](#), [SHRIMP](#), [LastZ](#), [MAQ](#) and many many others.

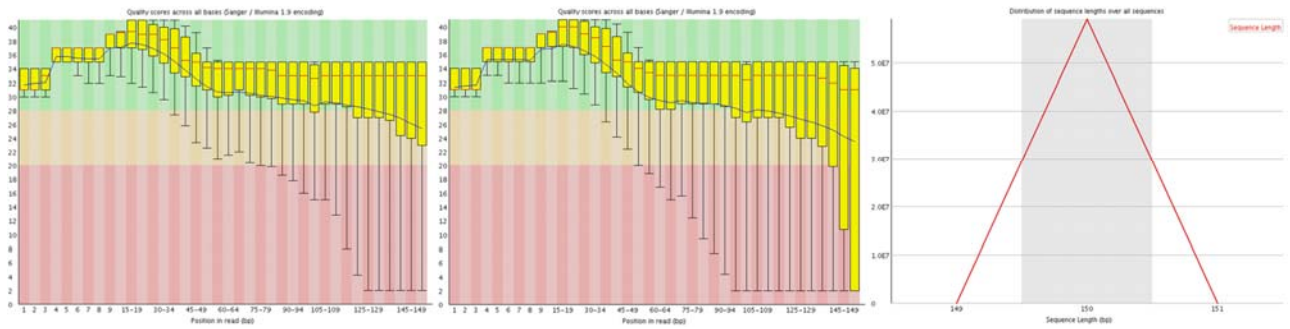
However, it is sometimes more productive to preprocess the FASTA/FASTQ files before mapping the sequences to the genome - manipulating the sequences to produce better mapping results.

The FASTX-Toolkit tools perform some of these preprocessing tasks.

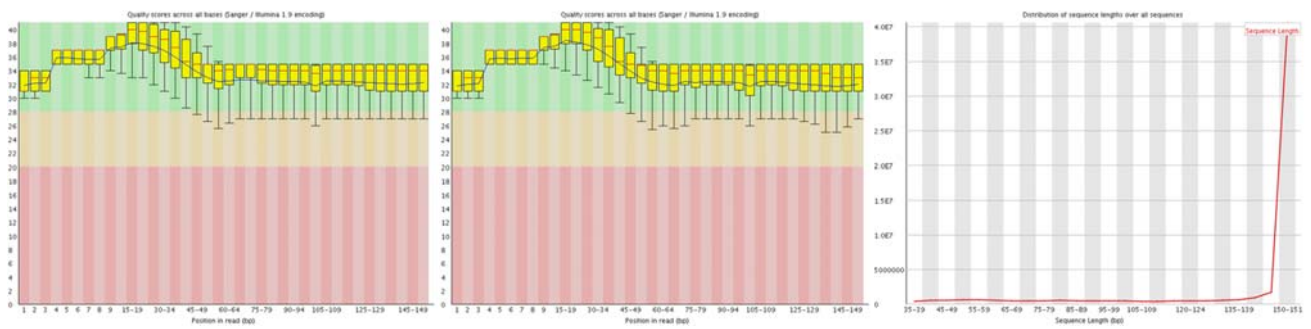
http://hannonlab.csh.edu/fastx_toolkit/

Adapter Trimming Result of HiSeq genomic PE Reads

PE 2*151nt (raw; R1 & R2)



Reads after adapter trimming by Trimmomatic



Public Resources

NGS data analysis *Data intensive biology for everyone.*

Galaxy is an open, web-based platform for data intensive biomedical research. Whether on the free public server or your own instance, you can perform, reproduce, and share complete analyses.

Galaxy is an open source, web-based platform for data intensive biomedical research. If you are new to Galaxy [start here](#) or consult our [help resources](#). You can install your own Galaxy by following the [tutorial](#) and choose from thousands of tools from the Tool Shed.

Try Galaxy on the Cloud

Now you can have a personal Galaxy within the infinite Universe

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The Galaxy Team is a part of the Center for Comparative Genomics and Bioinformatics at Penn State, the Department of Biology and at Johns Hopkins University and the Computational Biology Program at Oregon Health & Science University.

This instance of Galaxy is utilizing infrastructure generously provided by the CyVerse at the Texas Advanced Computing Center, with support from the National Science Foundation.

