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A Perspective:

Genomics and Bioinformatics

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Acknowledgement

- Dr. Matthew L. Settles

A Brief History

Sequencing Platforms

- 1986 - Dye terminator Sanger sequencing, peaking at about 900kb/day in early 2000s

‘Next’ Generation

- 2005 – ‘Next Generation Sequencing’ as Massively parallel sequencing, both throughput and speed advances. The first was the Genome Sequencer (GS) instrument developed by 454 life Sciences (later acquired by Roche), Pyrosequencing 1.5Gb/day

Discontinued

Illumina (Solexa)

- 2006 – The second ‘Next Generation Sequencing’ platform. Now the dominant platform with 75% market share of sequencer and and estimated >90% of all bases sequenced are from an Illumina machine, Sequencing by Synthesis > 1600Gb/day.

NovaSeq

HiSeq

Complete Genomics

- 2006 – Using DNA Nanoball sequencing, has been a leader in Human genome resequencing, having sequenced over 20,000 genomes to date. In 2013 purchased by BGI and became part of MGI.
- 2020 - Launched DNBSEQ sequencers, which integrate DNA Nanoball technology and PCR-free Rolling Circule Replication. Throughput on par with Novaseq.
- Comparative studies:
  - https://link.springer.com/article/10.1007%2Fs13258-021-01096-x
  - https://academic.oup.com/nargab/article/2/2/lqaa034/5836690

Bench top Sequencers

- Life Technologies
  - Ion Torrent
  - Ion Proton
  - Gene Studio S5



- Illumina
  - MiSeq
  - MiniSeq
  - iSeq 100



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The ‘Next, Next’ Generation Sequencers (3rd Generation)

- 2009 – Single Molecule Read Time sequencing by Pacific Biosystems, most successful third generation sequencing platforms, RSII ~2Gb/day, newer Pac Bio Sequel ~14Gb/day, near 100Kb reads
- 2020 - Sequel IIe ~150Gb/flow cell 30 hour run, ~5M HiFi reads.

SMRT Sequencing

Iso-seq on Pac Bio possible, transcriptome without ‘assembly’

Oxford Nanopore

- 2015 – Another 3rd generation sequencer, founded in 2005. The sequencer uses nanopore technology developed in the 90's to sequence single molecules. Throughput is about 500Mb per flowcell, capable of near 200kb reads.
- 2019 – PromethION over 7Tb in a single experiment.
- 2021 – New chemistry promises Q20+ raw sequencing quality.

