NGI Sweden

Next Generation Sequencing at the National Genomics Infrastructure

SciLifeLab

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Introduction to Bioinformatics Using NGS Data
Umeå, 2018-11-14

Overview

National Genomics Infrastructure

Sequencing Technologies

Sequencing Applications

Bioinformatics at the NGI

SciLifeLab

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The National Genomics Infrastructure

SciLifeLab NGI

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Research Programs

Technology Platforms

- National Genomics Infrastructure
- Proteomics
- Metabolomics
- Single-Cell Biology
- Cellular & Molecular Imaging
- Molecular Structure
- Chemical Biology
- Genome Engineering
- Diagnostic Development
- Drug Discovery & Development

National Bioinformatics Infrastructure

Data Office

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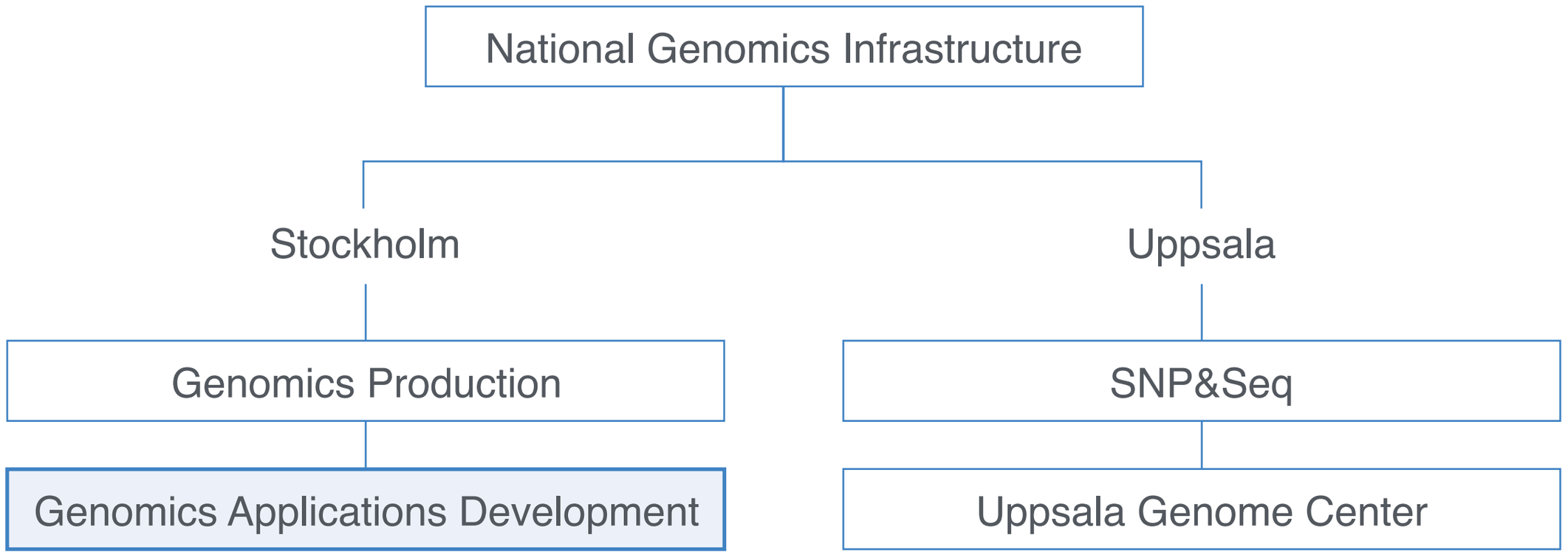

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SciLifeLab NGI

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INFRASTRUCTURE

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

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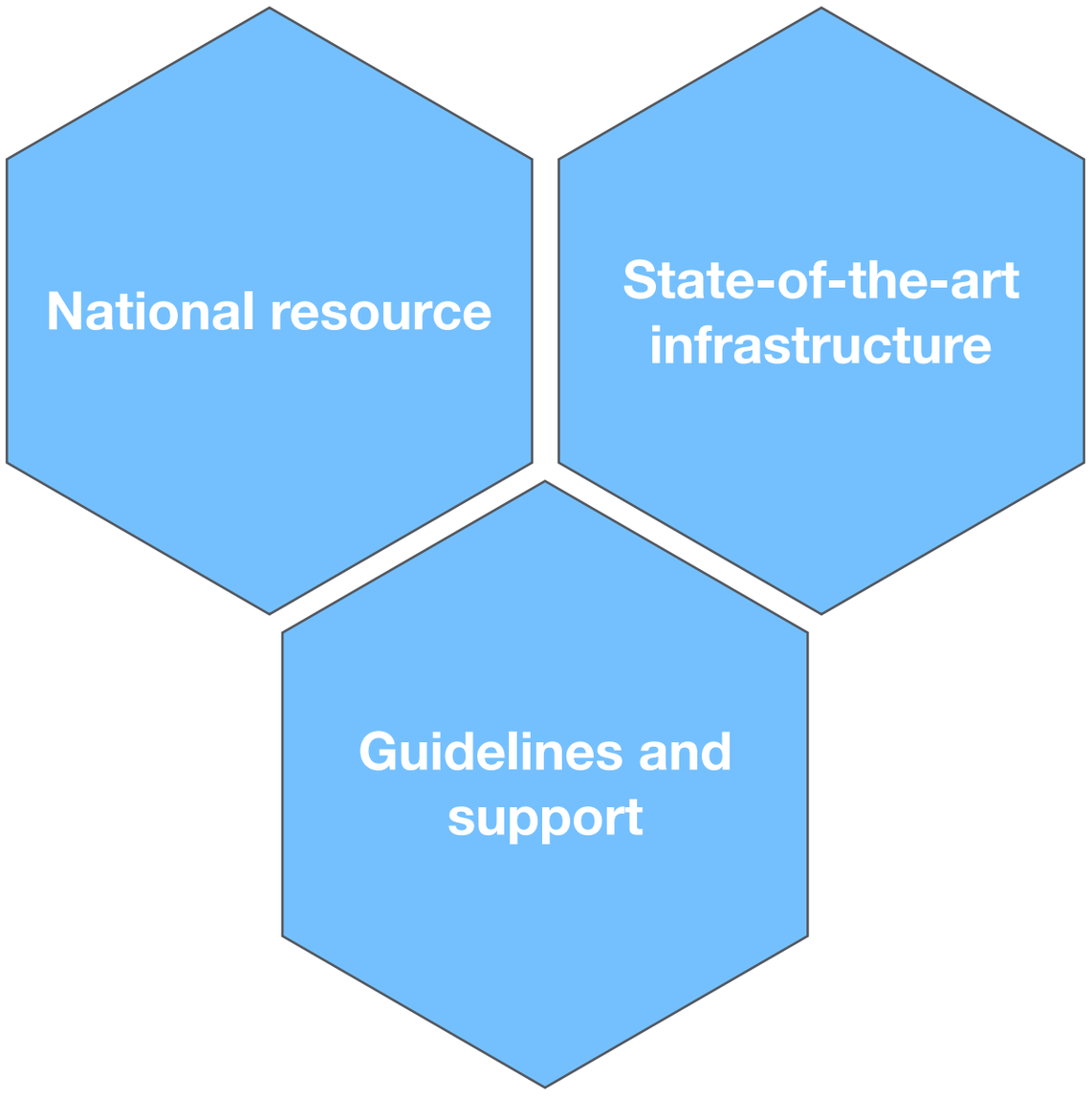

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We provide
guidelines and support
for sample collection, study design, protocol selection and bioinformatics analysis

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NGI Organisation

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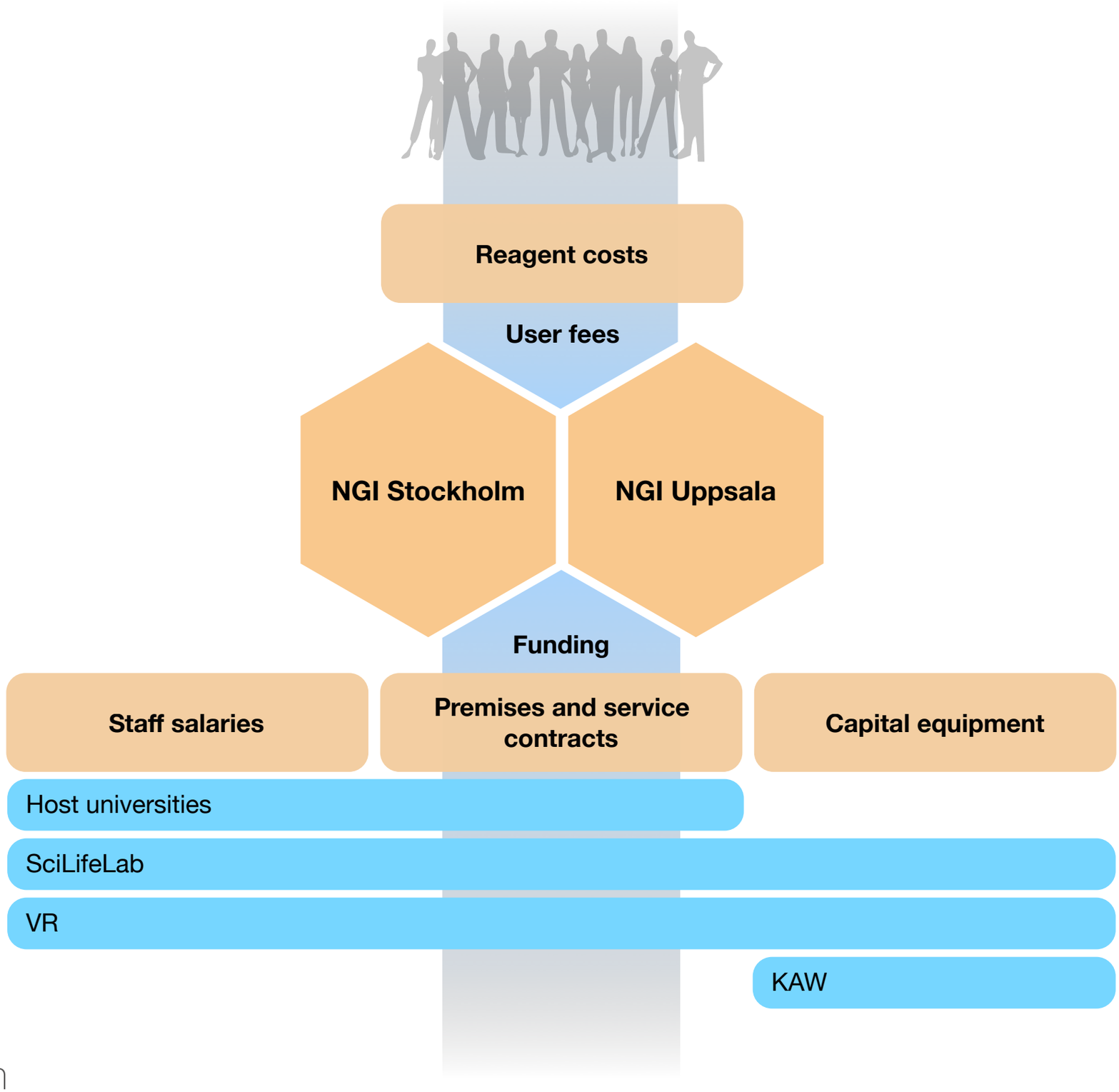

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Project timeline

Scientific support and project consultation

Sample QC

Library preparation, Sequencing, Genotyping

Data processing and primary analysis

Data delivery

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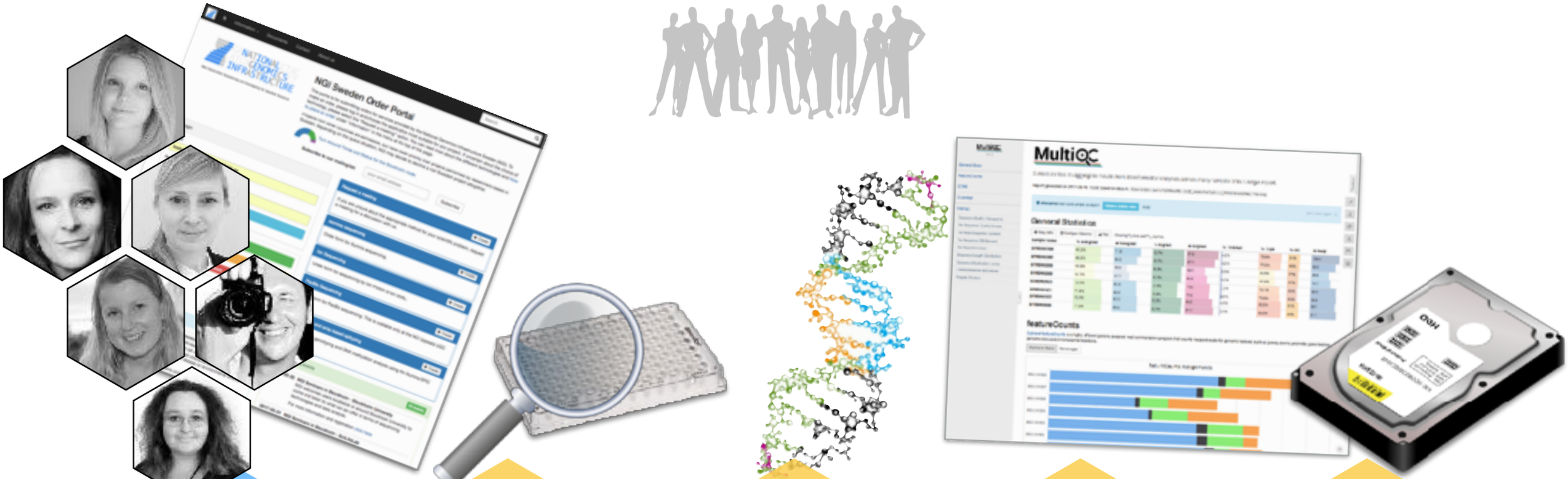
Project timeline



Scientific support and project consultation

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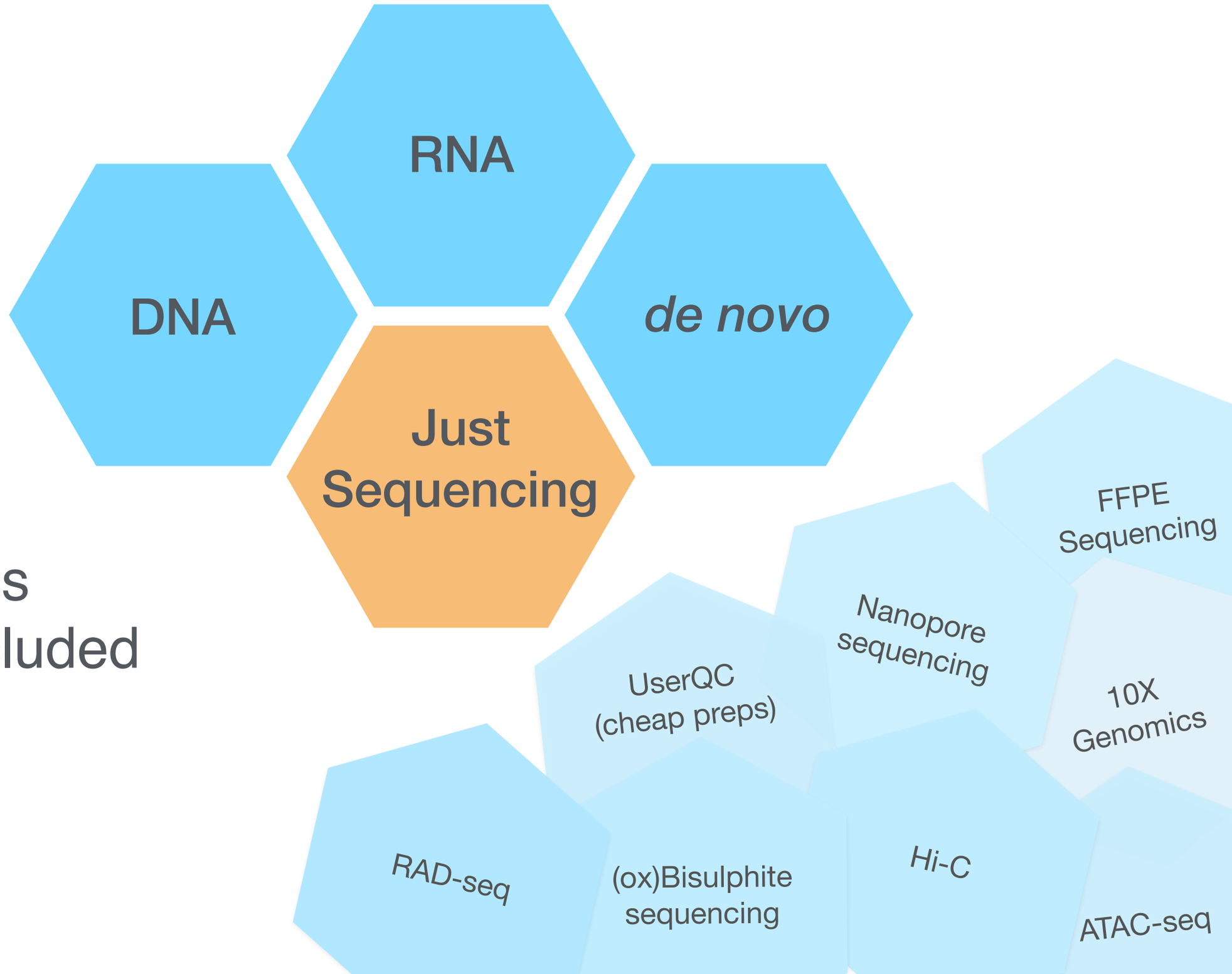
SWEDAC ACKREDITERING

