E NGI Sweden

Next Generation Sequencing at the National Genomics Infrastructure



Phil Ewels phil.ewels@scilifelab.se Introduction to Bioinformatics Using NGS Data Umeå, 2018-11-14



National Genomics Infrastructure

Sequencing Technologies

Sequencing Applications

Bioinformatics at the NGI



The National Genomics Infrastructure

- Scilifelab NGI



🗾 NGI stockholm

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Our mission is to offer a state-of-the-art infrastructure for massively parallel DNA sequencing and SNP genotyping, available to researchers all over Sweden



SciLifeLab NGI





We provide guidelines and support for sample collection, study design, protocol selection and bioinformatics analysis



- NGI Organisation





- NGI Organisation



- Project timeline



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- Project timeline



- Methods offered at NGI



- NGI Stockholm

RNA-seq is the most common project type



NGI Stockholm Projects in 2017

- NGI Stockholm

- RNA-seq is the most common project type
- In total, NGI Sweden processed 1068 NGS projects with almost 50 000 samples in 2017



NGI Stockholm Samples in 2017

NGI Stockholm

- Median turn around times from QC passed to data delivered for 2017
 - Sequencing only: 11.5 days
 - RNA: 6.5 weeks
 - WGS: 8 weeks

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NGI stockholm

https://ngisweden.scilifelab.se/ file/stockholm_dashboard



Sequencing Technologies

Sequencing Types

Illumina

PacBio

Oxford Nanopore

Ion Torrent



lumina®

- Illumina Sequencing

- Largest provider of sequencing technology
- NGS machines use "Sequencing-by-synthesis"
 - Developed at the University of Cambridge in 1990s
 - Spun into a company called Solexa in 1998
 - Solexa acquired by illumina in 2007
- Responsible for vast majority of DNA sequencing experiments worldwide



- Illumina Sequencing



https://youtu.be/fCd6B5HRaZ8



- Illumina iSeq 100







- Illumina MiniSeq 100







- Illumina MiSeq







- Illumina NextSeq







- Illumina HiSeq 2500



->12345ABXX	illumina
0	0
	6
	9





- Illumina HiSeq 3000





- Illumina HiSeq 4000





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- Illumina HiSeq X





- Illumina NovaSeq 6000





- Illumina at NGI

iSeq 100	Coming soon to NGI Uppsala Small cheap runs
MiSeq	Small runs, long reads (2x300bp)
HiSeq 2500	Primary machine for most of NGI's history
HiSeq X	Cheap, high throughput Only allowed to run WGS with > 15X coverage
NovaSeq 6000	Newest machine, both Stockholm & Uppsala Will eventually replace HiSeq 2500



- Illumina at NGI





- Illumina at NGI



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- How to choose

- Number of reads required
 - How many samples, how deeply sequenced?
- Type of reads required
 - Single End / Paired End, length?
- Urgency and cost
 - Sharing flow cells with other users
 - Best price for the project



- Patterned flow cells

- New type of flow cell
 - HiSeq 4000, HiSeq X, NovaSeq
- Single sequence per well
 - Higher density, more data
- Different side effects
 - Index hopping
 - Duplicate reads





BOCFAIL.COM

Articles about common next-generation sequencing problems

Phil Ewels Simon Andrews

BOCFAIL.COM

Illumina Patterned Flow Cells Generate Duplicated Sequences

Steven Wingett



https://sequencing.qcfail.com/articles/illuminapatterned-flow-cells-generate-duplicated-sequences/
Relative positioning of duplicates



X-displacement



flow cell



Patterned flow cell



- Regular duplicate removal works fine
 - Sequence alignment positions should be identical
- Can use Picard MarkDuplicate optical duplicate settings
 - May need to increase the default pixel threshold
- Specialised tools such as <u>EdinburghGenomics/</u> <u>well_duplicates</u> work directly with .bcl files
 - https://github.com/EdinburghGenomics/well_duplicates



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Illumina 2 colour chemistry can overcall high confidence G bases

Simon Andrews



https://sequencing.qcfail.com/articles/illumina-2colour-chemistry-can-overcall-high-confidence-g-bases/

- Older SBS used four different fluorophores
 - One for each nucleotide
- New machines use two
 - Faster and cheaper
 - NextSeq, NovaSeq, iSeq





4-colour chemistry

Base	G Filter	A Filter	T Filter	C Filter
G		×	×	×
A	×		×	×
Т	×	×		×
С	×	×	×	
Ν	×	×	×	×