Nanopore Sequencing

FYI: 4th generation sequencing is being described as In-situ sequencing

Bioinformatics

Old Way of thinking about Bioinformatics

Appro Cluster

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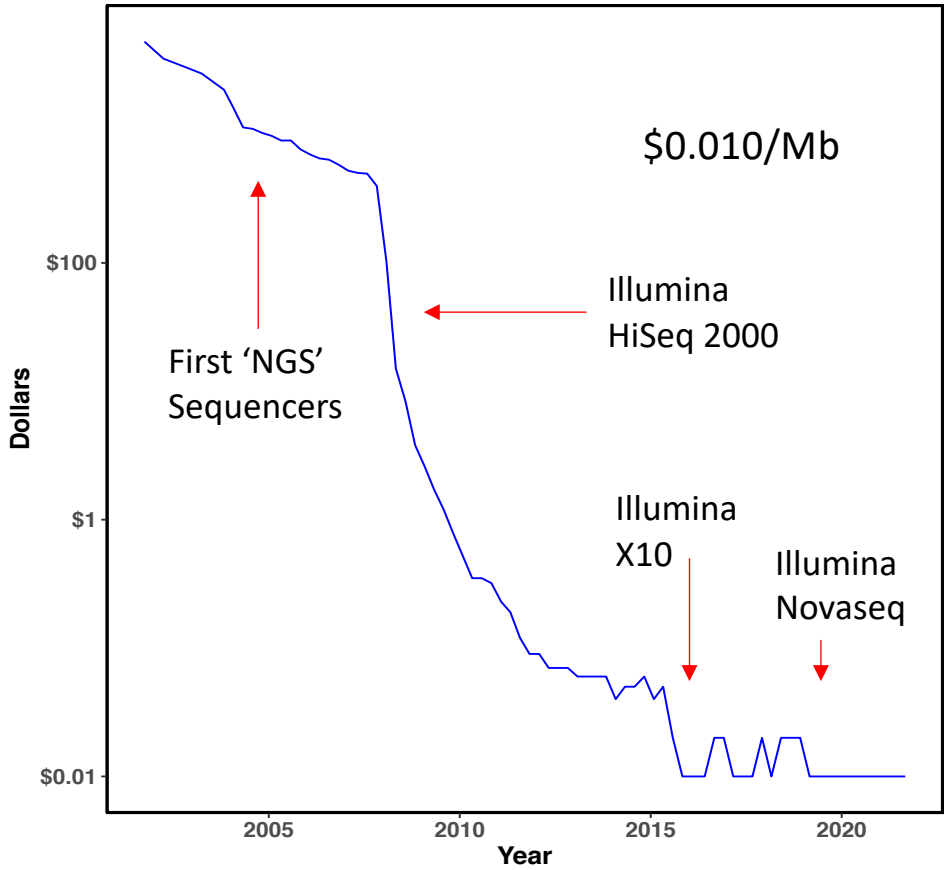
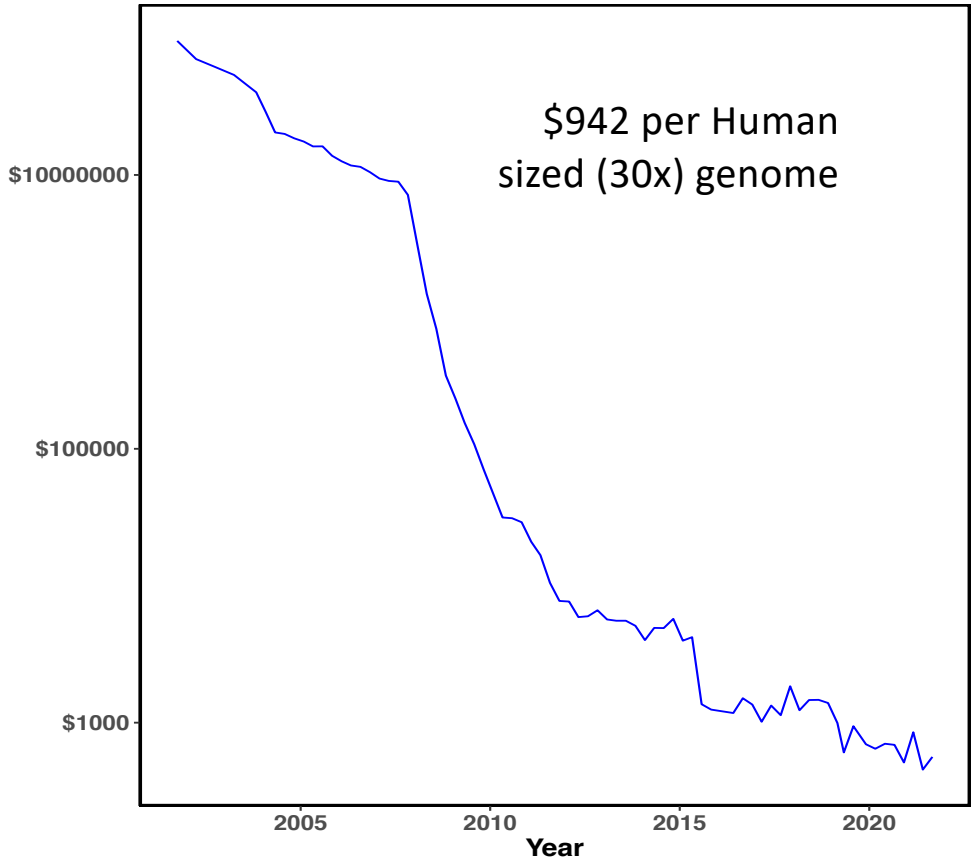
Sequencing Costs

Nov 2021





- Includes: labor, administration, management, utilities, reagents, consumables, instruments (amortized over 3 years), informatics related to sequence productions, submission, indirect costs.
- http://www.genome.gov/sequencingcosts/

