Next Generation Sequencing – An Overview

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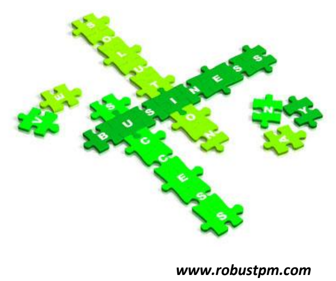
National Genomics Infrastructure hosted by ScilifeLab,
Uppsala Node (UGC)

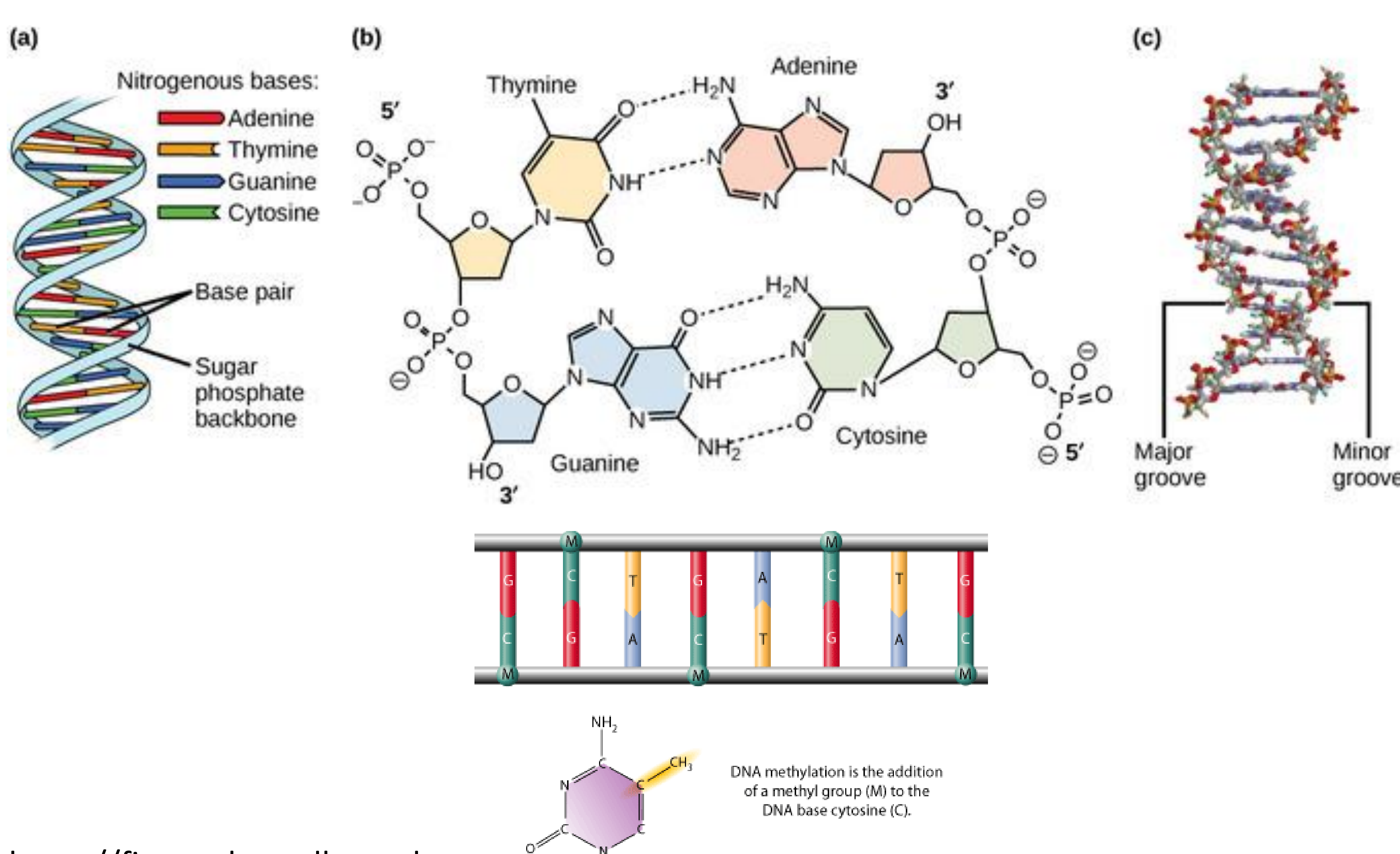
Version 6.3

Outline:



- A bit of history
- NGS technologies & sample prep
- NGS applications
- National Genomics Infrastructure – Sweden

What is sequencing?



https://figures.boundless-cdn.com

Once upon a time…

- Fredrik Sanger and Alan Coulson

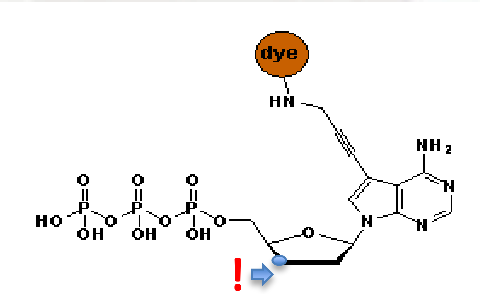
  Chain Termination Sequencing (1977)

  Nobel prize 1980

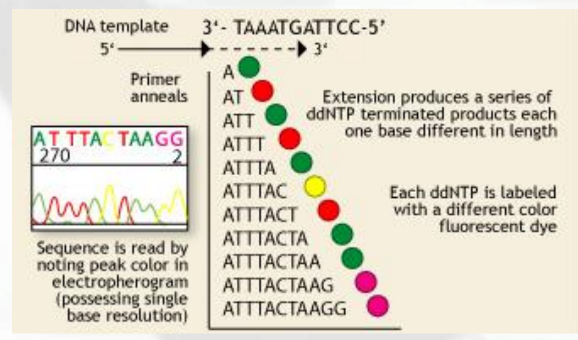
Principle:

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

nature International weekly journal of science

Journal home > Archive > Article > Full Text

Article

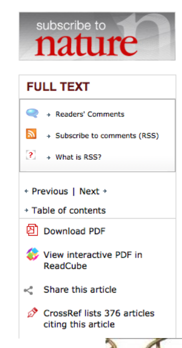
Nature **437**, 376-380 (15 September 2005) | doi:10.1038/nature03959; Received 6 May 2005; Accepted 10 June 2005; Published online 31 July 2005

There is a Corrigendum (26 January 2006) associated with this document.

There is a Corrigendum (4 May 2006) associated with this document.

Genome sequencing in microfabricated high-density picolitre reactors

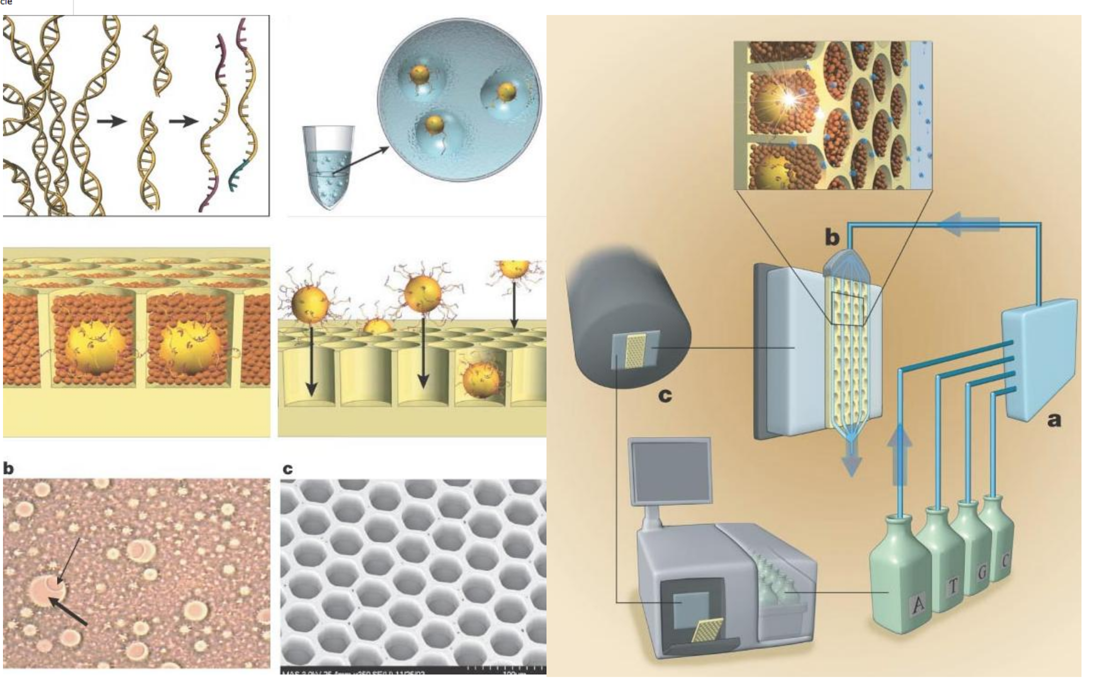
Marcel Margulies[1,5], Michael Egholm[1,5], William E. Altman[1], Said Attiya[1], Joel S. Bader[1], Lisa A. Bemben[1], Jan Berka[1], Michael S. Braverman[1], Yi-Ju Chen[1], Zhoutao Chen[1], Scott B. Dewell[1], Lei Du[1], Joseph M. Fierro[1], Xavier V. Gomes[1], Brian C. Godwin[1], Wen He[1], Scott Helgesen[1], Chun He Ho[1], Gerard P. Irzyk[1], Szilveszter C. Jando[1], Maria L. I. Alenquer[1], Thomas P. Jarvie[1], Kshama B. Jirage[1], Jong-Bum Kim[1], James R. Knight[1], Janna R. Lanza[1], John H. Leamon[1], Steven M. Lefkowitz[1], Ming Lei[1], Jing Li[1], Kenton L. Lohman[1], Hong Lu[1], Vinod B. Makhijani[1], Keith E. McDade[1], Michael P. McKenna[1], Eugene W. Myers[2], Elizabeth Nickerson[1], John R. Nobile[1], Ramona Plant[1], Bernard P. Puc[1], Michael T. Ronan[1], George T. Roth[1], Gary J. Sarkis[1], Jan Fredrik Simons[1], John W. Simpson[1], Maithreyan Srinivasan[1], Karrie R. Tartaro[1], Alexander Tomasz[3], Kari A. Vogt[1], Greg A. Volkmer[1], Shally H. Wang[1], Yong Wang[1], Michael P. Weiner[4], Pengguang Yu[1], Richard F. Begley[1] & Jonathan M. Rothberg[1]



Thousands of molecules sequenced in parallel

1 mln reads sequenced per run

Roche 454 GS FLX



Technologies

NGS technologies

Company	Platform	Amplification	Sequencing method
Roche	454 (until 2016)	emPCR	Pyrosequencing
Illumina	HiSeq, MiSeq NextSeq, X10	Bridge PCR	Synthesis
LifeTechnologies(Thermo Fisher)	Ion Torrent, Ion Proton, S5	emPCR	Synthesis (pH)
Pacific Biosciences	RSII SEQUEL	None	Synthesis (SMRT)
Complete genomics	Nanoballs	None	Ligation
Oxford Nanopore*	MinION GridION	None	Flow

RIP technologies: Helicos, Polonator, SOLiD, 454 etc.

In development: Tunneling currents, nanopores, etc.

