The NGS IT notes

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Talleres Internacionales de Bioinformática - UNAM- Enero 2012
Agenda

- NGS basic notions and history
- Outline of NGS IT challenges
- The storage part
- The networking part
- The computing part
- The big picture
How not to hit the NGS IT platform brick wall

Talleres Internacionales de Bioinformática - UNAM - Enero 2012

Sunday, January 15, 2012
NGS basics: The genome

- The genome is the entirety of an organism's hereditary information. It is encoded either in DNA or RNA. The genome includes both the genes and the non-coding sequences of the DNA/RNA.

- The DNA/RNA sequence is the "code". This code is used as the blueprint to construct proteins, the building blocks living things are made of.
NGS (why?)

Next Generation Sequencing (NGS, also known as High Throughput Sequencing) aims to help us:

- Record the “code” of life at a reasonable time and cost (sequencing).
- Understand how living things are made (transcriptome, proteome) and how the “code” works.
- Understand how the environment affects us (metagenomics) and how we differ from each other (comparative genomics)
- Assemble this information into a complete genome project
Sequencing throughput (ST)

\[ ST = \frac{\text{Sequences generated}}{\text{time (OR) cost}} \]

Gb - Gigabases  Mb - Megabases  kb - Kilobases

**Graph:**
- **Illumina AB/LifeTech Helicos**
- **454 (Roche)**
- **Capillary-based (AB)**

Length of Read (bp):
- \(~50\) Gb
- \(~300\) Mb
- \(~700\) kb
Reverse engineering workflow (software)

This program performs functions A and B by using algorithms X and Y.
NGS workflow

1. Prepare Genomic DNA Sample
2. Sequence Assembly
3. Determine 5. base
4. Data Analysis
   - SNP detection and error masking

Annotated Genome Project
NGS technologies

454 (Roche)
Illumina Genome Analyzer
Applied Biosystems SOLiD Analyzer
Single Molecule Sequencing Analyzers

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Illumina NGS workflow

Sequencing by Synthesis with the Illumina Genome Analyzer
1984
Leroy E. Hood builds the first semi-automated DNA sequencer

1987
ABI 370 Protein Sequencer by Applied Biosciences
NGS history

Walter Gilbert, Frederick Sanger, Paul Berg

1980 - Nobel Prize in Chemistry
"for their contributions concerning the determination of base sequences in nucleic acids" (Gilbert, Sanger)
"for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA" (Berg)
NGS history (3)

1996
Pål Nyrén and Mostafa Ronaghi invent pyrosequencing at the Royal Institute of Technology (KTH) Sweden

2004
454 Pyrosequencer
"We have an idea that could increase the rate and lower the cost of gene sequencing by [a factor of] 104 or 105"
The NGS IT challenge

- Life Scientists generate more data than they can analyze. They need the data, but they need IT to manage the NGS workflow.

- You need to know how to:
  - Store: How much and at what speed?
  - Move: NGS data needs to move around.
  - Process the data: CPU and memory requirements.
Enter the Petabyte era

Big data: Welcome to the petacentre

What does it take to store bytes by the tens of thousands of trillions? Cory Doctorow meets the people and machines for which it's all in a day's work.

Cory Doctorow

Ten seconds after I stepped into the roar of the data centre at the Wellcome Trust Sanger Institute, in rural Cambridgeshire, my video camera croaked: CARD FULL, Impossible. That morning, I'd tossed a handful of thumbnail-sized 32-GB memory cards into my pocket, each one good for a couple of hours' worth of high-definition video. Yet this one had filled up in seconds.

Published online 3 September 2008 | Nature 455, 16-21 (2008) | doi:10.1038/458016a

News Feature

Stories by subject

- Lab life
- Technology
- Science in culture

Stories by keywords

- Petabyte
- Terabyte
- Sanger Centre
- CERN
- WSSALL
- Internet Archive
- Cory Doctorow
- energy costs of computing
- Datacentres
- bioinformatics
- LHC
- data

This article elsewhere

The data centre at the Wellcome Trust Sanger Institute in Cambridge, UK, under development.

S. NORFOLK
## The Petabyte table

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# NGS devices data production

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<th>Library/template preparation</th>
<th>NGS chemistry</th>
<th>Read length (bases)</th>
<th>Run time (days)</th>
<th>Gb per run</th>
<th>Machine cost (US$)</th>
<th>Pros</th>
<th>Cons</th>
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<td>PS</td>
<td>330*</td>
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<td>Longer reads; improve mapping in repetitive regions; fast run times</td>
<td>High reagent cost; high error rates in homo-polymer repeats</td>
<td>Bacterial and insect genome de novo assemblies; medium scale (&lt;3 Mb) exome capture; 16S in metagenomics</td>
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<td>Illumina/Solexa’s GA</td>
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<td>RTs</td>
<td>75 or 100</td>
<td>4†, 9§</td>
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<td>Currently the most widely used platform in the field</td>
<td>Low multiplexing capability of samples</td>
<td>Variant discovery by whole-genome resequencing or whole-exome capture; gene discovery in metagenomics</td>
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<td>7†, 14§</td>
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<td>Two-base encoding provides inherent error correction</td>
<td>Long run times</td>
<td>Variant discovery by whole-genome resequencing or whole-exome capture; gene discovery in metagenomics</td>
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Courtesy Elliott H. Margulies PhD, NIH
Tiered storage