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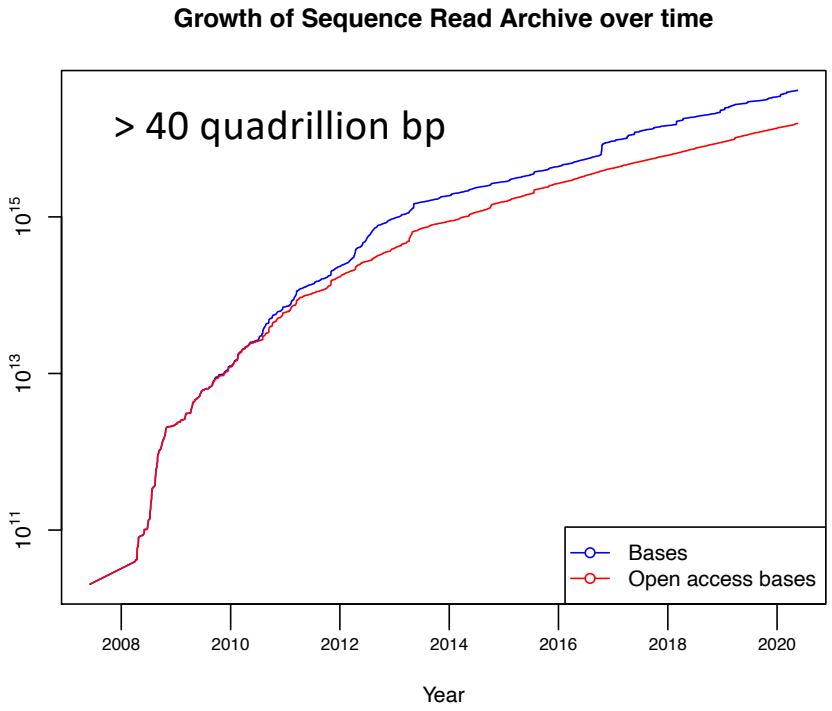
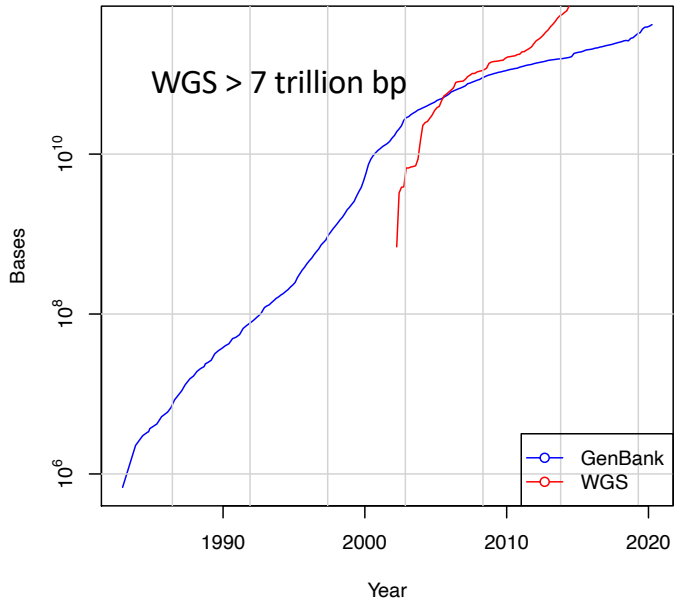
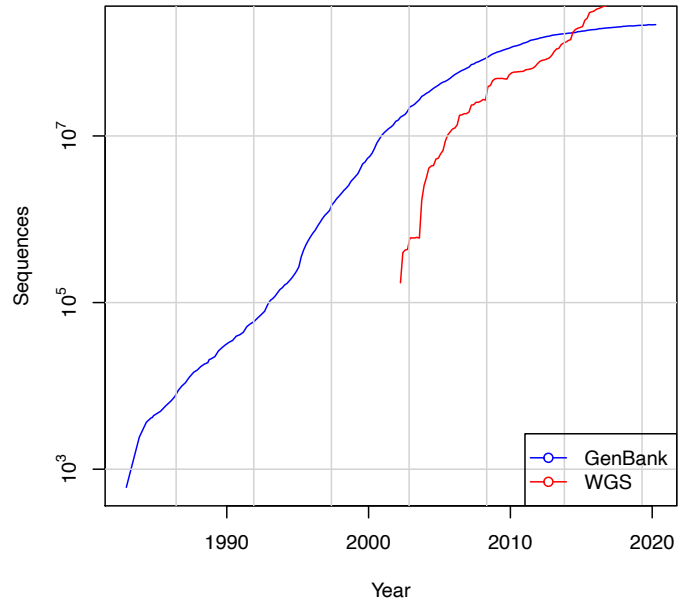
Growth in Public Sequence Database