Differences between platforms

- Technology: chemistry + signal detection
- Run times vary from hours to days
- Production range from Mb to Gb
- Read length from <100 bp to > 20 Kbp
- Accuracy per base from 0.1% to 15%
- Cost per base

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

Rd1 Seq Primer

Index Seq Primer

P5

P7

INDEX

Rd2 Seq Primer

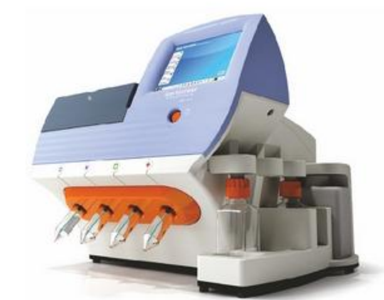
Sequence of Interest

3′

5′

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

Ion Torrent

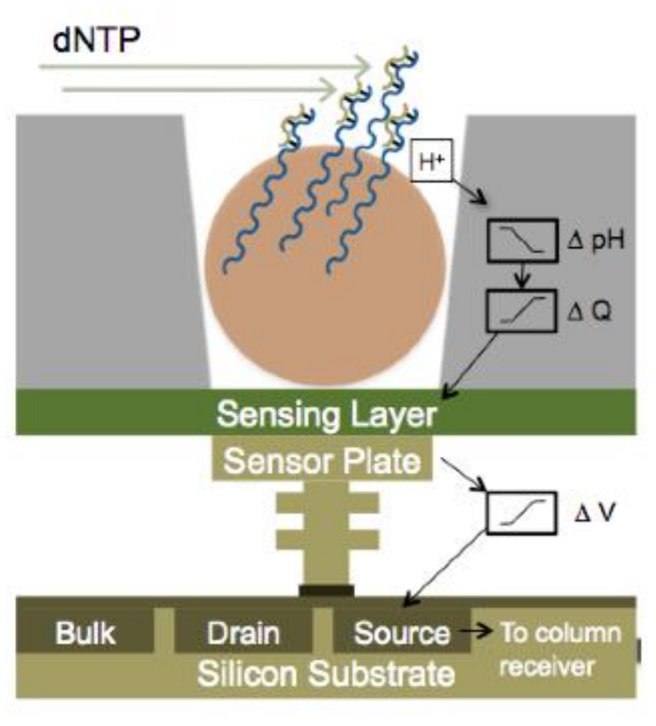


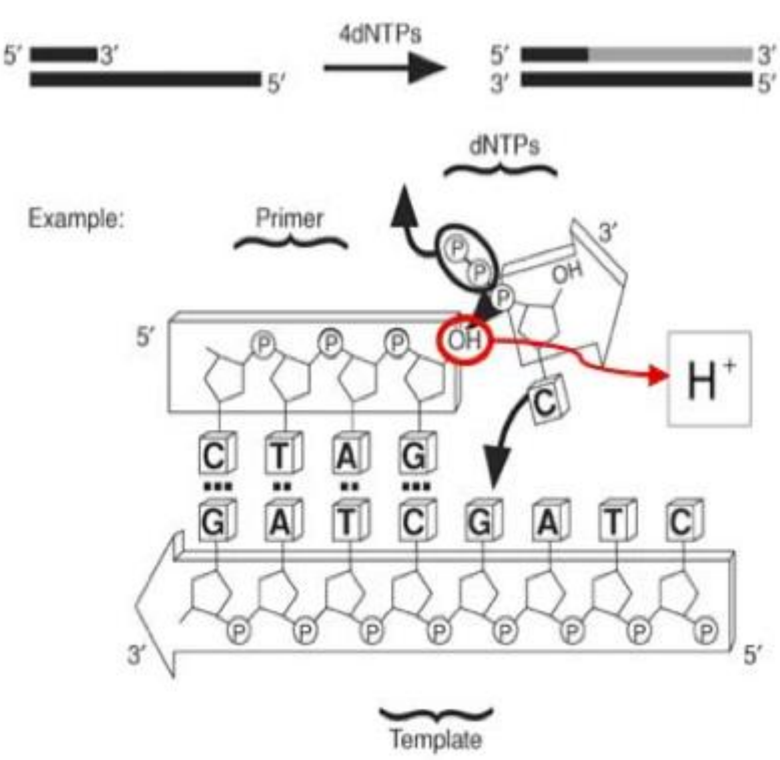
Chip	Yield - run time	Read Length
314, 316, 318 (**PGM**)	0.1 – 1 Gb Gb, 3 hrs	200 – 400 bp
P-I (**Proton**)	10 Gb 4 hrs	200 bp
520, 530, 540 (**S5**)	1 Gb – 10 Gb 3 hrs	400 (600) bp (except 540)

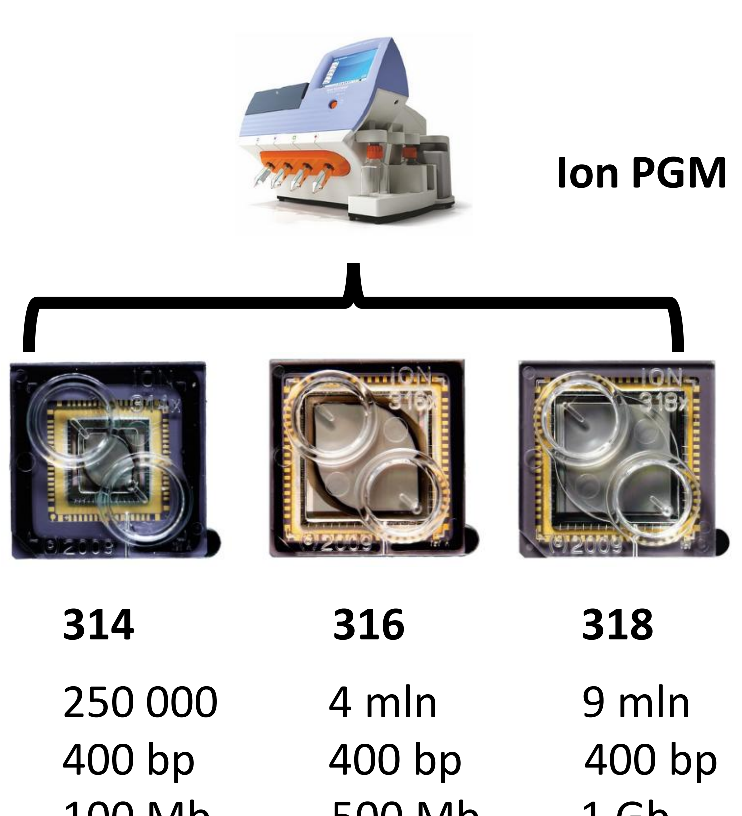
Main applications

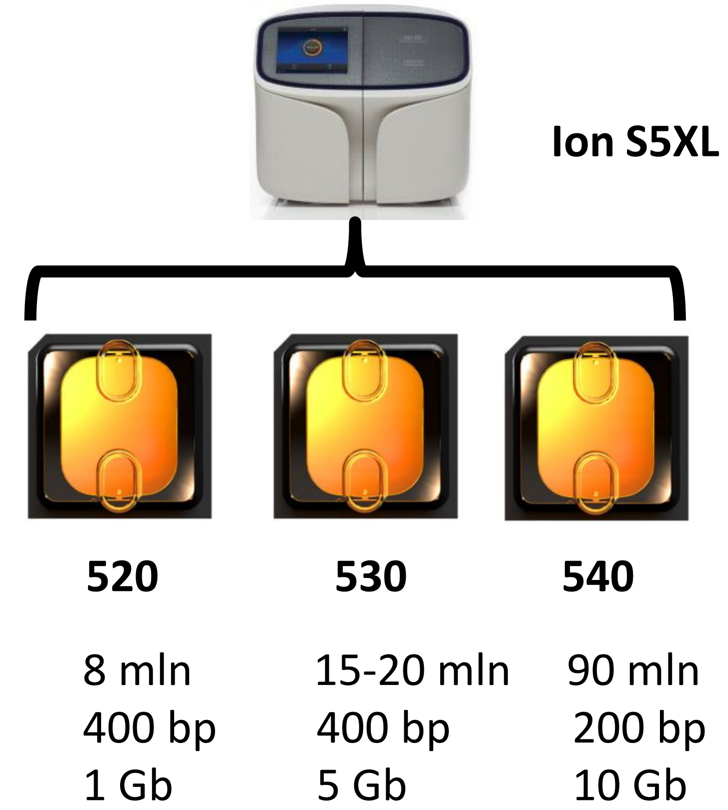
- Microbial and metagenomic sequencing
- Targeted re-sequencing (gene panels)
- Clinical sequencing

Ion Torrent - H^+ ion-sensitive field effect transistors









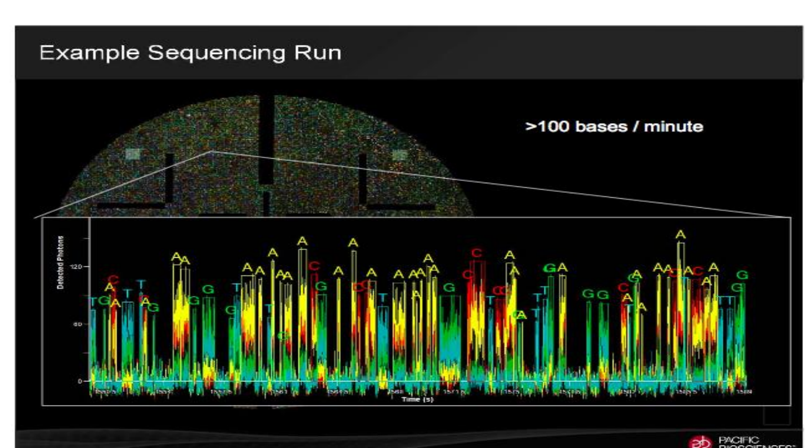
PI

90 mln
200 bp
10-18 Gb

PacBio SMRT-technology

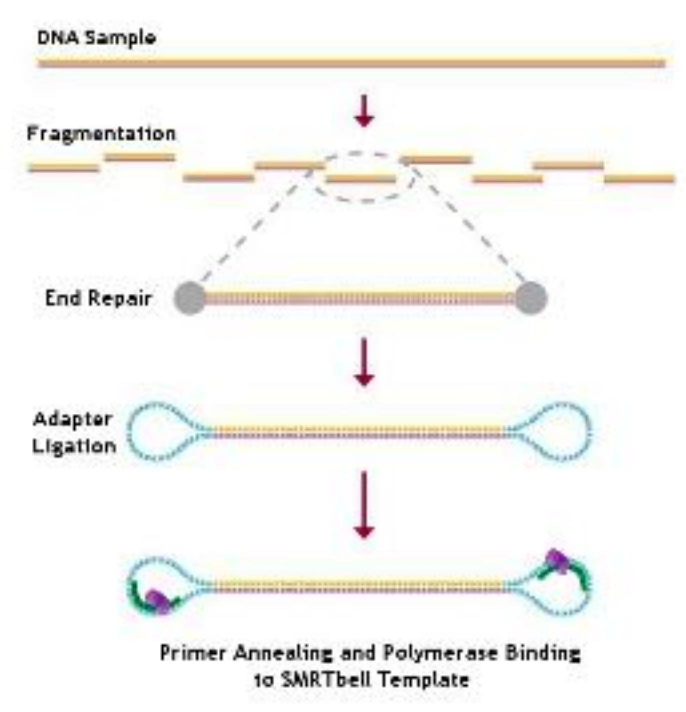
Instrument	Yield and run time	Read Length	Error rate	Error type
RS II	250 Mb – 1.3 Gb /30 - 360 min SMRTCell	250 bp – 30 kb *(74 kb)*	15% (on a single passage!)	Insertions , **random**
SEQUEL	2-6 Gb per SMRT 30-360 min	250 bp – 25 kb	as RSII	as RSII