Ackred. nr 1850
Provning
ISO/IEC 17025

Methods offered at NGI



Data analysis pipelines included

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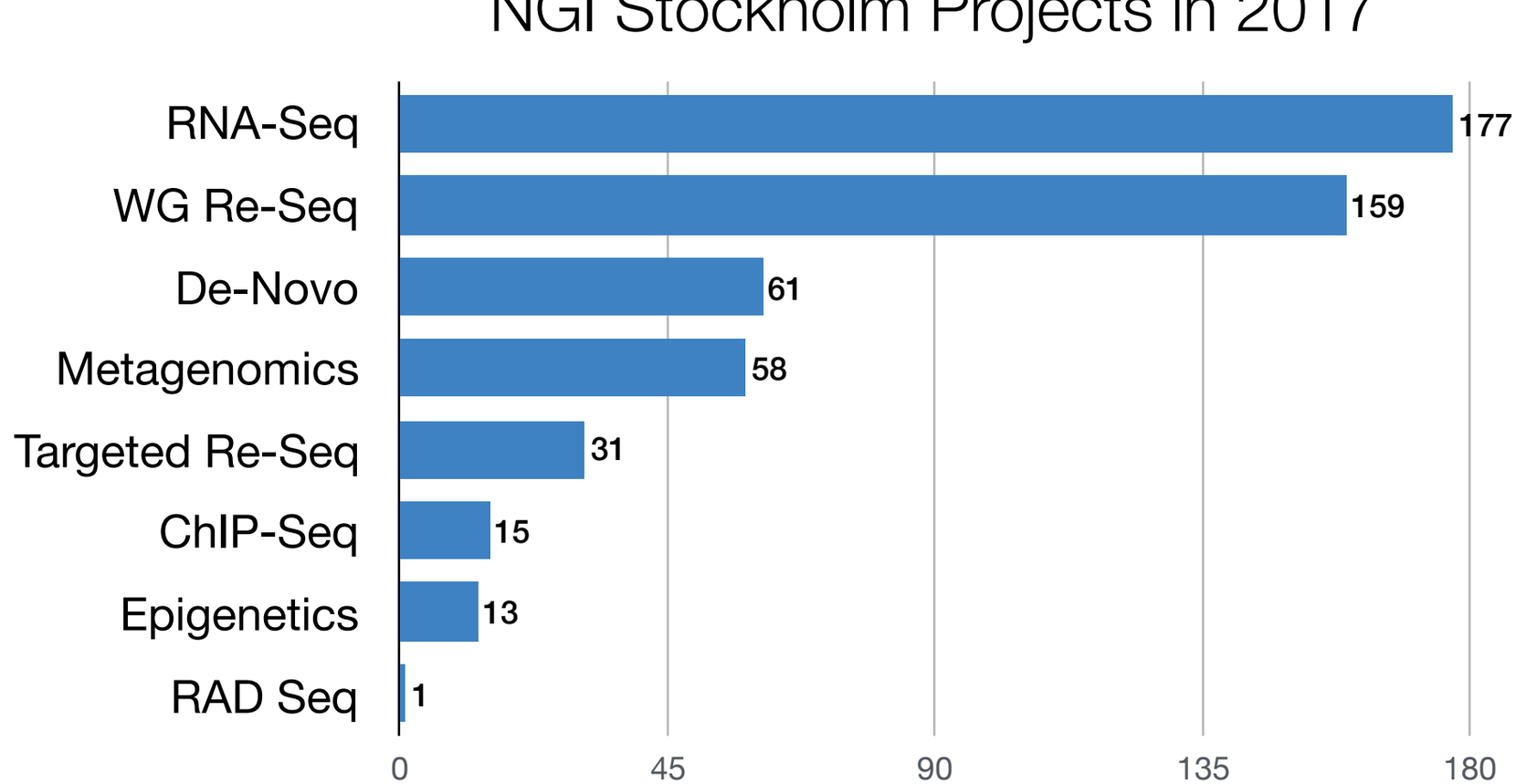
- RNA-seq is the most common project type



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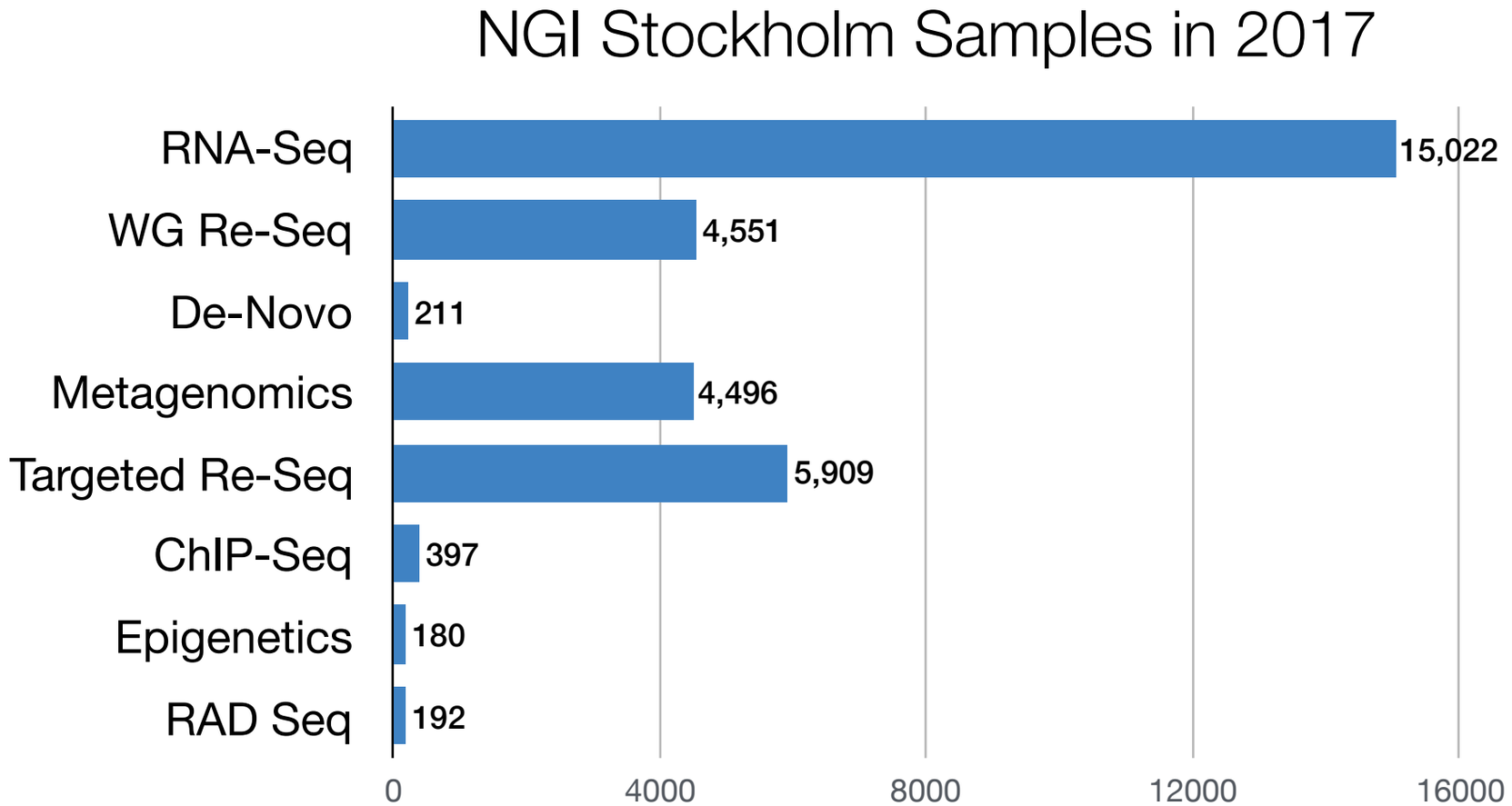
- RNA-seq is the most common project type
- In total, NGI Sweden processed 1068 NGS projects with almost 50 000 samples in 2017



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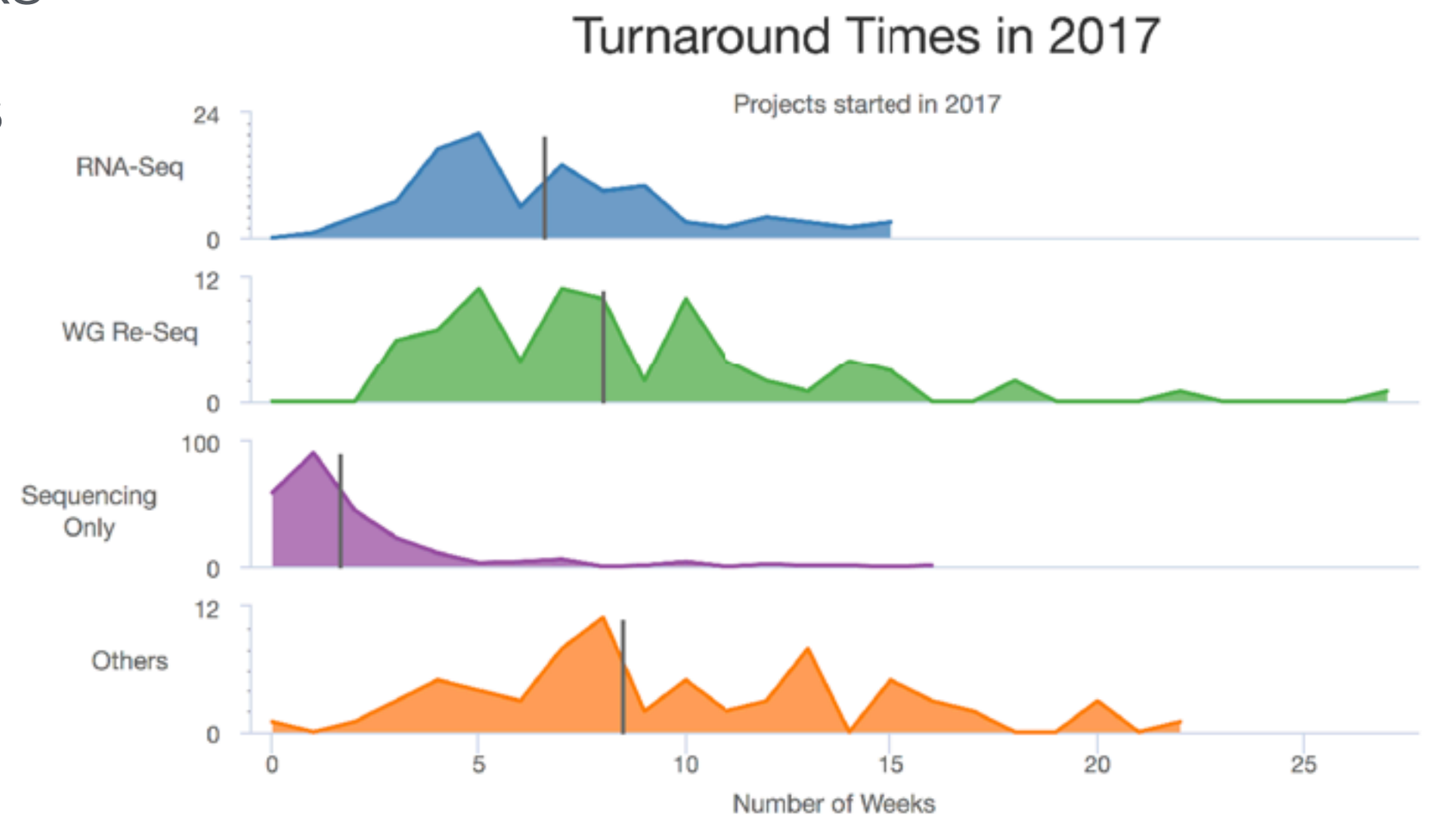
- Median turn around times from QC passed to data delivered for 2017
  - Sequencing only: 11.5 days
  - RNA: 6.5 weeks
  - WGS: 8 weeks

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



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Sequencing Technologies

Sequencing Types

Illumina

PacBio

Oxford Nanopore

Ion Torrent

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illumina®

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

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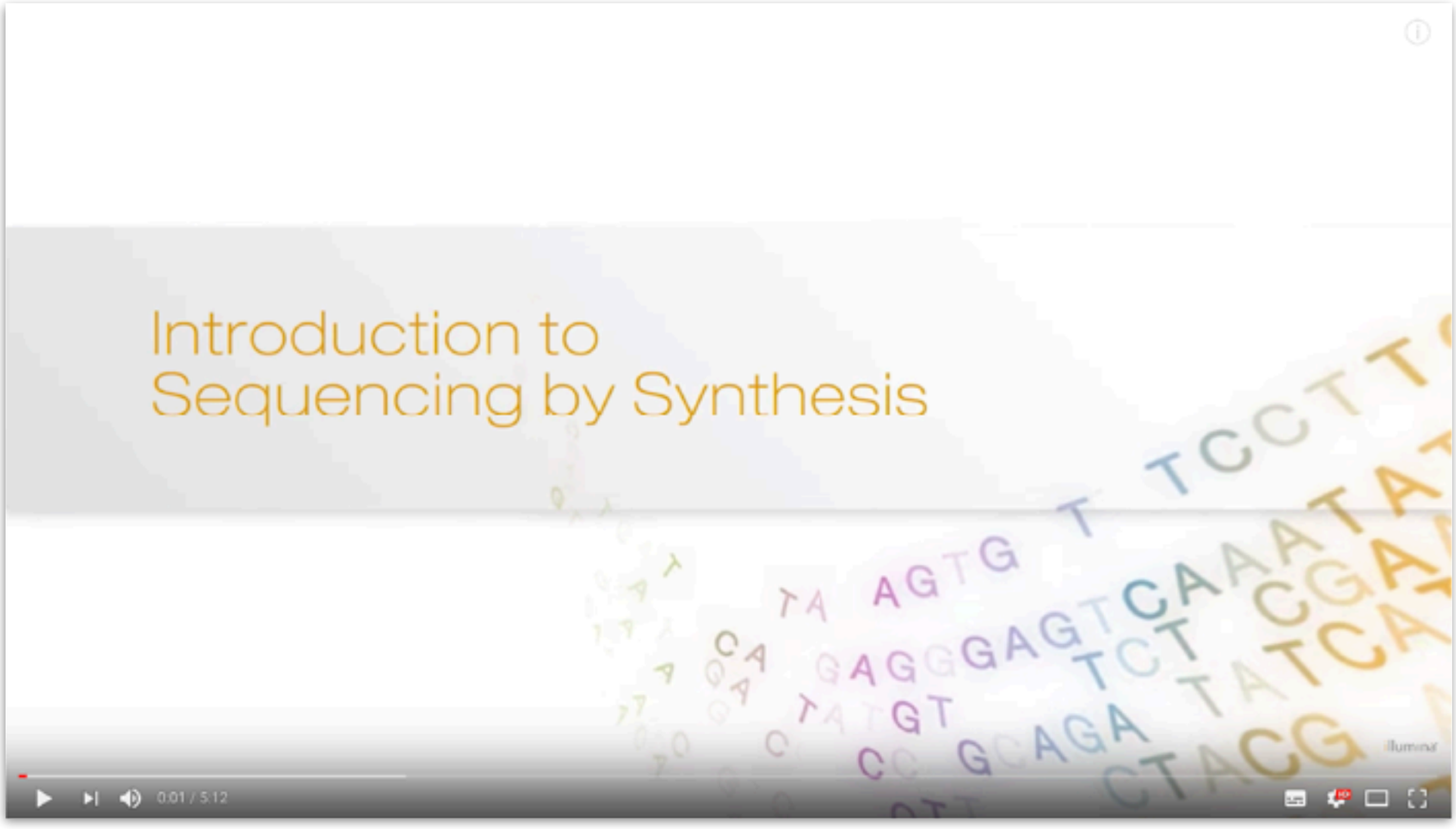
Illumina Sequencing



https://youtu.be/fCd6B5HRaZ8

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Illumina iSeq 100

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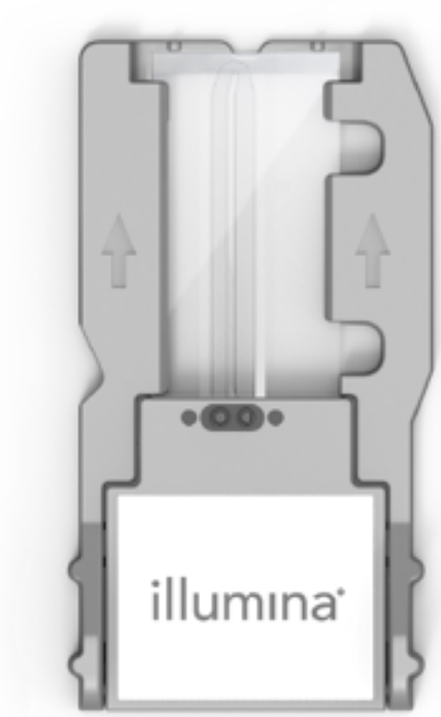
Illumina MiniSeq 100