2-colour chemistry

Base	Green Filter	Red Filter
G	×	×
Α		
Т		×
С	×	
Ν	×	×



AGCGATAG+ATTGTTGC	3 408 780	3.22%	
GGGGGGGGG+GGGGGGGG	3 051 380	2.89%	
TCCGCGAA+ATTGTTGC	2 844 020	2.69%	
GAGATTCC+ATTGTTGC	2 602 140	2.46%	
TAATGCGC+ATTGTTGC	2 578 560	2.44%	
TCTCGCGC+ATTGTTGC	2 405 340	2.28%	
ATTACTCG+ATTGTTGC	2 281 500	2.16%	
CGCTCATT+ATTGTTGC	2 279 700	2.16%	
ATTCAGAA+ATTGTTGC	2 252 180	2.13%	
CTGAAGCT+ATTGTTGC	2 249 940	2.13%	
TCCGGAGA+CGTTTACT	2 236 880	2.12%	
GGGGGGGGG+TGTTTCCC	2 160 120	2.04%	
CGGCTATG+ATTGTTGC	2 132 900	2.02%	
GAATTCGT+ATTGTTGC	2 080 700	1.97%	
TCCGGAGA+ATTGTTGC	2 070 820	1.96%	
ATTACTCG+CGTTTACT	2 017 160	1.91%	
TGCGATTG+TTTGTGGC	1 730 080	1.64%	
ATTCAGAA+CGTTTACT	1 640 140	1.55%	
GGGGGGGGG+TTTTGCCT	1 475 160	1.4%	
CGCTCATT+CGTTTACT	1 408 140	1.33%	

Total

%

Count

Index



Base	Green Filter	Red Filter
G	×	×
А		
т		×
С	×	
N	×	×

Sample 1GGTTSample 2GTTC



Base	Green Filter	Red Filter
G	×	×
Α		
т		×
С	×	
N	×	×

Sample 1GCATSample 2ATGC



- Poor quality reads may show up as G instead of N
 - For example, missing bases from short insert sizes
- Trimming tools such as <u>cutadapt</u> now updated to handle this
- Careful colour balancing of indexes can avoid problems with deduplication
 - This isn't new it's just more sensitive than before
- Check the illumina recommendations:
 - <u>http://emea.support.illumina.com/downloads/index-adapters-pooling-guide-100000041074.html?langsel=/se/</u>

- Balanced pooling

- New NovaSeqs make the S4 the best option
- Proper sample concentration normalisation more important than ever
 - Big (expensive) flow cells = high stakes!
- Our plans: always improving library quantitation and normalisation
 - Constant benchmarking of quant tools
 - More accurate automation





- PacBio

- Pacific Biosciences specialists in long reads
 - Also uses fluorescent nucleotides
 - Polymerases immobilised at the bottom of tiny wells give off pulses as the nucleotides are incorporated
- Each well is independent, doesn't use sequencing rounds like illumina
- Can work with much longer DNA fragments
 - 250 bp 60 kb (max ~160 kb)

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- PacBio



https://youtu.be/NHCJ8PtYCFc



- PacBio RS II



- PacBio Sequel



- PacBio Sequencing

- Long reads are excellent for *de-novo* genome assembly, haplotype phasing and isoform detection
- Output is expensive compared to illumina, but getting better
 - Small genomes are no problem. Larger genomes are now becoming more feasible.
- New amplification-free enrichment using CRISPR-Cas9





- Oxford Nanopore

- Newest contender in the sequencing world
 - Lots of hype and taken several years to become a reality
- Still developing very fast
 - Quality, yield and cost changing almost monthly
- High error rates (but better than they used to be)
 - Now 2-13% depending on sequencing type



- Oxford Nanopore





- MinION



- MinION



- GridlON





- PromethION









SmidglON

SmidgION





- Oxford Nanopore

- The best technology available for ultra long reads
 - Twitter users report getting reads over 1 Mbp long
 - "Whale spotting" finding the longest reads on the end of the distribution curve
 - Need to balance yield with read length
- Price dropping rapidly, but still expensive compared to illumina
- NGI has 2x MinIONs and a PromethION
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iontorrent by **Thermo Fisher Scientific**

- Ion Torrent

- Main application
 - Microbial and metagenomic sequencing
 - Targeted re-sequencing (gene panels)
 - Clinical sequencing
- Short, single-end reads
- Fast run times



- Ion Torrent PGM



- Yield
 - 0.1 1 Gbp
- Run time
 - 3 hrs
- Read length
 - 200 400 bp



- Ion Torrent Proton



- Yield
 - 10 Gbp
- Run time
 - 4 hrs
- Read length
 - 200 bp

- Ion Torrent S5 XL



- Yield
 - 1-13 Gbp
- Run time
 - 3 hrs
- Read length
 - 200 600 bp



- Sequencing Type

- No need to remember all of this
 - Many considerations, changing all the time
- We are experts come and speak to us!