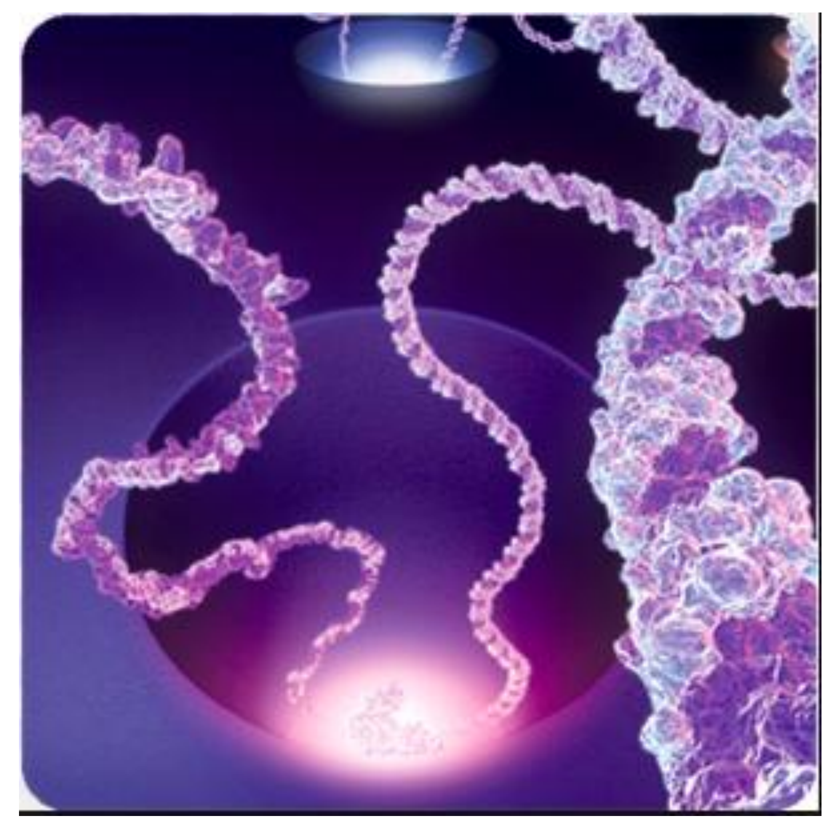
Single-Molecule, Real-Time DNA sequencing



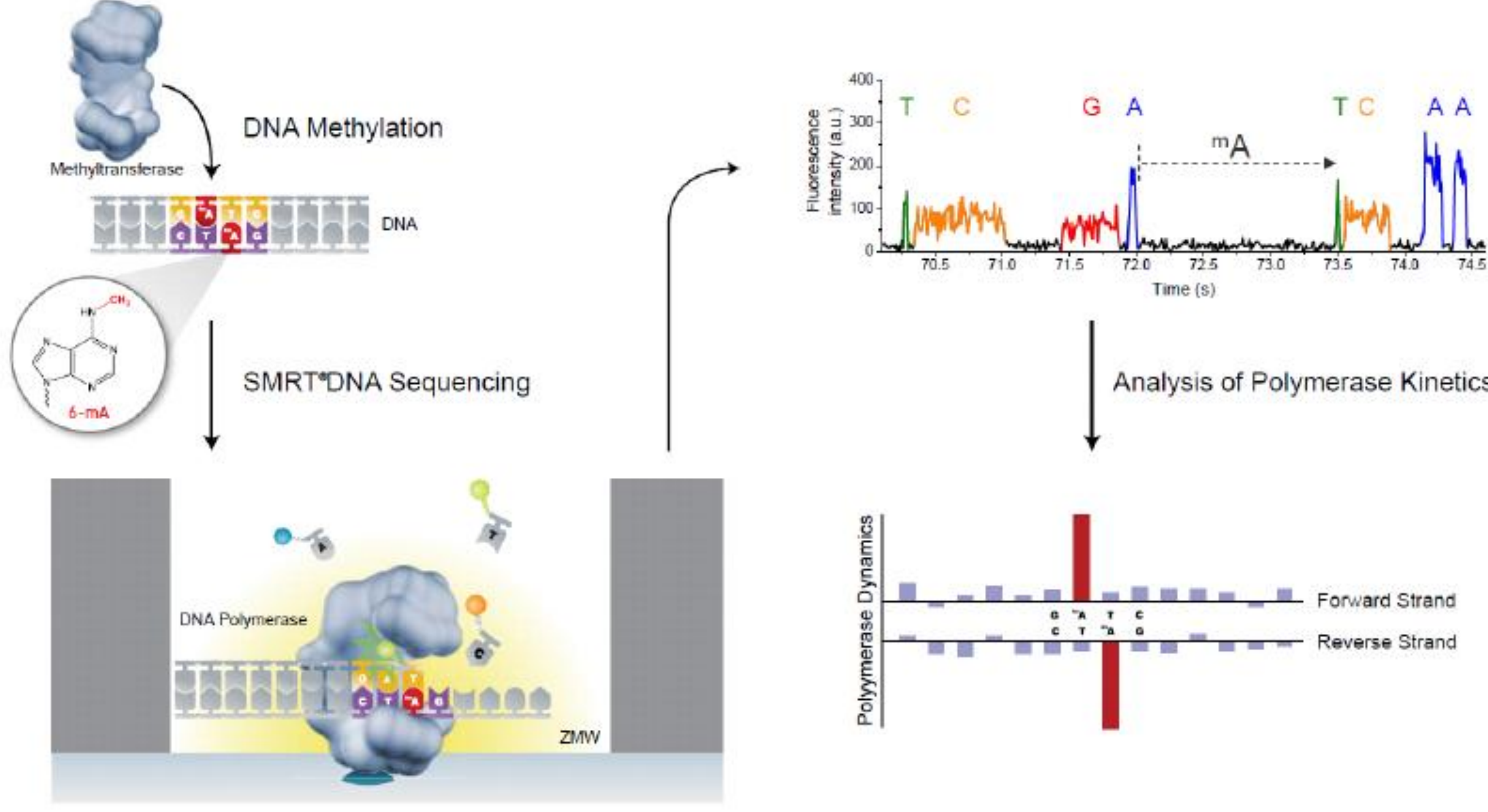
PacBio SMRT - technology

Single Molecule Real Time





Base Modification: Discover the Epigenome



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

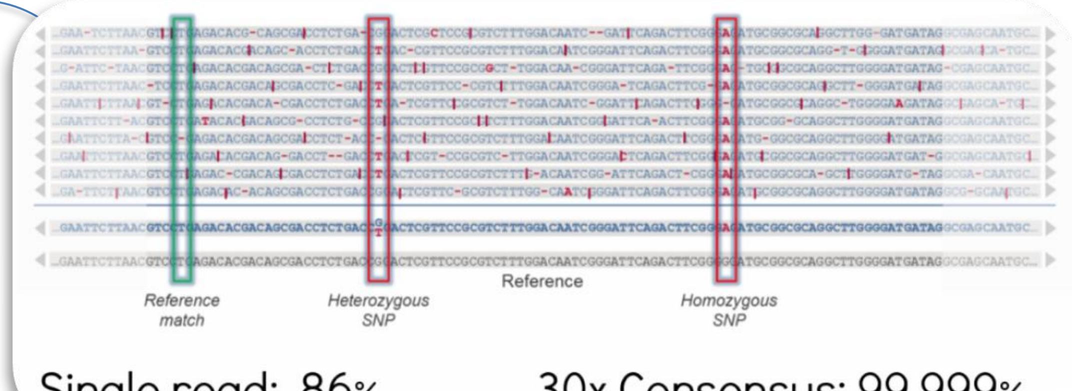
SMRT sequencing: common misconceptions

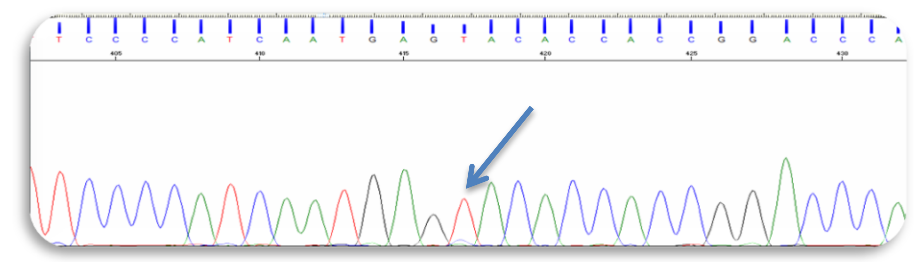
High error rate?

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

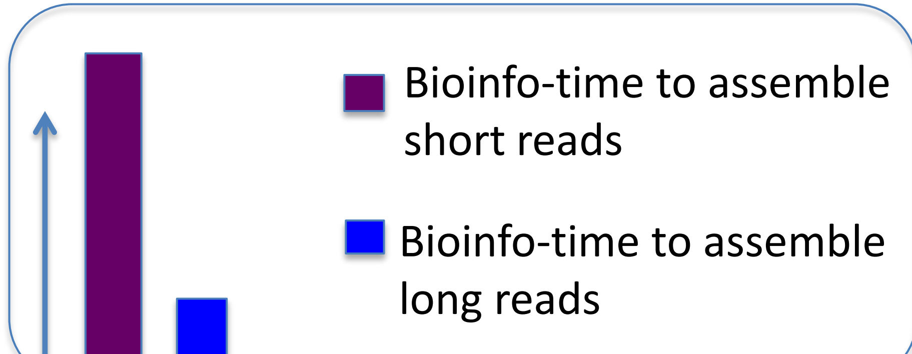




High price?

Not for small genomes

Better assembly quality
Single-molecule reads without PCR-bias



Oxford Nanopore MinION

DNA DOUBLE HELIX

❶ One protein unzips the DNA helix into two strands.

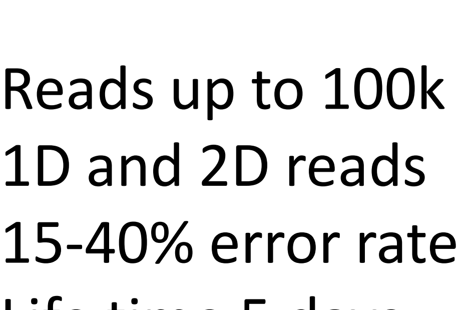
❷ A second protein creates a pore in the membrane and holds an "adapter" molecule.

❸ A flow of ions through the pore creates a current. Each base blocks the flow to a different degree, altering the current.

TGATATTGCTTTTGATGCCG

❹ The adapter molecule keeps bases in place long enough for them to be identified electronically.