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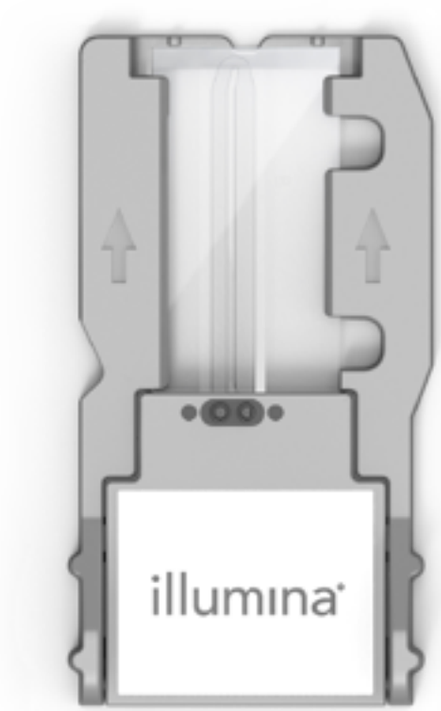
Illumina MiSeq



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Illumina NextSeq

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Illumina HiSeq 2500

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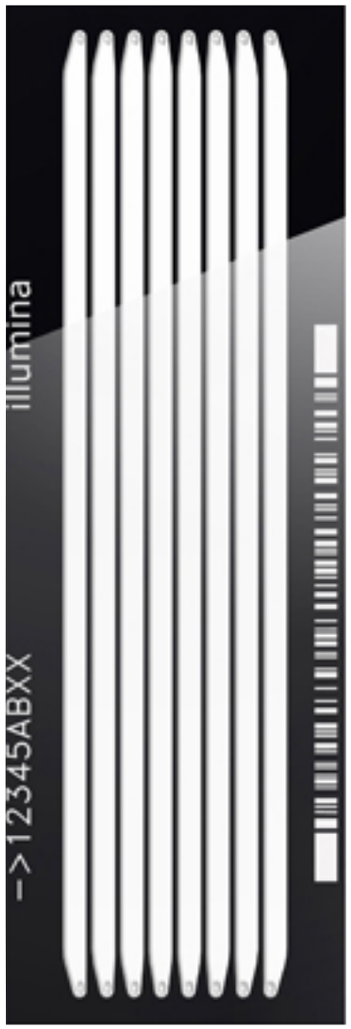
Illumina HiSeq 3000



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Illumina HiSeq 4000

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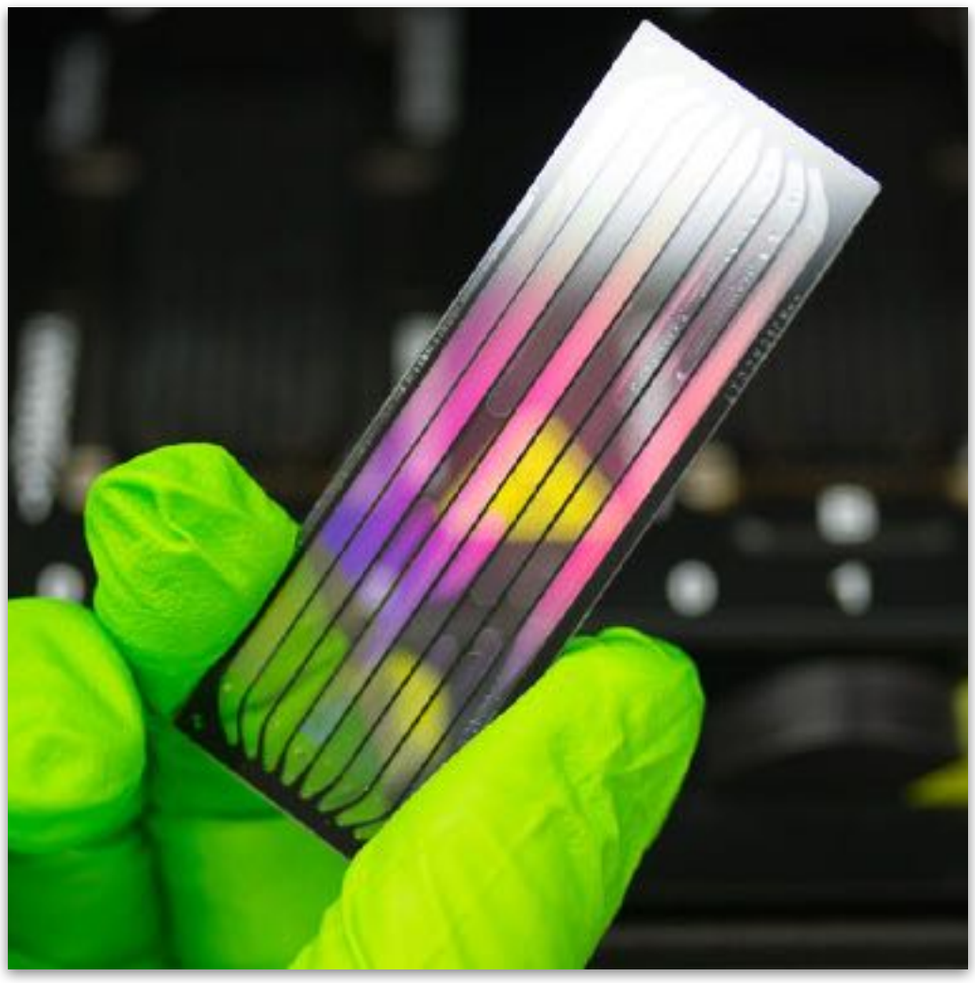
Illumina HiSeq X



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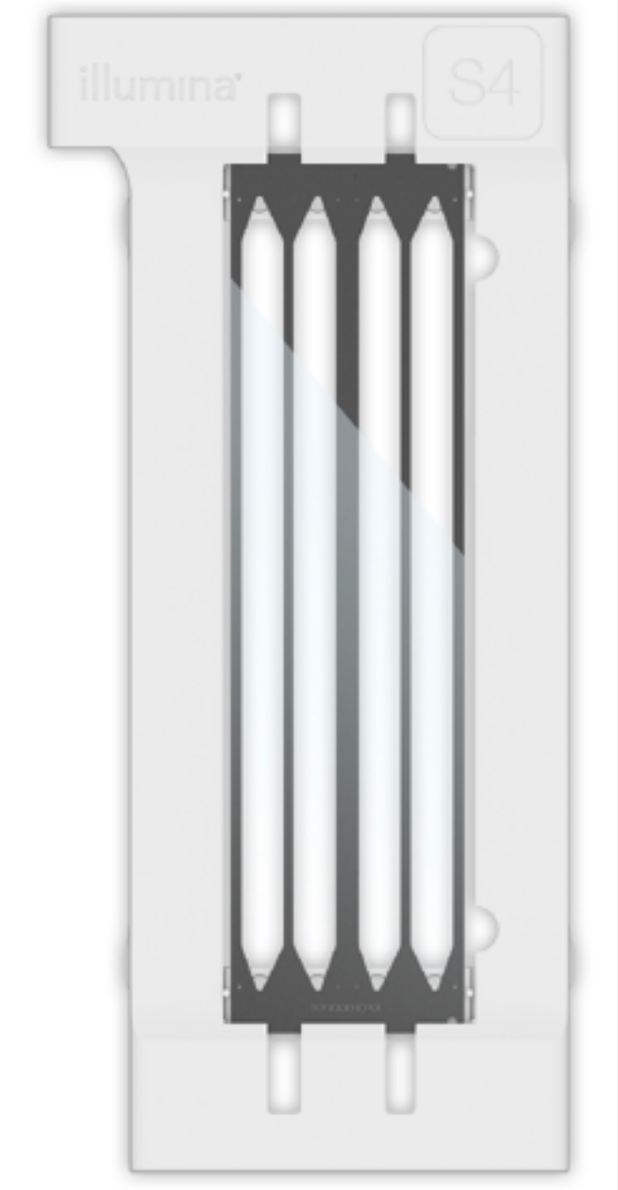
Illumina NovaSeq 6000



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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

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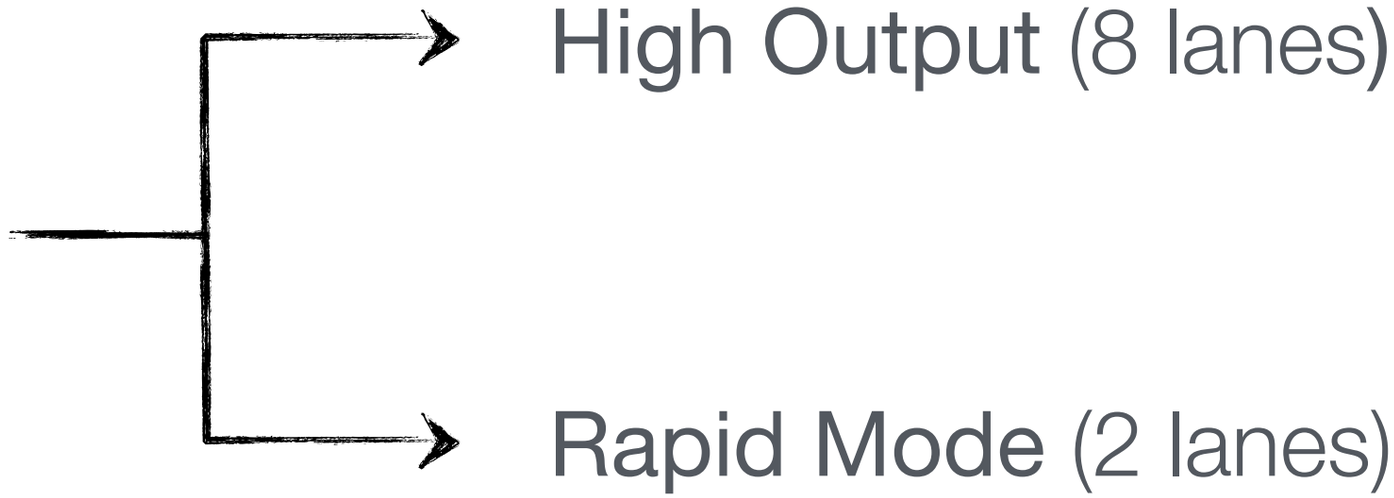
Illumina at NGI

- iSeq 100
- MiSeq
- HiSeq 2500
- HiSeq X
- NovaSeq 6000



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Illumina at NGI

- iSeq 100
- MiSeq
- HiSeq 2500
- HiSeq X
- NovaSeq 6000

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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

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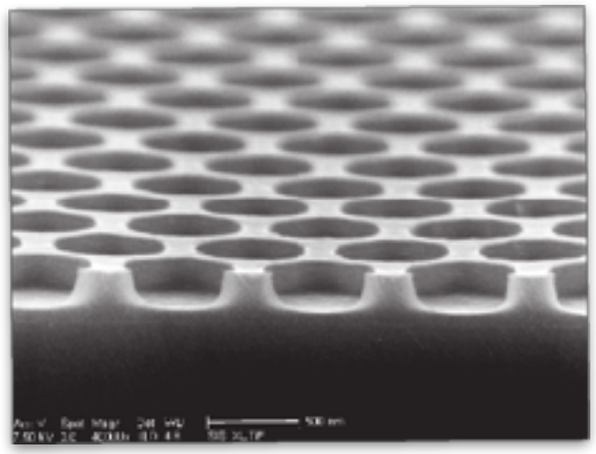
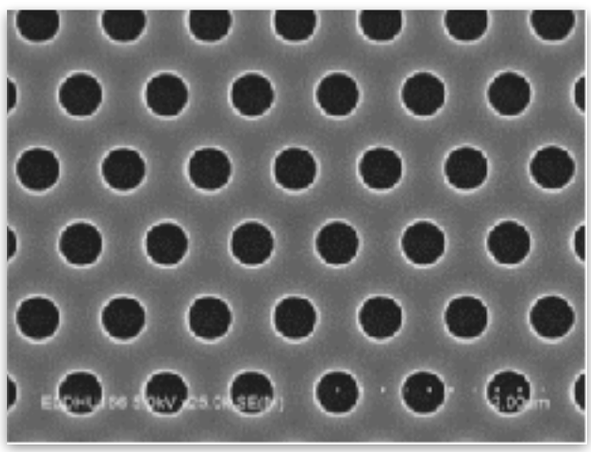
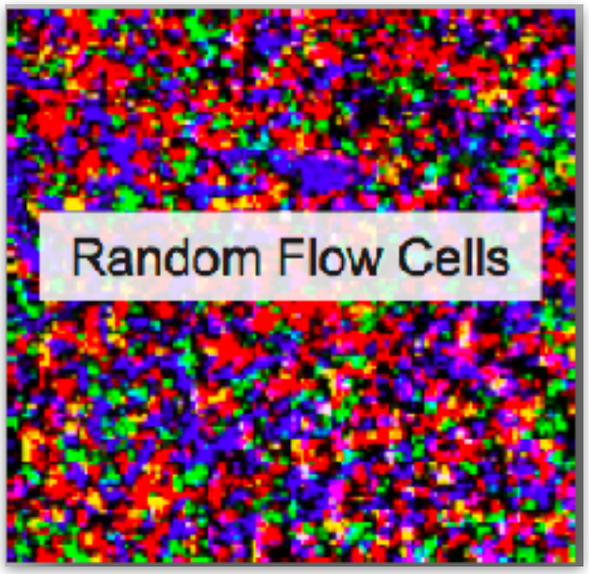
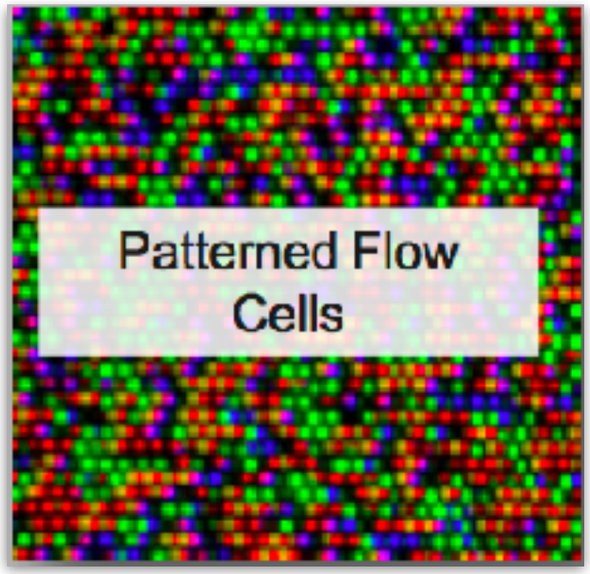
Patterned flow cells

