

Next Generation Sequencing – An Overview

Olga Vinnere Pettersson, PhD

National Genomics Infrastructure hosted by ScilifeLab,
Uppsala Node (UGC)

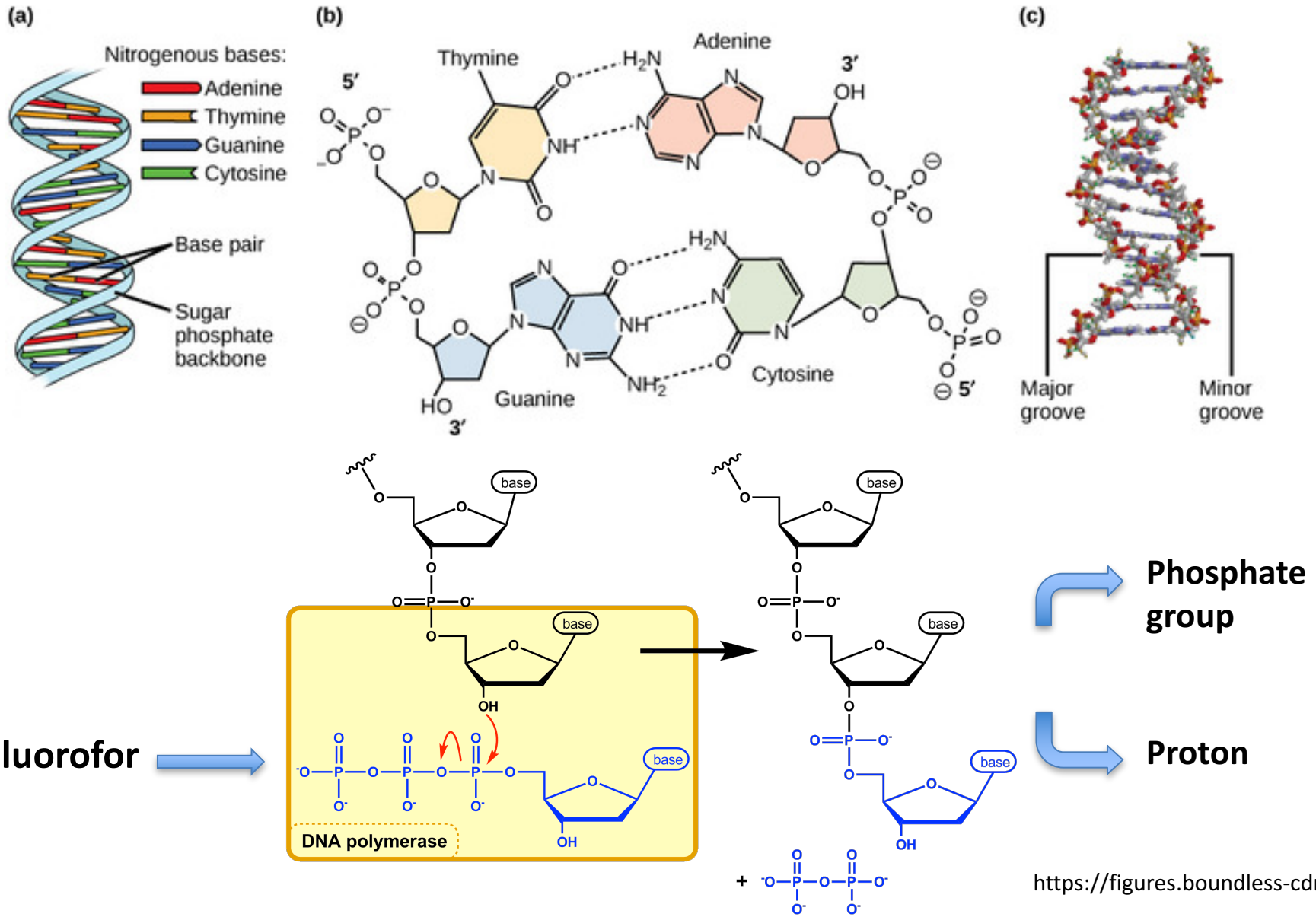
Outline



www.robustpm.com

- A bit of history
- NGS technologies
- NGS applications
 - De Novo
 - RNA-seq
 - Targeted enrichment (hybridization & amplicon-Seq)
- National Genomics Infrastructure – Sweden
- Auxiliary technologies (10x Chromium, BioNano)
- Sample prep for NGS

What is sequencing?



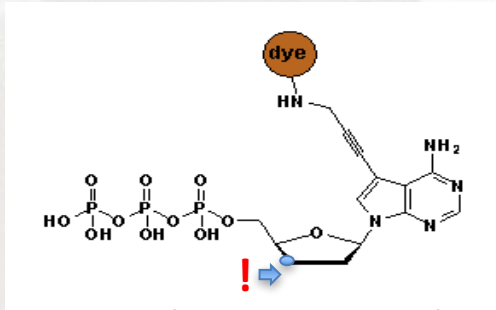
Once upon a time...

- Fredrik Sanger and Alan Coulson
Chain Termination Sequencing (1977)

Nobel prize 1980

Principle:

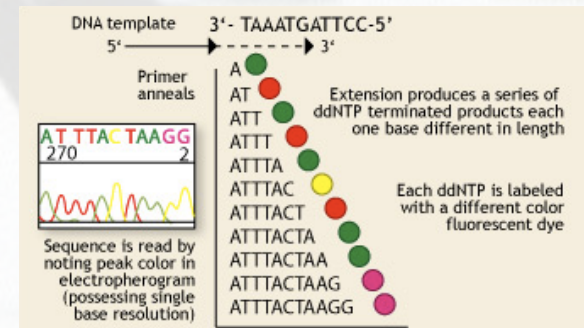
SYNTHESIS of DNA is randomly **TERMINATED** at different points
Separation of fragments that are 1 nucleotide different in size



Lack of OH-group at 3' position of deoxyribose

1 molecule sequenced at a time = 1 read

Capillary sequencer: 384 reads per run



2006 REVOLUTION



Journal home > Archive > Article > Full Text

Journal content

- Journal home
- Advance online publication
- Current issue
- Nature News
- Archive
- Supplements
- Web focuses
- Podcasts
- Videos
- News Specials

Journal information

- About the journal
- For authors
- Contact information

Article

Nature **437**, 376–380 (15 September 2005) | doi:10.1038/nature03959; Received 6 May 2005; Accepted 10 June 2005; Published online 31 July 2005

There is a [Correspondence](#) (26 January 2006) associated with this document.

There is a [Correspondence](#) (4 May 2006) associated with this document.

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies^{1,2}, Michael Egholm^{1,2}, William E. Altman¹, Said Attiya¹, Joel S. Bader¹, Lisa A. Bemben¹, Jan Berka¹, Michael S. Braverman¹, Yi-Ju Chen¹, Zhoutao Chen¹, Scott B. Dewell¹, Lei Du¹, Joseph M. Fierro¹, Xavier W. Gomes¹, Brian C. Godwin¹, Wen He¹, Scott Helgesen¹, Chun He Ho¹, Gerard P. Irzycki¹, Szilveszter C. Jando¹, Maria L. I. Alenquer¹, Thomas P. Jarvie¹, Kshama B. Jirage¹, Jong-Bum Kim¹, James R. Knight¹, Janna R. Lanza¹, John H. Leamon¹, Steven M. Lefkowitz¹, Ming Lei¹, Jing Li¹, Kenton L. Lohman¹, Hong Lu¹, Vinod B. Makhijani¹, Keith E. McDade¹, Michael P. McKenna¹, Eugene W. Myers², Elizabeth Nickerson¹, John R. Nobile¹, Ramona Plant¹, Bernard P. Puc¹, Michael T. Ronan¹, George T. Roth¹, Gary J. Sarkis¹, Jan Fredrik Simons¹, John W. Simpson¹, Muthureyan Srinivasan¹, Karrie R. Tartaro¹, Alexander Tomasz¹, Kari A. Vogt¹, Greg A. Volkmer¹, Shally H. Wang¹, Yong Wang¹, Michael P. Weiner¹, Pengguang Yu¹, Richard F. Beigley¹ & Jonathan M. Rothberg¹



FULL TEXT

- Readers' Comments
- Subscribe to comments (RSS)
- What is RSS?

• Previous | Next •

• Table of contents

• Download PDF

• View interactive PDF in ReadCube

• Share this article

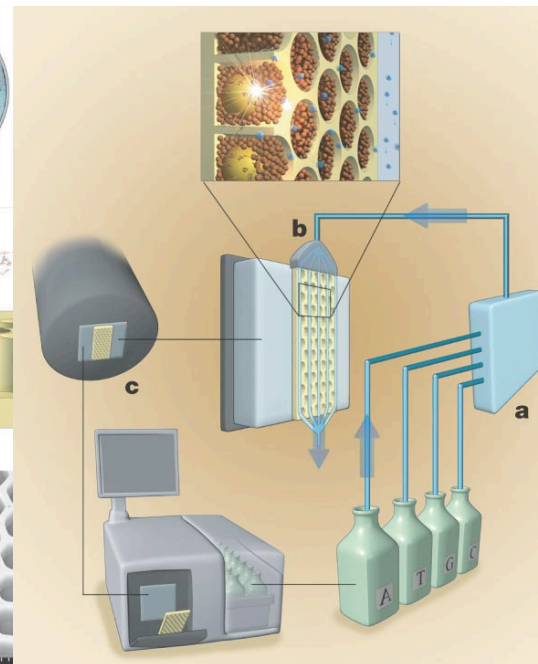
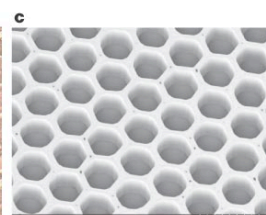
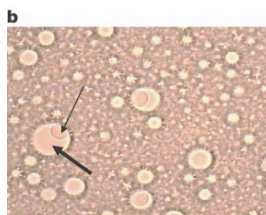
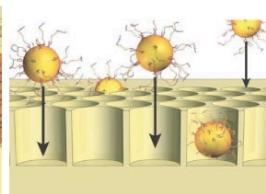
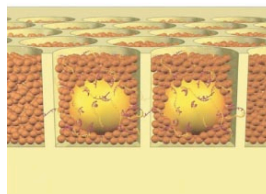
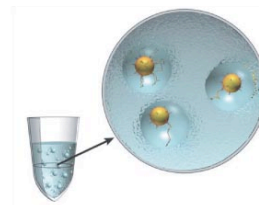
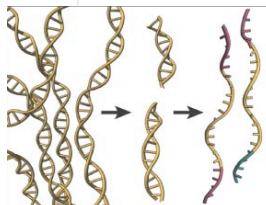
• CrossRef lists 376 articles citing this article

Thousands of molecules sequenced in parallel

1 mln reads sequenced per run



Roche 454 GS FLX



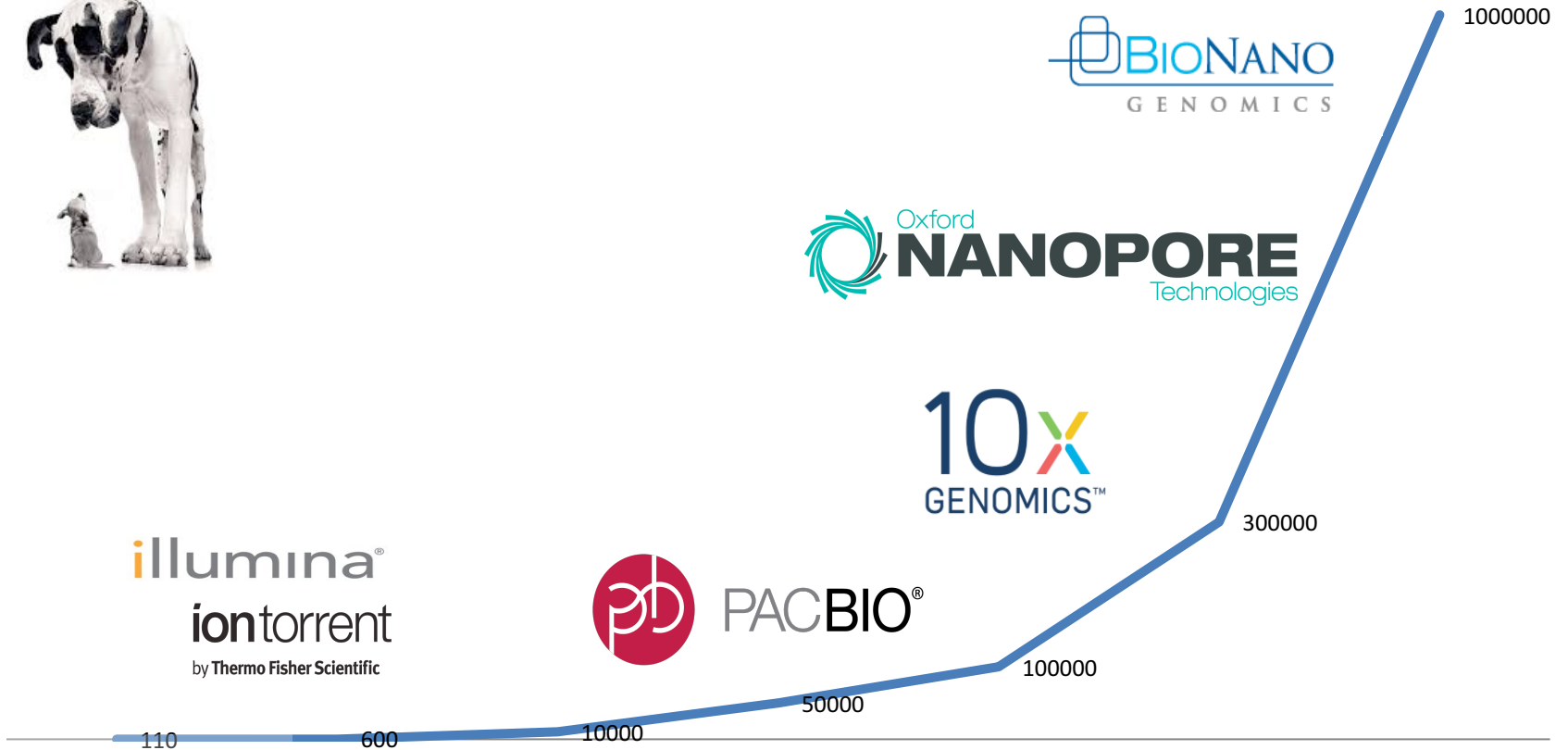
Technologies

Differences between platforms

- Technology: chemistry + signal detection
- Run times vary from hours to days
- Production range from Mb to Gb
- Accuracy per base from 0.1% to 15%
- Cost per base
- Library construction

Read length: from <100 bp to > 20 Kbp

Read length





Illumina

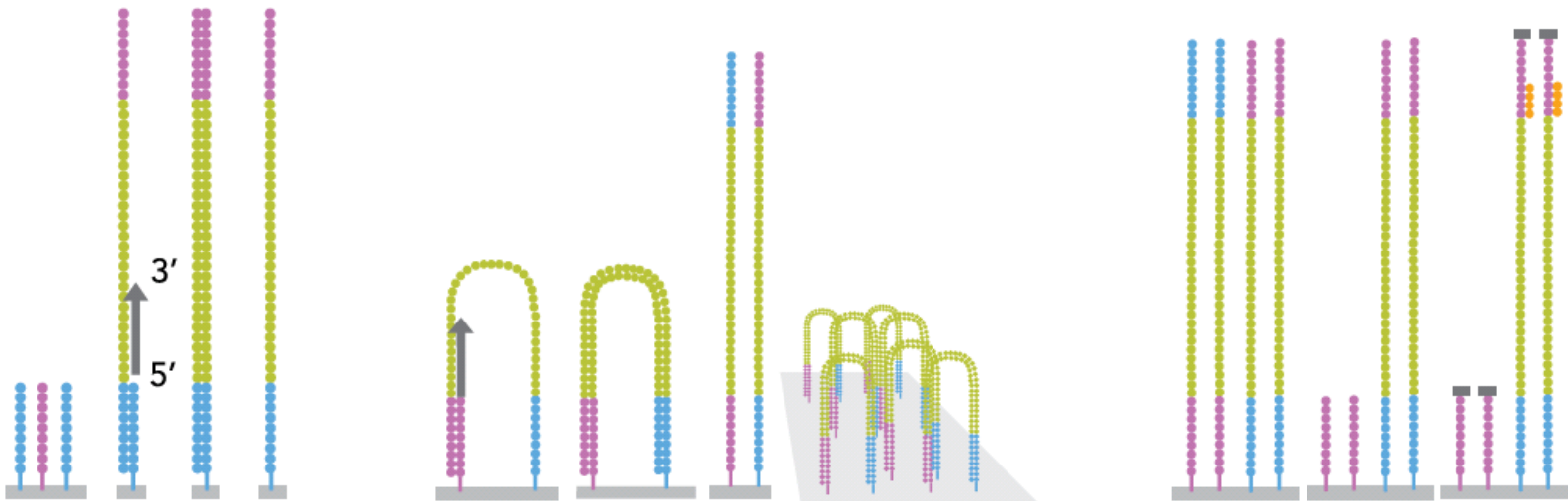
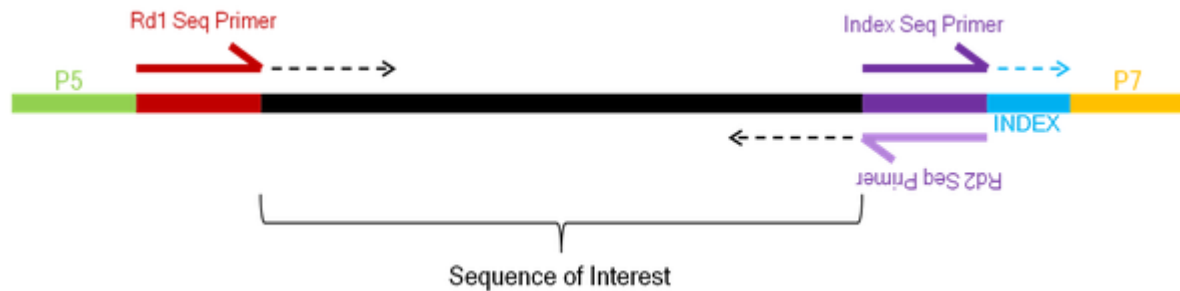
Instrument	Yield and run time	Read Length	Error rate	Error type
HiSeq2500	120 Gb – 600 Gb 27h or standard run	100x100 (250x250)	0.1%	Subst
MiSeq	540 Mb – 15 Gb (4 – 48 hours)	Up to 350x350	0.1%	Subst
HiSeqXten	800 Gb - 1.8 Tb (3 days)	150x150	“	“