MEMBRANE



Reads up to 100k
1D and 2D reads
15-40% 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

Ultra-long reads
FAST throughput

Applications

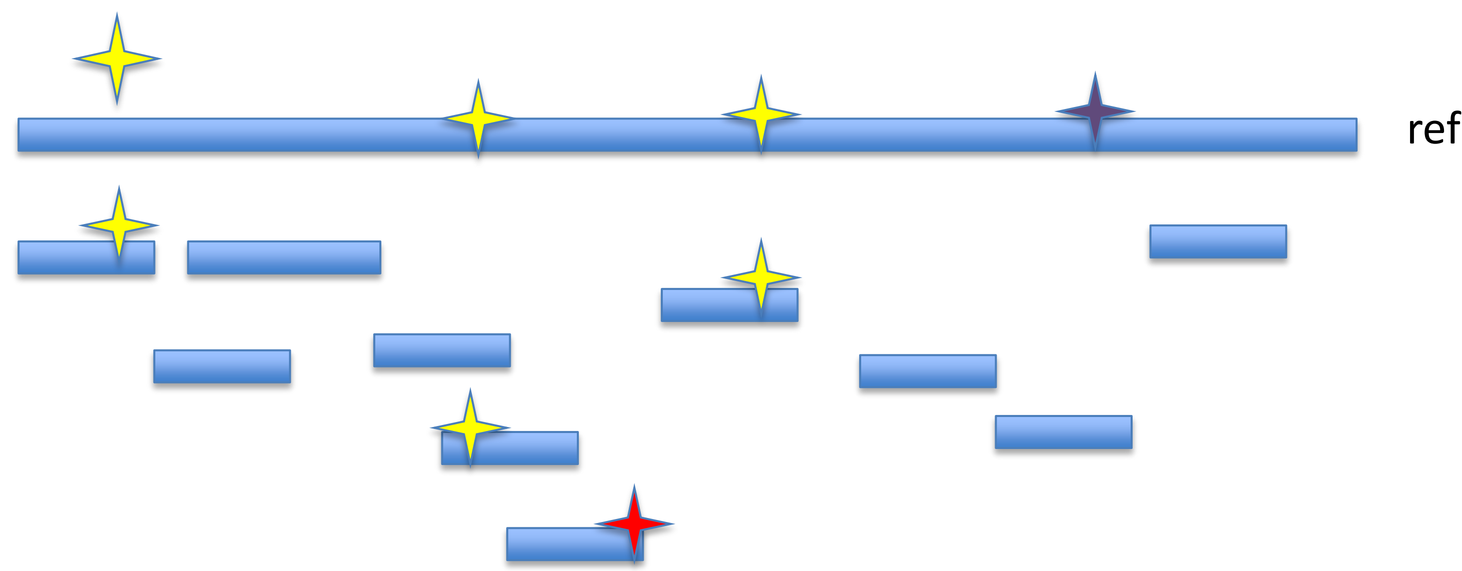
NGS/MPS applications

- Whole genome sequencing:
  - De novo sequencing
  - Re-sequencing
- Transcriptome sequencing:
  - **mRNA-se**q
  - **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 sequencing:

Illumina strategy

Sequencing:

- PE library with 350 bp
- PE library with 600 bp
- MP library with 2 kb
- MP library with 5-8-20 kb

PE: 50-100x, MP 10-15x

Analysis:

- ALLPATH

PacBio strategy

Sequencing:

- 10-20 kb library

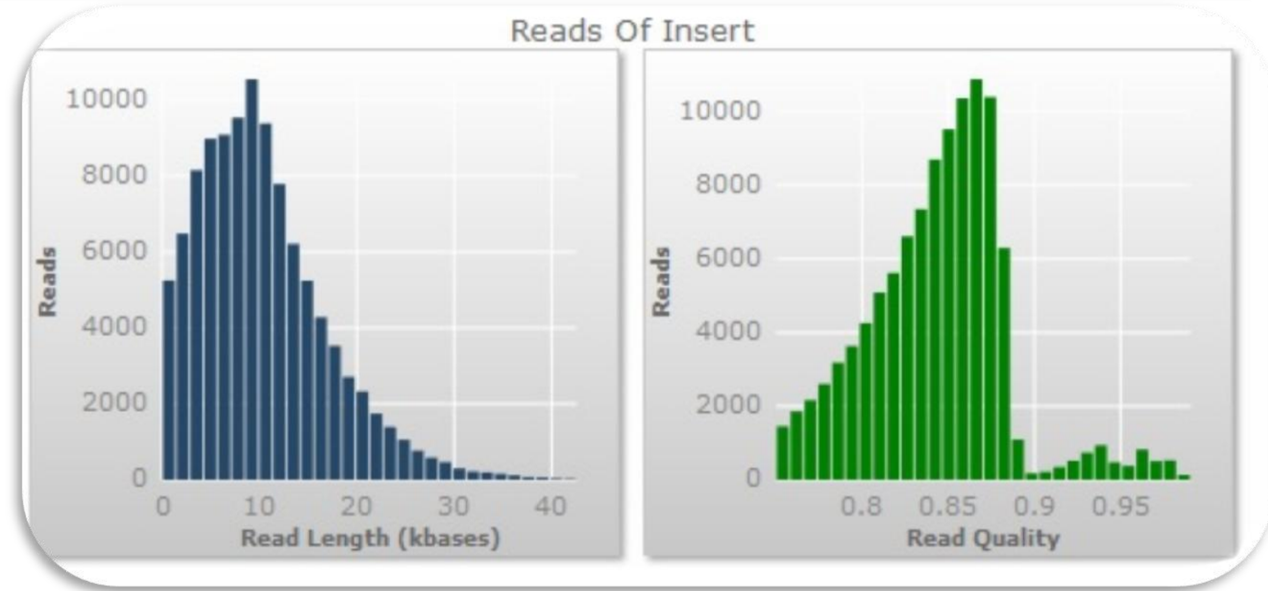
50-80x

(where 30x are reads above 10 kb)

Analysis:

- HGAP (haploid)
- FALCON (diploid)

Example: de novo PacBio; Crow



Sequencing results

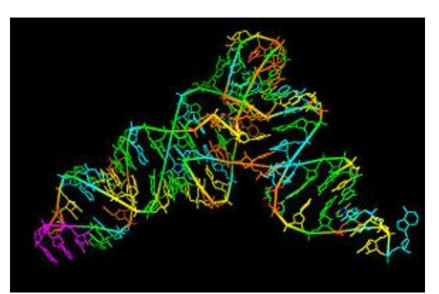
Number of SMRT cells: 70

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



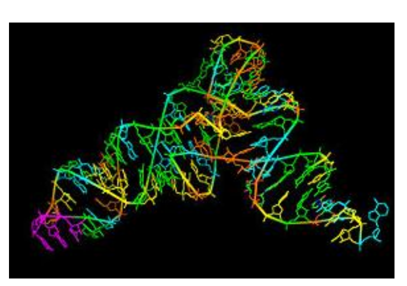
Transcriptome sequencing (RNA-seq)

TOTAL RNA

mRNA
- **Dif.ex.**
- Annotation

Non-codingRNA **miRNA**
- Transcriptional regulation

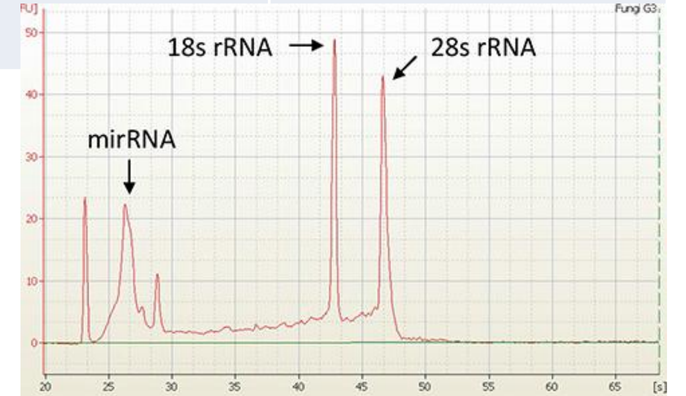
Splice isoforms

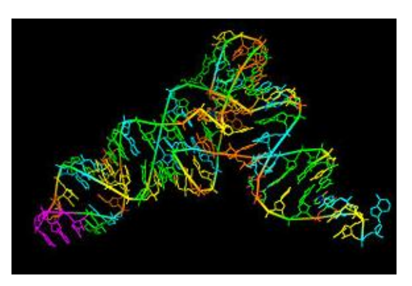


mRNA: rRNA depletion vs polyA selection

Method	Pros	Cons	Recommended
rRNA depletion	• Captures on-going transcription • Picks up non-coding RNA	• Does not get rid of all rRNA • Messy Dif.Ex. profile	20-40 mln reads (single or PE)
polyA selection	• Gives a clean Dif.Ex. profile	• Does not pick non-coding RNA	5-20 mln reads

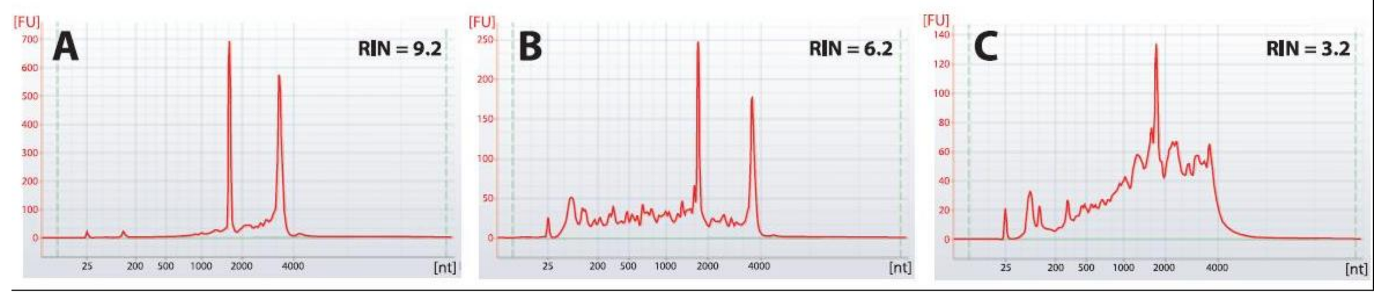
Alternative for **human** RNA-seq:
AmpliSeq Human Transcriptome panel:
- faster, cheaper, works fine with FFPE
- input: 50 ng **total** RNA
- dif.ex. ONLY



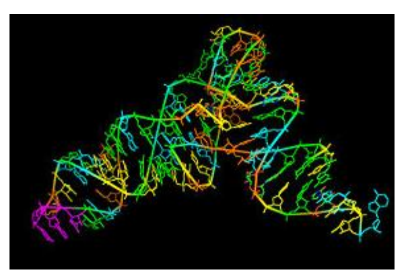


RNA-seq experimental setup

- 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

TRANSCRIPTOME

I'LL TAKE ALL OF THEM!

SHRED-O-MATIC

PASS THE TAPE, PLEASE

HERE YOU GO!