- 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









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QCFAIL.com

Articles about common next-generation sequencing problems

Phil Ewels
Simon Andrews

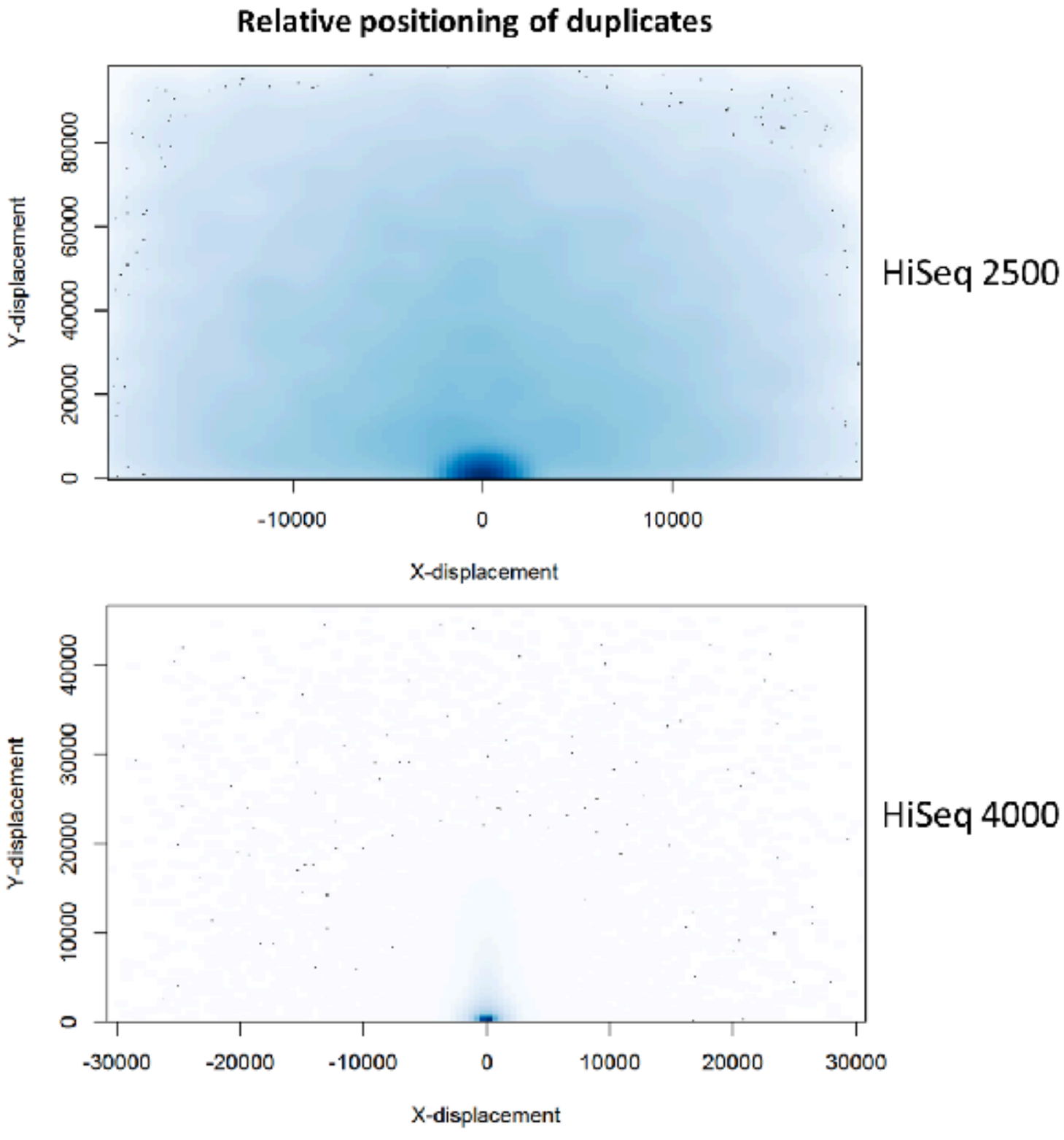
QCFAIL.com

Illumina Patterned Flow Cells Generate Duplicated Sequences

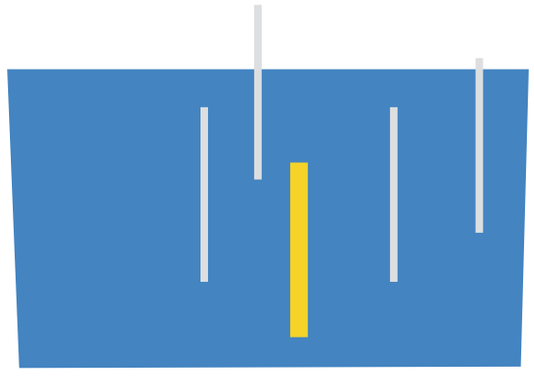
Steven Wingett

https://sequencing.qcfail.com/articles/illumina-patterned-flow-cells-generate-duplicated-sequences/