Main applications

- Whole genome, exome and targeted reseq
- Transcriptome analyses
- Methylome and ChIPSeq
- Rapid targeted resequencing (MiSeq)
- Human genome seq (Xten)



Illumina: bridge amplification



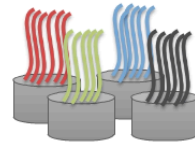
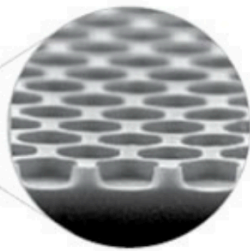
- 200M fragments per lane
- Bridge amplification
- Ends with blocking of free 3'-ends and hybridisation of sequencing primer



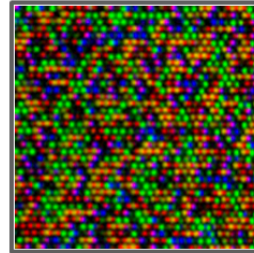
Illumina: ExAmp = black box

Nanowells on Patterned Flow Cell

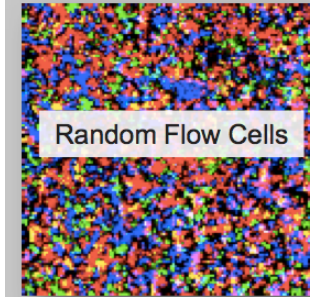
ExAmp on Patterned Flow Cell



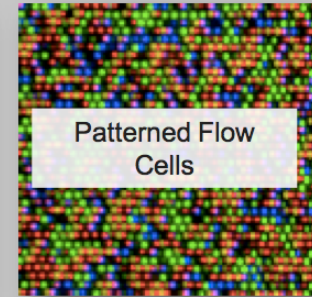
Monoclonal wells



Ordered cluster spacing



Random Flow Cells



Patterned Flow Cells



bioRxiv
beta
THE PREPRINT SERVER FOR BIOLOGY

HOME | A
| CHANN

Search

New Results

Index Switching Causes “Spreading-Of-Signal” Among Multiplexed Samples In Illumina HiSeq 4000 DNA Sequencing

Rahul Sinha, Geoff Stanley, Gunsagar Singh Gulati, Camille Ezran, Kyle Joseph Travaglini, Eric Wei, Charles Kwok Fai Chan, Ahmad N Nabhan, Tianying Su, Rachel Marie Morganti, Stephanie Diana Conley, Hassan Chaib, Kristy Red-Horse, Michael T Longaker, Michael P Snyder, Mark A Krasnow, Irving L Weissman

doi: <https://doi.org/10.1101/125724>

Affected platforms:

HiSeqXten,
HiSeq 3000 and 4000,
NovaSeq

Ion



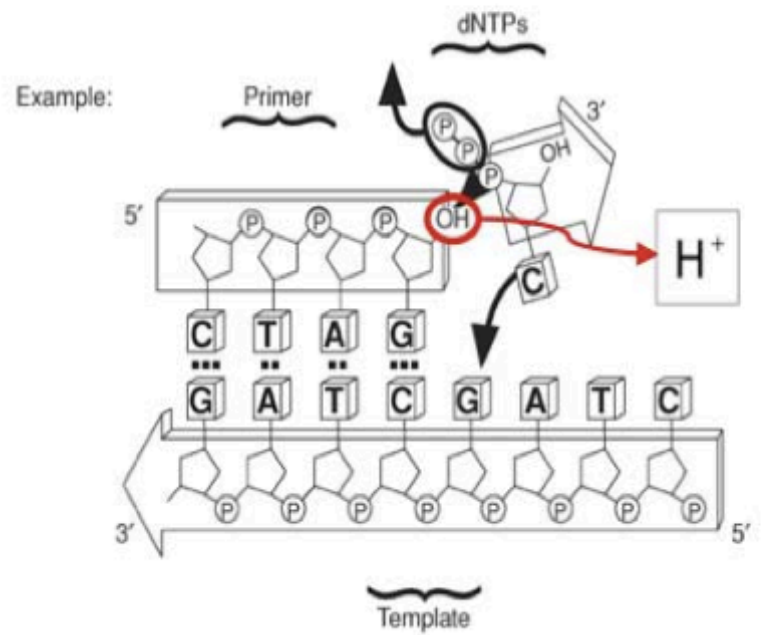
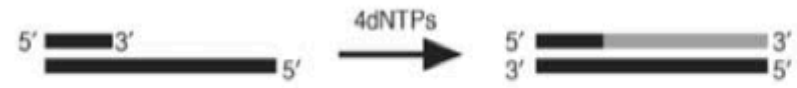
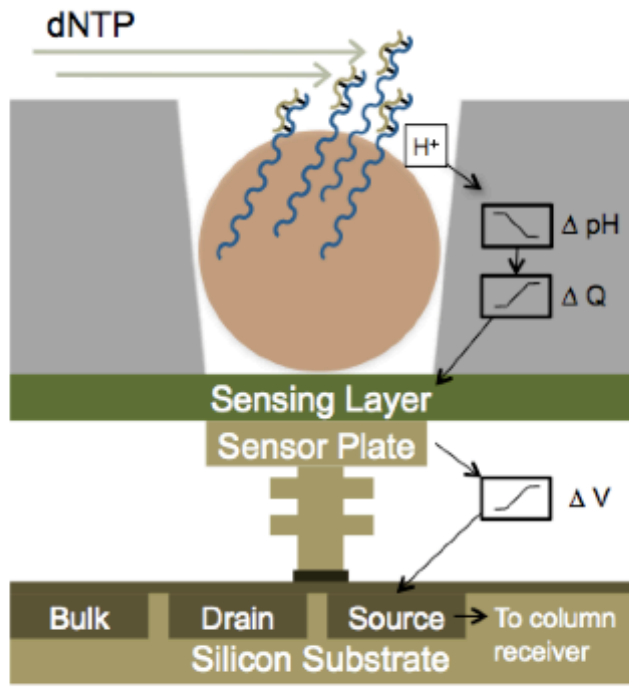
Chip	Yield - run time	Read Length
314, 316, 318 (PGM)	0.1 – 1 Gb Gb, 3 hrs	200 – 400 bp
P-I (Proton)	10 Gb 4 hrs	200 bp
520, 530, 540 (S5)	1 Gb – 10 Gb 3 hrs	200 - 600 bp (except 540)



Main applications

- Microbial and metagenomic sequencing
- Targeted re-sequencing (gene panels)
- Clinical sequencing

Ion Torrent - H⁺ ion-sensitive field effect transistors

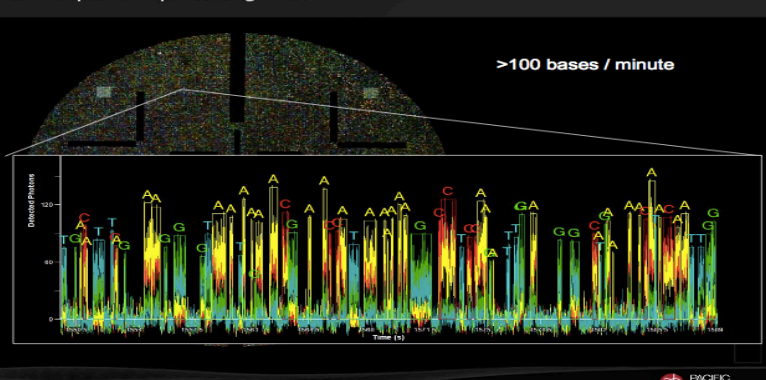


PacBio

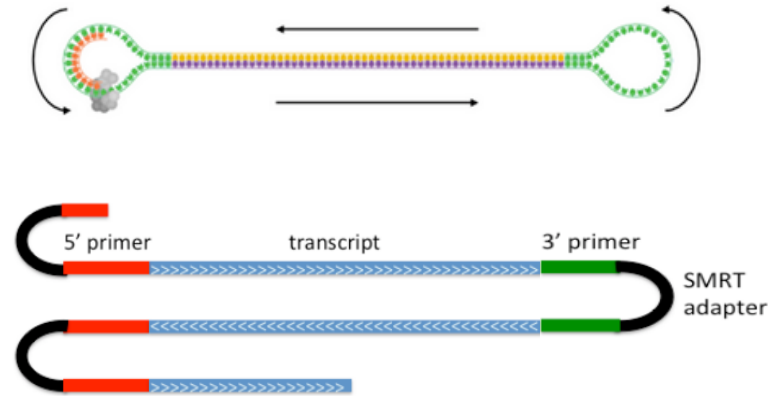
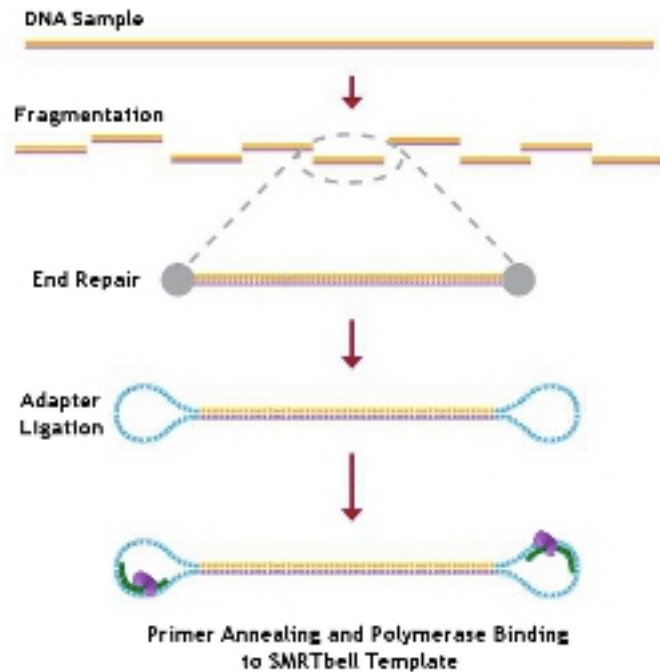
Instrument	Yield/cell and run time	Read Length	Error rate	Error type
RS II	250 Mb – 1.8 Gb 30 - 600 min	250 bp – 30 kb <i>(78 kb)</i>	15 % (single pass) 0.0001% (circular consensus)	Insertions, random
SEQUEL	2-6 Gb 30-600 min	250 bp – 25 kb	as RSII	as RSII

Single-Molecule, Real-Time DNA sequencing

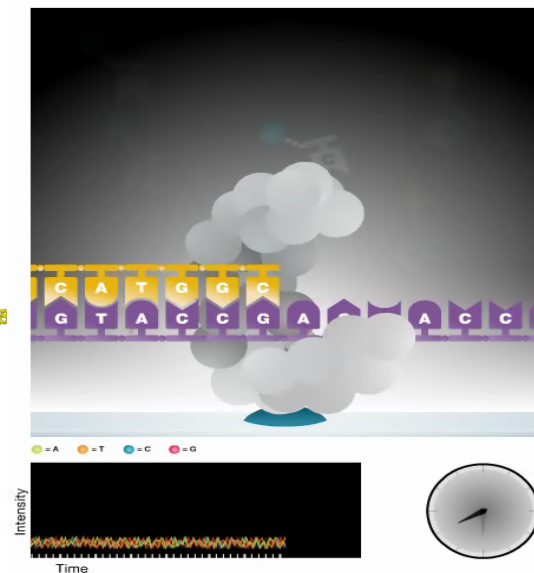
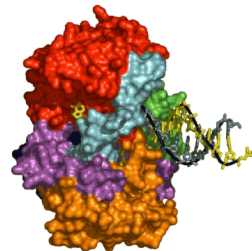
Example Sequencing Run



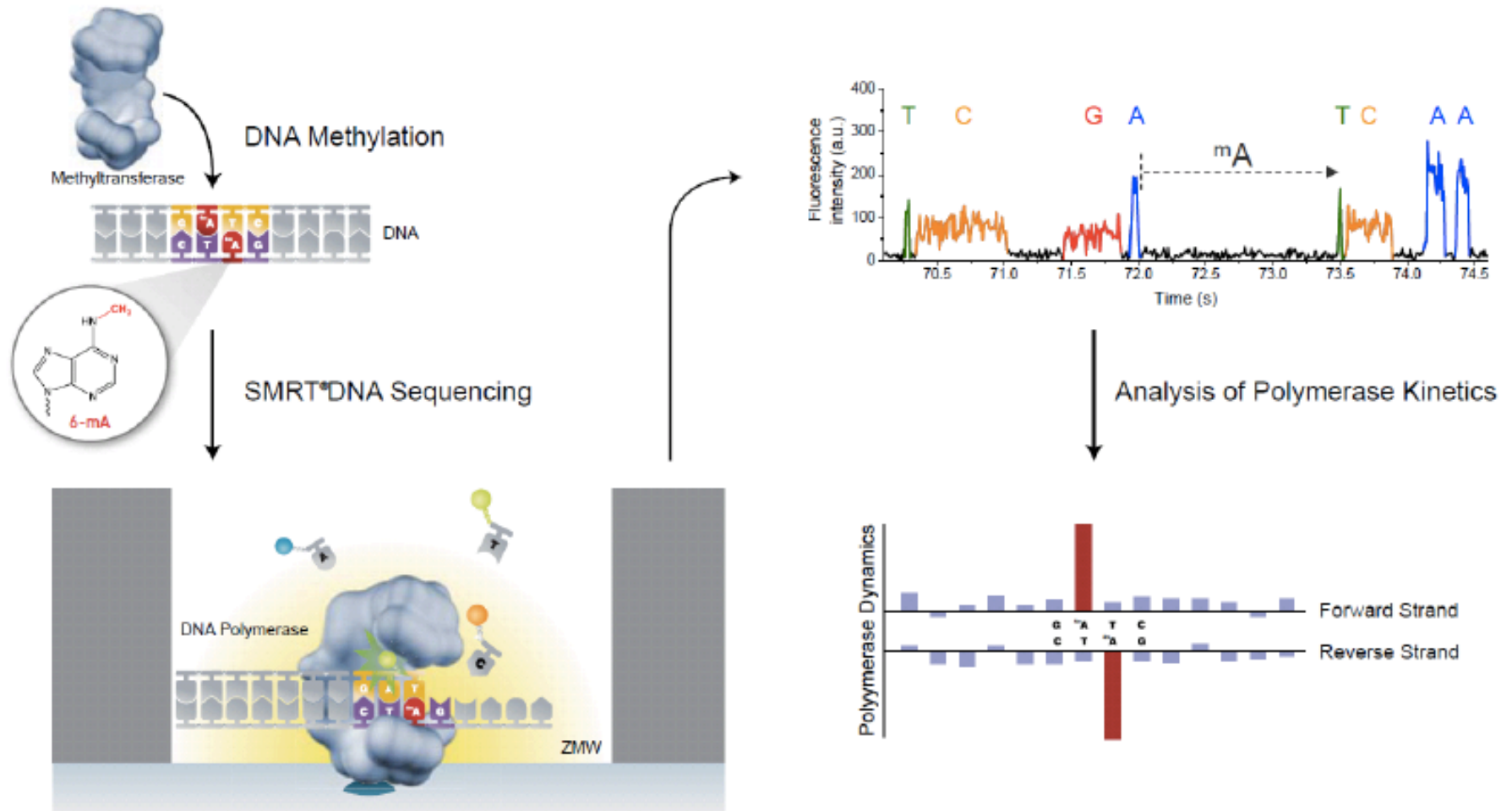
PacBio: SMRT - technology



SMRT =
Single Molecule Real Time



Base Modification: Discover the Epigenome



Detect base modifications using the kinetics of the polymerization reaction during normal sequencing