Gene

mRNA isoforms

Short-read technologies:

Insufficient Connectivity
Splice Isoform Uncertainty

Reads spanning splice junctions

PacBio's Iso-Seq solution:

Full-length cDNA Sequence Reads
Splice Isoform Certainty – No Assembly Required

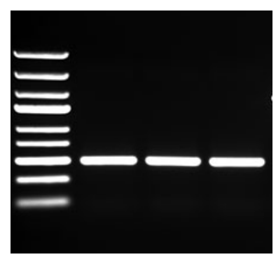
NATURE METHODS | NEWS AND VIEWS

Genomics: the state of the art in RNA-seq analysis

Ian Korf

Nature Methods **10**, 1165–1166 (2013) | doi:10.1038/nmeth.2735
Published online 26 November 2013

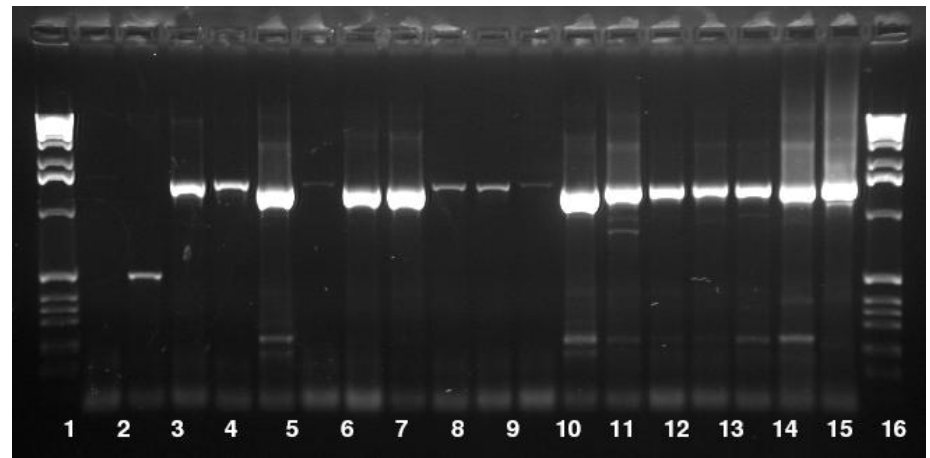
PacBio Iso-seq: full-length transcriptome seq



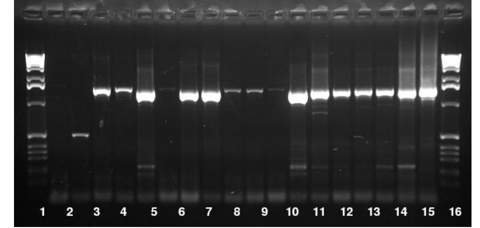
Amplicon sequencing

Used a lot in metagenomics

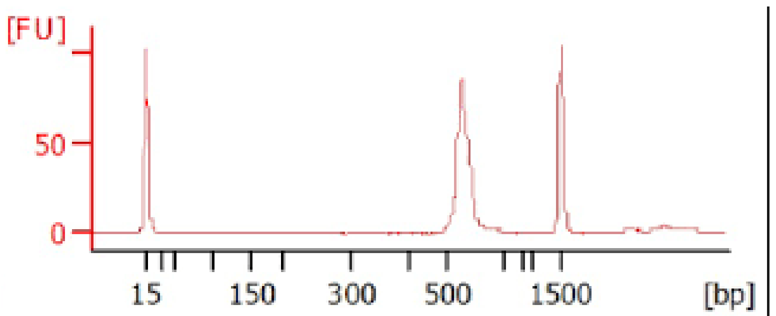
- **Community analysis**
  - rRNA genes & spacers (16S, ITS)
  - Functional genes
- Genotyping by sequencing



Amplicon sequencing



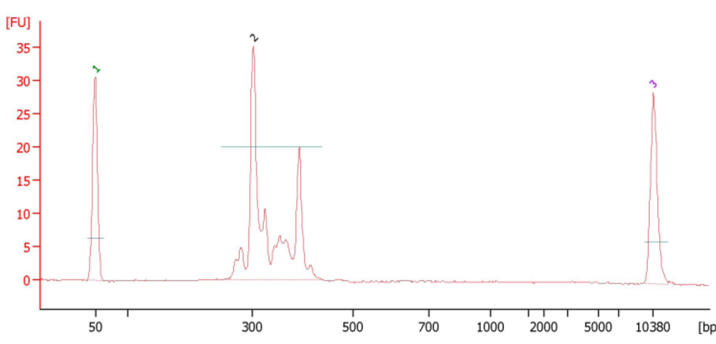
Example 1: tight peak, OK



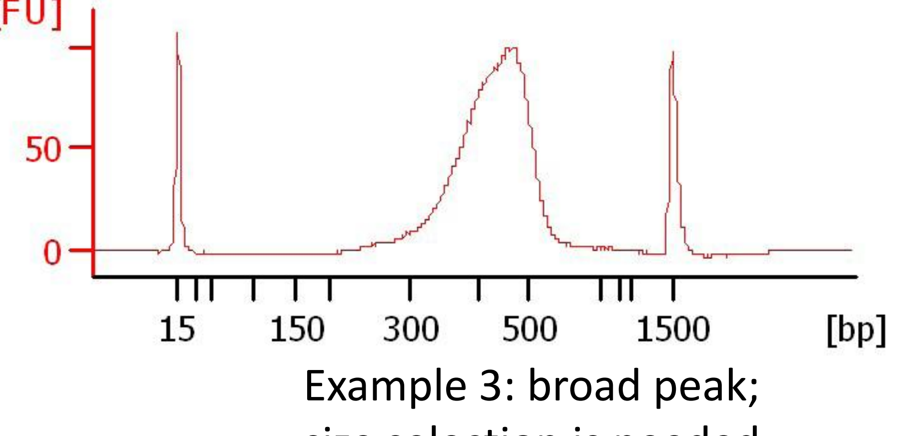
FOR ANY NGS TECHNOLOGY

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

Reason – preferential amplification of short fragments

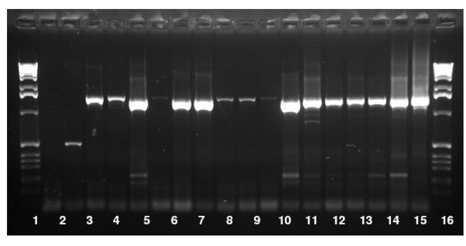


Example 2: several sizes, fractionation is needed
=> we HAVE to make several libraries

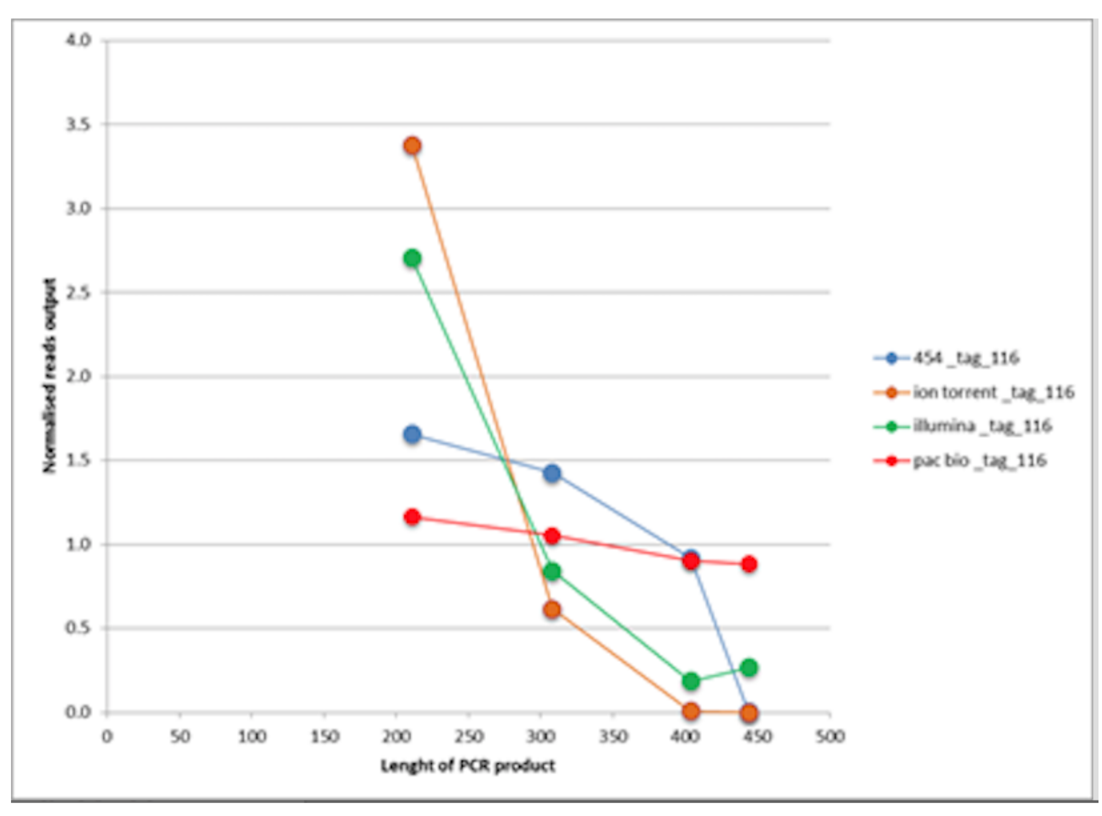


Example 3: broad peak; size selection is needed

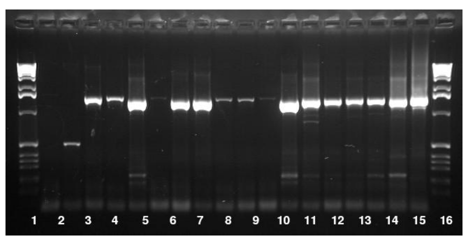
SIZE MATTERS…



Size-related bias in amplicon-seq



Courtesy Mikael Brandström Durling, Forest Mycology and Pathology, SLU



When you sequence an amplicon…

Forward reads

Reverse reads

Insertion
Deletion
SNP

Error rate (%)

Position in read

On MiSeq

FW read

RW read

On Ion

FW read

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

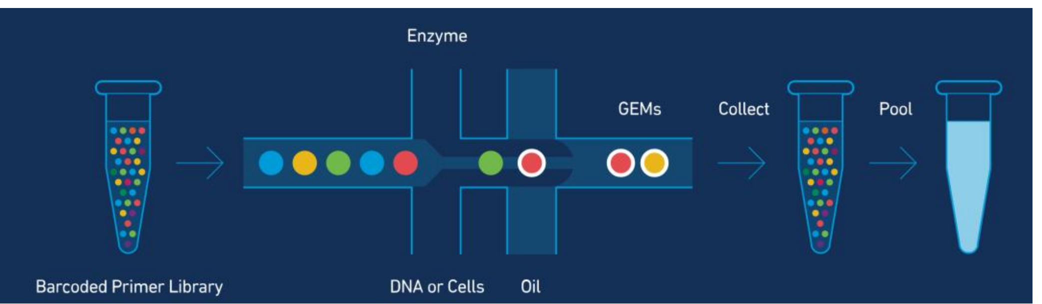
mRNA and miRNA
Exome
ChIP-seq
Short amplicons
Gene panels
Clinical samples

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

Other technologies for scaffolding of genomes



10x Chromium -> Illumina sequencing

BioNano Irys, optical mapping

What is “The BEST”?