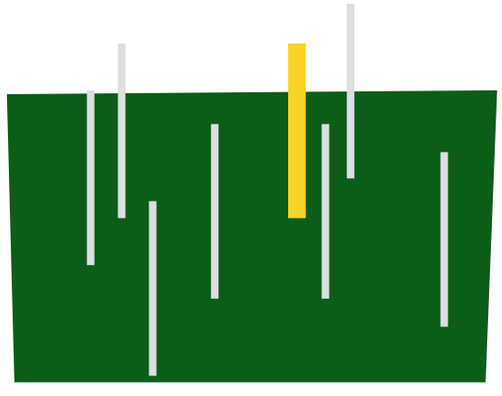
Patterned duplicates



Duplicates on different tiles

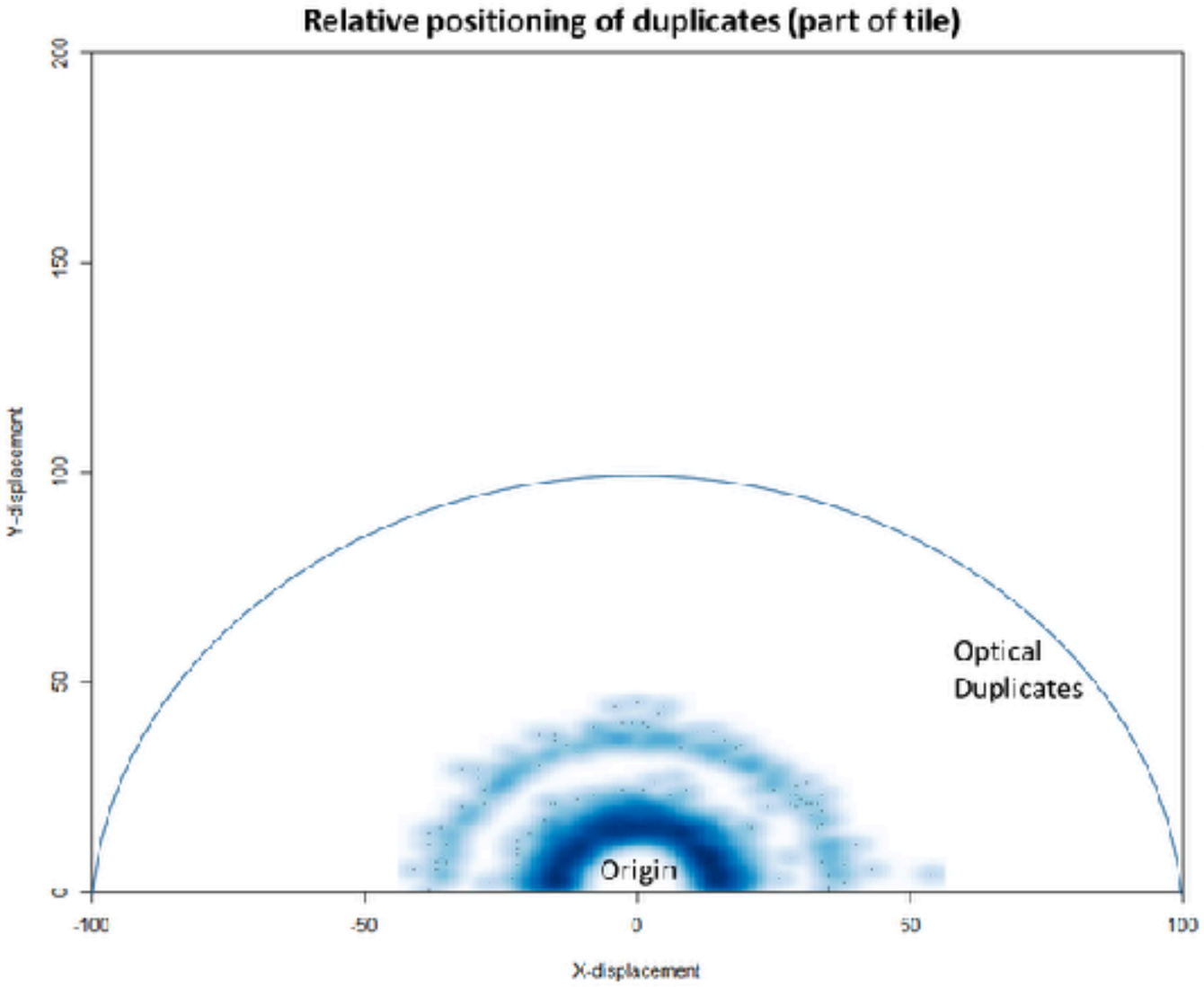


Tile A



Tile B

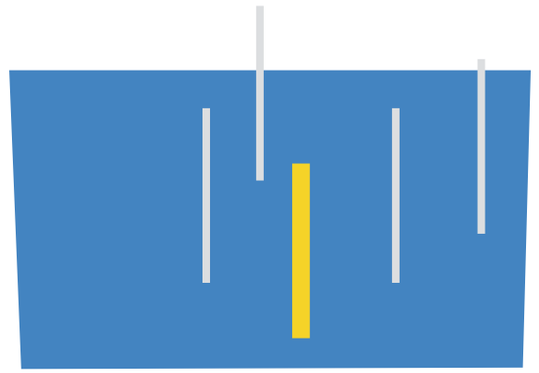
Patterned duplicates



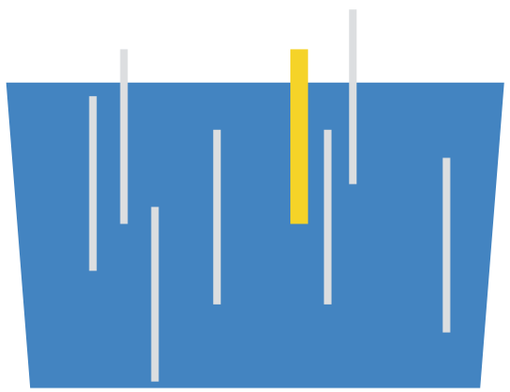
Unpatterned flow cell

Duplicates on the same tile

HiSeq 2500

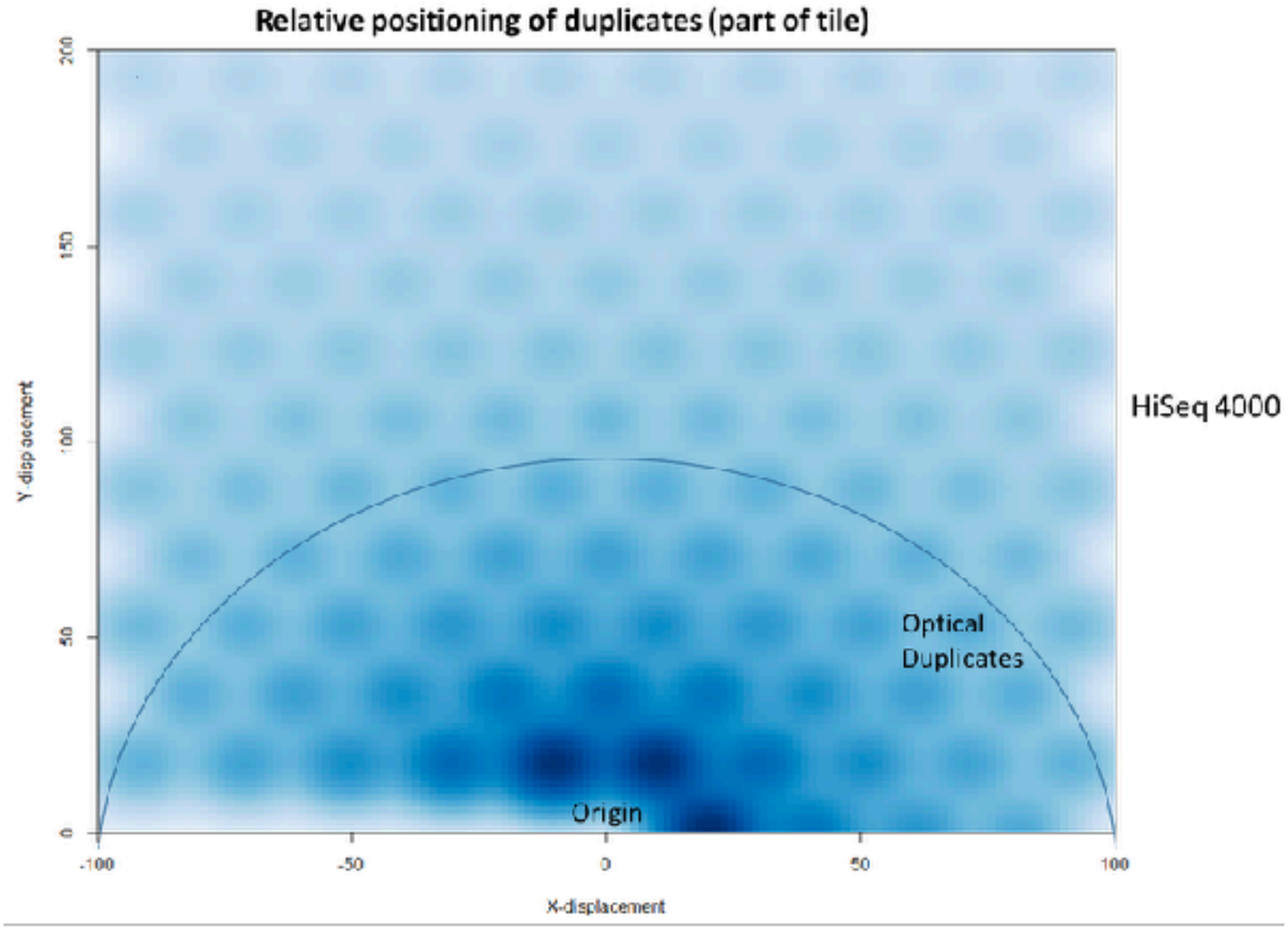


Tile A



Tile A

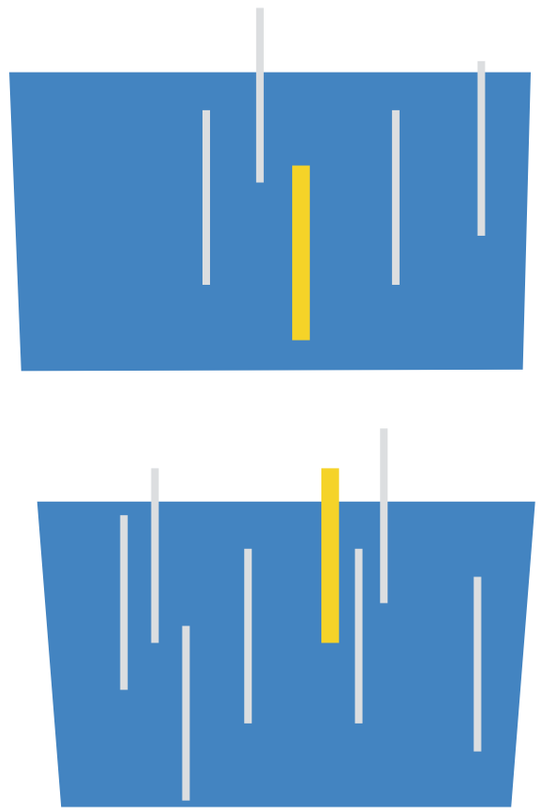
Patterned duplicates



HiSeq 4000

Patterned
flow cell

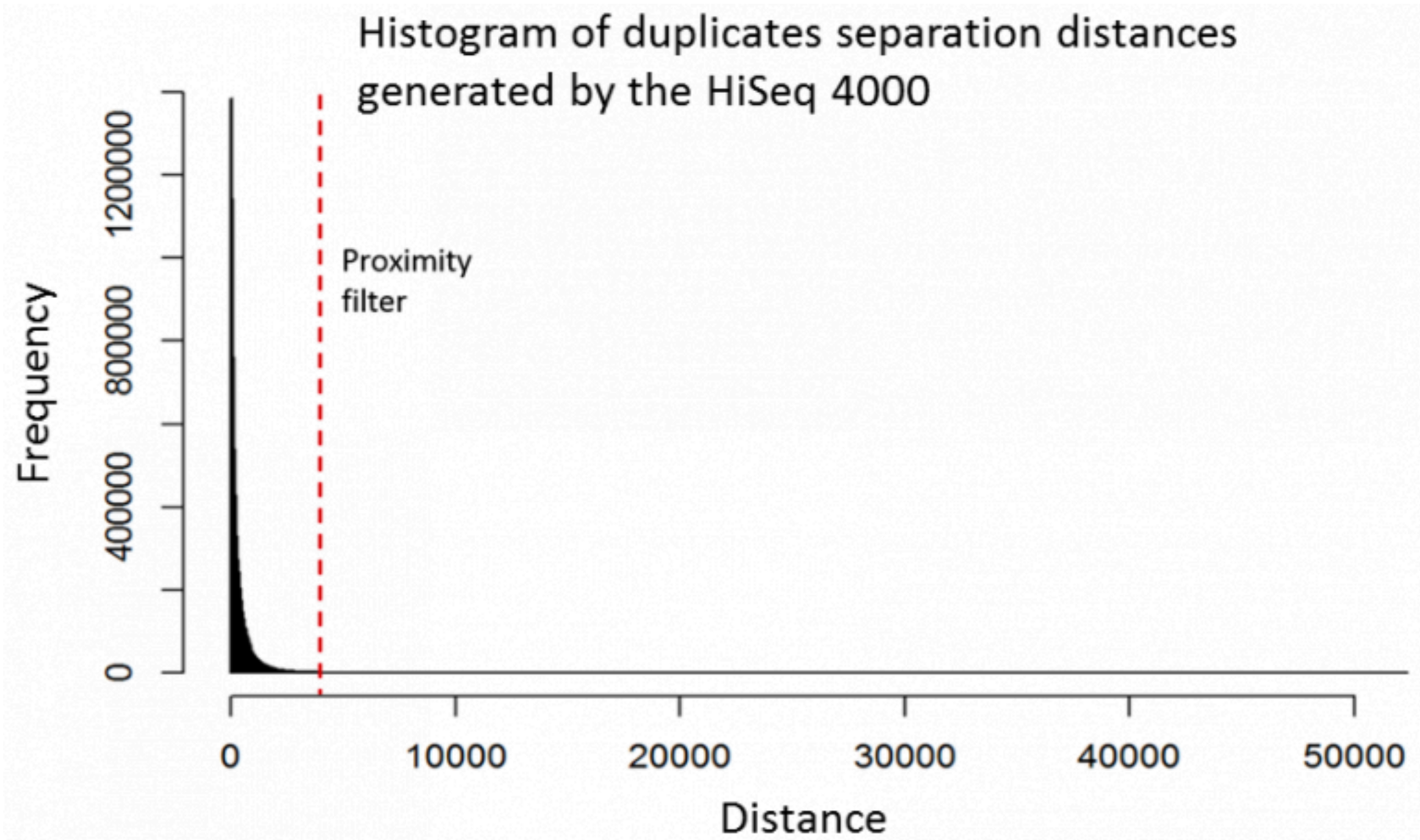
Duplicates on
the same tile



Tile A

Tile A

Patterned duplicates



Patterned duplicates

- 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 `EdinburghGenomics/well_duplicates` work directly with `.bcl` files
  - https://github.com/EdinburghGenomics/well_duplicates

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QCFAIL.com

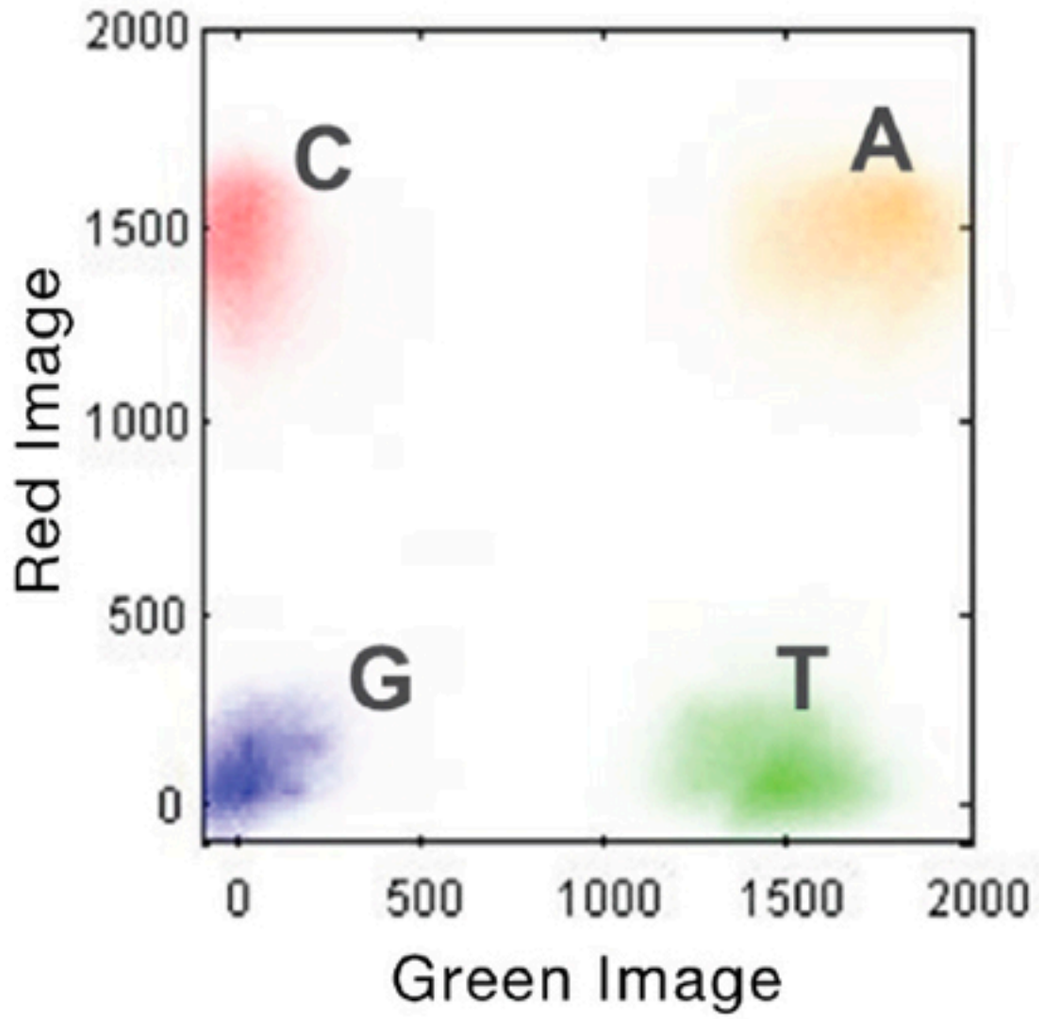
Illumina 2 colour chemistry can overcall high confidence G bases

Simon Andrews

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

Colour chemistry

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



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Colour chemistry

4-colour chemistry

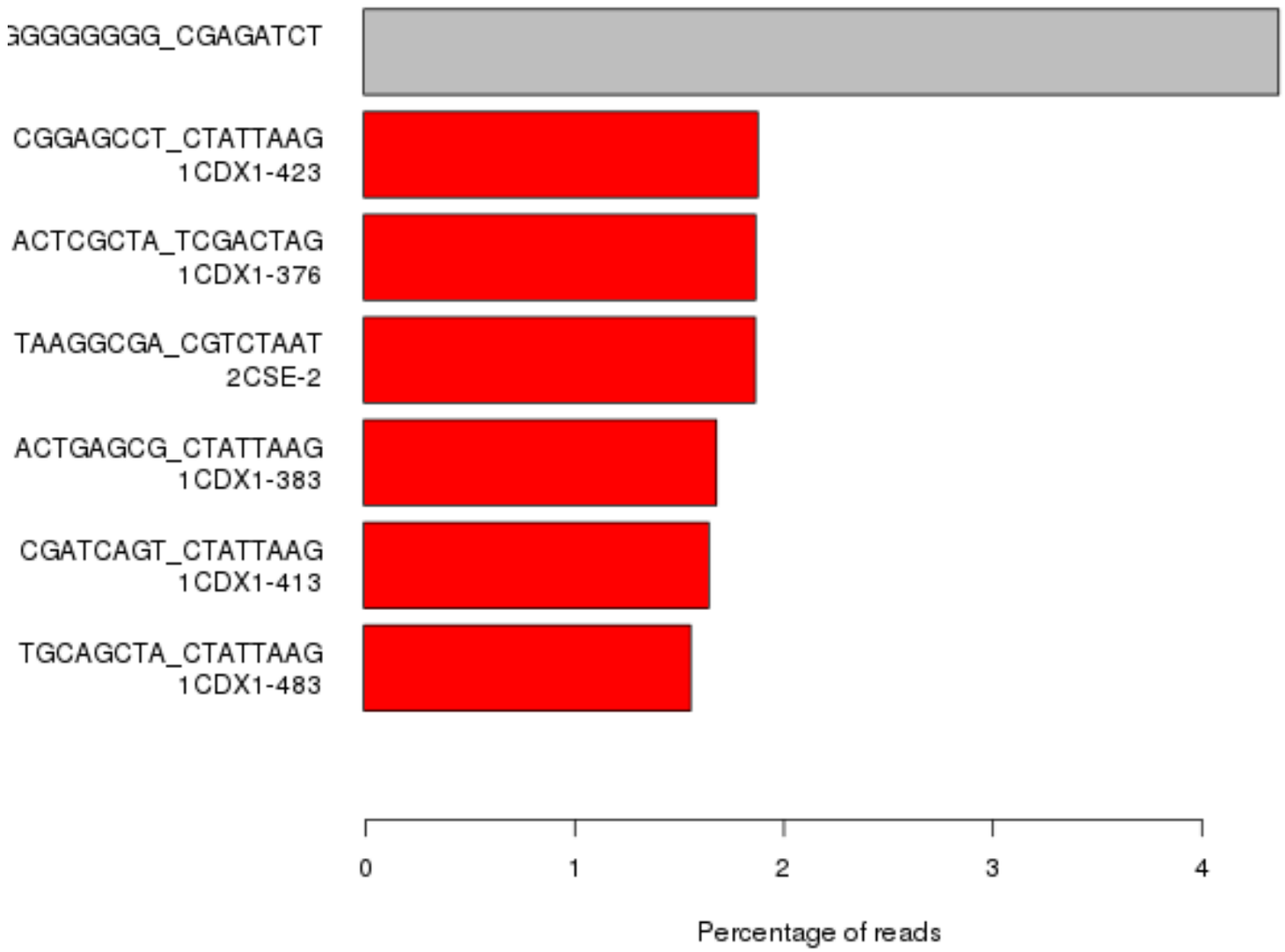
Base	G Filter	A Filter	T Filter	C Filter
G	✅	❌	❌	❌
A	❌	✅	❌	❌
T	❌	❌	✅	❌
C	❌	❌	❌	✅
N	❌	❌	❌	❌

Colour chemistry

2-colour chemistry

Base	Green Filter	Red Filter
G	❌	❌
A	✅	✅
T	✅	❌
C	❌	✅
N	❌	❌

Colour chemistry



Index	Count	Total %
AGCGATAG+ATTGTTGC	3 408 780	3.22%
GGGGGGGG+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%
GGGGGGGG+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%
GGGGGGGG+TTTTGCCT	1 475 160	1.4%
CGCTCATT+CGTTTACT	1 408 140	1.33%

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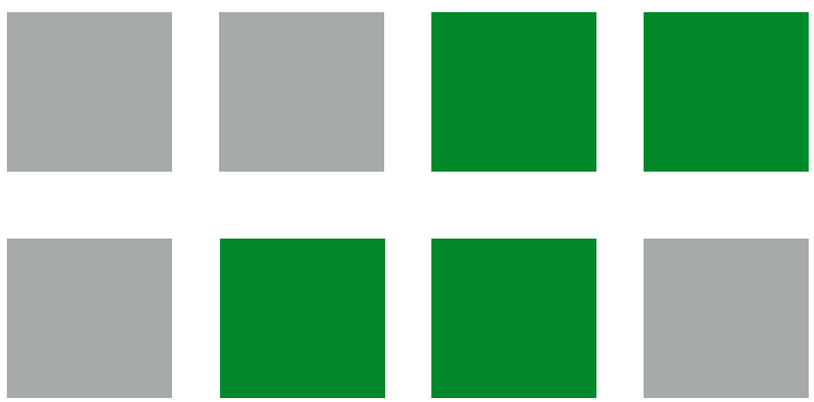
Colour chemistry

Base	Green Filter	Red Filter
G	❌	❌
A	✅	✅
T	✅	❌
C	❌	✅
N	❌	❌

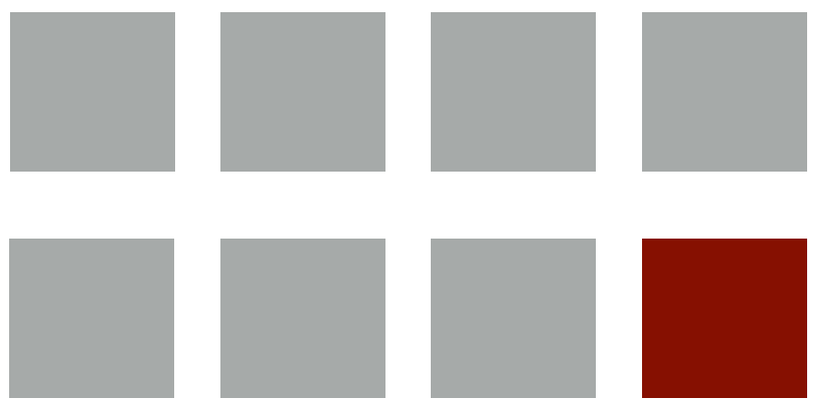
Sample 1 GGTT

Sample 2 GTTC

Green



Red



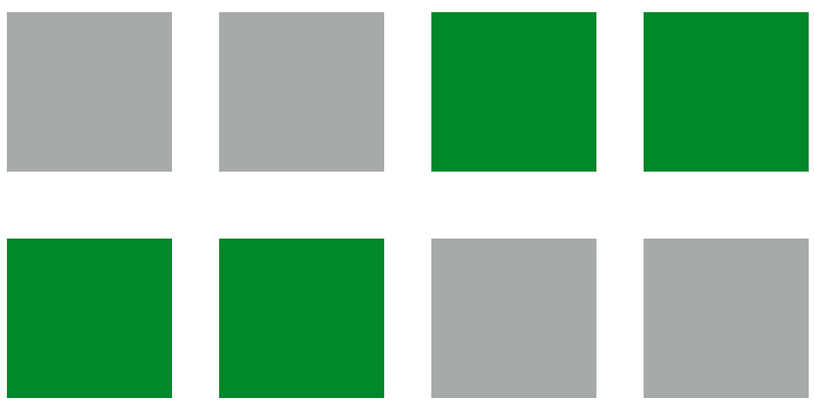
Colour chemistry

Base	Green Filter	Red Filter
G	❌	❌
A	✅	✅
T	✅	❌
C	❌	✅
N	❌	❌

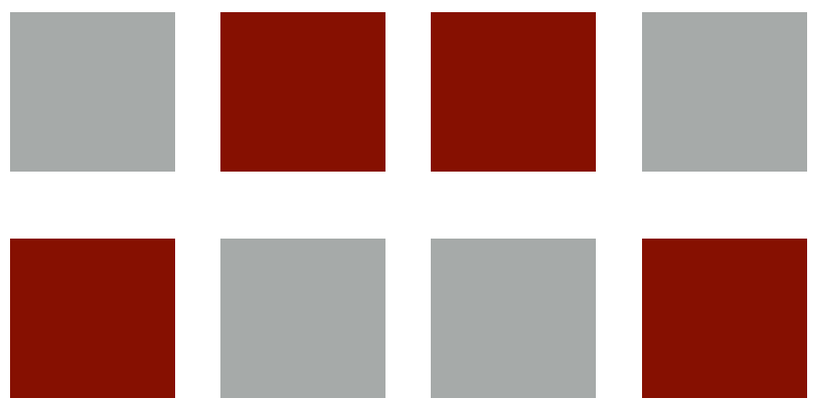
Sample 1 GCAT

Sample 2 ATGC

Green



Red



Colour chemistry

- Poor quality reads may show up as G instead of N
  - For example, missing bases from short insert sizes
- Trimming tools such as cutadapt 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:
  - http://emea.support.illumina.com/downloads/index-adapters-pooling-guide-1000000041074.html?langsel=/se/