http://www.ncbi.nlm.nih.gov/Traces/sra/



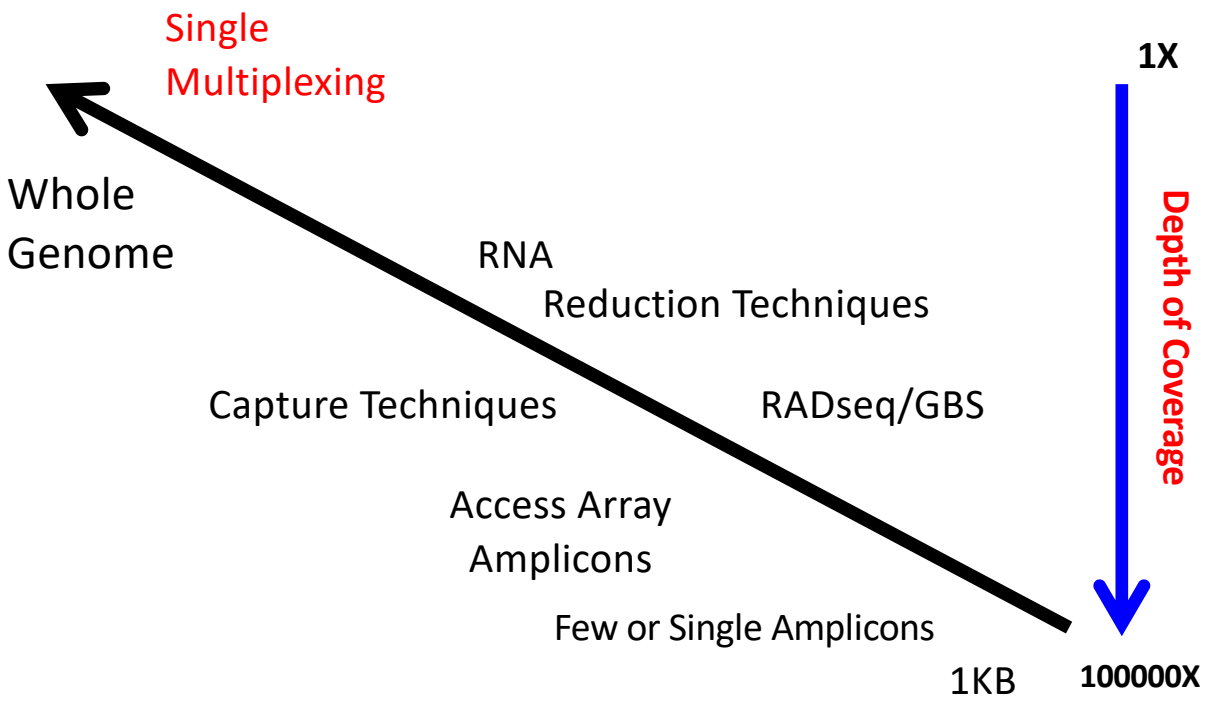


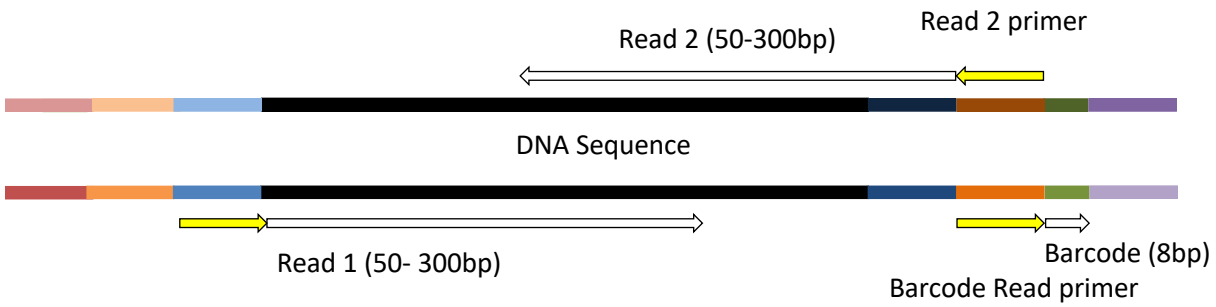
- http://www.ncbi.nlm.nih.gov/genbank/statistics

Illumina's Flexibility

Genomic reduction allows for greater coverage and multiplexing of samples.

You can fine tune your depth of coverage needs and sample size with the reduction technique





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Sequencing Libraries:

DNA-seq
RNA-seq
Amplicons
CHiP-seq
MeDiP-seq
RAD-seq
ddRAD-seq

DNase-seq
ATAC-seq
MNase-seq
FAIRE-seq
Ribose-seq
smRNA-seq

tagRNA-seq
PAT-seq
Structure-seq
MPE-seq
STARR-seq
Mod-seq

EnD-seq
Pool-seq
G&T-seq
Tn-Seq
BrAD-seq
SLAF-seq

MLA-seq is my favorite seq

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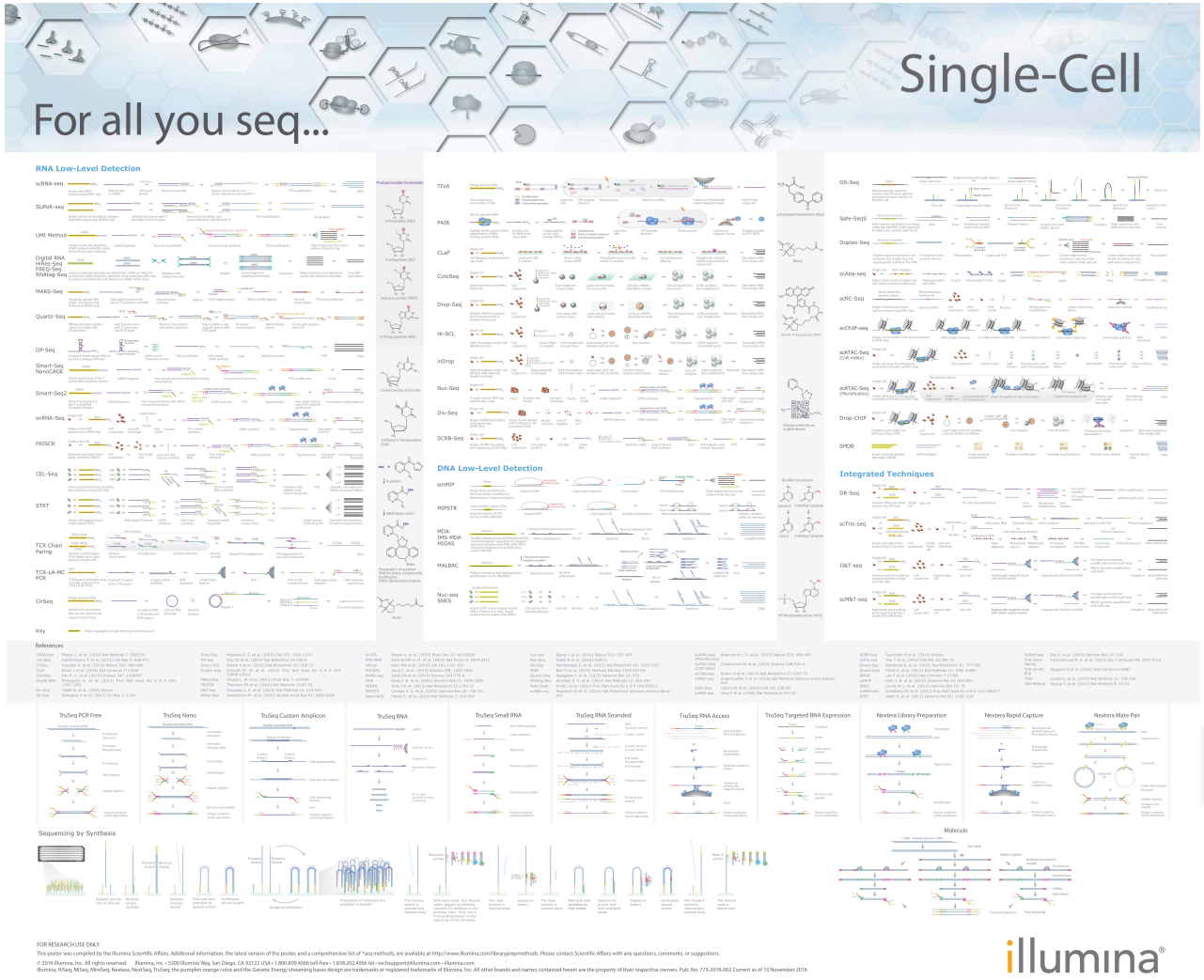
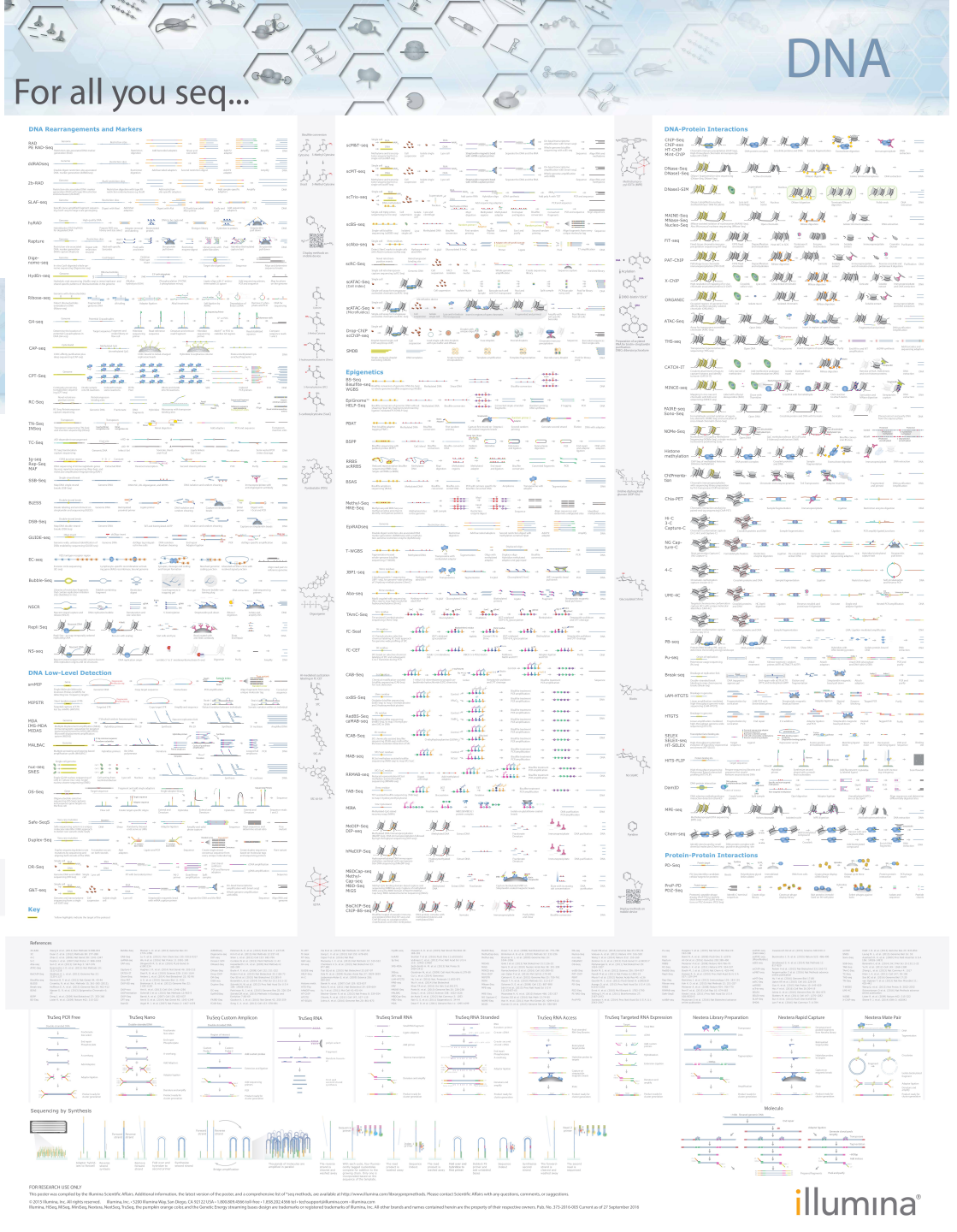
For all you seq