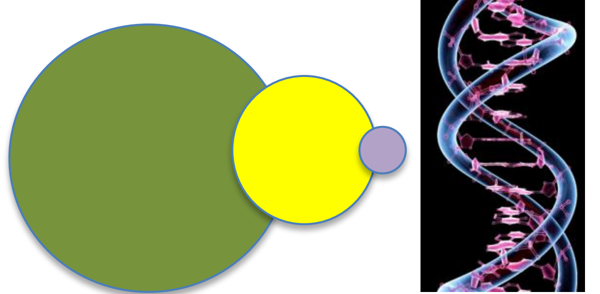
SAMPLE QUALITY REQUIREMENTS

Sample prep: take home message

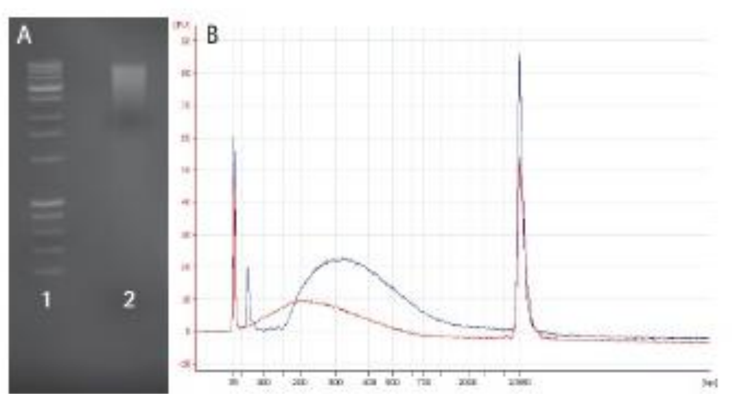
PCR-quality sample and

NGS-quality sample

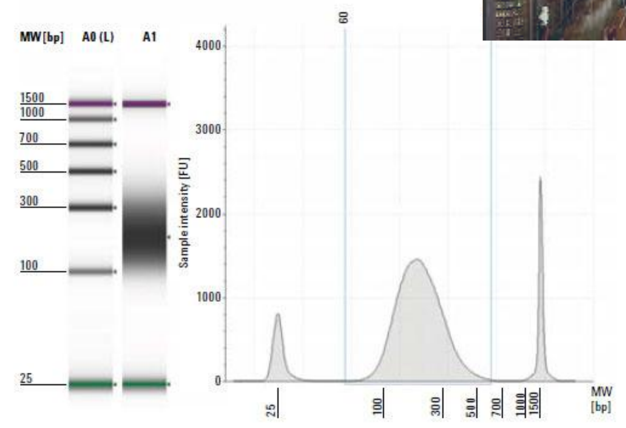
are two completely different things



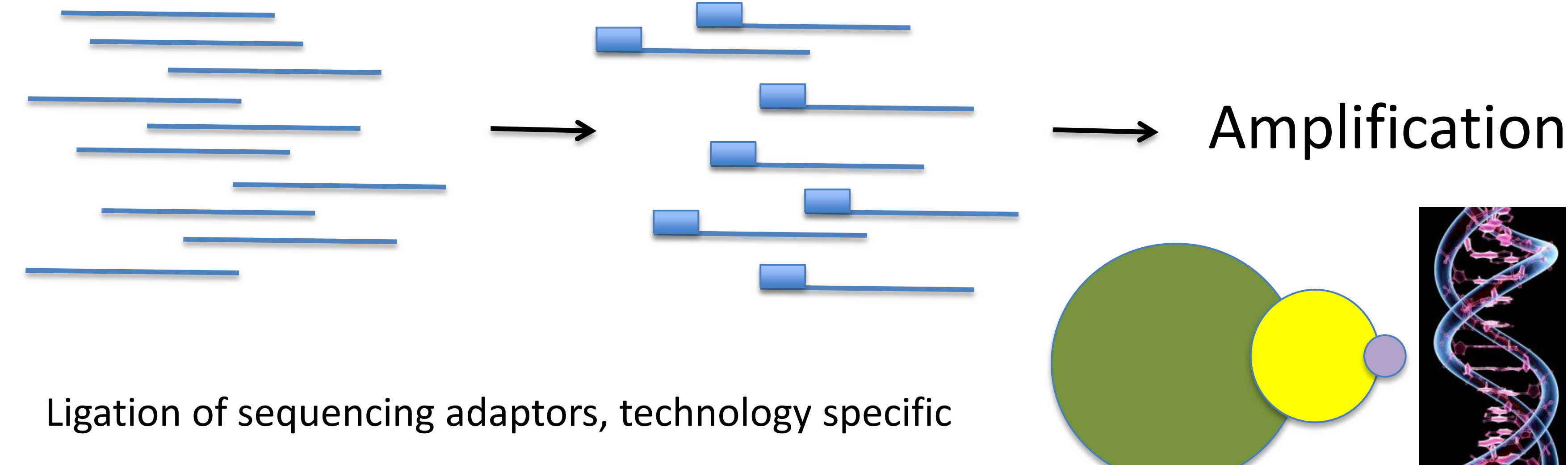
Making an NGS library



DNA QC – **paramount importance**



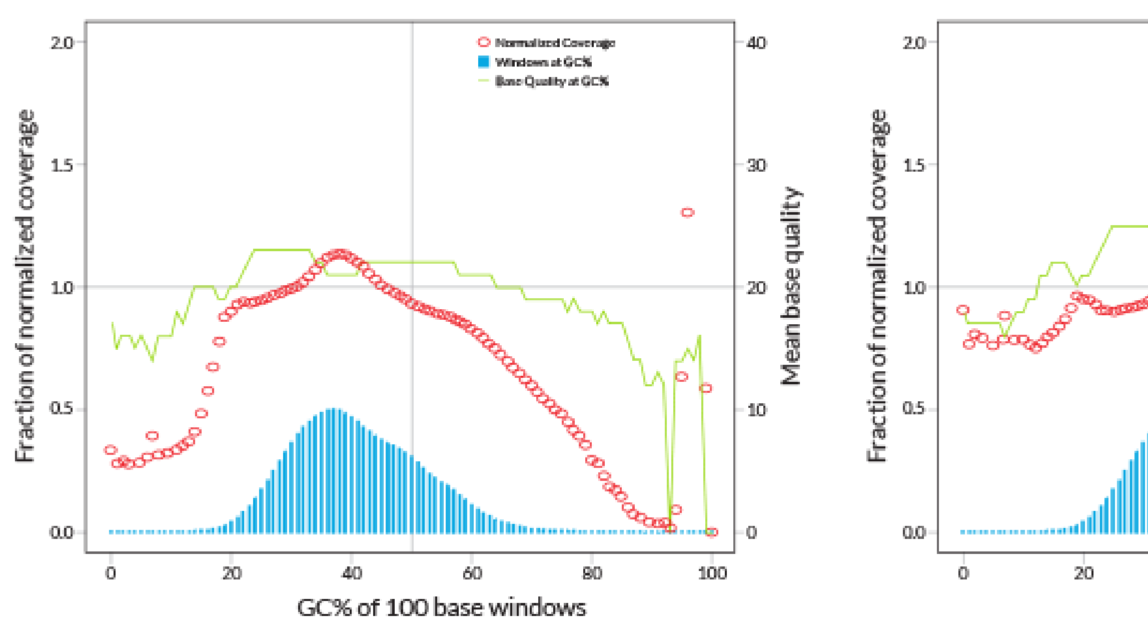
Sharing & size selection



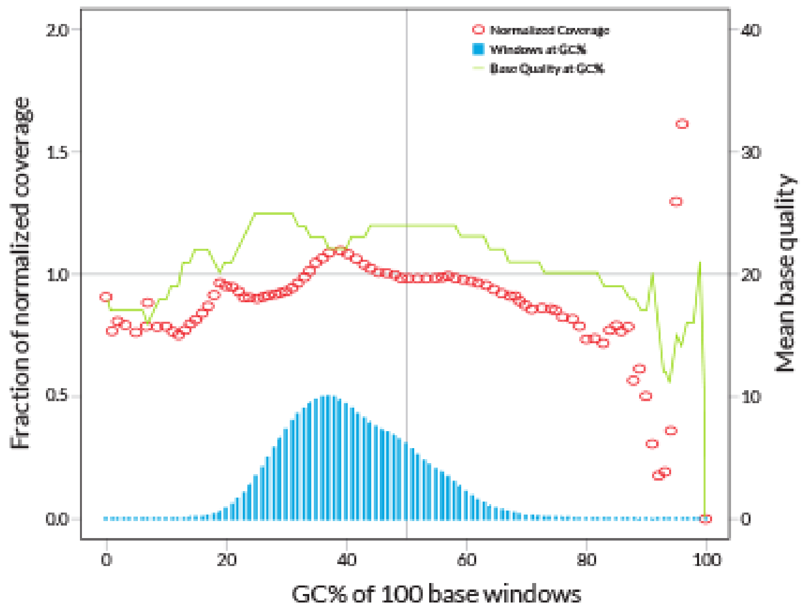
Amplification

Ligation of sequencing adaptors, technology specific

Library complexity



Suboptimal sample



Good sample

(source: https://www.kapabiosystems.com)

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

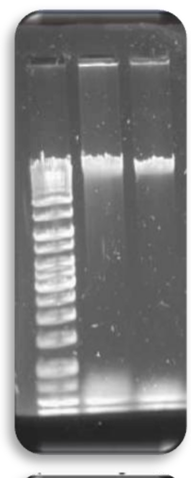
NanoDrop:

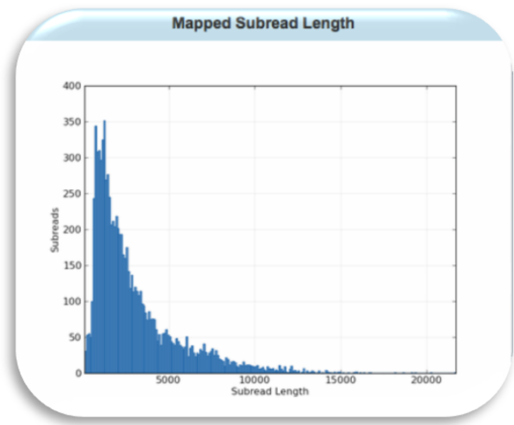
260/280 = 1.8 – 2.0
260/230 = 2.0 – 2.2

Qubit or Picogreen:

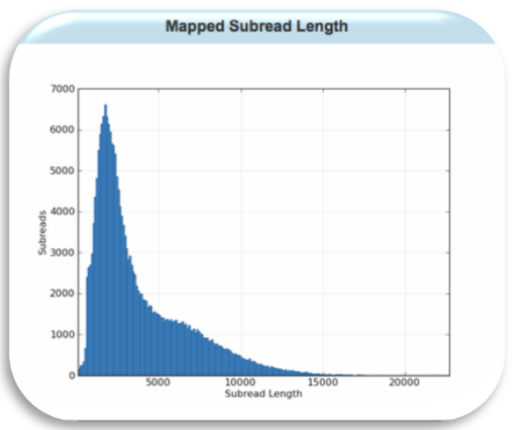
10 kb insert libraries: 3-5 ug
20 kb insert libraries: 10-20 ug

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

DNA solution

Low concentration

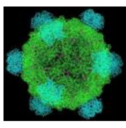
High concentration

- 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

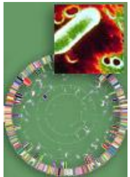
- **NANODROP is Bad**. Point.
- Use Qubit, or PicoGreen.