SMRT sequencing: common misconceptions

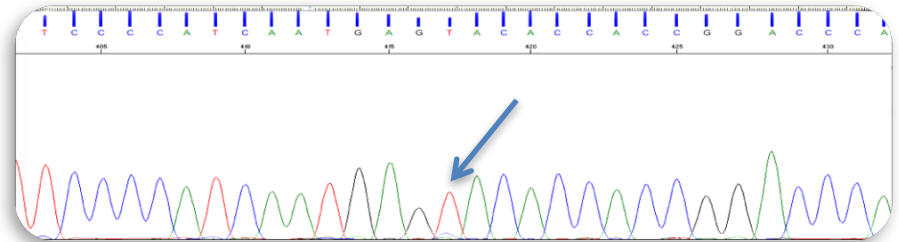
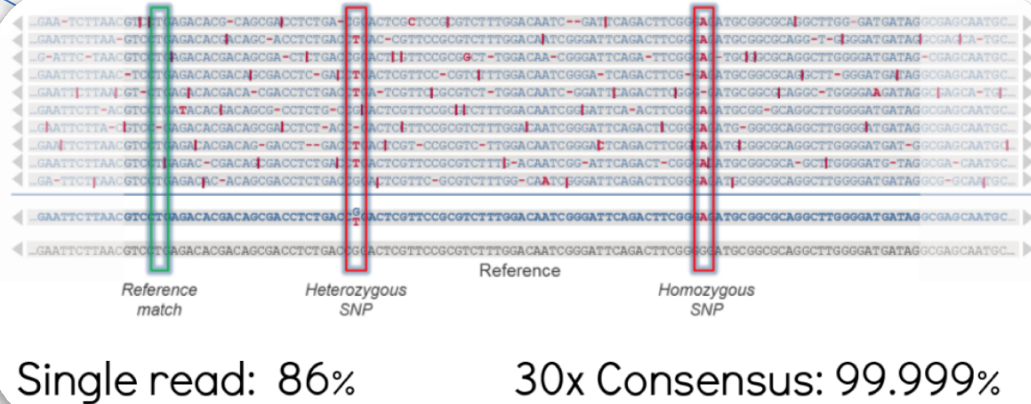
High error rate?

Irrelevant, because errors are random

Depending on coverage

Examples:

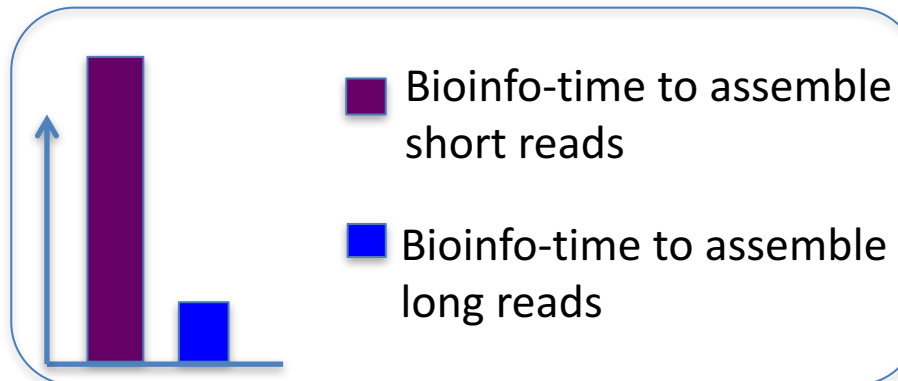
- 8 Mb genome, 8 SNPs detected
- 65 kb construct: 100% correct sequence
- Detection of low frequency mutations



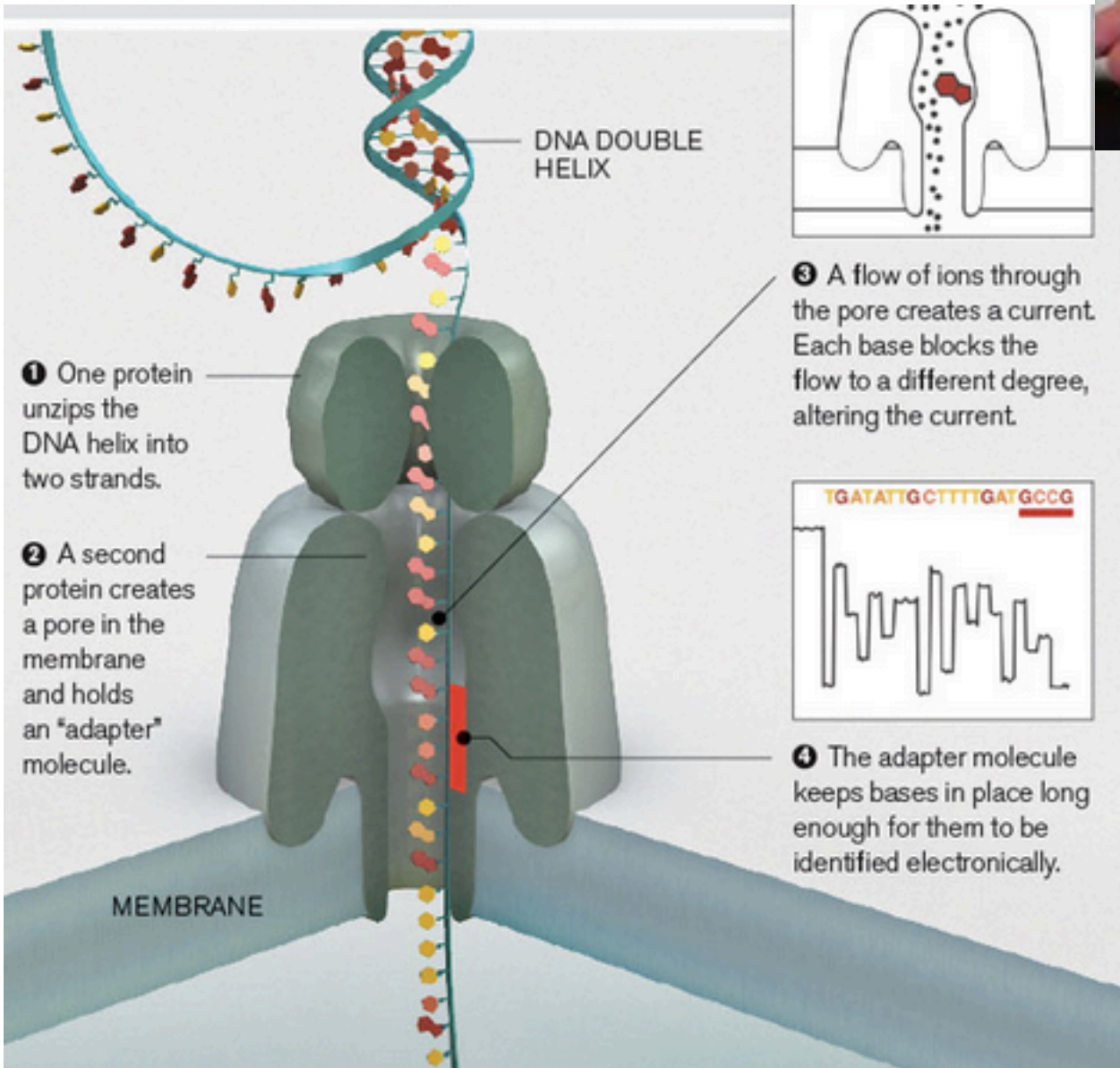
High price?

Not for small genomes

Better assembly quality
Single-molecule reads without PCR-bias



Oxford Nanopore MinION



Reads up to 800k
10-15% error rate
Life time 5 days



Main types of equipment



Illumina HiSeq
Illumina Xten
Illumina MiSeq

Short paired reads
HIGH throughput



Ion Torrent PGM
Ion Proton
Ion S5 XL

Short single-end reads
FAST throughput



PacBio RSII
PacBio Sequel

Ultra-long reads
FAST throughput

Applications

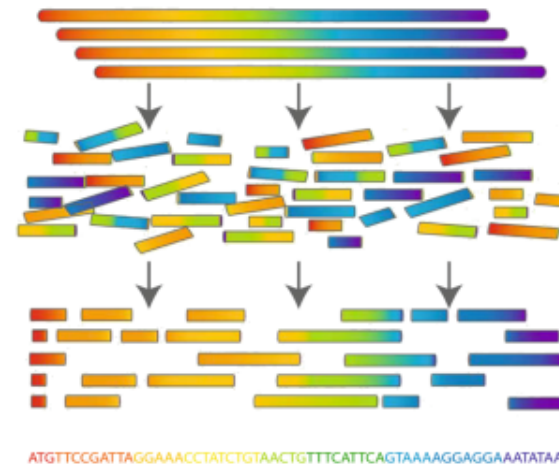
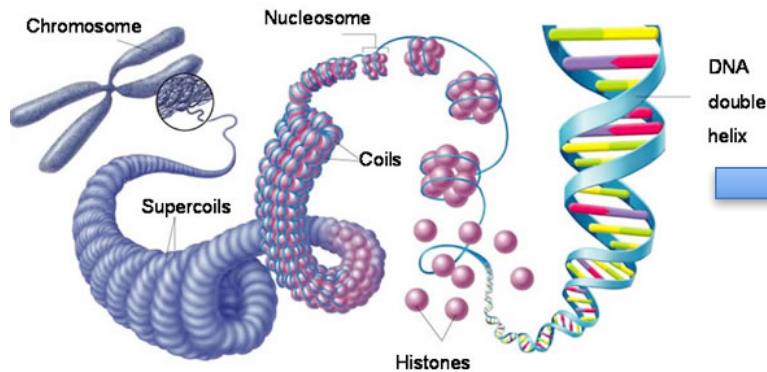
NGS/MPS applications

- Whole genome sequencing:
 - De novo sequencing
 - Re-sequencing
- Transcriptome sequencing:
 - **mRNA-seq**
 - **miRNA**
 - Isoform discovery
- Target re-sequencing
 - Exome
 - Large portions of a genome
 - Gene panels
 - **Amplicons**

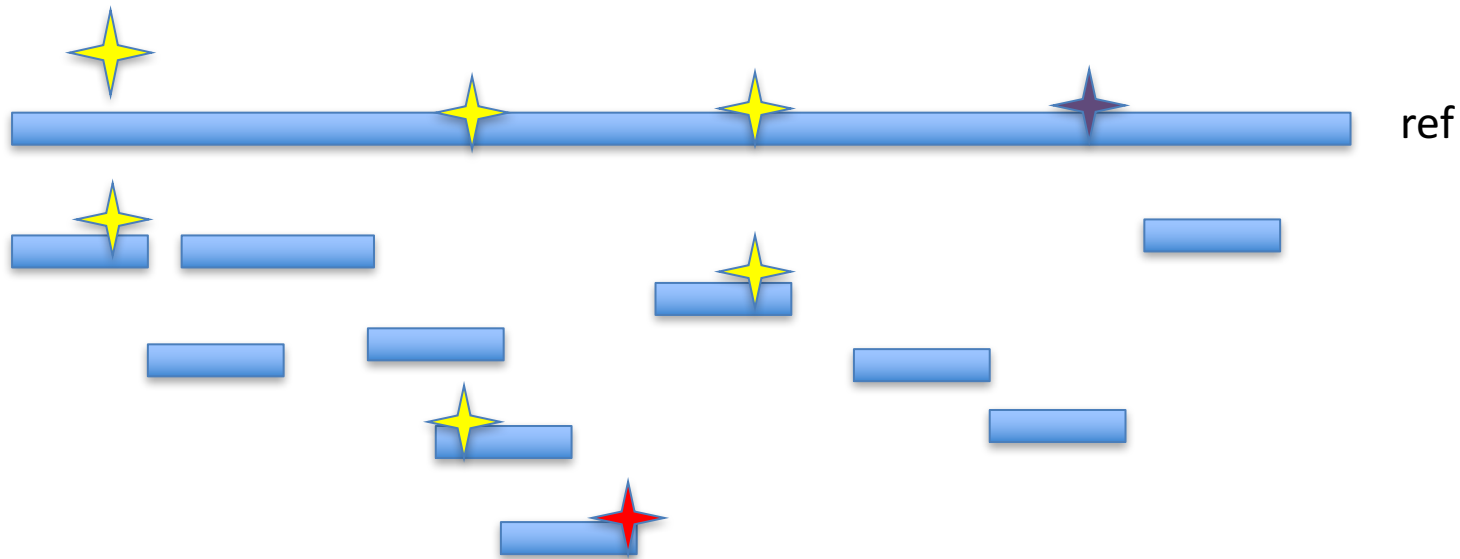


De novo sequencing

- Used to create a reference genome without previous reference



De novo vs re-sequencing



De novo

No bias towards a reference
No template to adapt to

Many contigs
Works best for large-scale events

Re-seq

Finding similarities to a reference
Easier to identify SNPs and minor events
Fewer contigs

Novel events are lost

De novo – do it with long reads!



Science AAAS

Home News Journals Topics Careers

Science Science Advances Science Immunology Science Robotics Science Signaling Science Translational Medicine

SHARE RESEARCH ARTICLE

Long-read sequence assembly of the gorilla genome

David Gordon^{1,2,*}, John Huddleston^{1,2,*}, Mark J. P. Chaisson^{1*}, Christopher M. Hill^{1*}, Zev N. Kronenberg^{1*}, Katherine ...

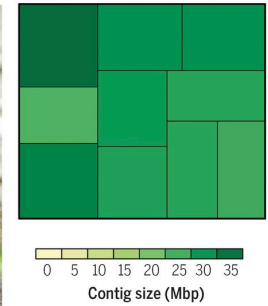
Science 01 Apr 2016; Vol. 352, Issue 6281, aae0344; DOI: 10.1126/science.aae0344

PRE Peer Reviewed

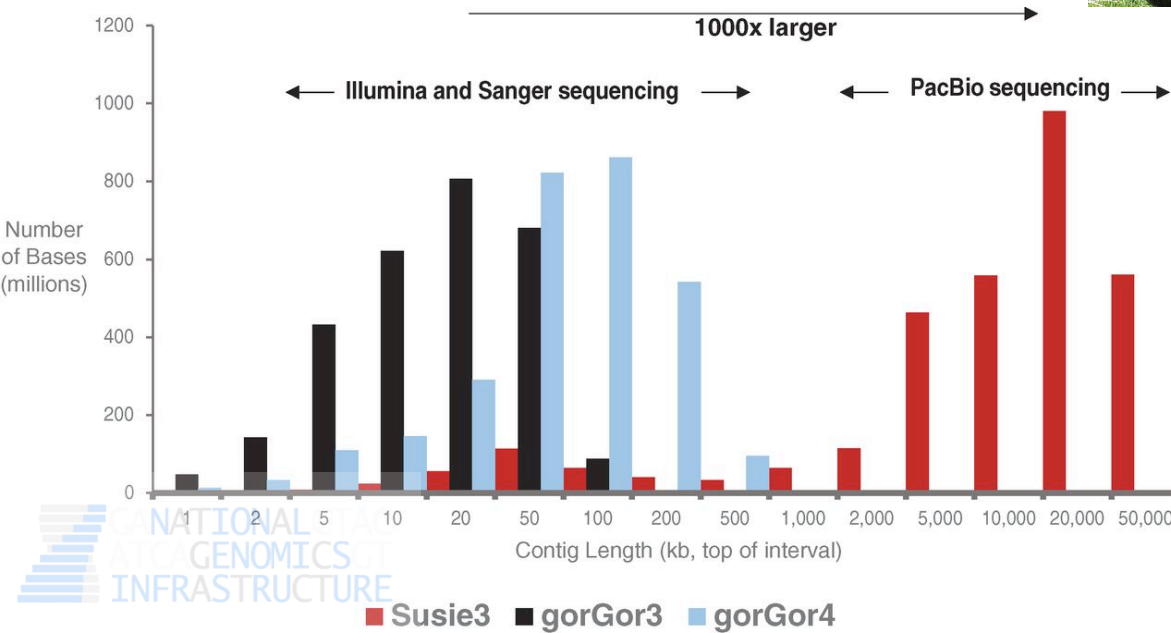
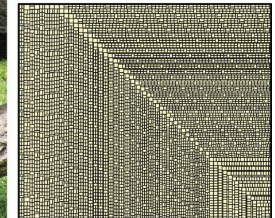
A Susie, reference sample



B Long-read assembly (Susie3)