The data deluge

- Plucking the biology from the Noise

Reality

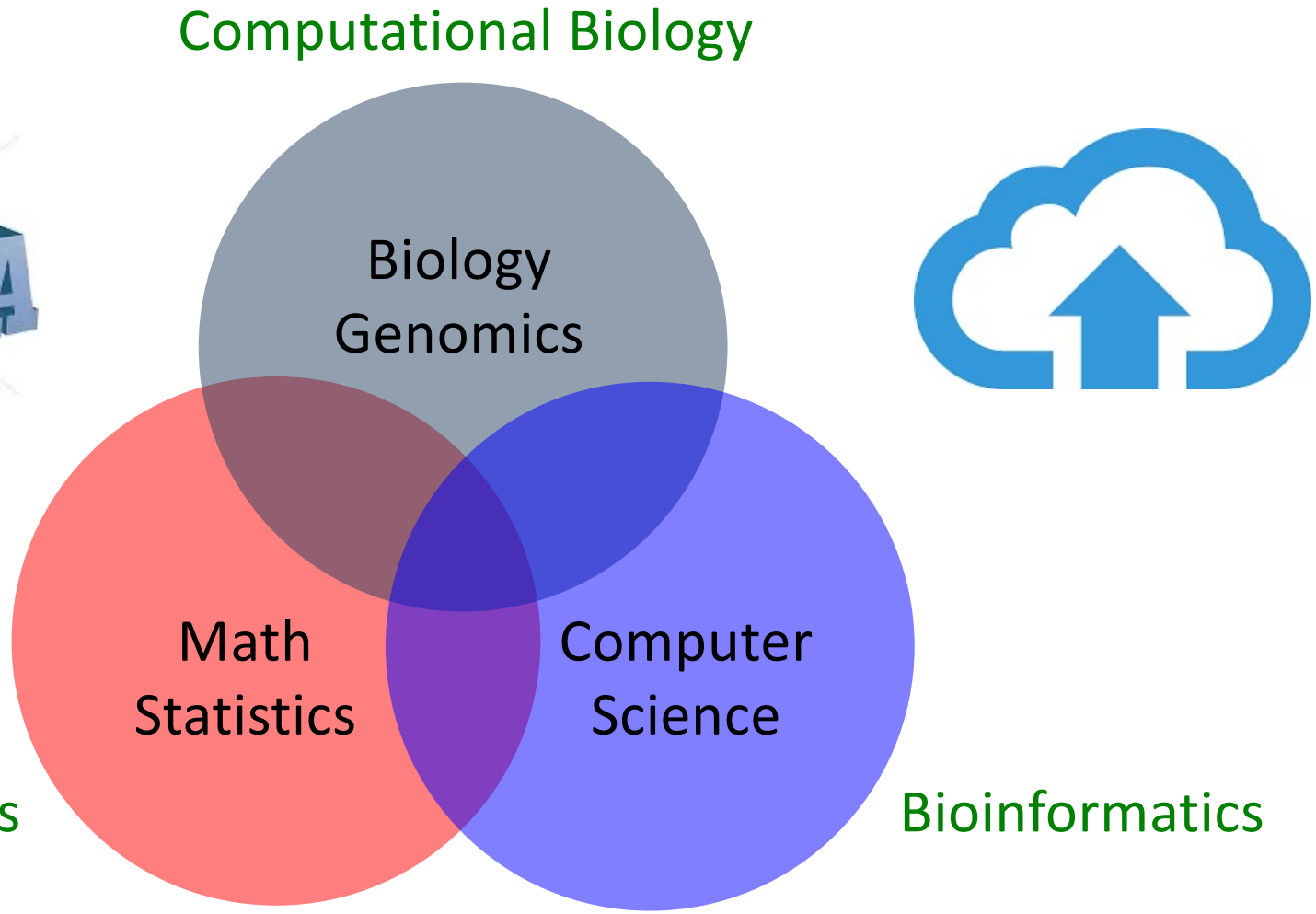
- Its much more difficult than we may first think