Let’s get philosophical

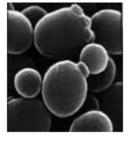
Since the beginning of Genomics:



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)

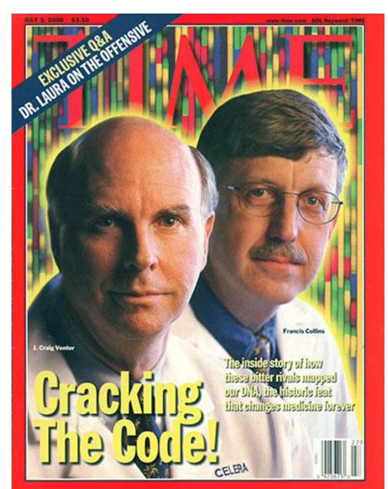
First multicellular organism: *Cenorhabditis elegans* - 100 MB (1998-2002)

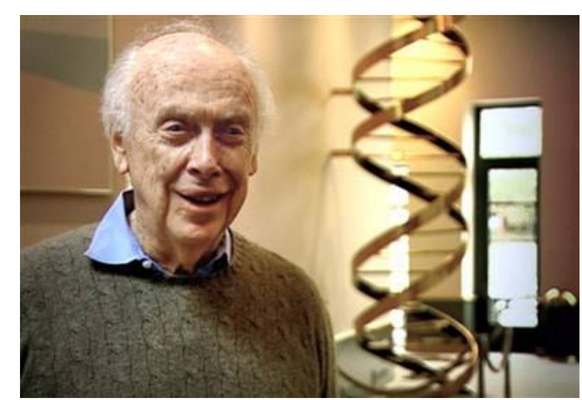
First plant: *Arabidopsis thaliana* - 157 Mb (2000)

… prices go down

- Human genome project, 2007
  - Genome of Craig Wenter costs 70 mln $
    - Sanger’s sequencing
  - Genome of James Watson costs 2 mln $
    - 454 pyrosequencing
  - Ultimate goal: 1000 $ / individual

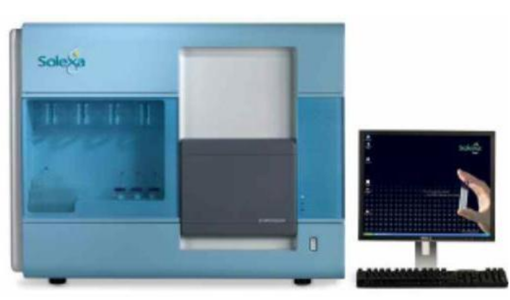
    Almost there! (1200 $)

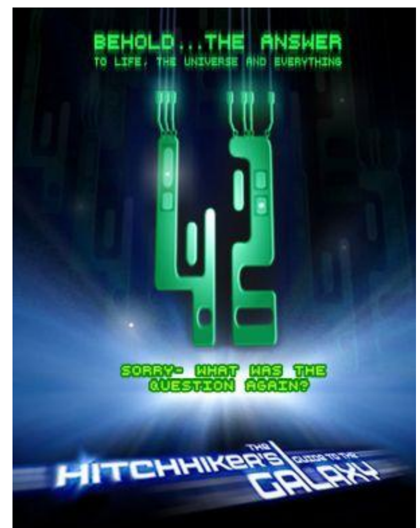




… paradigm change

- 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

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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

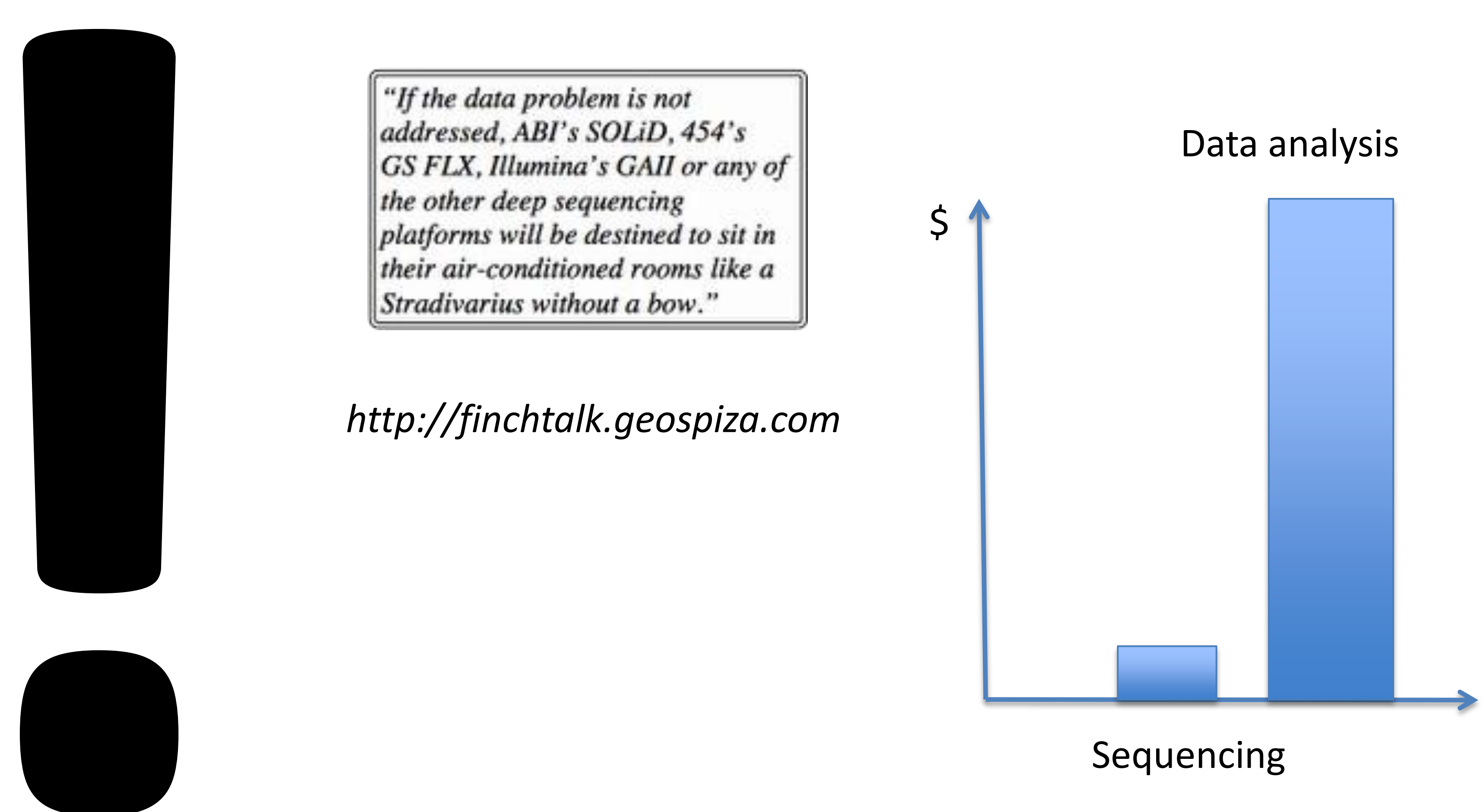
IF 2.9

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 Schneiker-Bekel[a], Daniel Wibberg[a], Thomas Bekel[b], Jochen Blom[b], Burkhard Linke[b], Heiko Neuweger[b], Michael Stiens[a, c], Frank-Jörg Vorhölter[a], Stefan Weidner[a], Alexander Goesmann[b], Alfred Pühler[a] and Andreas Schlüter[a],

Main challenge - DATA **ANALYSIS** and DATA **STORAGE**

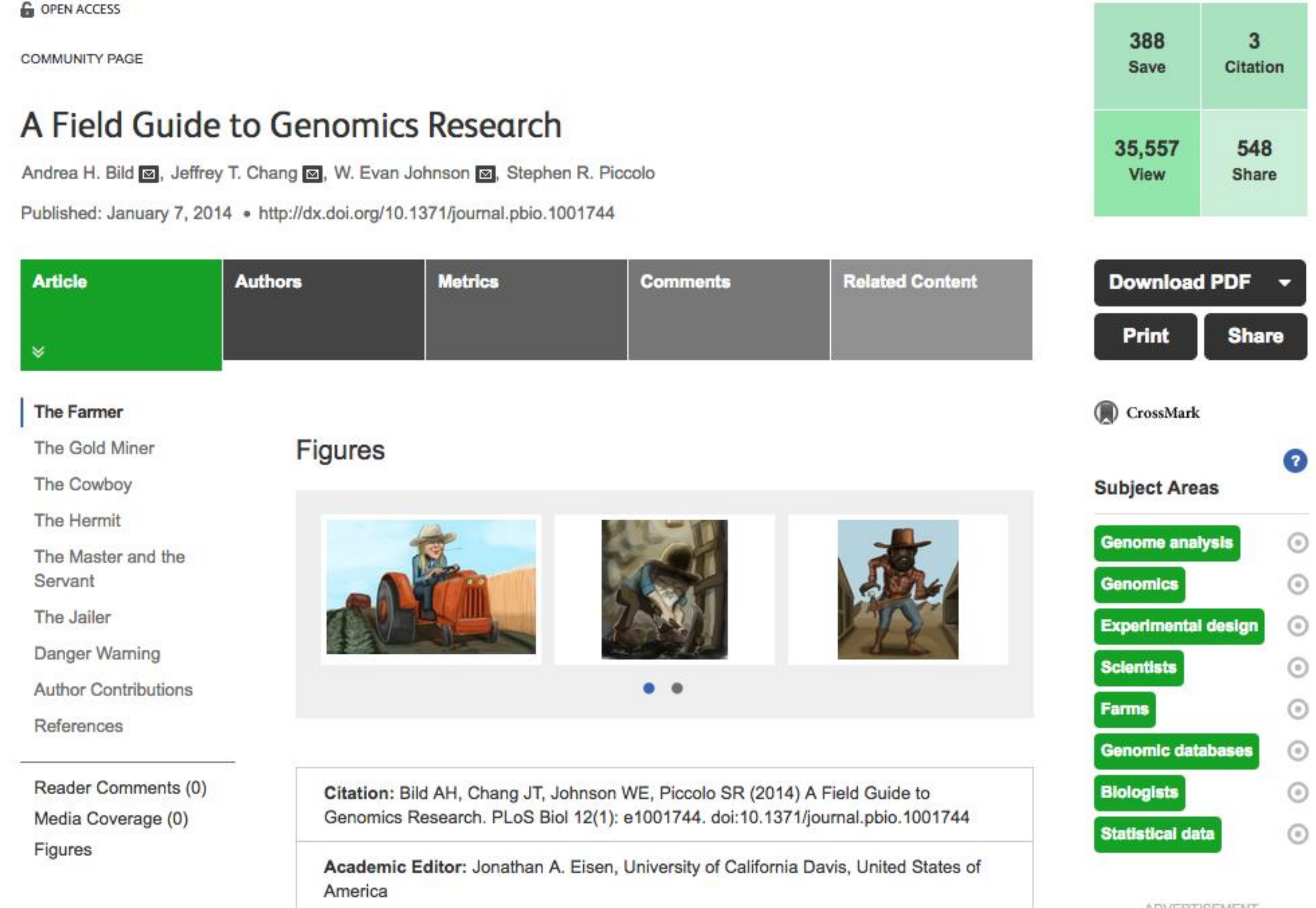


=> More bioinformaticians to people!