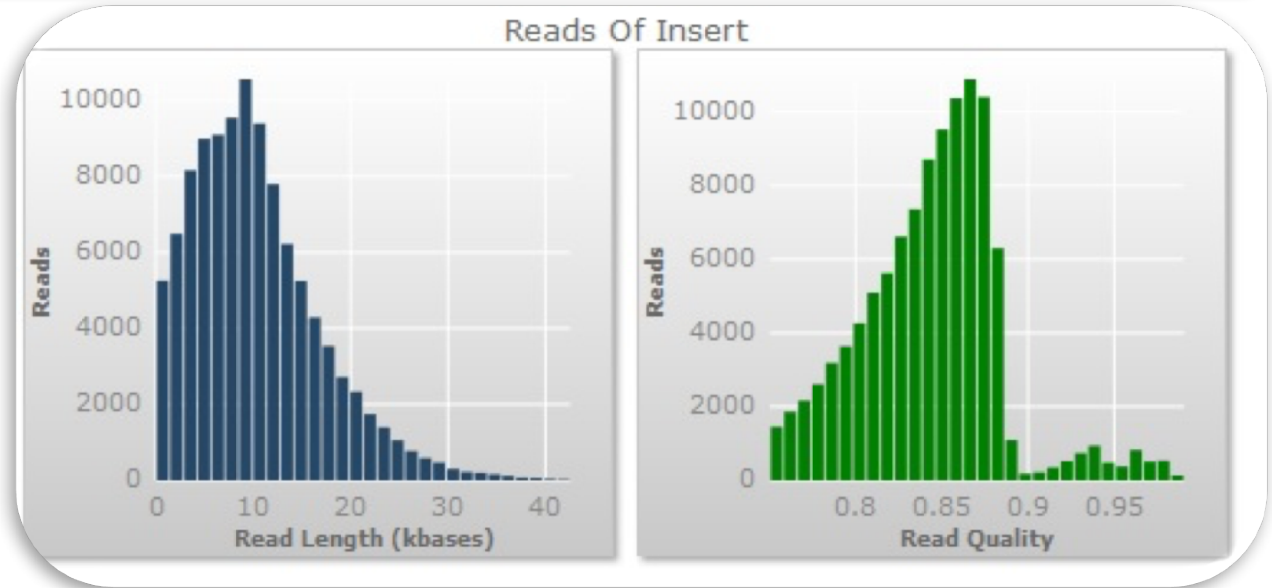


C Short-read assembly (gorGor3)



TEMPORA
MVTANTVR
ET NOS
MVTAMVR
IN ILLIS

Example: de novo PacBio; Crow



Sequencing results

Number of SMRT cells: 70

Total bases per SMRT: 1.39 Gb

Total reads per SMRT: 106 833

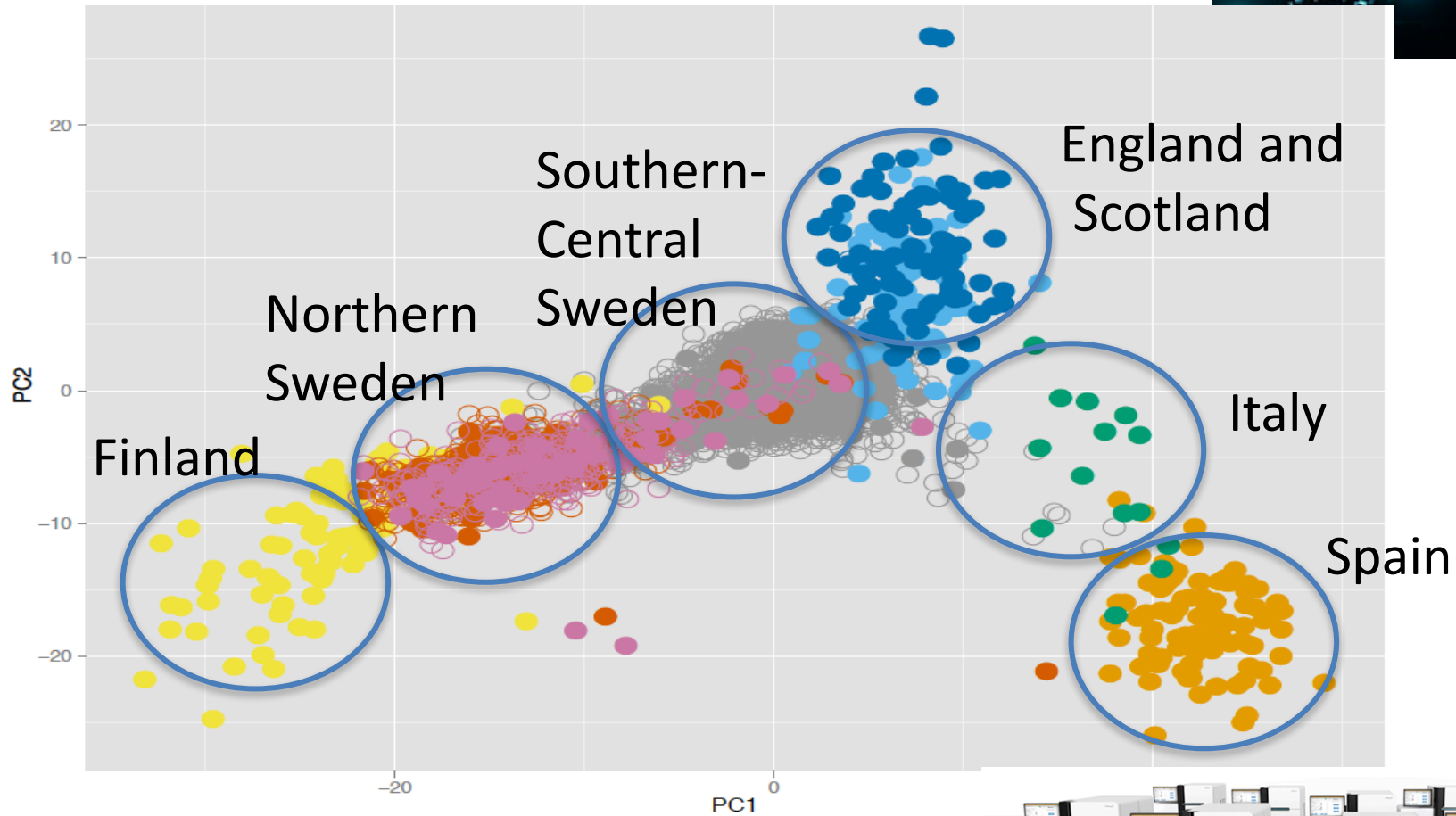
Assembly results, FALCON

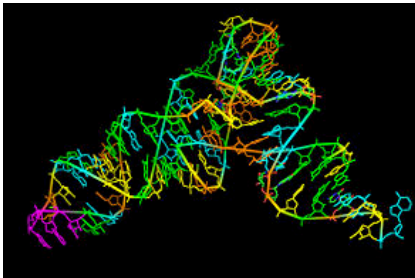
	PRIMARY	ALTERNATIVE
N50	8.5 Mb	23 kb
N75	3.9 Mb	18 kb
Nr contigs	4375	2614
Longest contig	36 Mb	121 kb
Total length	1.09 Gb	45 Mb

Re-sequencing



Population studies: Illumina HiSeq is **The Best**





Transcriptome sequencing (RNA-seq)



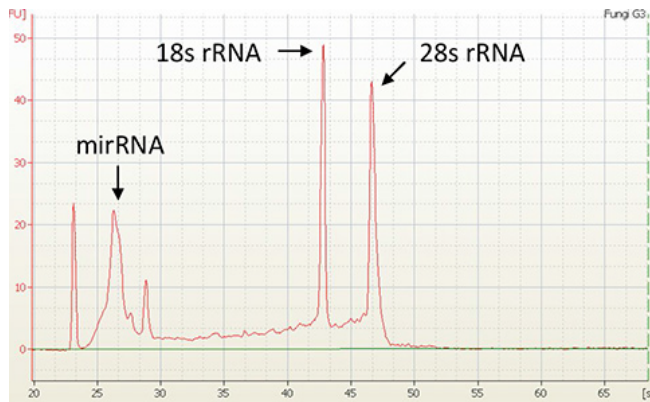
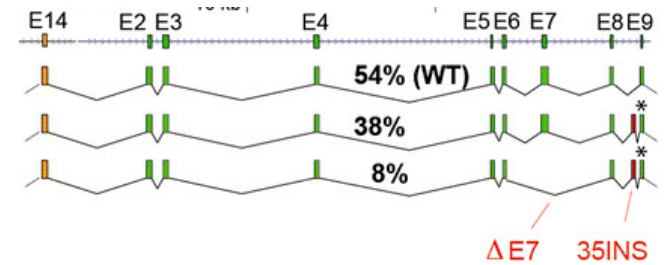
mRNA

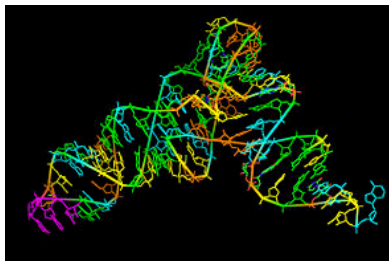
- **Dif.ex.**
- Annotation

Non-codingRNA miRNA

- Transcriptional regulation

Splice isoforms





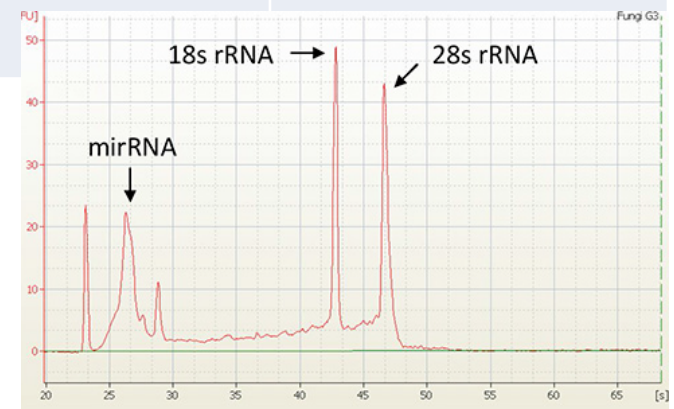
mRNA: rRNA depletion vs polyA selection

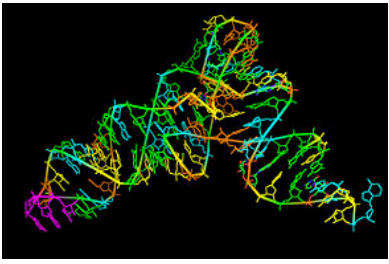
Method	Pros	Cons	Recommended
rRNA depletion	<ul style="list-style-type: none"> • Captures on-going transcription • Picks up non-coding RNA 	<ul style="list-style-type: none"> • Does not get rid of all rRNA • Messy Dif.Ex. profile 	20-40 mln reads (single or PE)
polyA selection	<ul style="list-style-type: none"> • Gives a clean Dif.Ex. profile 	<ul style="list-style-type: none"> • Does not pick non-coding RNA 	5-20 mln reads

Alternative for **human** RNA-seq:

AmpliSeq Human Transcriptome panel:

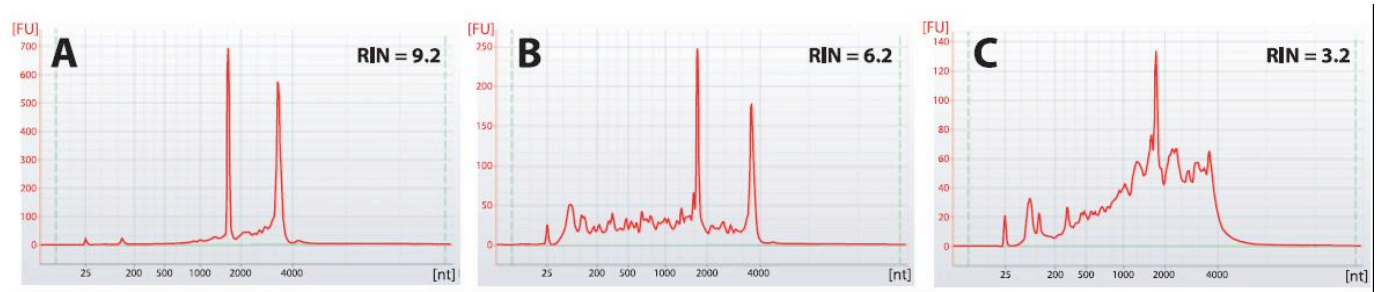
- faster, cheaper, works fine with FFPE
- input: 50 ng **total** RNA
- dif.ex. ONLY



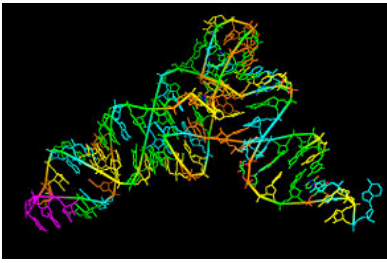


RNA-seq experimental setup

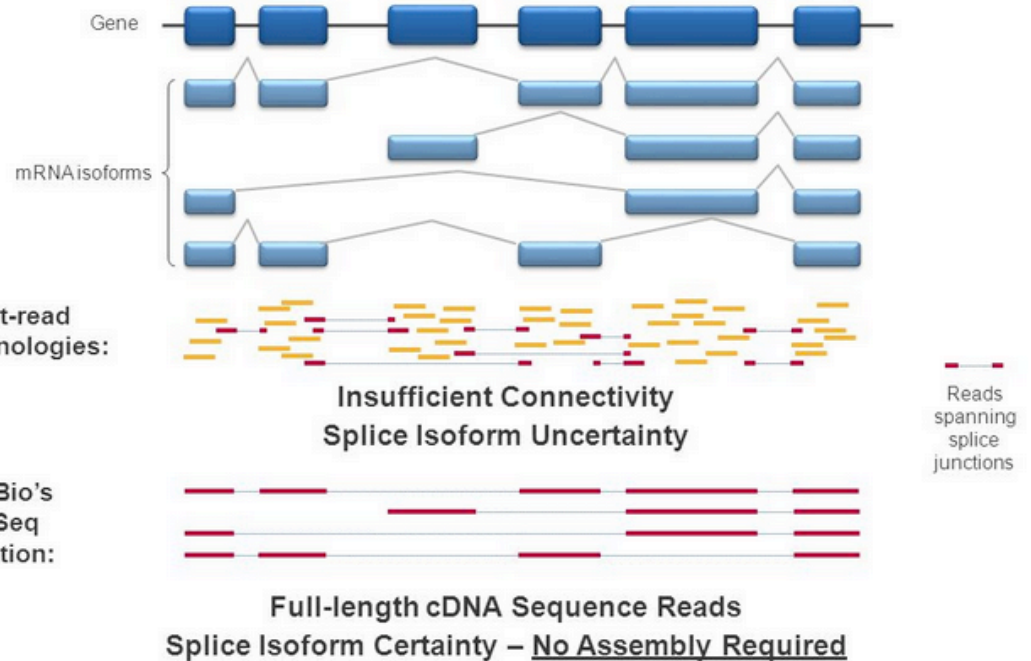
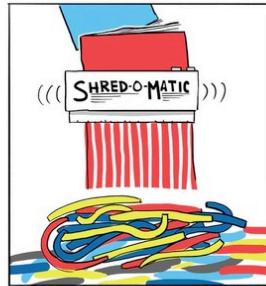
- mRNA only: any kit
- mRNA **and** miRNA: only specialized kits
- Always use DNase!
- RIN value above 8.