support@ngisweden.se

https://ngisweden.scilifelab.se/



Sequencing Applications
- Library Preparation

- All high throughput sequencing requires some kind of library preparation
 - Add adapters for sequencing chemistry
 - Adjust DNA fragment lengths
 - Incorporate biological signal into sequence
 - Add required enzymes
- Different library preps enable different applications



- RNA Sequencing

- Choose a type of RNA
 - Protein coding mRNA (poly-A)
 - All RNA (rRNA depletion)
 - Small RNA
- Choose your question
 - Differential gene expression
 - Differential isoform detection & quantification
 - Fusion gene detection

- Define your limitations
 - Low-input material
 - Low quality material (eg. FFPE)





- RNA Sequencing

- Illumina sequencing RNA library prep kits
 - Illumina TruSeq RNA
 - Illumina RiboZero
 - Illumina TruSeq RNA Exome
 - Clontech SMARTER Pico
 - Illumina TruSeq Small RNA

- Protein-coding poly-A
 - rRNA depletion
 - FFPE / low quality
 - low input
 - small RNA

• Oxford Nanopore, PacBio, IonTorrent



DNA Sequencing

- Choose your question
 - SNP, SNV, indel calling
 - Structural variant detection
 - *De-novo* genome assembly
- Choose your priorities
 - Sequencing accuracy
 - Sequencing depth
 - Ultra-long reads

- Define your requirements
 - Low-input material
 - Low quality material (eg. FFPE)

GGAGTTTTTGGGTGAGAACATATCCAAC	CTTTCTTTCCTTAGCTGGCAATACTT/
GGAGTTTTTGGGTGAGAACATATCCAAG	CTTTCTTTCCTTAGCTGGCAATACTT/
GGAGTTTTTGGGTGAGAAGATATGGAA	ATTERTICUT AUCTOUCAATACTE
GGAGTTTTTGGG <mark>G</mark> GAGAACATATCCAA	CTT TGGCAATACTT
GGAGTTTTTGG CATATCCAA	CTTTCTTCCTTAGCTGGCAATACTT
GGAGCTTTTGGGTGAGAACATATCCAA(CTTTCTTTCCTTAGCTGGCAATACTT/
GGAGTTTTCGGGTGAGAACATATCCAA	CTCTCTTTCCCTAGCTGGCAATACTT/
GGAGTCTTTGGGTGAGAACATATCCAA	CTTTCTTTCCTTAGCTGGCAATACTT/
GGAGITTTTGGGTGAGTAC CCA/	ACCTCCTTCCTTCGCTGGCAATACCT/
GGAGGTTTTGGGTGAGAACATATCCAA	CTTTCTTCCTTAGCTGGCAA
GGACAGTTAGGGAGAGAACATATCCAA	ETTTCTTTCCTTAGCTGGCAATACT
GGAGTTTTTGGGTGAGGACATATCCAA	CTTTCTTTCCAGATCGGAAGAGCT
GGAGTTTTTGGGTGAGAACATATCCAA	ATTTCTTCCTTAGCT CCTT/
GGAGGTTTTGGGAGAGAACATATCCAA	ATTTGTATCCTAAGATG
GMANTGTTTGGGTGAGAACATATCCAA	ATTTCTTCCTTAGCTGGCAATGCTT
GAGGTTGTGGGTGAGAACATATCCAA	ATTTCTTCCTTAGCTGGCAATACTT
CCACTTTTTCCCTCACAACATATCCAA	MTTTCTTTCCTTACCTCCCAATACTT.



- DNA Sequencing

- Illumina sequencing DNA library prep kits
 - Illumina TruSeq DNA PCR Free
 - Rubicon ThruPLEX
 - Illumina Nextera XT
 - Illumina Nextera Flex
 - 10X Genomics

- Best quality
 - Low input
- Cheap (plate format)
 - Fast and simple
 - Linked reads

• Oxford Nanopore, PacBio, IonTorrent



- 10X Genomics

- Chromium instrument uses droplet emulsion technology for nanoliter reaction volumes
- Linked-read sequencing
 - Large molecules fragmented in droplets and barcoded
 - Normal short-read illumina sequencing used
 - Long fragments (20-100+ Kbp) reassembled from barcodes
- Regular illumina sequencing libraries produced





10X Genomics



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10X Genomics

- Single cell RNA sequencing
 - Thousands of cells captured in droplets
 - Each RNA molecule tagged with droplet barcode