Tweets by @galaxyproject

Galaxy Project Retweeted
Nicola Bonzanni @nbonzanni
New #galaxy pipeline for analysis and visualization of TCR and BCR sequencing data from @ErasmusMC. bit.ly/2o3XEol

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History

search datasets

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历史信息: You can load your own data or get data from an external source

<https://usegalaxy.org/>

OMICS Tools: High Throughput Sequencing

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WGS analysis 1932 tools	WES analysis 154 tools
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BS-seq analysis 201 tools	Metagenomic sequencing analysis 402 tools
MeRIP-seq analysis 9 tools	16S rRNA-seq analysis 101 tools
CLIP-seq analysis 46 tools	Hi-C analysis 129 tools
Amplicon sequencing analysis 14 tools	GBS analysis 8 tools
Pool-seq analysis 83 tools	RAD-seq analysis
Rep-seq analysis 41 tools	Strand-seq analysis 1 tool

Pool sequencing data analysis bioinformatics software tools

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<http://omictools.com/sga-s1518.html>

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HIGH-THROUGHPUT SEQUENCING > WGS ANALYSIS

Whole-genome sequencing data analysis bioinformatics...

WGS ANALYSIS STEPS

Preprocessing & quality control

- Base calling: 23 tools
- Error correction: 56 tools
- Adapter trimming: 47 tools
- k-mer counting: 16 tools
- Variant recalibration: 5 tools
- Read quality control: 58 tools
- Duplicate read removal: 18 tools
- Read clustering: 10 tools
- Depth of coverage: 4 tools

Data analysis

- Read alignment: 115 tools
- Somatic SNV detection: 39 tools
- Indel detection: 47 tools
- Somatic CNA detection: 24 tools
- Insertion detection: 42 tools
- Duplication detection: 29 tools
- Transposon detection: 31 tools
- Germline SNP detection: 85 tools
- De novo mutation detection: 10 tools
- CNV detection: 40 tools
- Deletion detection: 65 tools
- Inversion detection: 34 tools
- Microsatellite detection: 43 tools
- Intra-chromosomal translocation detect...: 17 tools

RELATED CATEGORIES

- De novo sequencing analysis: 260 tools
- Metagenomic sequencing analysis: 402 tools
- WES analysis: 154 tools

RELATED WEBSITES

- DNA sequencing theory: Wikipedia
- Whole genome sequencing: Wikipedia

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RELATED WEBSITES

- DNA sequencing theory: Wikipedia
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Public NGS Databases

NIH Short Read Archive

NCBI Site map All databases PubMed Search

Short Read Archive

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Announcements Provisional SRA Tracking History About

The Short Read Archive (SRA) stores raw sequencing data from the "next" generation of sequencing platforms including Roche 454 GS System[®], Illumina Genome Analyzer[®], Applied Biosystems SOLID[®] System, Helicos Heliscope[®], Complete Genomics[®], and others.

Current capabilities include:

- [Run Browser](#)
- [Study/Sample/Experiment/Analysis](#) browsers
- [Download facility](#)
- [Search SRA \(using Entrez\)](#)
- [Interactive submissions facility](#)
- [Automated submissions](#)

ERA (European Short Read Archive)

EMBL-EBI EB-eye Search All Databases Enter Text Here Go Reset ? Advanced Search Give us feedback

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EMBL-Bank Home Access Documentation

EBI > Databases > EMBL-Bank > Documentation

European Nucleotide Archive - Reads

1. NCBI SRA is a repository for NGS sequenced reads
2. Data is stored in association with basic metadata explaining experimental techniques and inter-sample relationships
3. Data format is NCBI-specific SRA/and SRA-lite format. “Universal” lossless format.
4. Upload and download is offered via FTP and HTTP.

JGI Genome Portal

Search for Genomic Data [Advanced Search](#)

Open Calls for Collaborative Science Proposals

The "Facilities Integrating Collaborations for User Science" (FICUS) initiative was established to encourage and enable researchers to more easily integrate the expertise and capabilities of multiple user facilities into their research.

New on the portal:

KBase is a platform enabling systems biology research and comparative functional genomics of microbes and plants while promoting the sharing of results and methods with other scientists.