Data Science

Data science is the process of formulating a quantitative question that can be answered with data, collecting and cleaning the data, analyzing the data, and communicating the answer to the question to a relevant audience.

Five Fundamental Concepts of Data Science
statisticsviews.com November 11, 2013 by Kirk Borne

Bioinformatician as a Data Scientist



‘The data scientist role has been described as “part analyst, part artist.”’
Anjul Bhambhri, vice president of big data products at IBM

7 Stages to Data Science

1. Define the question of interest

2. Get the data
3. Clean the data
4. Explore the data

5. Fit statistical models

6. Communicate the results
7. Make your analysis reproducible

1. Define the question of interest

Begin with the end in mind!

what is the question
how are we to know we are successful
what are our expectations

dictates

the data that should be collected
the features being analyzed
which algorithms should be use

2. Get the data
3. Clean the data
4. Explore the data

Know your data!

know what the source was
technical processing in producing
data (bias, artifacts, etc.)
“Data Profiling”

Data are never perfect but love your data anyway!

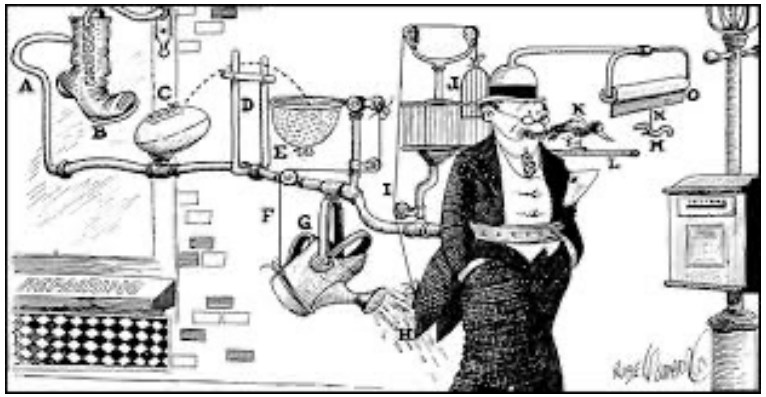
the collection of massive data sets often leads to unusual , surprising, unexpected and even outrageous.

5. Fit statistical models Machine Learning

Over fitting is a sin against data science!

Model's should not be over-complicated

- If the data scientist has done their job correctly the statistical models don't need to be incredibly complicated to identify important relationships
- In fact, if a complicated statistical model seems necessary, it often means that you don't have the right data to answer the question you really want to answer.



6. Communicate the results
7. Make your analysis reproducible

Remember that this is 'science'!

We are experimenting with data selections, processing, algorithms, ensembles of algorithms, measurements, models. At some point these ***must all be tested for validity and applicability*** to the problem you are trying to solve.

Data science done well looks easy – and that's a big problem for data scientists

simplystatistics.org
March 3, 2015 by Jeff Leek

Bad data science (bioinformatics) also looks easy

The Data Science in Bioinformatics

Bioinformatics is not something you are taught,

it's a way of life

*"The best bioinformaticians I know are **problem solvers** – they start the day not knowing something, and they enjoy finding out (themselves) how to do it. It's a great skill to have, but for most, it's not even a skill – it's a passion, it's a way of life, it's a thrill. It's what these people would do at the weekend (if their families let them)."*

Mick Watson – Rosland Institute

Genomics and Bioinformatics

Following data science principles, 2 stages in bioinformatics

- **Data reduction**

  Sequence data (raw data) to summarized form.

  * Command line, shell scripting, and programming.
  * Requires an understanding of the technology, molecular biology.
  * Removing technical noise from data.

- **Data analysis**

  Summarized data to biological interpretation

  * R/Python statistical programming
  * Requires an understanding of the biological question, statistics.