- Hi-C

- Now testing Hi-C in NGI Stockholm
 - Proximity ligation assay to detect physical colocation of DNA fragments within cell nuclei
- Multiple applications for data
 - Epigenetics

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- De-novo genome assembly
- Structural variation detection





- Methylation Sequencing

- Bisulphite sequencing detects Cytosine methylation in genomic DNA
 - Unmethylated Cs converted to Uracil by bisulfites and sequenced as T
 - Methylated Cs are protected and sequenced as C
- Oxidative bisulphite informs about hydroxy-methylation
 - Current under development at NGI Stockholm
- PacBio and Oxford Nanopore able to detect some native base modifications



- RAD Sequencing

- Restriction-site Associated DNA sequencing, also known as GBS (Genotyping By Sequencing)
 - Genome fragmented using a restriction enzyme
 - Narrow size range purified same regions of genome for all individuals
- Allows cheap high-depth variant calling for large numbers of samples, without a reference genome
 - Excellent for population genomics and ecology



- Amplicon Sequencing

- 16S / 18S / Custom amplicons
- High sample throughput
 - Plates of 96 samples processed using liquid handling automation
 - Large numbers of index combinations allow large pools
- Cheap and convenient for metagenomics and metabarcode sequencing projects
 - Contact us to talk about a pilot project

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Bioinformatics at the NGI

- Bioinformatics at NGI

- Raw sequencing data management
 - Demultiplexing, data transfers, backups, delivery
- Quality control
 - Every project is checked against quality criteria
- Automated analysis pipelines
 - Standardised pipelines give reproducible results
- Software development

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- NGI Data Handling



- Grus Deliveries

- UPPMAX tool for NGI data deliveries
 - NGI creates a SNIC Supr "delivery project" for each NGI sequencing project
 - Project PI and contact person given access, according to what was put on the order form
 - Email sent with project ID and instructions
- Grus is for secure short term storage only
 - Requires two-factor authentication





- Analysis Pipelines

- Initial data analysis for major protocols
- Internal QC and standardised starting point for users
- All software open source and on GitHub
 - <u>http://opensource.scilifelab.se/</u>
 - <u>http://github.com/SciLifeLab/</u>
- Accredited facility



Ackred. nr 1850 Provning ISO/IEC 17025



- Analysis Requirements





Sarek

GitHub https://github.com/SciLifeLab/Sarek

- Tumour/Normal pair WGS analysis based on GATK best practices
 - SNPs, SNVs and indels
 - Structural variants
 - Heterogeneity, ploidy and CNVs
- Works with regular WGS and Exome data too

Barntumörbanken



Sarek

- Tool split into subworkflows
- Preprint available on bioRxiv
 - https:// www.biorxiv.org/ content/early/ 2018/05/09/316976
- Will soon be main DNA pipeline at NGI







Barntumörbanken

- nf-core

- A community effort to collect a curated set of Nextflow analysis pipelines
 - GitHub organisation to collect pipelines in one place
 - No institute-specific branding
 - Strict set of guideline requirements
 - Automated testing for code style and function





- nf-core

<page-header>

For facilities

Highly optimised pipelines with excellent reporting. Validated releases ensure reproducibility.

For users

Portable, documented and easy to use workflows. Pipelines that you can trust.

For developers

0

Companion templates and tools help to validate your code and simplify common tasks.

Nextflow is an incredibly powerful and flexible workflow language. **nf-core** pipelines adhere to strict guidelines - if one works, they all will.

Documentation

Extensive documentation covering installation, usage and description of output tiles ensures that you wont be left in the dark.

CI Testing

Every time a change is made to the pipeline code, nf-core pipelines use continuousintegration testing to ensure that nothing has broken. Travis Cl

Stable Releases

nt-core pipelines use GitHub releases to tag statile versions of the code and software, making pipeline runs totally reproducable.



https://nf-co.re

Easy to run pipelines

- Helpful community
- Super reproducible
 results

- Quality Control

- Every project has some level of quality control checks
 - Sequencing quality
 - FastQC, FastQ Screen
- Analysis pipelines give application-specific QC
 - Qualimap, RSeQC
- Reporting is done using MultiQC





- MultiQC

- Reporting tool, parses logs from completed analysis
- Creates single HTML report for all samples & steps in a project
- Interactive plots for data exploration
- Current version now has 67 supported tools
- Works with anything from tens \rightarrow thousands of samples
- Highly customisable







v1.0

P1234: Test_NGI_Project

General Stats

NGI-RNAseq

Sample Similarity

MDS Plot

STAR

Cutadapt

FastQC

Sequence Quality Histograms

Per Sequence Quality Scores

Per Base Sequence Content

Per Sequence GC Content

Per Base N Content

Sequence Length Distribution

Sequence Duplication Levels

Overrepresented sequences

Adapter Content



5



P1234: Test_NGI_Project

This is an example project. All identifying data has been removed.