- CONTROL vs experimental conditions
- Biological replicates: 4 strongly recommended



RNA-seq experimental setup



Targeted re-sequencing



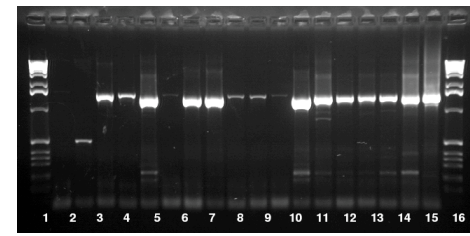
Suitable applications for target-seq

- Metagenomics
- Resolving complex regions
- Low frequency mutations
- Human re-sequencing
- Clinical diagnostics
-

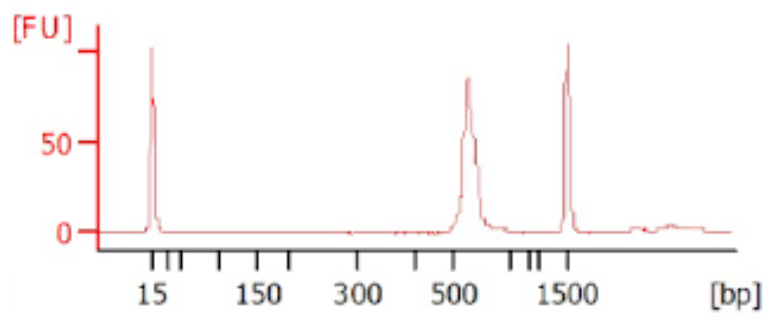
Approaches

- Hybridization capture
(Agilent, NimbleGen, MyBaits)
- PCR (Amplicon sequencing)
 - Long-range
 - Conventional
 - Multiplex
- *Experimental:*
 - *TLA, Samplix, CRISPR-Cas9)*

Amplicon sequencing



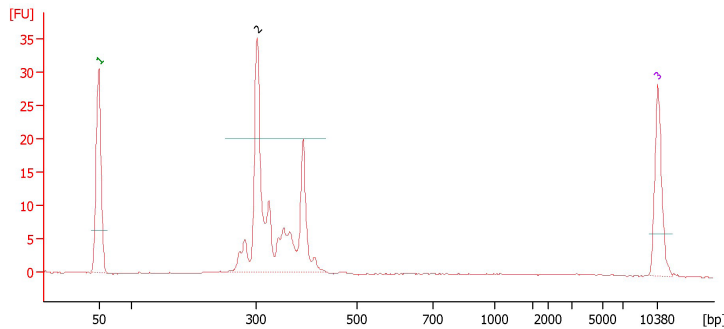
Example 1: tight peak, OK



FOR ANY NGS TECHNOLOGY

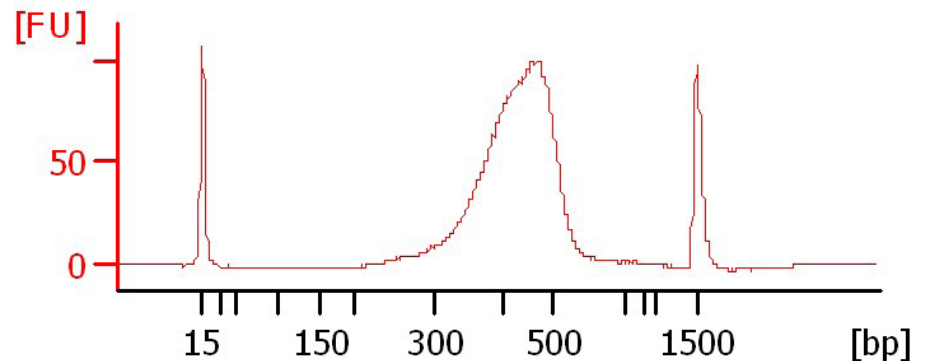
Size difference among fragments **must not** exceed 80 bp (or 20% in length)

Reason – preferential amplification of short fragments



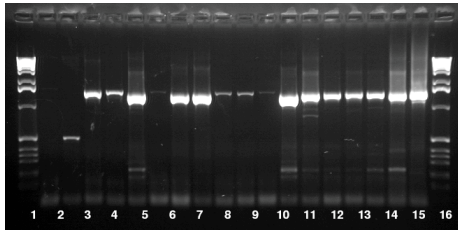
Example 2: several sizes,
fractionation is needed

=> we HAVE to make several libraries

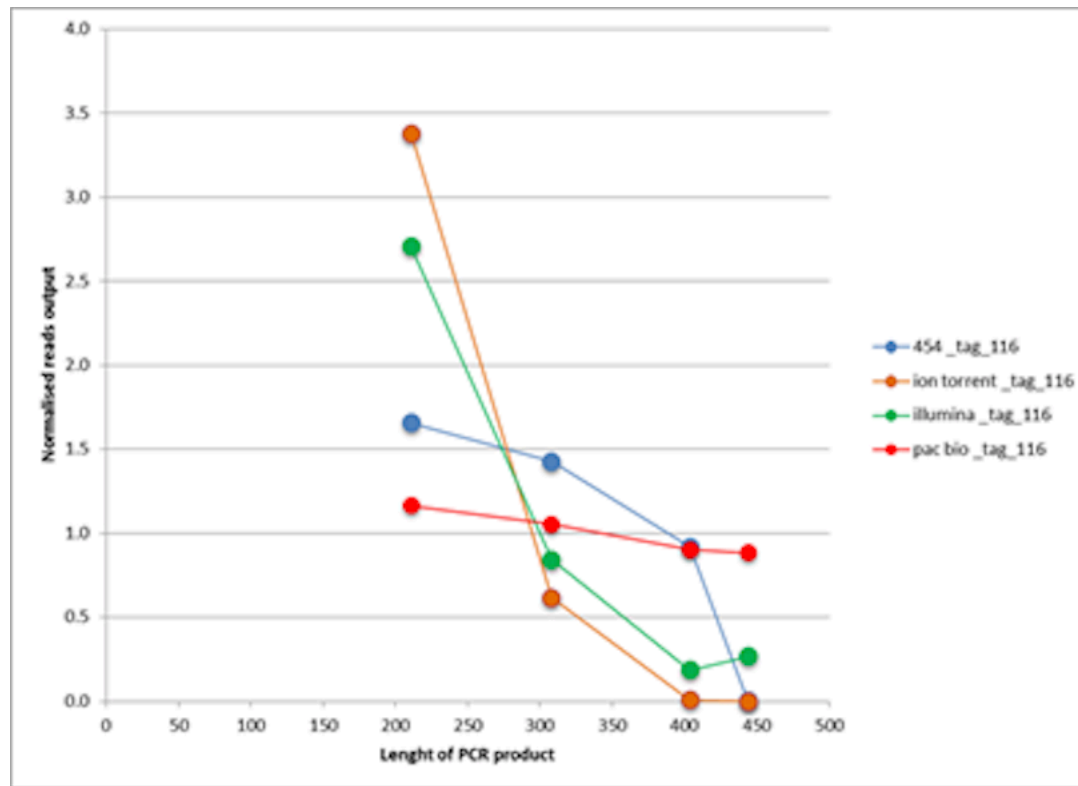


Example 3: broad peak;
size selection is needed

SIZE MATTERS...



Size-related bias in amplicon-seq

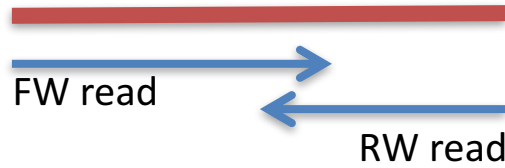


Courtesy Mikael Brandström Durling, Forest Mycology and Pathology, SLU

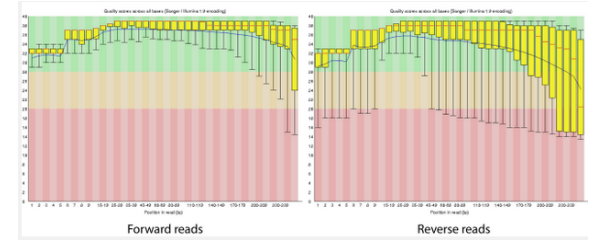
Amplicon sequencing: Technologies



Illumina MiSeq



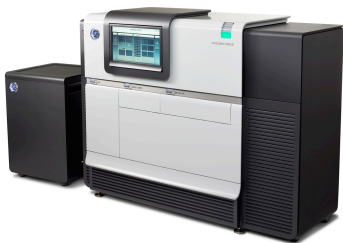
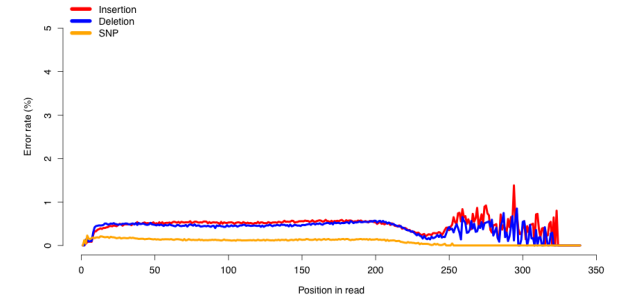
Paired-end reads



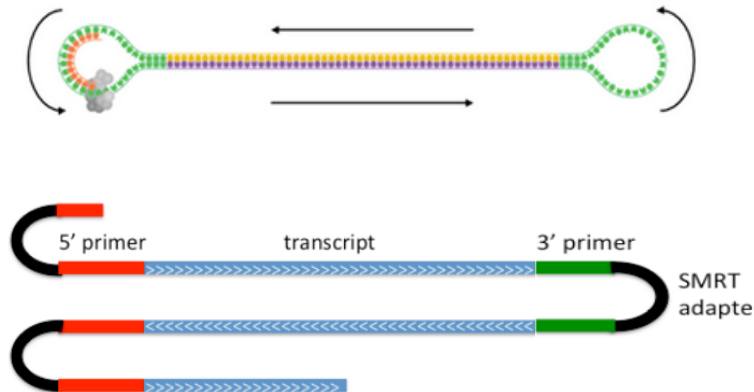
Ion S5XL



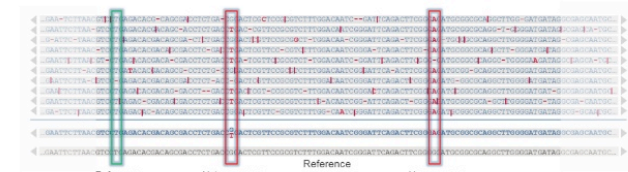
Single-end reads



PacBio RSII



Circular consensus reads



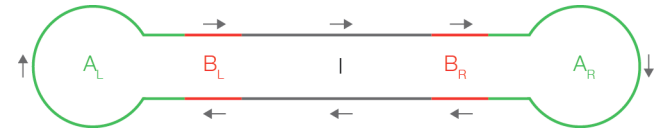
Single read: 86%

30x Consensus: 99.999%

Amplicon sequencing: Barcoding strategies



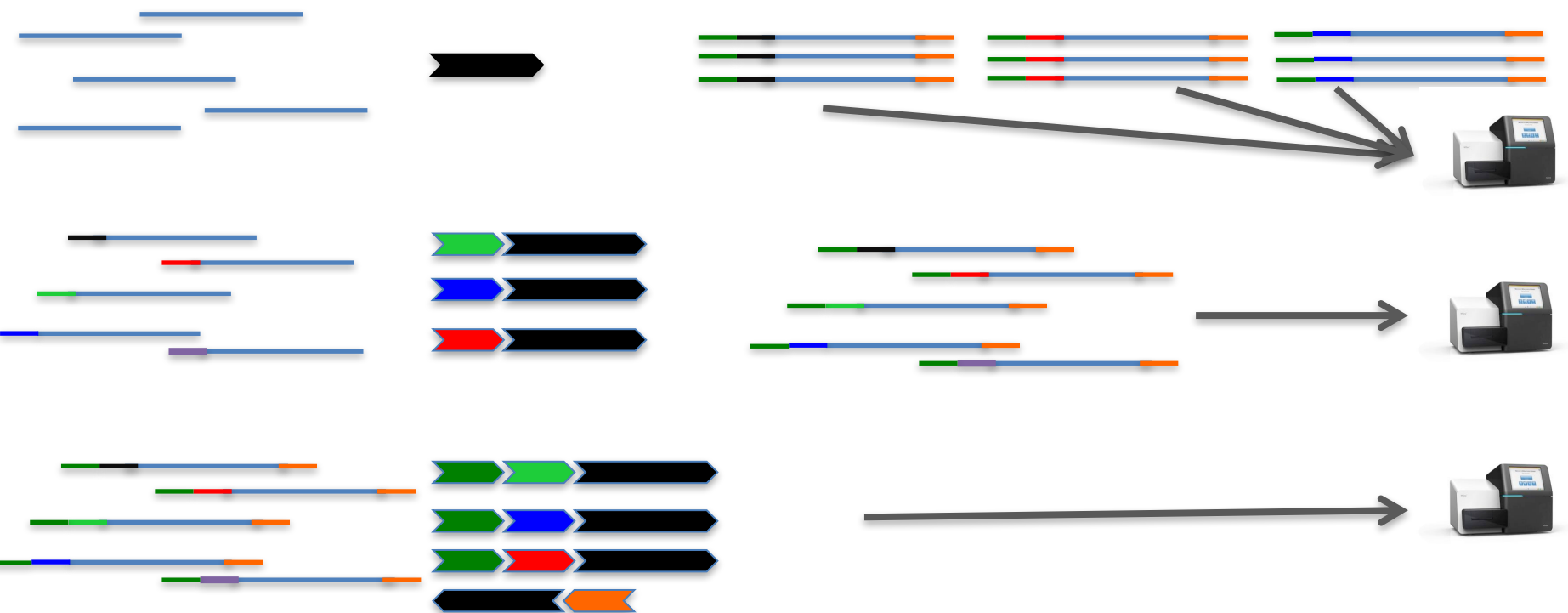
Illumina and Ion



PacBio

USER

NGI



Main types of equipment & applications



Illumina HiSeq
NextSeq, X10, MiSeq,
MiniSeq, NovaSeq

Short paired reads
HIGH throughput

Human WGS
Re-sequencing 30x
mRNA and miRNA
De novo transcriptome
Exome
ChIP-seq
Short amplicons
Methylation



Ion Torrent PGM
Ion Proton
Ion S5 XL

Short single-end reads
FAST throughput

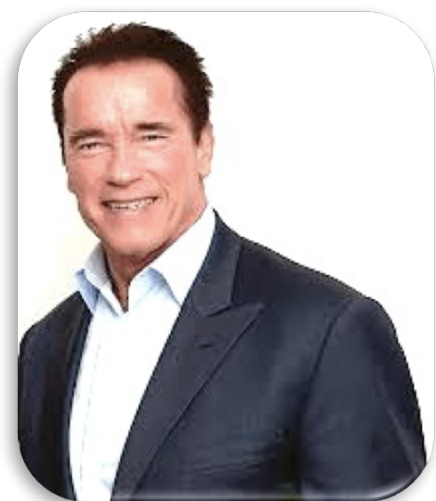
mRNA and miRNA
Exome
ChIP-seq
Short amplicons
Gene panels
Clinical samples



PacBio RSII
SEQUEL

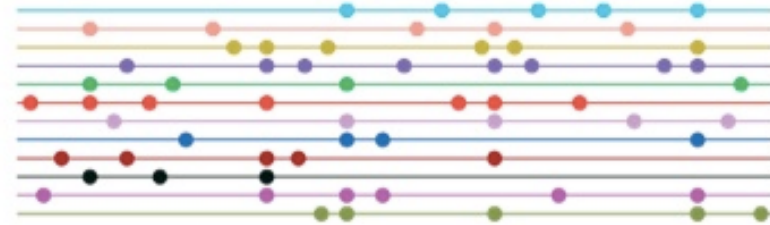
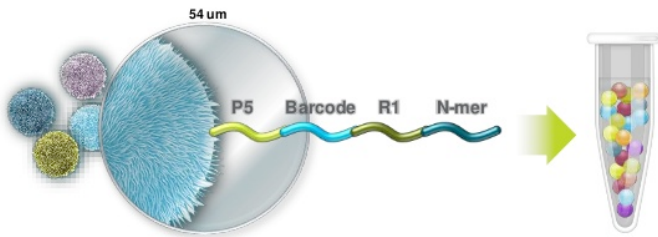
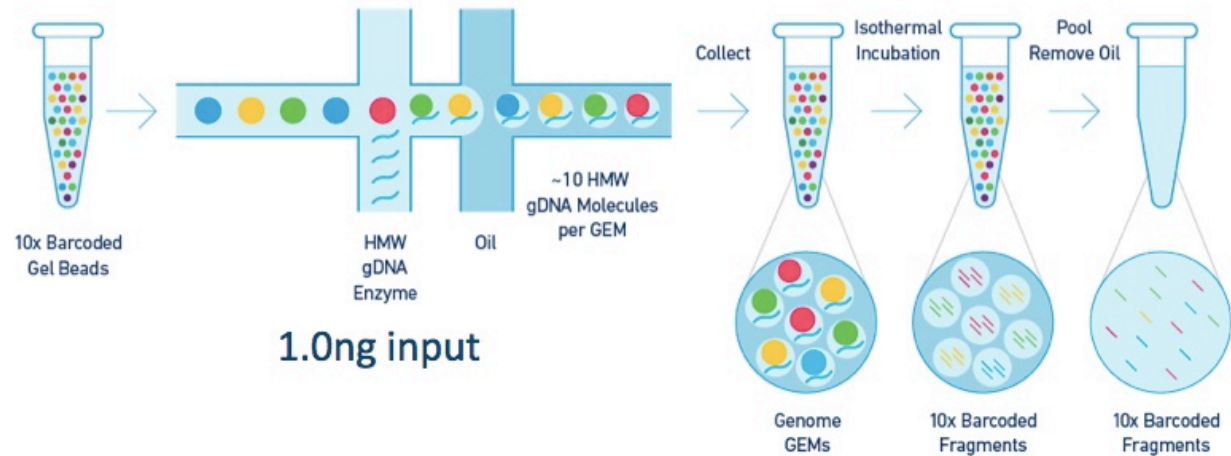
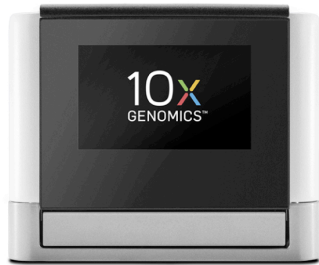
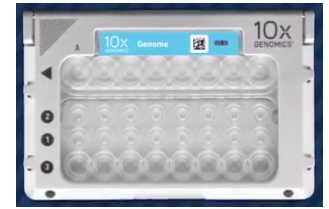
Ultra-long reads
FAST throughput