Genome Releases

- [Fungal Releases](#)
- [Metagenomics Releases](#)
- [Microbial Releases](#)
- [Plant Releases](#)

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Scientific Divisions



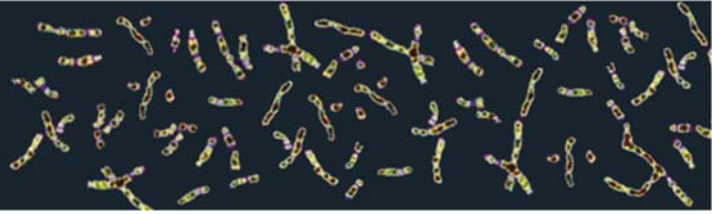
Mammoth Genome Project

Pennsylvania State University



1000 Genomes

A Deep Catalog of Human Genetic Variation



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International consortium announces the 1000 Genomes Project.

Any two people have 99% identical DNA.



Genome sequences are important

1001 Genomes

A Catalog of *Arabidopsis thaliana* Genetic Variation



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Welcome to the 1001 Genomes Project

The 1001 Genomes Vision

The 1001 Genomes Project, launched at the beginning of 2008, has a simple goal: to discover the whole-genome sequence variation in 1001 strains (accessions) of the reference plant *Arabidopsis thaliana*. The resulting information will pave the way for a new era of genetics that combines large-scale association studies in wild strains with forward genetic analyses in experimental crosses, in order to identify alleles underpinning phenotypic diversity across the entire genome and the entire species. The analyses enabled by this project will have broad implications for areas as diverse as evolutionary sciences, plant breeding and human genetics.

This 1001 Genomes Project is particularly timely because the current technological revolution in sequencing means that it is now feasible to resequence large numbers of genomes. Indeed, a **1000 Genomes** project for humans has just been launched in early 2008 as well. There are, however, several important differences between the two projects. The most important one is that each of the accessions in the *Arabidopsis* 1001 Genomes project is an inbred line with seeds that will be freely available from the stock centre to all our colleagues. Unlimited numbers of plants with identical genotype can be grown and phenotyped for each accession, in as many environments as desired, and so the sequence information we collect can be used directly in association studies at biochemical, metabolic, physiological, morphological, and whole plant-fitness levels.

As of early 2010, the complete genome sequences of over 80 accessions have already been released by the Max Planck Institute. There are commitments for the remaining accessions, primarily from the Salk Institute, the Gregor Mendel Institute and Monsanto, and we are hoping for completion of the 1001 Genomes project in the first half of 2011.

Progress as of June 2, 2010:

C o m m i t m e n t s : 1 0 0 1
S e q u e n c i n g u n d e r w a y : 9 9
F i n i s h e d g e n o m e s : 1 5 7
R e l e a s e d g e n o m e s : 9 1

Links

[NCBI SRA Genomes Project](#)

[Map resource for 1001 Genomes](#)

News

Mai 3, 2010

A collection of 80 *A. thaliana* accessions sequenced as part of the 1001 Genomes Project is available from ABRC. Each of the accessions is an inbred line that can be ordered as an individual line or as a set (CS76427). These stocks can be found using the [ABRC catalog](#).

February 2, 2010

The Weigel laboratory has just released 80 *Arabidopsis thaliana* genomes sequenced with paired end Illumina short reads. SNPs and structure variants (SVs) are now available online. For more details, please read the [README file](#).

[>> News archive...](#)

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National Human Genome Research Institute
National Institutes of Health

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Division of Genome Sciences

- Centers of Excellence in Genomic Science
- Division Staff
- ENCODE Project**
- Functional Analysis Program
- Genetic Variation Program
- Genome Informatics and Computational Biology Program
- Genome Technology Program
- NHGRI Genome Sequencing Program (GSP)

The ENCODE Project: ENCyclopedia Of DNA Elements

Overview

- [Publications, Features and Press Releases](#)
- [Consortium Membership](#)
- [Data Release Policy](#)
- [Accessing ENCODE Data](#)
- [ENCODE Tutorials](#) new
- [Common Cell Types](#)
- [Requests for Application \(RFAs\)](#)
- [Program Staff](#)

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ENCODE Overview

The National Human Genome Research Institute (NHGRI) launched a public research consortium named ENCODE, the **ENCyclopedia Of DNA Elements**, in September 2003, to

See Also:

- [Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project](#) PDF
Nature, June 13, 2007
- [Major Findings from The ENCODE Pilot Project](#)
June 2007
- [The modENCODE Project](#)
- [Grants Home](#)