Contact E-mail:	phil.ewels@scilifelab.se
Application Type:	RNA-seq
equencing Platform:	HiSeq 2500 High Output V4
Sequencing Setup:	2x125
Reference Genome:	hg19

Report generated on 2017-05-17, 18:43 based on data in: /Users/philewels/GitHub/MultiQC_website/public_html/examples/ngi-rna/data

General Statistics

donoral otationoo									
🔏 Copy table	III Configure Columns	II Plot Showing 22/2	$_2$ rows and $^{6}\!/_{9}$ columns.						
Sample Name	% Aligned	M Aligned	% Trimmed	% Dups	% GC	M Seqs			
P1234_1001	68.2%	22.8	10.3%	71.3%	49%	33.7			
P1234_1002	67.9%	20.9	10.7%	70.1%	50%	31.1			
P1234_1003	64.7%	21.7	11.0%	72.3%	50%	33.7			
P1234_1004	55.2%	17.0	13.2%	73.4%	5196	31.2			
P1234_1005	53.0%	17.7	15.9%	75.8%	52%	33.8			
P1234_1006	52.7%	16.1	14.1%	73.8%	52%	30.8			
P1234_1007	33.0%	7.0	32.0%	80.5%	52%	21.8			
P1234_1008	27.5%	4.3	44.2%	79.1%	50%	16.7			
P1234_1009	52.3%	10.5	20.9%	64.2%	46%	20.5			

- Getting MultiQC

ewels / MultiQC BIOCONDA

🕜 http://multiqc.info ျှ





Python or pip not installed? See the full installation instructions.

Conclusions

- If you have a project

- Visit our order portal
 - Create projects
 - Request meetings
- Send us an email

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sit our order portal	Hometon - Documents Contact Ab Antional Contact Ab Contact Ab	NGI Sweden Order Portal				
Create projects	INFRASTRUCTURE Bast Concretion Sequencing and Concrypting for Dendlish Research	make an order, please log in and cho technology, please select the "Requir to place an order under "Information Projects from other countries are ad Sweden. Depending on the cueve si	peers for services provided by the National Genomics Intrastructure Sweden (MG), 10 nd choose the application most suitable for your project. If uncertain about the choice of "Request a meeting" option. You can read more about the different technologies and How mation" in the menu at the top of the page. are admissible, but have lower priority than projects performed by researchers based in usue situation, NOI may decide to decline a non-Swedish project altogether.			
Request meetings	1 Login	Subscribe to our mailing list	t your email address	Sitsoite	+ Create	
nd us an email	Email Email address of account Password -OLogin		you are unsure about the appropriate meth meeting for a discussion with us. umina Sequencing rder form for illumina sequencing. In Bequencing	od for your scientific proble	 Fraguesi Create Create 	
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support@ngisw	eden.se	summer, Library	preparation and sequencing will continue a	it a reduced pace.	Al nava	
ab	 Sample submission will be closed from June 291 Director and Co-Director positions for SciLifeLab ge Are you a visionary researcher within genomics with exp SciLifeLab now seeks a Director and a Co-Director to h both national and international candidates are welcome Hood more at SciLifeLab website. 	e August 6. nomics and high-throughput sequent perience of operating or supervising infa and the national Genomics Platform and cf	cing (2016-04-16) sstructures? Are you ready to take a mejor d high-throughput secuencing. An expressi	escierahio roles in cenomi ons of interest to these por	ca? illions from	

cies: Forskningsingenjör (vikariet) at NGI Uppsale (2018-04-16)

År du en utbilded biotekniker, molekylärbiolog eller liknende med hög noggrennhat, ansverskänsle och god samarbetsförmåga? Har du prektisk orfarenhet av storskeligt isboratoriearbete, gilms i ackrediterad miljó och med datorstyrda isboratorieinstrument? NGI Uppsala söker nu en forskningsingenjör till sekvenseringsenheten av SNP&SCOsknologiplattformen. Arbetet omfattar DNA-sekvensering med modern instrumentering hän Illumina säsom HiSegX, NovsSeg, HiSeg2500 och MiSeg.

Las mer om tjänsten och ansök på UU hemsida

Vacancies: 2 Bioinformaticians at NGI Uppsala (2018-04-11)

- Find our tools

- View our open-source software
- All code available on GitHub

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- Acknowledgements

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- ewels
- 🕑 tallphil

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NGI Sweden



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http://opensource.scilifelab.se

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