Long amplicons
Re-sequencing
De novo sequencing
Novel isoform discovery
Fusion transcript analysis
Haplotype phasing
Clinical samples



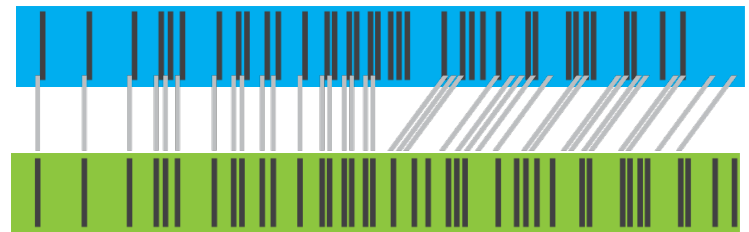
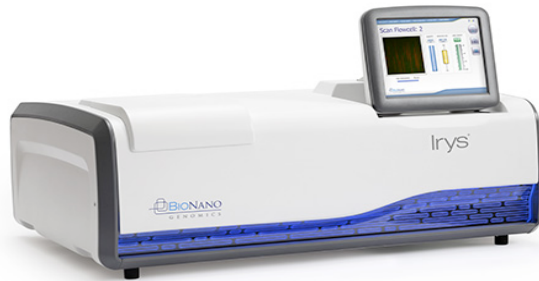
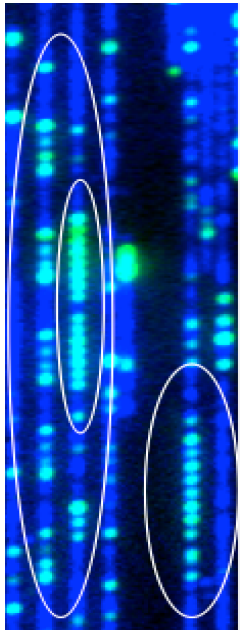
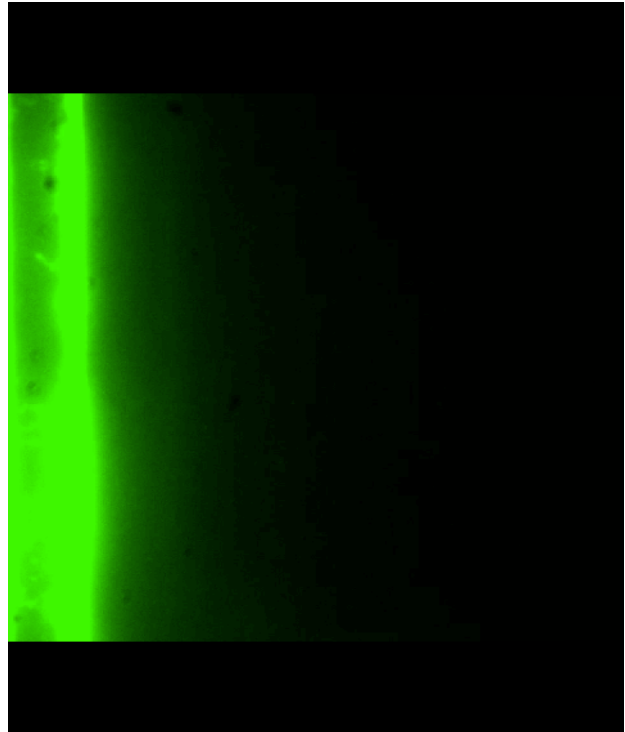
But there is more!

10x Genomics (Chromium)



Fragment length: 50 kb – 100+ Kb

BioNano Genomics (Irys)



Fragment length: 100 kb – 3 Mb

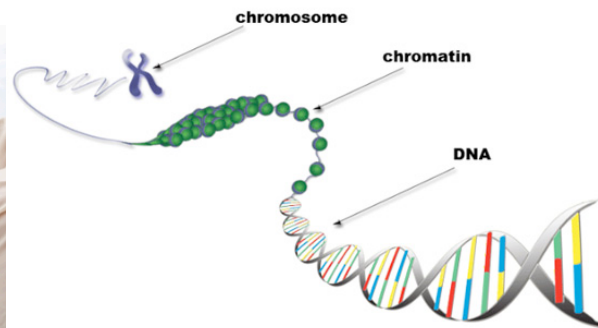
SAMPLE QUALITY REQUIREMENTS

Sample prep: take home message

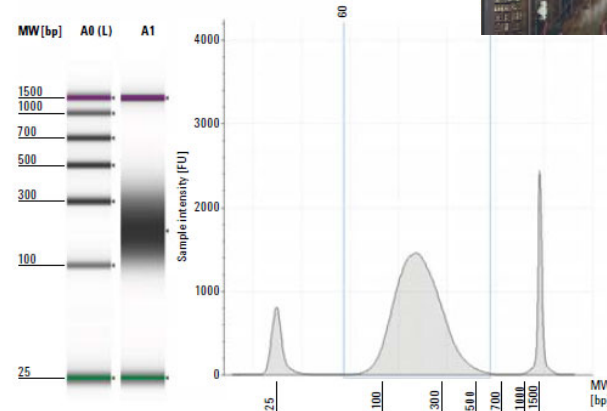
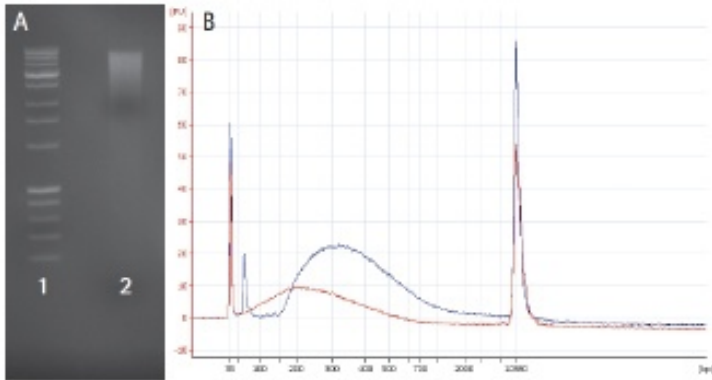
PCR-quality sample and

NGS-quality sample

are two completely different things

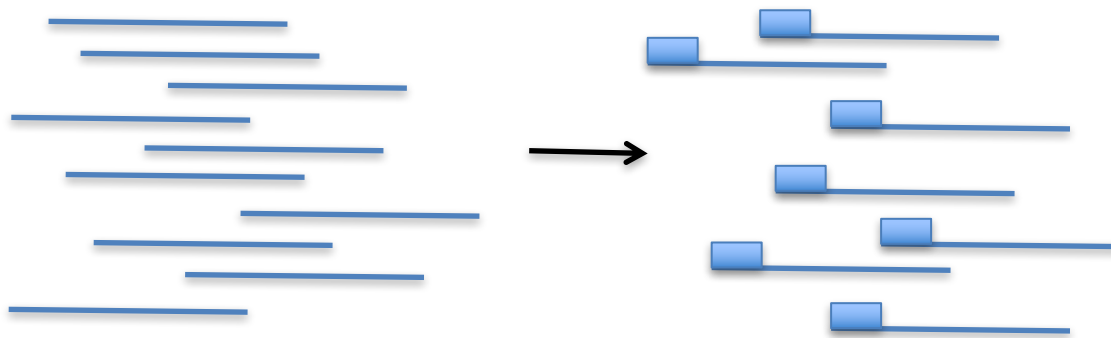


Making an NGS library



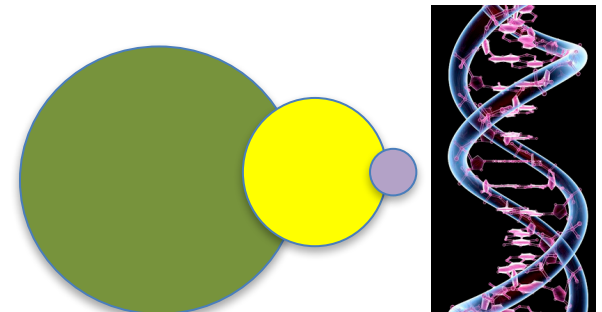
DNA QC – **paramount importance**

Sharing & size selection

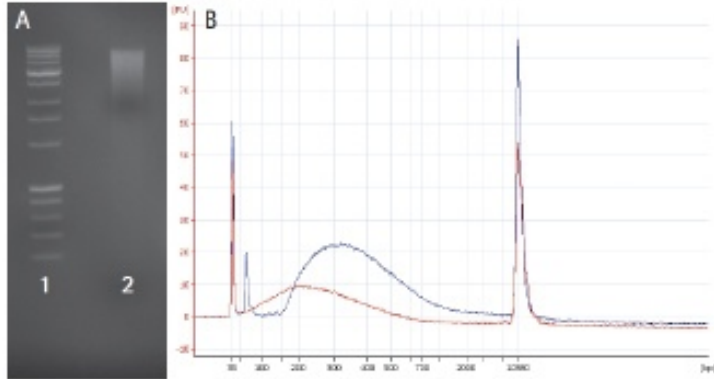


Amplification

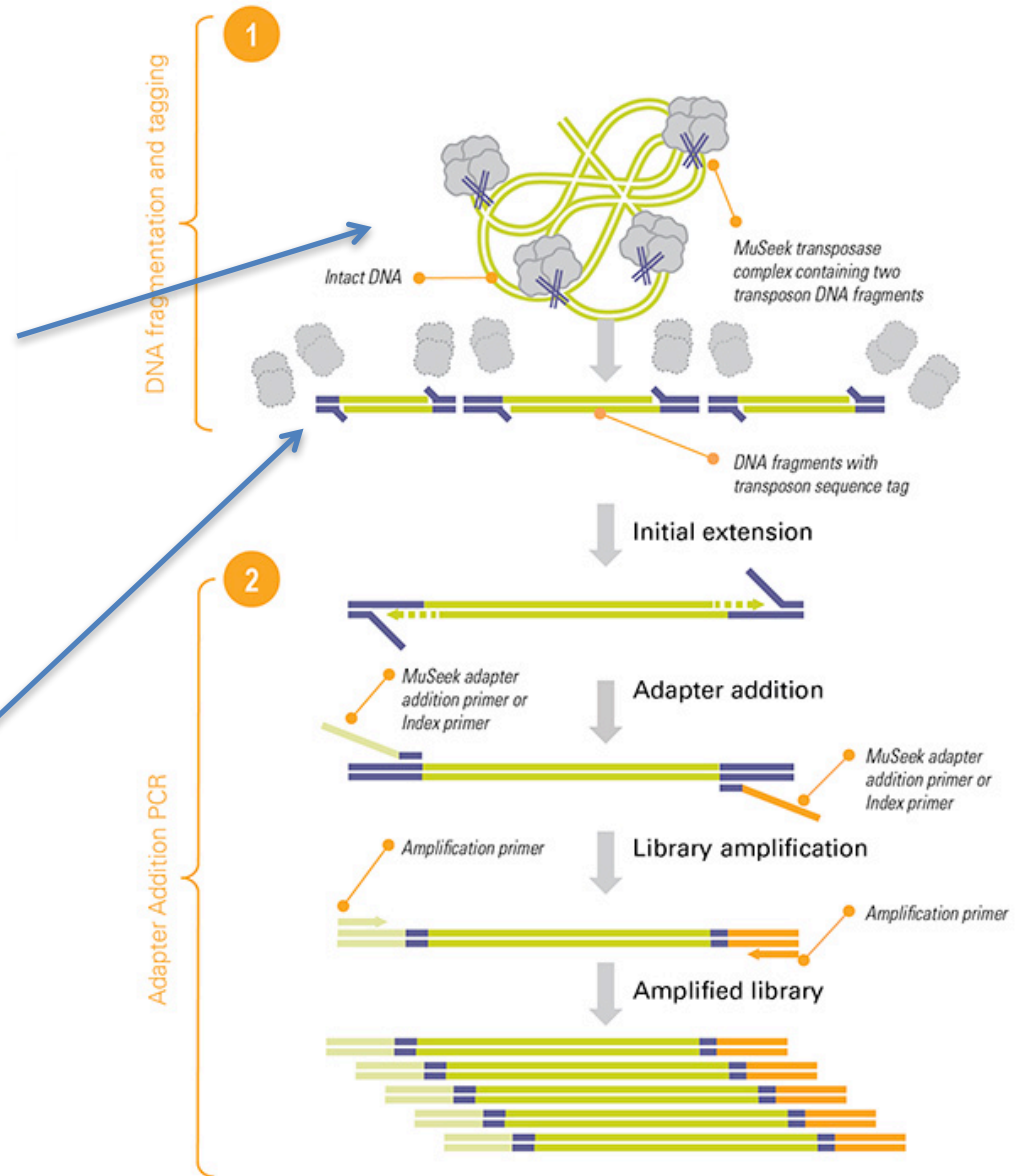
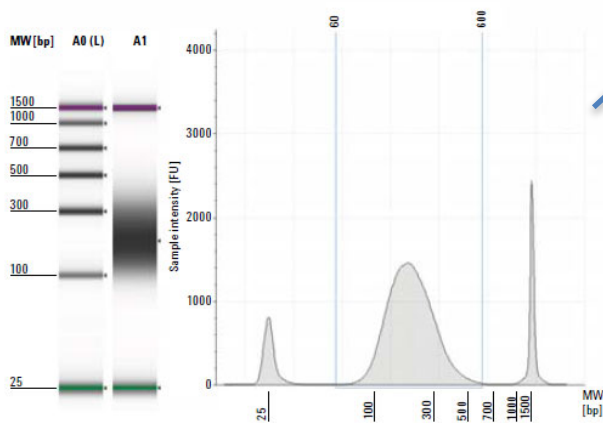
Ligation of sequencing adaptors, technology specific



NGS library

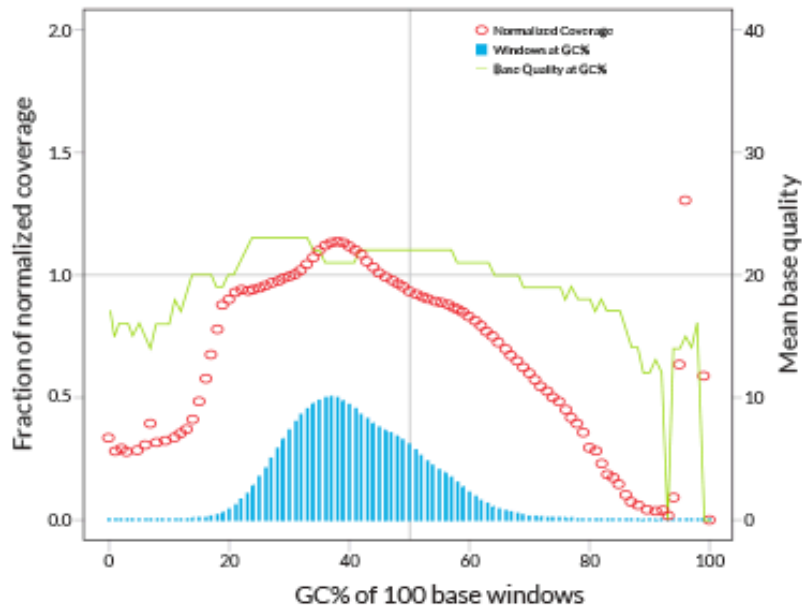


DNA QC – **paramount importance**

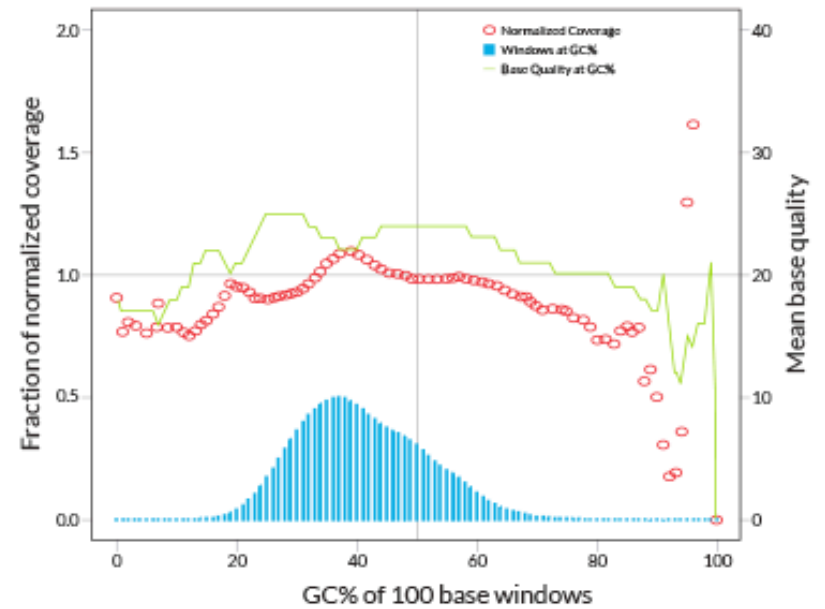


Sharing & size selection

Library complexity

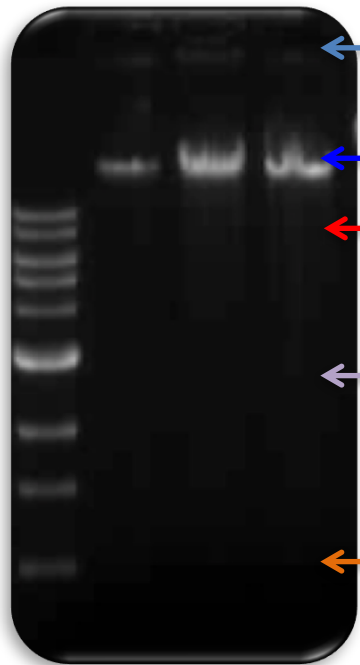


Suboptimal sample

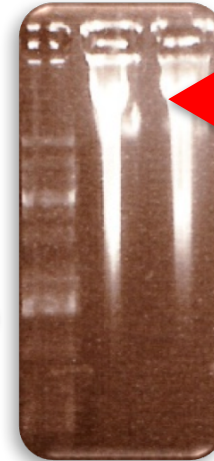


Good sample

DNA quality requirements



- Some DNA left in the well
- Sharp band of 20+kb
- No sign of proteins
- No smear of degraded DNA
- No sign of RNA