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

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illumina®

PACIFIC BIOSCIENCES®

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

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PacBio RS II

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PacBio Sequel

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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

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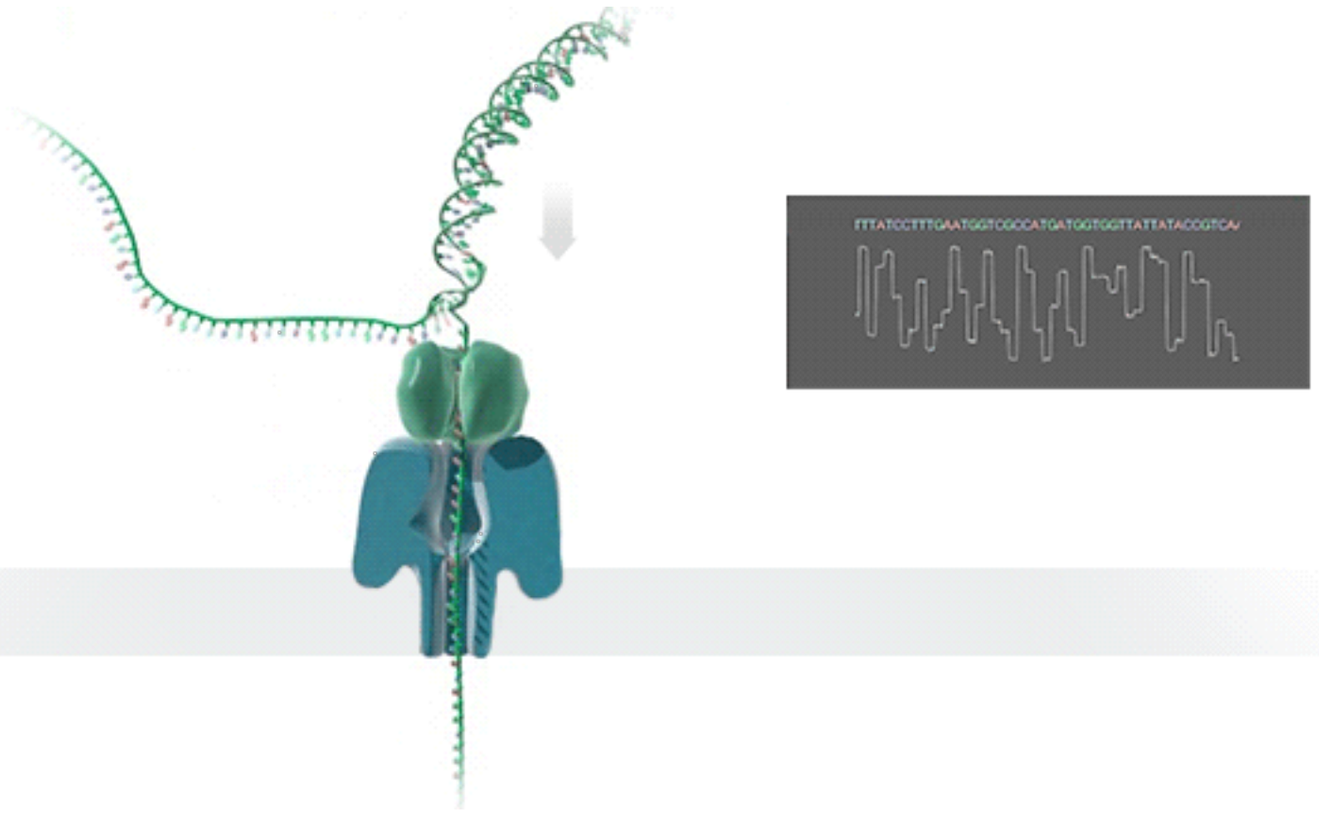
Oxford
NANOPORE
Technologies

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



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MinION

SciLifeLab

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MinION

SciLifeLab

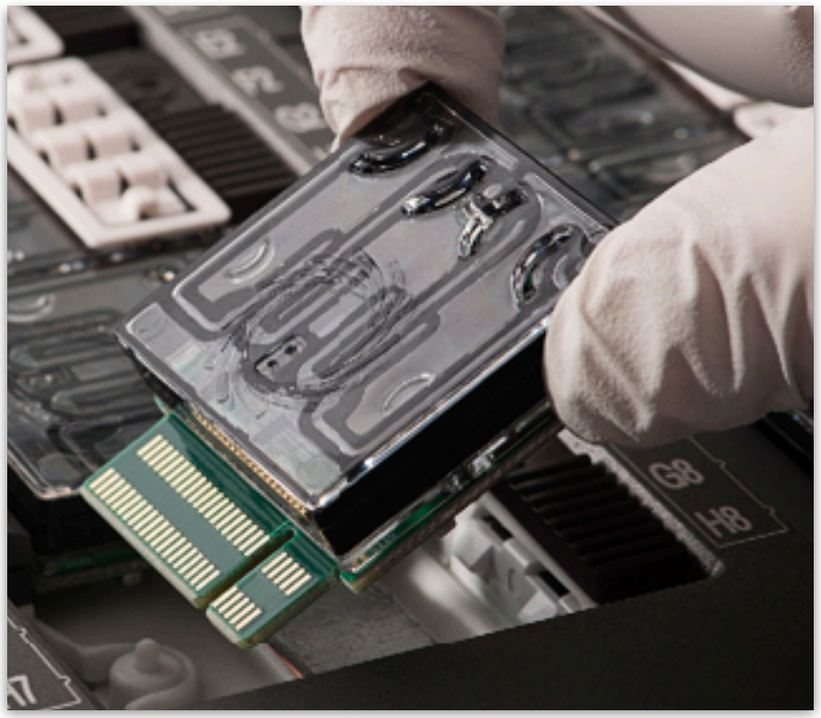
NGI stockholm

GridION

SciLifeLab

NGI stockholm

PromethION



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SmidgION

(not yet released)

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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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ion torrent

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

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Ion Torrent PGM

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

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Ion Torrent Proton

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

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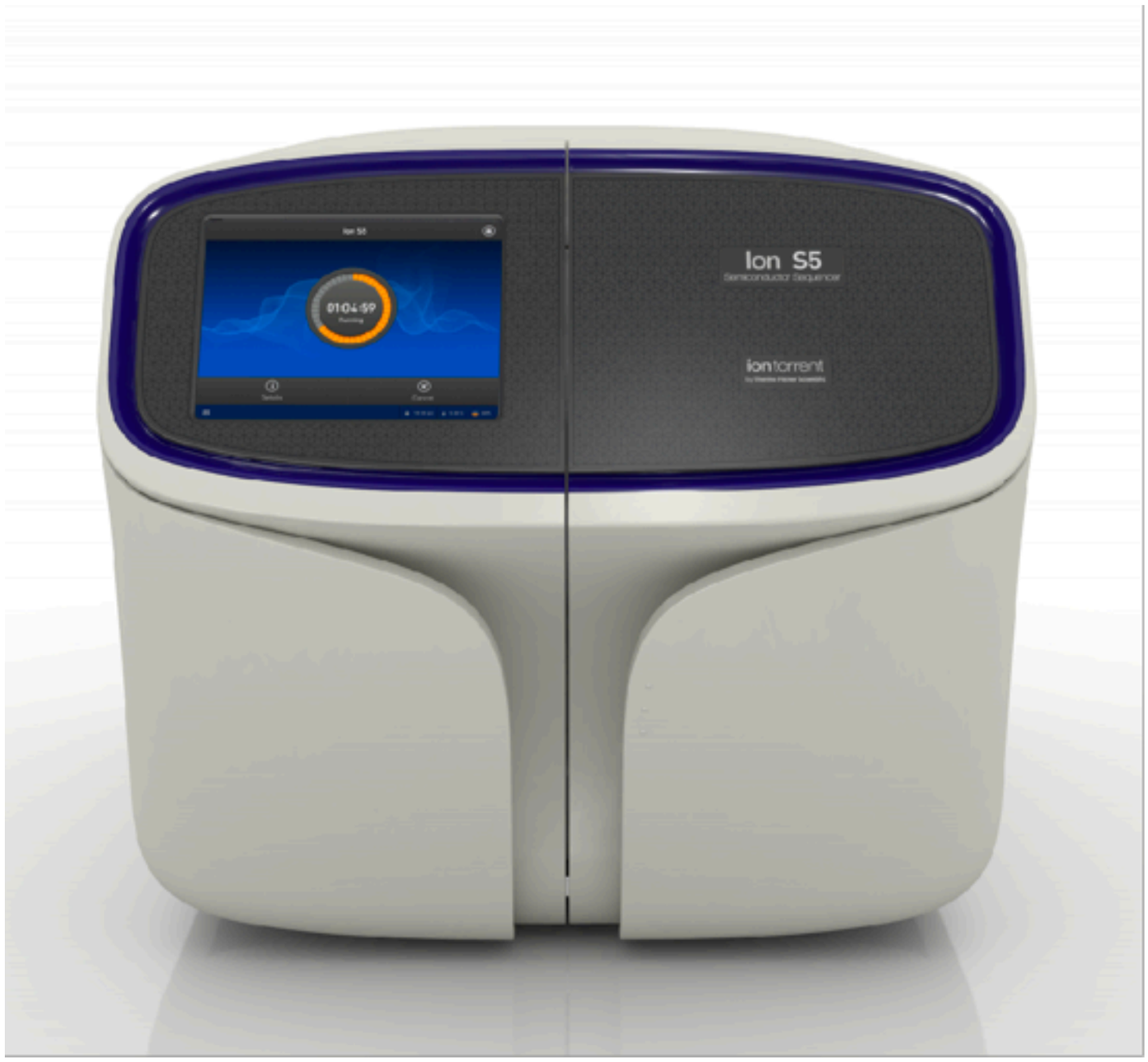
Ion Torrent S5 XL



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

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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/

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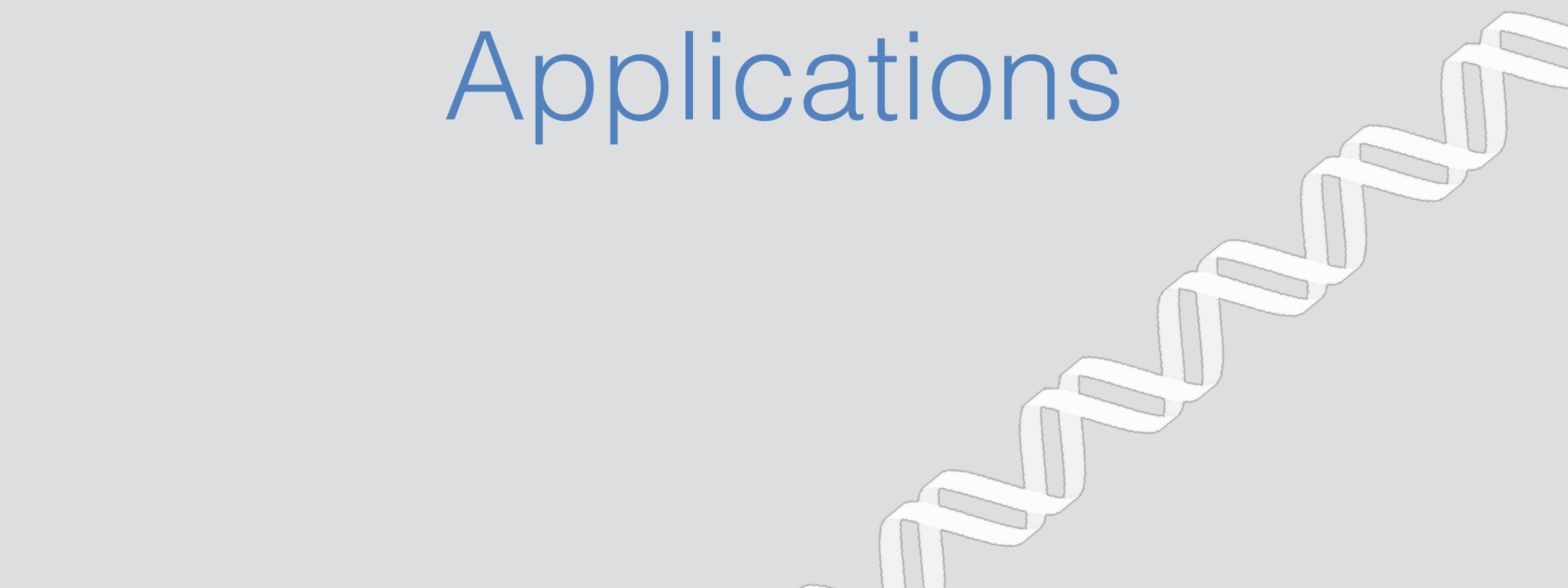
Sequencing Applications



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

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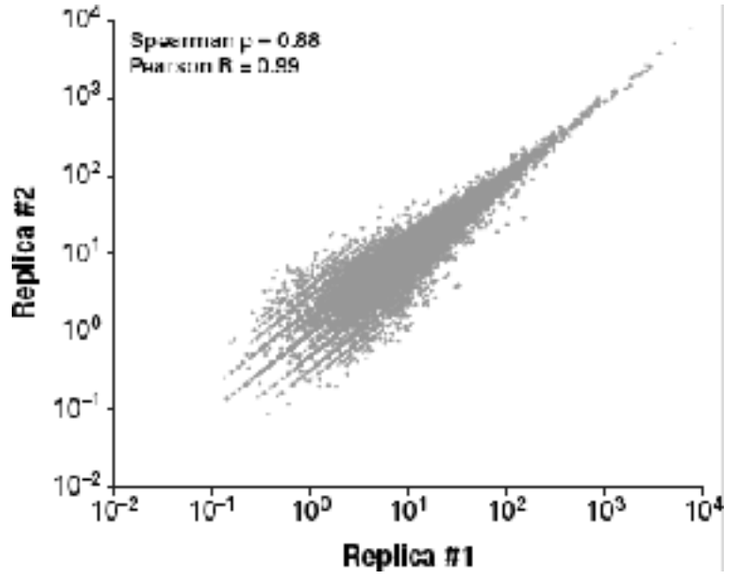
RNA Sequencing

- Choose a type of RNA
  - Protein coding mRNA (poly-A)
  - All RNA (rRNA depletion)
  - Small RNA
- Define your limitations
  - Low-input material
  - Low quality material (eg. FFPE)

- Choose your question
  - Differential gene expression
  - Differential isoform detection & quantification
  - Fusion gene detection