What we sequenced at SciLifeLab

16S amplicon, Acinetobacter baumanii, Acrasis kona, Acridotheres javanicus, Actinobacillus succinogenes, African swine fever virus, Agaricomycotina sp, **Alces alces**, **Alligator mississippiensis**, Amphiura filiformis, Apis mellifera, Aquila chrysaetus, Arabidopsis thaliana, Arabis alpina, Archaeorhizomycetes finlayi, Arctocephalus gazella, artificial sequences, Arvicola amphibius, Ascaridia galli, Aspergillus oryzae, Astrapia stephaniae, Atlantic herring, Atlantic salmon, **Avena sativa**, Baccharis breviseta, Baccharis dracunculifolia, Bacteriophages, Balaenoptera musculus, Balaenoptera physalus, Balanus improvisus, Baltic Sea microorganisms, Bathynomus sp, Bifidobactrium sp, **Borrelia burgdorferi**, Borrelia garinii, Bos taurus, Bovine viral diarrhea virus, Brachyspira suanatina, Brassica sp, Brettanomyces naardensis, Caenorhabditis elegans, Callosobruchus maculatus, Candida intermedia, Candida parapsilosis, Candidatus Neoehrlichia mikurensis, **Canis lupus**, Capreolus capreolus, Capsella bursa-pastoris, Capsella grandiflora, Capsella orientalis, Capsella rubella, Ceanothus thyrsiflorus, Cervus dama, Cervus elaphus, Childia submaculatum, Clonostachys rosea, Clostridium ultunense, Coelodonta antiquitatis, Colias crocea, Collinsia heterophylla, Coregonus lavaretus, Coronavirus, Corvus corone, Corvus monedula, Crassostrea gigas, Cricetulus griseus, Cryptococcus tephrensis, Cubanola dominguensis, Cytomegalovirus, Danio rerio, Datisca glomerata, Deformed wing virus, Dekkera bruxellensis, Dicerorhinus sumatrensis, Dictyostelium discoideum, Diopisthoporus gymnopharyngeus, Diopistoporus longitubus, Drosophila melanogaster, Drosophila paulistorum, Electrophorus electricus, Enterobacter cloacae, Enterococcus faecium, **Equus caballus**, Escherichia coli, Eumecynostomum macrobursalium, Euphorbia lathyris, Euphorbia peplus, Euplectes afer, Euplectes ardens, Euplectes aureus, Euplectes hordeaceus, Euplectes macrourus, Euplectes orix, **Felis catus**, Ficedula albicollis, Ficedula hypoleuca, **Fragaria ananassa**, Freshwater microbial communitites, Fucus radicans, Fucus vesiculosus, Fumaria sp, Galerucella, Gallus gallus, Geospiza magnirostris, Giardia muris, Globodera rostochiensis, Gnetum gnemon, Gnetum luofuense, Gnetum montanum, Gnetum parvifolium, Gnetum pendulum, Gonyostomum semen, Gonzalagunia, Gut microbiota, Hamelia marantha, Heterobasidion abietinum, Heterobasidion 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 pyrrhopterus, **Lynx lynx**, Malassezia sympodialis, **Malus domestica**, Malus sylvestris, **Mammuthus primigenius**, Marchantia polymopha, Marine bacteria whole community, **Meligethes aeneus**, Metagenomes, Methanoculleus sp, Metschnikowia andauensis, Metschnikowia hawaiiensis, Metschnikowia lopburiensis, Metschnikowia pulcherrima, Metschnikowia saccharicola, Mixornis gularis, Moorella thermoacetica, Mus musculus, Mycobacterium malmoense, Mycobacterium marinum, **Mytilus edulis**, Nemertoderma westbladi, Nesophontes sp, Neurospora crassa, Neurospora hispaniola, Neurospora intermedia, Neurospora metzenbergii, Neurospora perkinsii, 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, Penicillium sp, **Perca fluviatilis**, Peridinium aciculiferum, Philomachus pugnax, Phoca sibirica, Phylloscopus collybita, Phylloscopus trochilus, Wolbachia persica, Physcomitrella patens, Phytophthora infestans, **Picea abies**, Pieris napi, Pieris rapae, Pinus pinaster, Pinus sylvestris, **Pisum sativum**, Planctomycetes sp, Plasmodiophora brassicae, Plasmodium falciparum, Podospora anserina, Polystachya paniculata, Pomatoschistus minutus, Populus maximowiczii, Populus tremula, Populus trichocarpa, Posoqueria sp, Pseudomonas aeruginosa, Pseudomonas brassicacearum, Pseudomonas chlororaphis, Pseudomonas putida, Pteridophora alberti, Ptiloris paradiseus, Puccinia striiformis, Pythium oligandrum, Quelea quelea, 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, Spironucleus barkahanus, Spironucleus salmonicida, Spironucleus vortens, Staphylococcus aureus, Staphylococcus pseudintermedius, Stemmadenia sp, Streptococcus pneumoniae, Streptococcus pyogenes, Streptomyces coelicolor, Struthio camelus, Sulfolobus acidocaldarius, **Sus scrofa**, Synthetic DNA, Syntrophaceticus schinkii, Taphrina betulina, Tepidanarobacter aceatoxydans, Thamnolia vermicularis, Theileria parva, Trypanosoma cruzi, Trypanosoma rangeli, **Ursus spelaeus**, Vitex agnus-castus, Yarrowia lipolytica, Zalophus californianus, Zalophus wollebaeki, Zygopetalum crinitum, Zygosaccharomyces bailii

Good reading:



http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001744

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PLA Proteomics
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Fluorescence Correlation Spectroscopy

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Bioinformatics Compute and Storage (UPPNEX)
Bioinformatics Long-term Support (WABI)
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Chemical Biology Consortium Sweden
Laboratories for Chemical Biology Umeå (LCBU)
The Laboratories for Chemical Biology at Karolinska Institutet (LCBKI)
Uppsala Drug Optimization and Pharmaceutical Profiling (UDOPP)

Clinical Diagnostics
Clinical Biomarkers
Clinical Genomics
Clinical Sequencing

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ADME (Absorption Distribution, Metabolism Excretion) of Therapeutics (UDOPP)
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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
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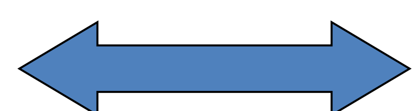
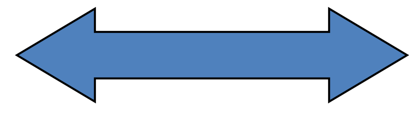
NGI Stockholm (Genomics Production)
NGI Uppsala (SNP&SEQ Technology Platform)
NGI Uppsala (Uppsala Genome Center)

Structural Biology
Protein Science Facility

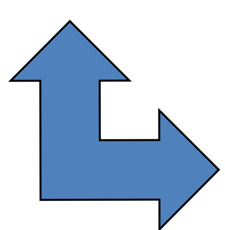
National Genomics Infrastructure

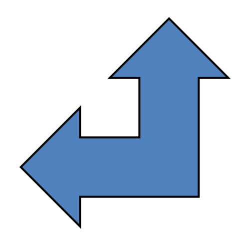
SciLifeLab, Stockholm



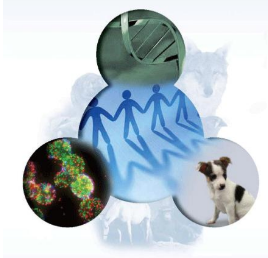


SciLifeLab, Uppsala





Uppmax, Uppsala



NGI-SciLifeLab is one of the most well-equipped NGS sites in Europe

10 Illumina HiSeq Xten
17 Illumina HiSeq 2500/4000
3 Illumina MiSeq
1 Illumina NextSeq
2 Ion Torrent
1 Ion S5
5 Ion Proton
2 PacBio RSII
1 PacBio SEQUEL
1 Sanger ABI3730
1 Argus Whole Genome Map. Syst.
1 BioNano Irys
2 Oxford Nanopore MinIon
2 Chromium 10x

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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.

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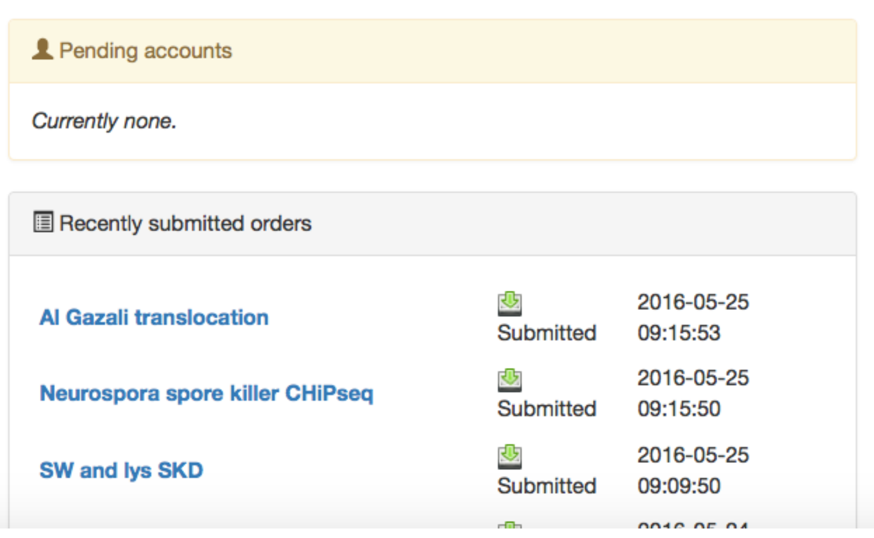

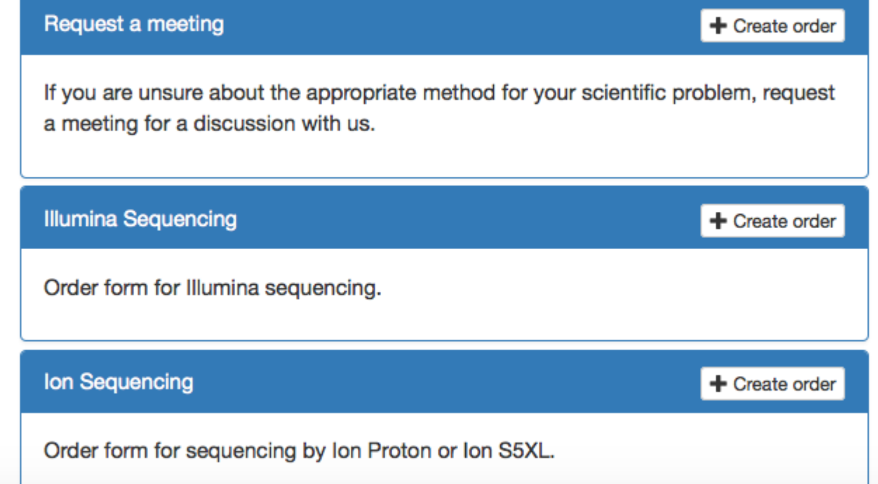

https://ngisweden.scilifelab.se/

What happens then?

NGI Project coordinators meet twice a week via Skype

Ulrika
Liljedahl

Ellenor
Devine

SNP&SEQ, Uppsala node

Mattias
Ormestad

Beata
Werne Solenstam

Stockholm Node

Olga
Vinnere Pettersson

UGC, Uppsala Node

Project distribution is based on:

1. Wish of PI
2. Type of sequencing technology
3. Type of application
4. Queue at technology platforms

Project is then assigned to a certain node and a coordinator contacts the PI

Project meeting

What we can help you with:

- Design your experiment based on the scientific question.
- Chose the best suited application for your project.
- Find the most optimal sequencing setup.
- Answer all questions about our technologies and applications, as well as bioinformatics.

- In special cases, we can give extra-support with bioinformatics analysis – development of novel methods and applications

QUESTIONS?