NanoDrop:

$260/280 = 1.8 - 2.0$

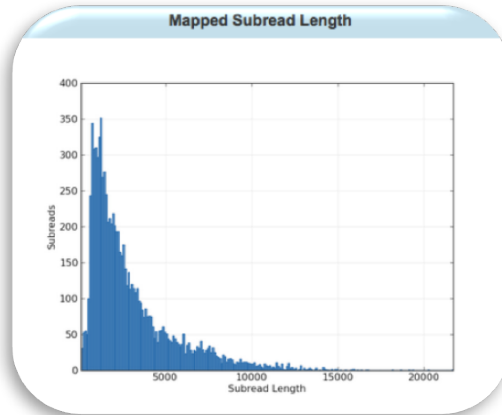
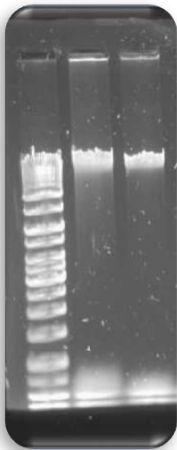
$260/230 = 2.0 - 2.2$

Qubit or Picogreen:

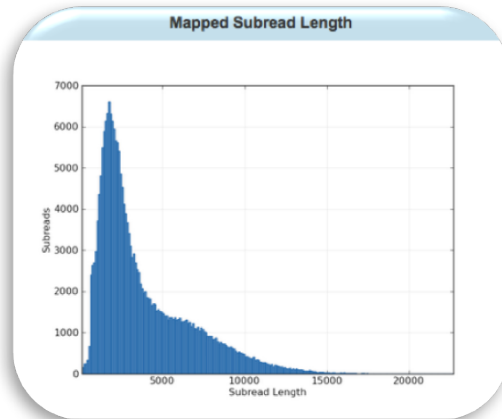
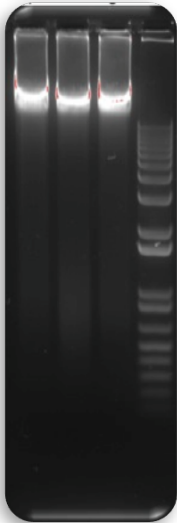
10 kb insert libraries: 3-5 ug

20 kb insert libraries: 10-20 ug

Example:



Polished Contigs	223	Max Contig Length	36,298
N50 Contig Length	2,932	Sum of Contig Lengths	480,087



Polished Contigs	9	Max Contig Length	1,508,929
N50 Contig Length	1,353,702	Sum of Contig Lengths	7,813,244

What do absorption ratios tell us?

Pure DNA 260/280: 1.8 – 2.0

< 1.8:

Too little DNA compared to other components of the solution; presence of organic contaminants: proteins and phenol; glycogen - **absorb at 280 nm**.

> 2.0:

High share of RNA.

Pure DNA 260/230: 2.0 – 2.2

<2.0:

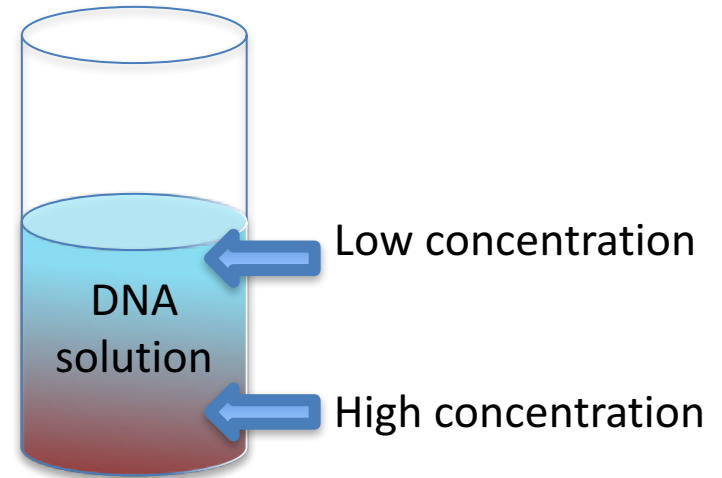
Salt contamination, humic acids, peptides, aromatic compounds, polyphenols, urea, guanidine, thiocyanates (latter three are common kit components) – **absorb at 230 nm**.

>2.2:

High share of RNA, very high share of phenol, **high turbidity**, dirty instrument, wrong blank.

*Photometrically active contaminants:
phenol, polyphenols, EDTA, thiocyanate, protein,
RNA, nucleotides (fragments below 5 bp)*

How to make a correct measurement

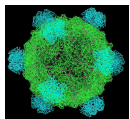


- Thaw DNA completely
- Mix gently (**never vortex!**)
- Put the sample on a thermoblock: 37°C, 15-30 min
- Mix gently
- **Dilute 1:100** (if HMW)
- Mix gently
- Make a measurement with an appropriate blank

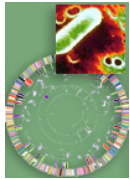
- **NANODROP is Bad.** Point.
- Use Qubit, or PicoGreen.

Let's get philosophical

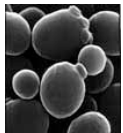
Since the beginning of Genomics:



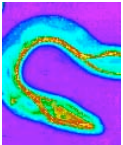
First genome: virus ϕ X 174 - 5 368 bp (1977)



First organism: *Haemophilus influenzae* - 1.5 Mb (1995)



First eukaryote: *Saccharomyces cerevisiae* - 12.4 Mb (1996)



First multicellular organism: *Cenorhabditis elegans* - 100 MB (1998-2002)



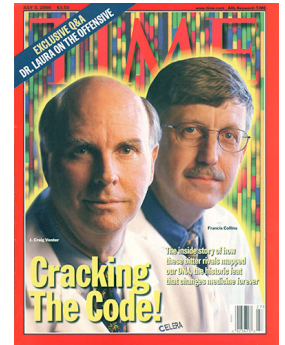
First plant: *Arabidopsis thaliana* - 157 Mb (2000)

... prices go down

Human genome sequencing:

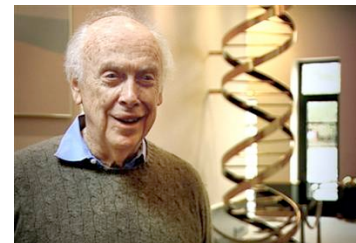
2004: Genome of Craig Wenter costs 70 mln \$

- Sanger's sequencing



2007: Genome of James Watson costs 2 mln \$

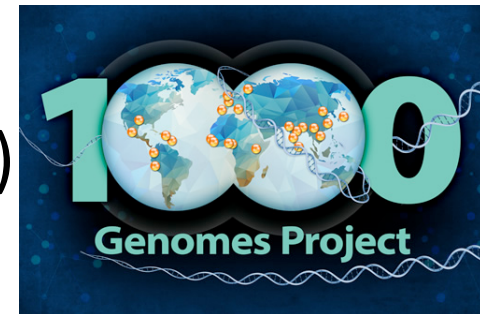
- 454 pyrosequencing



2014: Ultimate goal: 1000 \$ / individual

2016: Illumina Xten: Almost there! (1200 \$)

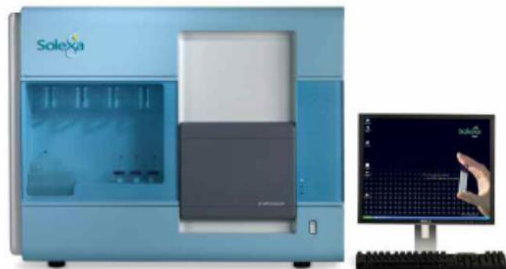
2017: NovaSeq: "Hold my beer..." (100 \$)



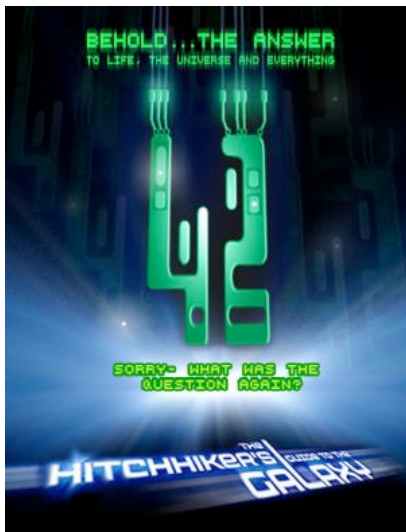


... paradigm changes

- From single genes to complete genomes
- From single transcripts to whole transcriptomes
- From single organisms to complex metagenomic pools
- From model organisms to the species you are studying
- Personal genome = personalized medicine



... scientific value diminishes



Science 5 September 1997:
Vol. 277 no. 5331 pp. 1453-1462
DOI: 10.1126/science.277.5331.1453

IF 31.6

[< Prev](#) | [Table of Contents](#) | [Next >](#)

ARTICLES

The Complete Genome Sequence of *Escherichia coli* K-12

Frederick R. Blattner^{*}, Guy Plunkett III^{*}, Craig A. Bloch, Nicole T. Perna, Valerie Burland, Monica Riley, Julio Collado-Vides, Jeremy D. Glasner, Christopher K. Rode, George F. Mayhew, Jason Gregor, Nelson Wayne Davis, Heather A. Kirkpatrick, Michael A. Goeden, Debra J. Rose, Bob Mau and Ying Shao

Journal of Biotechnology
Article in Press, Corrected Proof - Note to users

IF 2.9



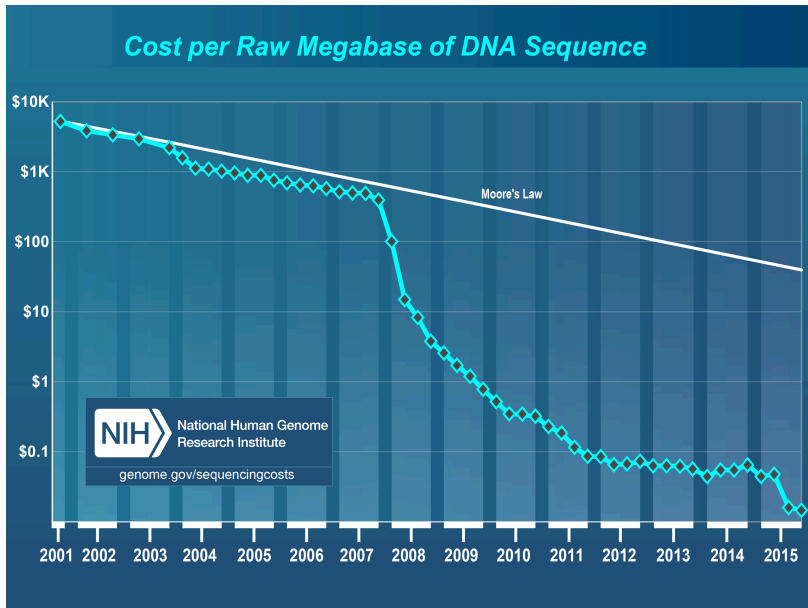
doi:10.1016/j.jbiotec.2010.12.018 | [How to Cite or Link Using DOI](#)

[Permissions & Reprints](#)

The complete genome sequence of the dominant *Sinorhizobium meliloti* field isolate SM11 extends the *S. meliloti* pan-genome

Susanne Schneider-Bekel^a, Daniel Wibberg^a, Thomas Bekel^b, Jochen Blom^b, Burkhard Linke^b, Helko Neuweger^b, Michael Stiens^{a, c}, Frank-Jörg Vorhölter^a, Stefan Weidner^a, Alexander Goesmann^b, Alfred Pühler^a and Andreas Schlüter^a, , 

... demand for bioinformatics and data storage is unprecedented



2007:

"If the data problem is not addressed, ABI's SOLiD, 454's GS FLX, Illumina's GAII or any of the other deep sequencing platforms will be destined to sit in their air-conditioned rooms like a Stradivarius without a bow."

<http://finchtalk.geospiza.com>

Big Data: Astronomical or Genomical?

Zachary D. Stephens, Skylar Y. Lee, Faraz Faghri, Roy H. Campbell, Chengxiang Zhai, Miles J. Efron, Ravishankar Iyer, Michael C. Schatz , Saurabh Sinha , Gene E. Robinson

Published: July 7, 2015 • <https://doi.org/10.1371/journal.pbio.1002195>

By 2025, between 100 million and 2 billion human genomes could have been sequenced. The data-storage demands for this alone could run to as much as 2–40 exabytes (1 exabyte is 10^{18} bytes).

Stay tuned!

NGI SEMINAR SERIES

Epigenetics

The National Genomics Infrastructure (NGI) hosted by SciLifeLab is welcoming you to register for a half-day event given within the new NGI series of scientific symposia. An opportunity to interact, meet experts, get inspired, and learn more about the latest advances in the broad range of Next-Generation Sequencing (NGS) and genotyping technologies offered at NGI, this time focusing solely on epigenetic research.

Program	Welcoming remarks
13:00	Joakim Lundberg, director of NGI
13:05	Introduction Presentation of available sequencing and genotyping services at National Genomics Infrastructure for epigenetic studies.
13:45	Keynote speaker: Elin Grumdborg <i>Capture the Human Epigenome by High-Throughput Sequencing Technologies for Insight Into Common Disease Risk</i> Associated professor at McGill University in Montreal, Canada. She is a co-author of several Nature Genetics, Nature and PLoS Genetics papers addressing the role of epigenetic changes on different aspects of human health. Several of her publications are based on genotyping, transcriptome and methylation analysis by means of next NGS and genotyping arrays.
14:15	Coffee and poster session
14:40	Åsa Johansson, Uppsala University <i>Variation in DNA methylation in a human population</i>
15:00	Dominic Wright, Linköping University <i>Mapping methylation and gene expression variation in the chicken</i>
15:20	Christopher Wheat, Stockholm University <i>Patterns of methylation underlying aging in a butterfly</i>
15:40	Karl Ekwall, Karolinska Institutet <i>Tot</i>
16:00	Snacks and poster session

When
27 October

Where
SciLifeLab Stockholm
Conference room Åky Fine
Tomtebodavägen 23A, Solna



More information and registration at www.scliflab.se

The NGI Seminar series is a new initiative by NGI to provide researchers in Sweden the opportunity to interact, meet experts, get inspired, and learn more about next-generation sequencing (NGS) and genotyping technologies through theme-based half-day events.



The National Genomics Infrastructure Sweden (NGI) is hosted by Science for Life Laboratory (S4L) (SciLifeLab). NGI is supported by SciLifeLab, the Swedish Research Council (Forskningsrådet), VTI and four universities (U, KTH, SU, UG).

NGI Seminar Series

Metagenomics, metabarcoding and eDNA

The National Genomics Infrastructure (NGI) is welcoming you to register for a half-day event given within the NGI series of scientific symposia: an opportunity to interact, meet experts, get inspired, and learn more about the latest advances in the broad range of technologies offered at NGI, this time focusing solely on metagenomic research.