Prerequisites for doing Bioinformatics

- Access to a multi-core (24 cpu or greater), ‘high’ memory 64Gb or greater Linux server.
- Familiarity with the ’command line’ and at least one programming language.
- Basic knowledge of how to install software
- Basic knowledge of R (or equivalent) and statistical programming
- Basic knowledge of Statistics and model building

Substrate

Cloud Computing

Cluster Computing

LINUX

BAS™

Laptop & Desktop

Environment

“Command Line” and “Programming Languages”

VS

Bioinformatics Software Suite

Training - Models

- Workshops
  - Often enrolled too late
- Collaborations
  - More experience persons
- Apprenticeships
  - Previous lab personnel to young personnel
- Formal Education
  - Most programs are graduate level
  - Few Undergraduate

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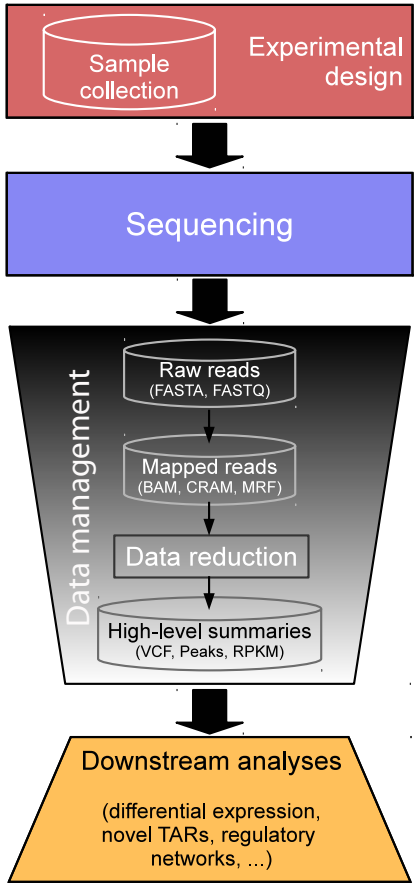
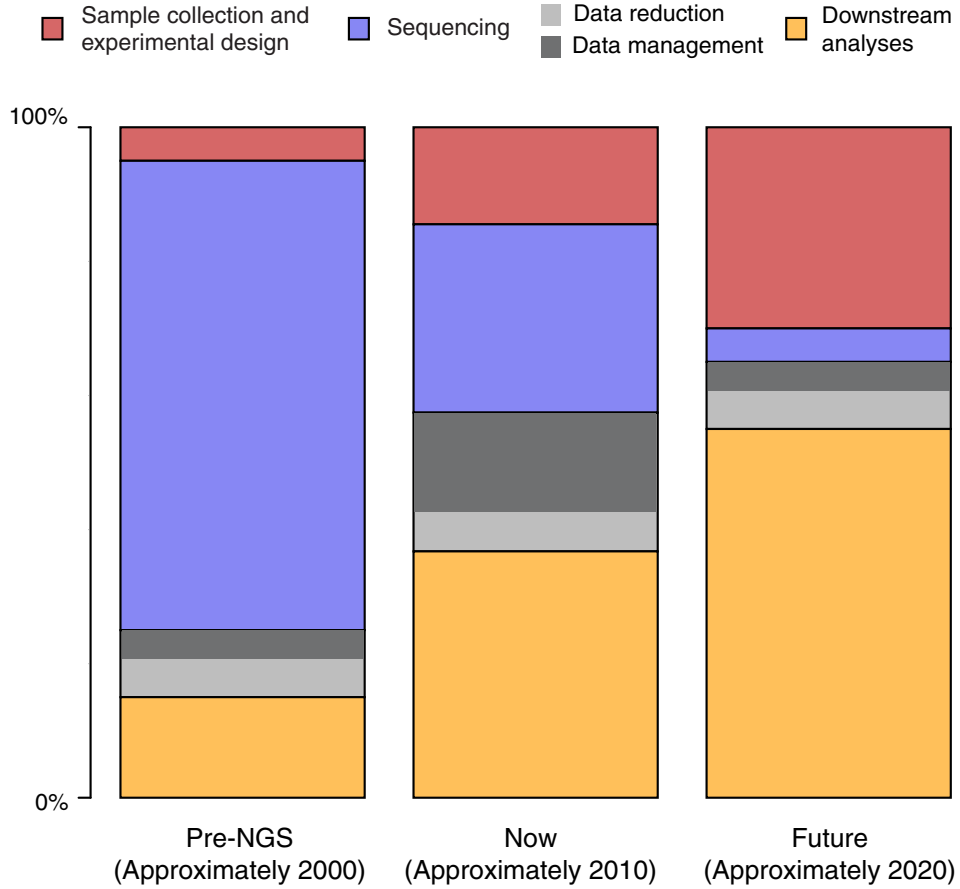
“The real cost of sequencing”





Sboner et al. Genome Biology 2011 12:125 doi:10.1186/gb-2011-12-8-125

The last mile

http://www.bikeblanket.com/blog/suisse

The Bottom Line:
In Genomics

Spend the time (and money) planning and producing **good quality, accurate and sufficient data.**

Get to know to the data, develop and test expectations, explore and identify patterns.

Result, **spend much less time** (and less money) extracting biological significance and results with fewer failures and reproducible research.