- **Tier 1**: raw unprocessed data as they come out from the instrument (mostly images)

- **Tier 2**: including base (or colour) calls, intensities and first pass quality scores

- **Tier 3**: Includes aligned and analyzed data (alignments of all the reads to a reference or de-novo assembly, if required)

- **Tier 4**: Backup up off-site of Tiers 2 and 3, in order to provide disaster recovery/regulatory requirements.
Tiered storage equations

\[ \text{Tier1}_{\text{store}} = \sum (N_{hts} \times G_{bpr} + (N_{hts} \times G_{bpr})/4) \times N_{\text{runs}} \]

where:
- \( N_{hts} \) = number of per type HTS devices
- \( G_{bpr} \) = Gigabytes per run

\[ \text{Tier2,3}_{\text{store}} = \sum (N_{\text{runs}} \times G_{\text{analysis}} + (N_{\text{runs}} \times G_{\text{analysis}})/3) \]

where:
- \( N_{\text{runs}} \) = expected number of runs per year
- \( G_{\text{analysis}} \) = Gigabytes per run for Tiers 2 and 3 (Table 1)

\[ \text{Tier4}_{\text{store}} = \text{Tier2,3}_{\text{store}} + R_{\text{period}} \times \text{Tier2,3}_{\text{store}} \]

where:
- \( R_{\text{period}} \) = number of years to keep the data
tiered storage example

- 2 x Illumina
- 2 x 454
- 1 x SOLiD
- 3 year data retention period
Filesystems

A filesystem is a key component of the Operating System that dictates how the files are stored and accessed.

**Commonly used disk filesystems:** ext3/4 (Linux), NTFS (Windows), HFS+ (MACOSX), ZFS (Sun), XFS (SGI)

**Shared/clustered/SAN filesystems:** GFS (RedHat), XSAN (Apple)

**Distributed File Systems (Network File Systems):** NFS(9), CIFS/SMB

**Distributed parallel fault-tolerant file systems:** GPFS (IBM), XtreemeFS, OneFS (Isilon), PanFS(Panasas), Lustre
Filesystem Requirements

- **Next Gen Sequencing (NGS) filesystems need to:**
  - **Be scalable in size**
  - **Be Scalable in the number of IOPS for read/writes/nested directory access**
  - **Allow concurrent access**
  - **Have file redundancy/replication features**
The data network and storage (2)

SAN
FiberChannel, iSCSI, or AoE

NAS
SMB, NFS, AFS
The data network and storage (3)

Questions for your IT architect/system administrator(s):

- Can you afford the pure Fiber Channel solutions today?

- How many storage interconnects you have (GigE, FC, Infiniband).

- Would it not be nice to have a smaller number of storage interconnects (consolidation)?
The data network and storage (4)

FCoE
The data network and storage - FCOE(5)
NGS Computing

- Multi-core CPU intensive jobs: Image processing, Quality Score
- Multi-core memory intensive jobs: Sequence Assembly
- Sequence assembly is the most challenging computational task in NGS, besides data storage
Sequence assembly graphs

Sequencing Reads

Graph Data Structure

R₁  ACGATAACCGTAGA
R₂  TACCGTAGATGCA
R₃  GTAGATGCAGTAC
De Bruijn Graph


How to get the complete sequence from short read overlapping sequences
Common Sequence Assemblers

- **Velvet**: [http://www.ebi.ac.uk/~zerbino/velvet/](http://www.ebi.ac.uk/~zerbino/velvet/)
- **SOAPdenovo**: [http://soap.genomics.org.cn/soapdenovo.html](http://soap.genomics.org.cn/soapdenovo.html)
- **ABYSS**: [http://www.bcgsc.ca/platform/bioinfo/software/abyss](http://www.bcgsc.ca/platform/bioinfo/software/abyss)
- **Curtain**: [http://code.google.com/p/curtain/](http://code.google.com/p/curtain/)
Sequence assembly requirements

- Installation: OpenMP, other middleware and libraries
- Large RAM requirement:
  - Get at least one memory fat node: RAM $\geq$ 256 Gbytes (512 Gb to 1Tb of RAM are common)
  - Standalone or part of a queue/batch system
  - Multi/core$\leftrightarrow$Multi-thread
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```

```
root@biotin:/storage/tools/lexandole/ra/ra_run_illumina
```
Tier 1 Architecture

http://ctdb.samba.org/
Bill of materials (data network)

Cisco Nexus Switch 5000 series

QLE8152 Dual Port 10GbE Ethernet to PCIe Converged Network Adapter (CNA).

www.qlogic.com
Bill of materials (storage and computing)

- Dell | EMC CX4-960 (8Gbit and 4Gbit FC/10 with FCoE support modules)
- Dell R815 Memory fat node - 32 cores - 512 Gbytes of RAM
- Dell 1950, 64 Gbytes of RAM/Qlogic CN cards (as access/front end nodes), 8 cores.
Questions?

admin@embnet.uio.no

and

http://www.embnet.org/join/ContactRegistration