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RNA Sequencing

- Illumina sequencing RNA library prep kits
  - Illumina TruSeq RNA — *Protein-coding poly-A*
  - Illumina RiboZero — *rRNA depletion*
  - Illumina TruSeq RNA Exome — *FFPE / low quality*
  - Clontech SMARTER Pico — *low input*
  - Illumina TruSeq Small RNA — *small RNA*
- Oxford Nanopore, PacBio, IonTorrent

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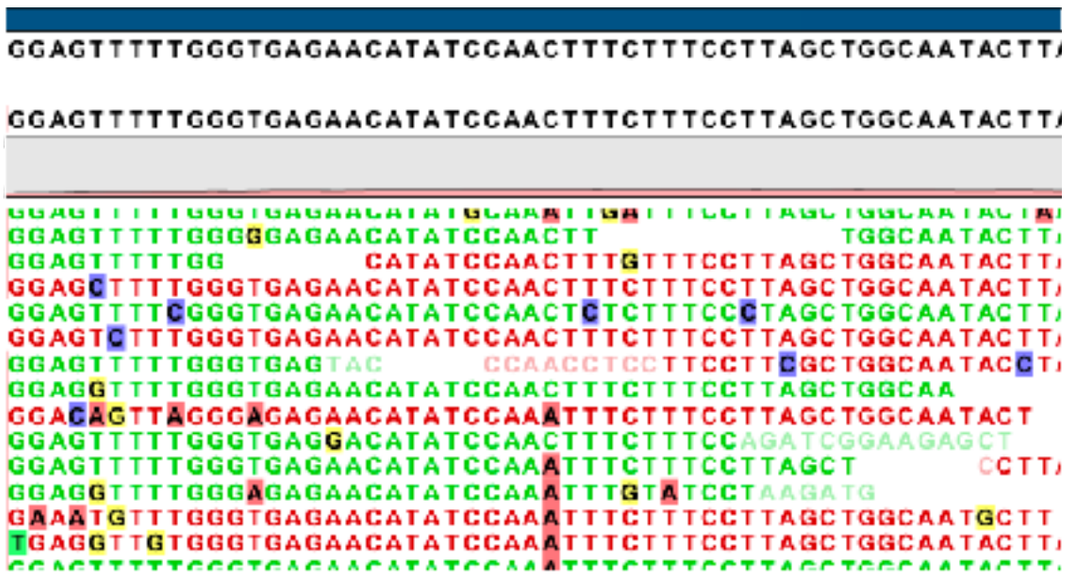
DNA Sequencing

- Choose your question
  - SNP, SNV, indel calling
  - Structural variant detection
  - *De-novo* genome assembly
- Define your requirements
  - Low-input material
  - Low quality material (eg. FFPE)
- Choose your priorities
  - Sequencing accuracy
  - Sequencing depth
  - Ultra-long reads



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DNA Sequencing

- Illumina sequencing DNA library prep kits
  - Illumina TruSeq DNA PCR Free — *Best quality*
  - Rubicon ThruPLEX — *Low input*
  - Illumina Nextera XT — *Cheap (plate format)*
  - Illumina Nextera Flex — *Fast and simple*
  - 10X Genomics — *Linked reads*
- Oxford Nanopore, PacBio, IonTorrent

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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

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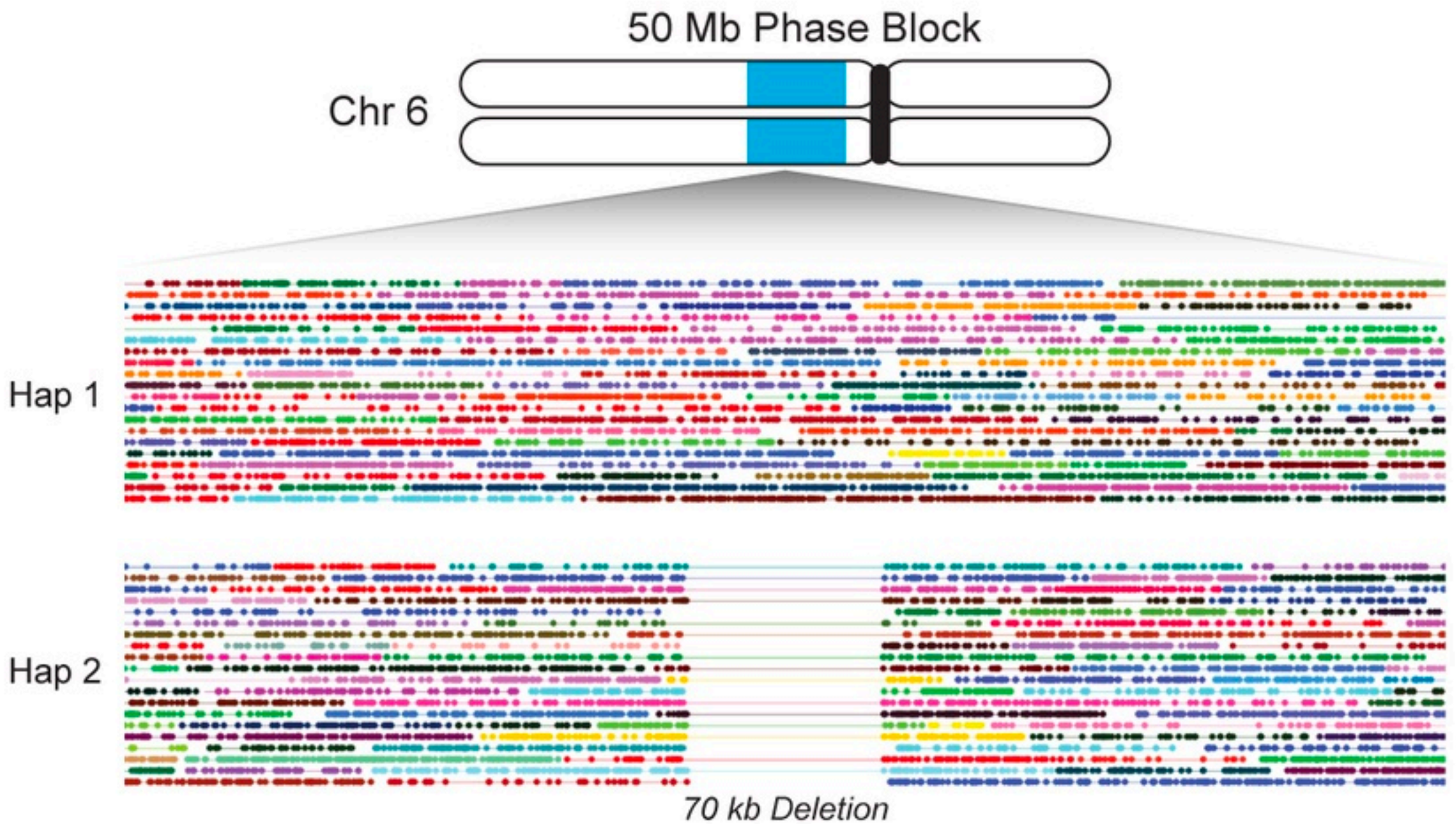
10X Genomics



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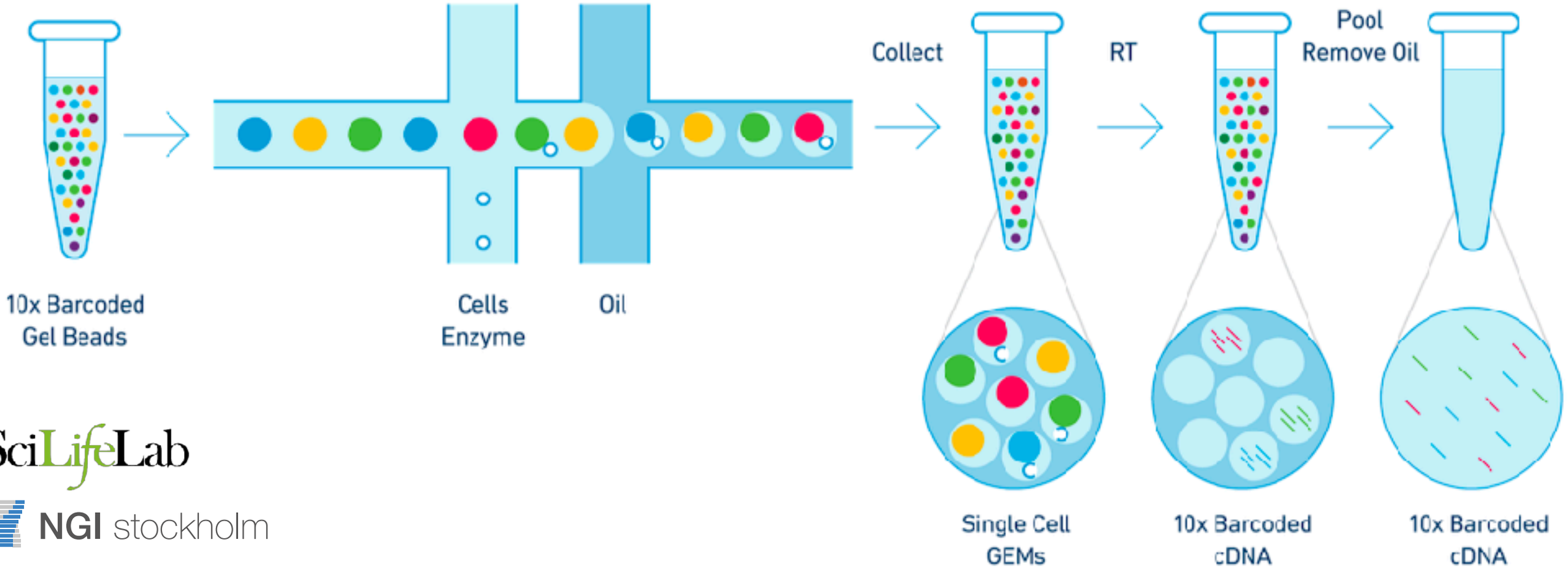
10X Genomics

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



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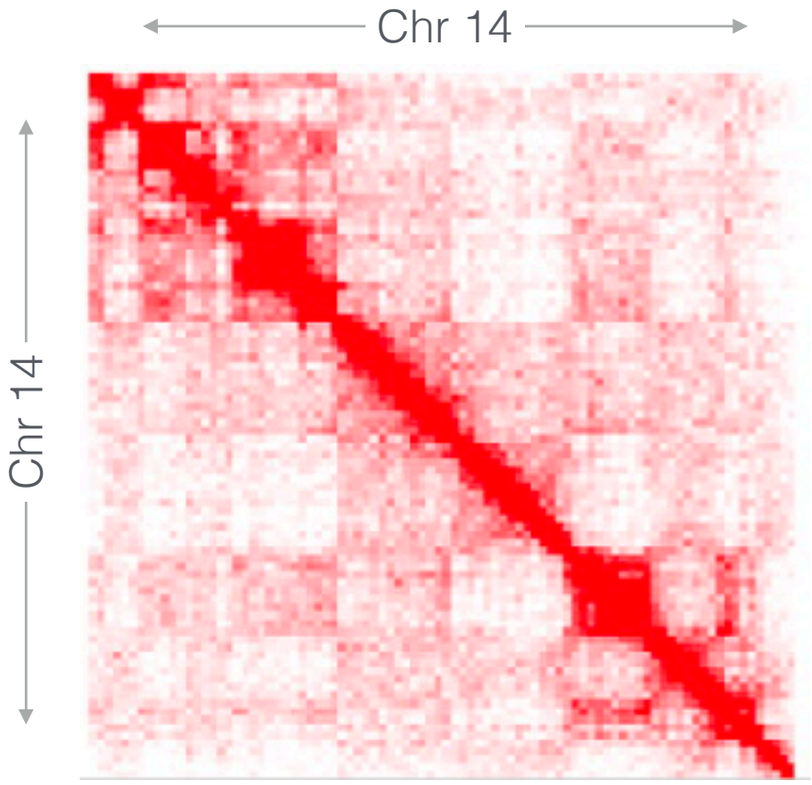
Hi-C

- 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
  - De-novo genome assembly
  - Structural variation detection



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ARIMA
Dovetail GENOMICS

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

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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

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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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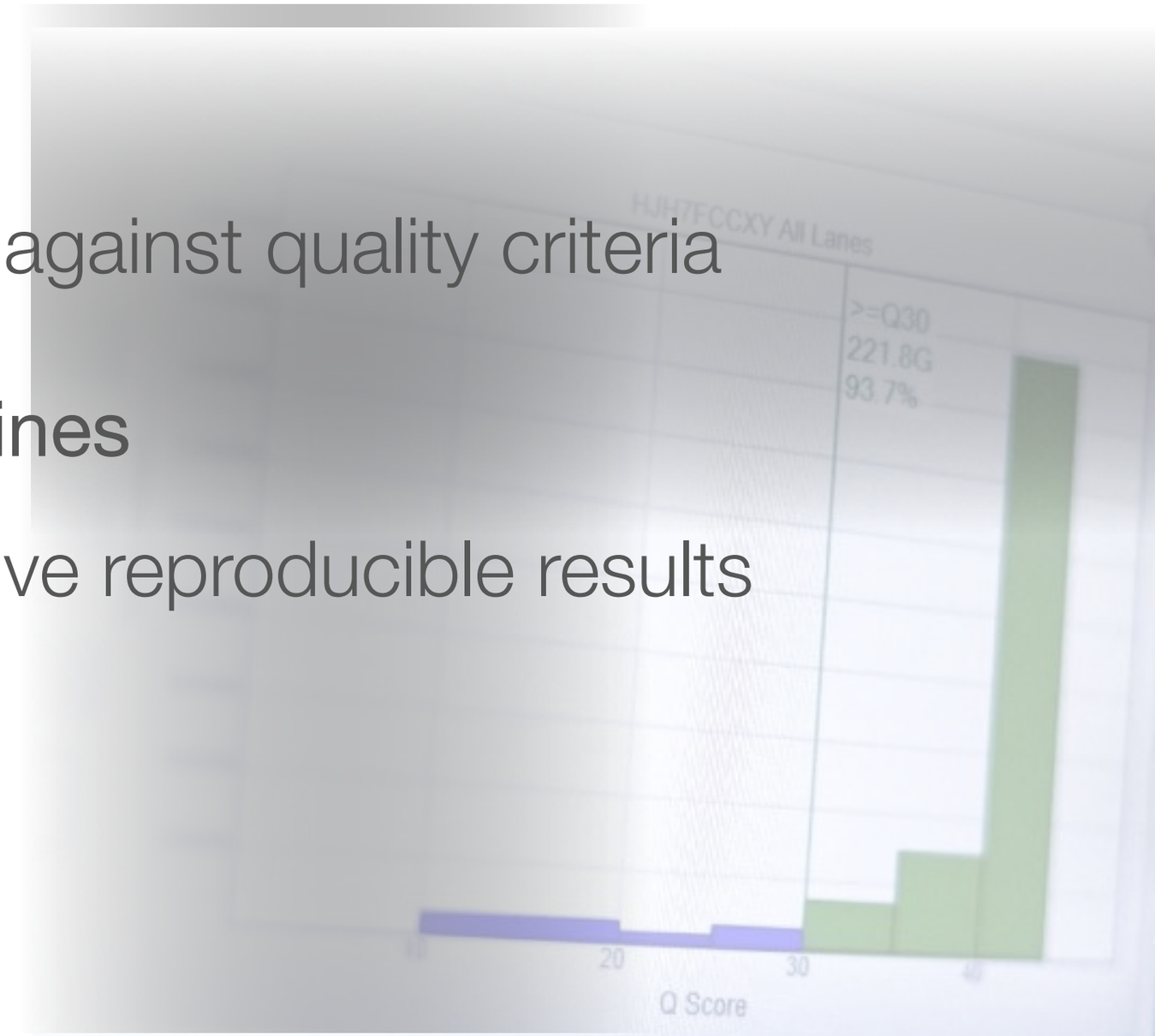
Bioinformatics at the NGI