On Other Sites:

- [The ENCODE \(ENCyclopedia Of DNA Elements\) Project](#), *Science*, October 22, 2004
- [Nature ENCODE explorer](#)
Sept. 5, 2012

ENCODE Resources:

- [ENCODE Web Focus](#)

The screenshot shows the Nature ENCODE website interface. At the top, there's a navigation bar with 'nature' and 'ENCODE' logos, a search bar, and links for 'Home', 'Research', 'Threads', 'Additional Research', 'News and Comment', 'About', and 'Sponsor'. Below this is the 'nature ENCODE explorer' section, which features a circular 'THREADS' navigation menu and a 'PAPERS' section. A 'THREAD OVERVIEW' pop-up is displayed, detailing the thread 'Characterization of intergenic regions and gene definition' by Djebali, S. and Davis, C. A. et al. The overview includes a brief description: 'The prevalence and analysis of ENCODE data are changing the definition and characterization of intergenic and genic regions' and a 'Read Thread' button. A 'nature' pop-up window shows the paper's title, authors, journal name, and DOI (10.1038/nature11233 (2012)). At the bottom of the explorer, there are three buttons: 'What is ENCODE?', 'Threads: a new approach', and 'Guide to the ENCODE explorer'.

<http://www.nature.com/encode/>

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NGS Reviews

Next-generation DNA sequencing

Jay Shendure¹ & Hanlee Ji²

Nature Biotechnology 26, 1135 - 1141 (2008)

APPLICATIONS OF NEXT-GENERATION SEQUENCING

Sequencing technologies – the next generation

Michael L. Metzker^{*†}

Nature Review Genetics 11, 31-46 (2010)

NIH Public Access

Author Manuscript

J Genet Genomics. Author manuscript; available in PMC 2011 April 13.

Published in final edited form as:

J Genet Genomics. 2011 March 20; 38(3): 95–109. doi:10.1016/j.jgg.2011.02.003.

The impact of next-generation sequencing on genomics

Jun Zhang^{a,b,*}, Rod Chiodini^c, Ahmed Badr^a, and Genfa Zhang^d

^a COE for Neurosciences, Department of Anesthesiology, Texas Tech University Center El Paso, TX 79905, USA



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Journals Search Alerts

Adv Wound Care (New Rochelle). 2015 Jan 1; 4(1): 50–58.
doi: 10.1089/wound.2014.0542

PMCID: PMC4281878

Next-Generation Sequencing: A Review of Technologies and Tools for Wound Microbiome Research

Brendan P. Hodkinson and Elizabeth A. Grice^{*}

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NATURE REVIEWS GENETICS | REVIEW

ARTICLE SERIES: [Applications of next-generation sequencing](#)

Coming of age: ten years of next-generation sequencing technologies

Sara Goodwin, John D. McPherson & W. Richard McCombie

[Affiliations](#) | [Corresponding author](#)

Nature Reviews Genetics 17, 333–351 (2016) | doi:10.1038/nrg.2016.49
Published online 17 May 2016

Video Clips

- [Sanger Sequencing of DNA](#) [HD Animation]
 - <https://www.youtube.com/watch?v=nudG0r9zL2M>
- [Pyro Sequencing](#)
 - <https://www.youtube.com/watch?v=nFfgWGFe0aA>
- [Illumina Sequencing Technology](#)
 - <https://www.youtube.com/watch?v=womKfikWlxM>
- [Ion Torrent™](#) next-gen sequencing technology
 - <https://www.youtube.com/watch?v=WYBzbxIfuKs>
- [Single Molecule Real Time Sequencing - Pacific Biosciences](#)
 - <https://www.youtube.com/watch?v=v8p4ph2MAvI>
- [Oxford Nanopore Technologies](#)
 - <https://www.youtube.com/watch?v=3UHw22hBpAk>
- [Next-Generation Sequencing Technologies - Elaine Mardis \(2014\)](#)
 - <https://www.youtube.com/watch?v=6Is3W7JkFp8>
- [PCR](#) (Polymerase Chain Reaction)
 - <https://www.youtube.com/watch?v=iQsu3Kz9NYo>
- [Polymerase Chain Reaction](#) [HD Animation]
 - <https://www.youtube.com/watch?v=0HCWmD7Mv8U>

Applications of Next-Generation Sequencing