Program	Welcoming remarks
13:00	Introduction
13:05	Olof Vinster Pettersson, NGI
13:35	Introduction Presentation of available sequencing services at NGI for metagenomics and eDNA studies.
13:35	eDNA Networks
13:45	SLU Metabarcoding lab
13:45	Åsa Olsson, SLU
13:45	Keynote speaker: Thijs Ettema, UU Thijs Ettema, who obtained a doctoral degree at Wageningen University, focuses his research on exploring biodiversity of microbial communities using the latest technological advances. One of the main topics of research of Thijs and his colleagues is to shed light upon early evolution of the Three domains of life and emergence of the eukaryotic cell.
14:40	Coffee and poster session
15:00	Topic Water: Anders Andersson, KTH
15:20	Topic Soil: Karina Engdahlbeck Clemmensen, SLU
15:40	Topic Animal Health: Oskar Karlsson, SLU
16:00	Mingle and poster session

When
11 Maj

Where
BMC, Uppsala
seminars@bmc.uu.se
Entrance A11
from Ög. Hammersköldsväg



More information and registration at ngiseminars.wixsite.com/outreachvt2017

The NGI Seminar series is a new initiative by NGI to provide researchers in Sweden the opportunity to interact, meet experts, get inspired, and learn more about next-generation sequencing (NGS) and genotyping technologies through theme-based half-day events.



The National Genomics Infrastructure Sweden (NGI) is hosted by Science for Life Laboratory (S4L) (SciLifeLab). NGI is supported by SciLifeLab, the Swedish Research Council (Forskningsrådet), VTI and four universities (U, KTH, SU, UG).



NGI Seminar Series

Long-read workshop in Uppsala

2017: December 6-7

Long-Read Single-Molecule Sequencing at NGI - SciLifeLab

March 17-18
Navet, BMC
Uppsala



Long Read Single-Molecule Real-Time (SMRT) Sequencing

November 16-17
Navet, BMC
Uppsala

It is with great pleasure we announce the second SMRT meeting to take place on **November 16-17** in **Uppsala**, aiming to provide information about state-of-the-art **PacBio** applications, as well to inspire the scientific community to apply advances of **SMRT** technology in research!

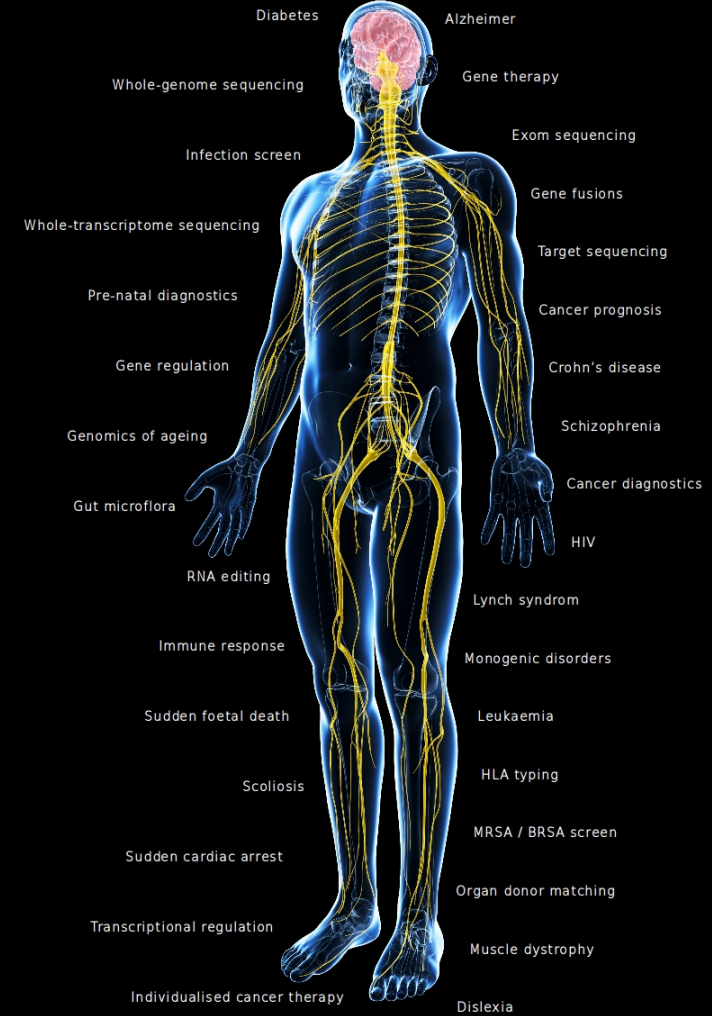
For more information please visit: <https://goo.gl/YMu2SO>

Registration form: <https://goo.gl/forms/VvFpOF5hbdsz3Lac2>

OR scan the QR codes:



What we sequenced at SciLifeLab



16S amplicon, Acinetobacter baumannii, Acrasis kona, Acridotheres javanicus, Actinobacillus succinogenes, African swine fever virus, Agaricomycota sp., **Alces alces**, **Alligator mississippiensis**, Amphipura filiformis, Apis mellifera, Aquila chrysaetos, Arabidopsis thaliana, Arabis alpina, Archaeorhizomycetes finlayi, Arctocepalus gazella, artificial sequences, Arvicola amphibius, Ascaridia galli, Aspergillus oryzae, Astrapla stephaniae, Atlantic herring, Atlantic salmon, **Avena sativa**, Baccharis brevifolia, Baccharis dracunculifolia, Bacteriophages, Balaenoptera musculus, Balaenoptera physalus, Balanus improvisus, Baltic Sea microorganisms, Bathynomus sp., Bifidobacterium sp., **Borrelia burgdorferi**, Borrelia garinii, Bos taurus, Bovine viral diarrhoea virus, Brachyspira suanatina, Brassica sp., Brettanomyces naardensis, Caenorhabditis elegans, Callosobruchus maculatus, Candida intermedia, Candida parapsilosis, Candidatus Neoehrlichia mikurensis, **Canis lupus**, Caprellus caprellus, Capsella bursa-pastoris, Capsella grandiflora, Capsella orientalis, Capsella rubella, Ceanothus thyrsiflorus, Cervus dama, Cervus elaphus, Chlidia submaculatum, Clonostachys rosea, Clostridium ultunense, Coelodonta antiquitatis, Colla crocea, Collinsia heterophylla, Coregonus lavaretus, Coronavirus, Corvus corone, Corvus monedula, Crassostrea gigas, Cricetulus griseus, Cryptococcus tephrensis, Cubanola domingensis, Cytopegalovirus, Danio rerio, Datisca glomerata, Deformed wing virus, Dekkera bruxellensis, Dicerorhinus sumatrensis, Dictyostelium discoideum, Diophtroporus gymnothoracis, Diophtroporus longitubus, Drosophila melanogaster, Drosophila pallidistoma, Electrophorus electricus, Enterobacter cloacae, Enterococcus faecium, **Equus caballus**, Escherichia coli, Eumecynostomum macrobursatum, Euphorbia lathyris, Euphorbia peplis, Euplectes afer, Euplectes ardens, Euplectes aeneus, Euplectes hordeaceus, Euplectes macrorhis, Euplectes orix, **Felis catus**, Ficedula albicollis, Ficedula hypoleuca, **Fragaria ananassa**, Freshwater microbial communities, Fucus radicans, Fucus vesiculosus, Fumaria sp., Galarrucella, Gallus gallus, Geopiza magnirostris, Glardia muris, Globodera rostochiensis, Gnetum gnemon, Gnetum luofuense, Gnetum montanum, Gnetum pendulum, Gonystomum semen, Gonzalagula, Gut microbiota, Hamelia marantha, Heterobasidium abietinum, Heterobasidium annosum, **Hippophae rhamnoides**, **Homo sapiens**, Human Immunodeficiency Virus, Huperzia selago, Hymenoscyphus albidus, Hymenoscyphus pseudoalbidus, Idotea baltica, **Influenza A virus**, Klebsiella pneumoniae, Laccaria bicolor, Lactobacillus, Lepidium campestre, **Leptidea sinapis**, Letharia rugosa, Letharia vulpina, Littorina saxatilis, Lycocorax pyrropterus, **Lynx lynx**, Malassezia sympodialis, **Malus domestica**, Malus sylvestris, **Mammuthus primigenius**, Marchantia polymorpha, Marine bacteria whole community, **Meligethes aeneus**, Methanococcus, Methanococcus sp., Metschnikowia andauensis, Metschnikowia hawaiiensis, Metschnikowia lophurensis, Metschnikowia pulcherrima, Metschnikowia saccharicola, Mixorhis gularis, Moorella thermoacetica, Mus musculus, Mycobacterium malmoense, Mycobacterium marinum, **Mytilus edulis**, Nemertoderma westbladi, Nesophontes sp., Neurospora crassa, Neurospora hispaniola, Neurospora intermedia, Neurospora metzenbergii, Neurospora perkinsi, Neurospora sitophila, Neurospora tetrasperma, Nora Virus, Nothoprocta ornata, Nothoprocta perdicaria, **Notophthalmus viridescens**, Nyctereutes procyonoides, Ogataea pini, **Oryctolagus cuniculus**, Rana arvalis, **Oryzias latipes**, **Pacifastacus leniusculus**, Paenibacillus polymyxa, **Panthera leo**, Panthera pardus, Paradisaea rubra, Parus major, Passer montanus, Paxillus involutus, Penidillium sp., **Pera fluviatilis**, Peridinium adiculiferum, Philomachus pugnax, Phoca sibirica, Phylloscopus collybita, Phylloscopus trochilus, Wolbachia persica, Physcomitrella patens, Phytophthora infestans, **Picea abies**, Pteris napa, Pteris rapae, Pinus pinaster, Pinus sylvestris, **Pisum sativum**, Planctomyces sp., Plasmidophora brassicae, Plasmidium falci-parum, Podospora anserina, Polystachya paniculata, Pomatoschistus minutus, Populus maximowiczii, Populus tremula, Populus trichocarpa, Pseudoherosia sp., Pseudomonas aeruginosa, Pseudomonas brassicacearum, Pseudomonas chlororaphis, Pseudomonas putida, Pteridophora alberti, Ptiloris paradiseus, Puccinia striformis, Pythium oligandrum, Qualea qualea, Rangifer tarandus, Rattus rattus, Rhizoctonia sp., Saccharomyces cerevisiae, Salix purpurea, Salix viminalis, Salmonella enterica, Salmonella typhimurium, Salmo salar, Salmo trutta, Schizophyllum commune, Schizosaccharomyces pombe, Scrippsiella hangoei, **Semibalanus balanoides**, Setaria digitata, Silene conoidea, Silene latifolia, Silene viscaria, Sindbis virus, Siphocampylus, Siphocampylus retrorsus, Siphoviridae phage, Skeletonema marinoi, **Solanum tuberosum**, Sorghum sp., Spiroplasma karyanum, Spiroplasma salmonicida, Spiroplasma vortense, Staphylococcus aureus, Staphylococcus pseudintermedius, Stemmadienia sp., Streptococcus pneumoniae, Streptococcus pyogenes, Streptomyces coelicolor, Struthio camelus, Sulfobolus acidodanians, **Sus scrofa**, Synthetic DNA, Syntrophactinicus schinkii, Taphrina betulina, Tepidanaerobacter acetatoxidans, Thammolia vermicularis, Thelazia parva, Trypanosoma cruzi, Trypanosoma rangeli, **Ursus spelaeus**, Vitex agnus-castus, Yarrowia lipolytica, Zalophus californianus, Zalophus wollebaeki, Zygotetium crinitum, Zygosaccharomyces bailii



SciLifeLab

SciLifeLab

TECHNOLOGIES & SERVICES ▾

RESEARCH ▾

EDUCATION ▾

COLLABORATION ▾

Find more information and search for what you need on the page for Technologies & Services

What is the difference between national and regional facilities?

🔍 Search for Technologies & Services

National facilities

Affinity Proteomics

Biobank Profiling
Cell Profiling
Fluorescence Tissue Profiling
PLA Proteomics
Protein and Peptide Arrays
Tissue Profiling

Bioimaging

Advanced Light Microscopy
Fluorescence Correlation Spectroscopy

Bioinformatics

Bioinformatics Compute and Storage (UPPNEX)
Bioinformatics Long-term Support (WABI)
Bioinformatics Short-term Support and Infrastructure (BILS)

Chemical Biology Consortium Sweden

Laboratories for Chemical Biology Umeå (LCBU)
The Laboratories for Chemical Biology at Karolinska Institutet (LCBK1)
Uppsala Drug Optimization and Pharmaceutical Profiling (UDOPP)

Clinical Diagnostics

Clinical Biomarkers
Clinical Genomics
Clinical Sequencing

Drug Discovery and Development

ADME (Absorption Distribution, Metabolism Excretion) of Therapeutics (UDOPP)
Biochemical and Cellular Screening
Biophysical Screening and Characterization
Human Antibody Therapeutics
In Vitro and Systems Pharmacology
Medicinal Chemistry – Hit2Lead
Medicinal Chemistry – Lead Identification
Protein Expression and Characterization

Functional Genomics

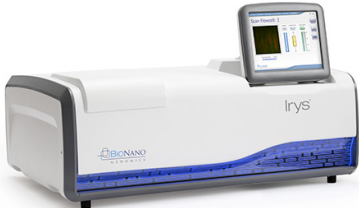
Karolinska High Throughput Center (KHTC)

National Genomics Infrastructure

NGI Stockholm (Genomics Applications)
NGI Stockholm (Genomics Production)
NGI Uppsala (SNP&SEQ Technology Platform)
NGI Uppsala (Uppsala Genome Center)

Structural Biology

Protein Science Facility



Operational principles of NGI

User community

- Open to all Swedish academic scientists on equal terms.
- Consultation and introduction of new protocols.
- Workshops, courses, etc.

Cost basis

- Academic users of NGI only cover their own agent cost.
- Staff salaries at NGI covered by SciLifeLab, VR, and host universities.
- Premises and service costs covered by SciLifeLab, VR and host universities.
- Capital equipment covered by KAW, VR, SciLifeLab.

Quality

- Emphasis on data quality and needs of the users.
- Illumina sequencing and genotyping processes accredited by SWEDAC, ISO/IEC 17025
- Ion and PacBio: accreditation due (1)

We are non-profit

**We have technology and knowledge
We want to help you to do GREAT
research**

We do not want co-authorship

Let us help YOU





Next-Generation Sequencing and Genotyping for Swedish Research

NGI Sweden Order Portal

This portal is for submitting orders for services provided by the National Genomics Infrastructure Sweden (NGI). [Edit](#)
To make an order, please log in and choose the application most suitable for your project. If uncertain about the choice of technology, please select the "Request a meeting" option. You can read more about the different technologies and [How to place an order](#) under "Information" in the menu at the top of the page.

Projects from other countries are admissible, but have lower priority than projects performed by researchers based in Sweden. Depending on the queue situation, NGI may decide to decline a non-Swedish project altogether.

Summer Order & Sample Submission Dates

All NGI facilities will be closed for sample submission over the summer from **1 July to 8 August**. To make sure you will be able to submit your samples before 1 July your order must be submitted no later than **24 June**. Orders submitted from 24 June to 8 August will not be processed until after 8 August.

Subscribe to our mailing list:

Pending accounts

Currently none.

Recently submitted orders

Al Gazali translocation	Submitted	2016-05-25 09:15:53
Neurospora spore killer CHIPseq	Submitted	2016-05-25 09:15:50
SW and lys SKD	Submitted	2016-05-25 09:09:50
		2016-05-24

Request a meeting [+ Create order](#)

If you are unsure about the appropriate method for your scientific problem, request a meeting for a discussion with us.

Illumina Sequencing [+ Create order](#)

Order form for Illumina sequencing.

Ion Sequencing [+ Create order](#)

Order form for sequencing by Ion Proton or Ion S5XL.

<https://ngisweden.scilifelab.se/>

Contact NGI

Place an order or request a meeting:

<https://ngisweden.scilifelab.se/>

NGI Stockholm Illumina

NGI Uppsala Illumina

NGI Uppsala PacBio, Ion



Email: support@ngisweden.se.

Email: seq@medsci.uu.se

Email: uppsala_orders@ngisweden.zendesk.com.

Project Coordinators:
Mattias Ormestad
Beata Werne Solnestam
Karin Gillner

Project Coordinators:
Ellenor Devine
Johanna Lagensjö

Project Coordinators:
Olga Vinnere Pettersson
Susana Häggqvist

QUESTIONS?

Pricing



Illumina MiSeq



Ion S5XL



PacBio RSII

Instrument/seq unit	Read length, bp	Mln reads /unit	Library cost, SEK	Sequencing cost, SEK
Illumina MiSeq, Flow cell (FC)	300+300	18	1100	16 000
Illumina HiSeq, Rapid run (FC)	250+250	220	1100	60 000
Ion S5XL				
chip 520	200 – 400 – 600	3	1100	6 500
chip 530	200 – 400 – 600	18	1100	7 300
chip 540	200	80	1100	7 900
PacBio RSII, SMRT cell	250 – 13 000	0,5	1800	3 000