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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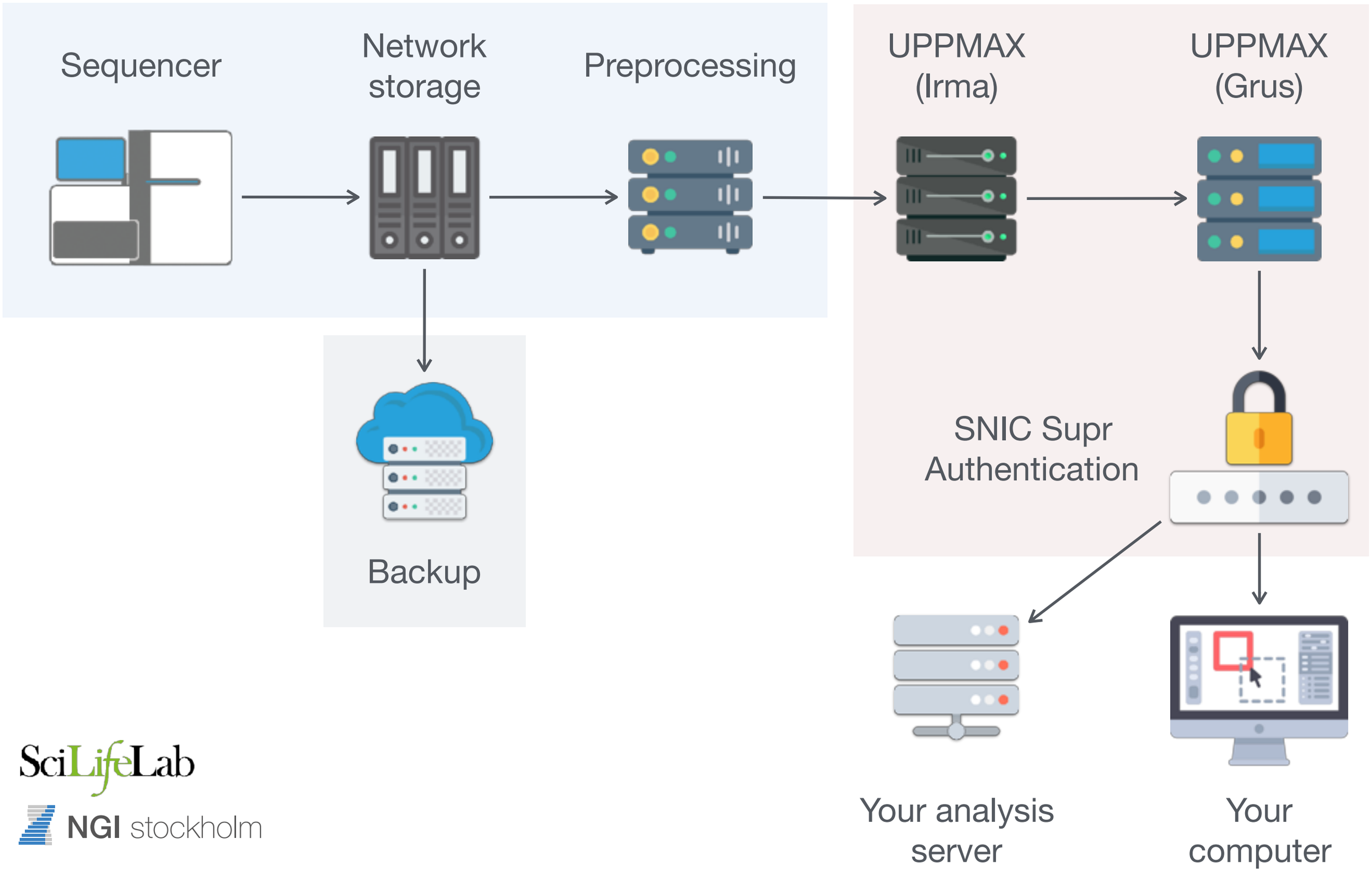
NGI Data Handling



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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

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Analysis Pipelines

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

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SWEDAC. ACKREDITERING

Ackred. nr 1850
Provning
ISO/IEC 17025

Analysis Requirements

Automated

Reliable

nextflow

Easy for others to run

Reproducible results

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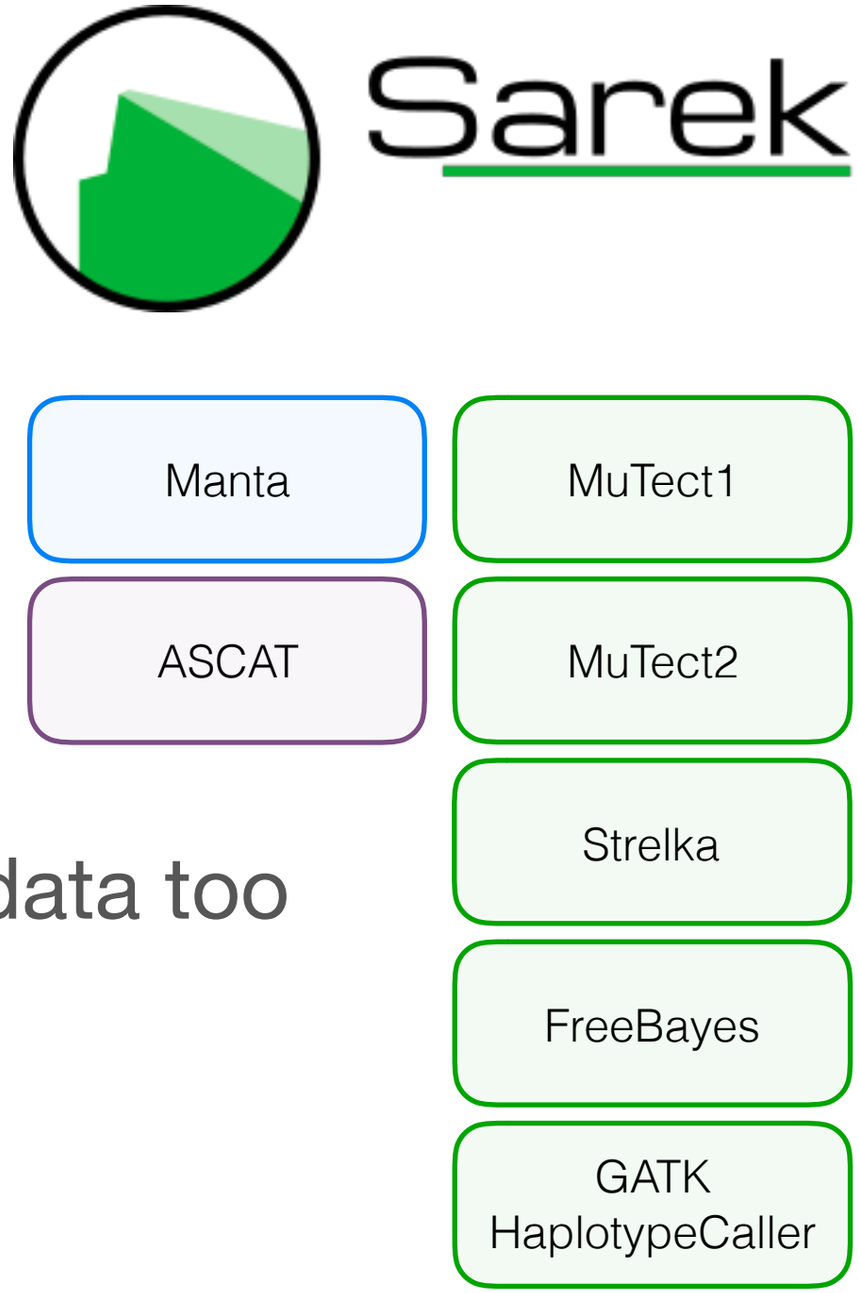
Sarek

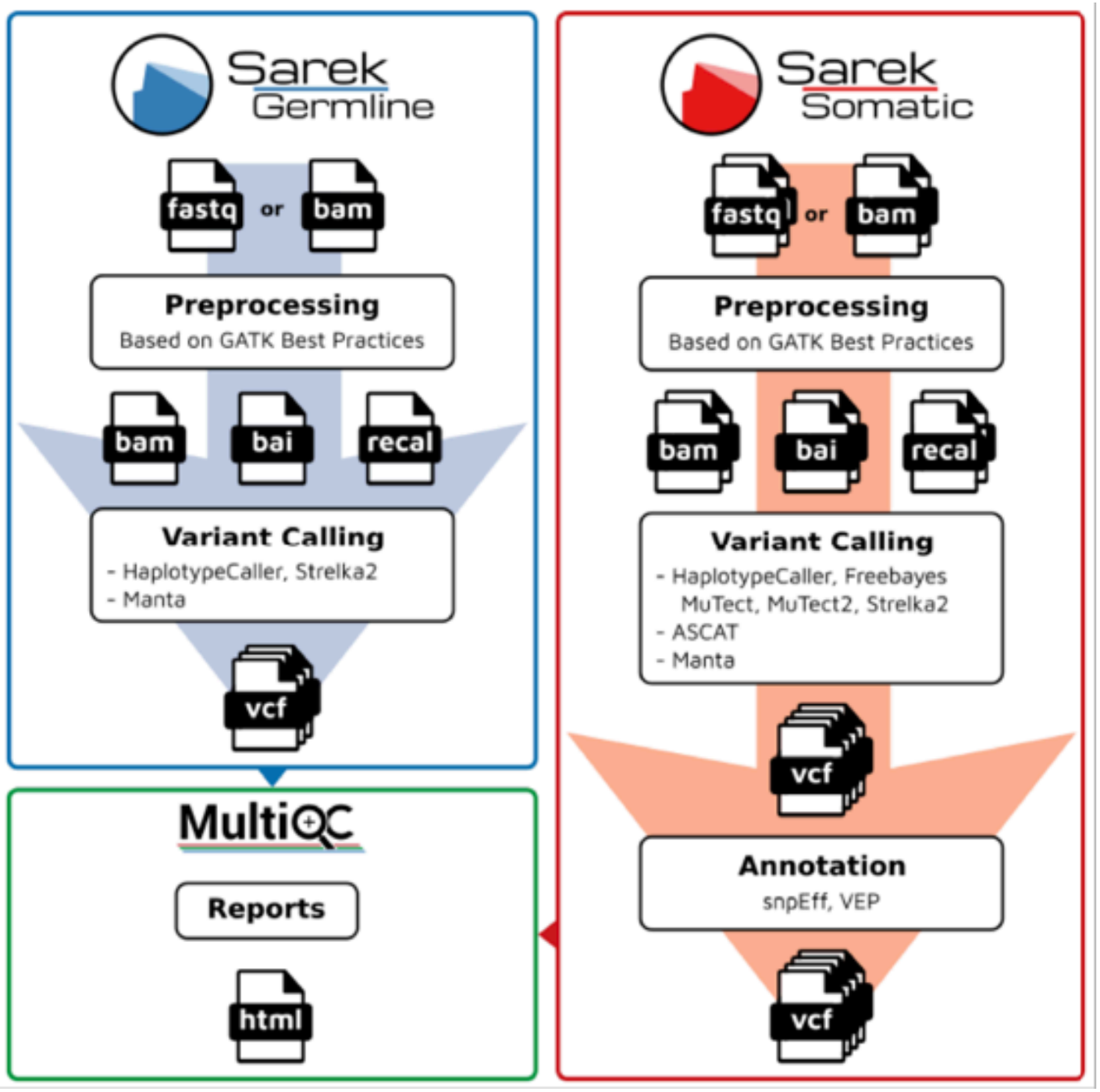
GitHub https://github.com/SciLifeLab/Sarek

- 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



Sarek

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



NATIONAL GENOMICS INFRASTRUCTURE
NBIS NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN
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

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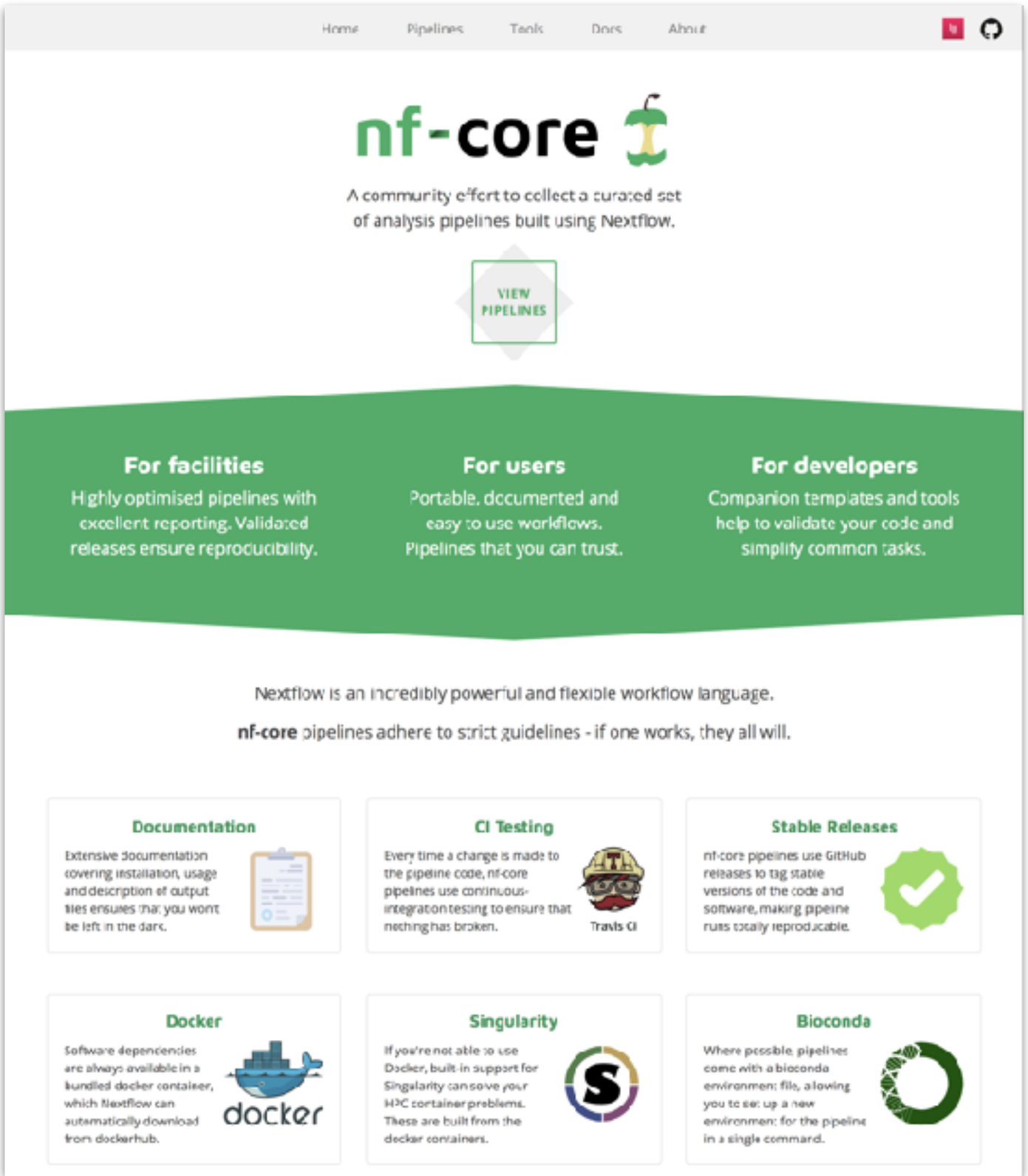
nf-core

https://nf-co.re

nf-core



nf-core

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

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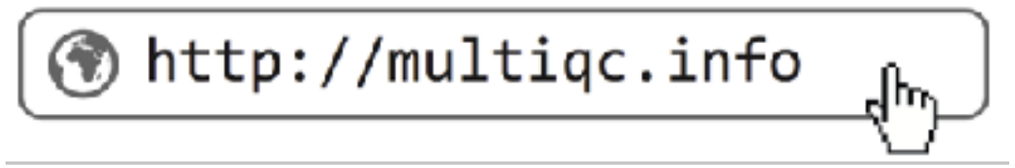
MultiQC

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 → thousands of samples
- Highly customisable



MultiQC

MultiQC

NATIONAL GENOMICS INFRASTRUCTURE

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
Sequencing 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`

NGI names | User supplied names

General Statistics

Copy table | Configure Columns | Plot | Showing 22/22 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%	51%	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%	60.5%	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

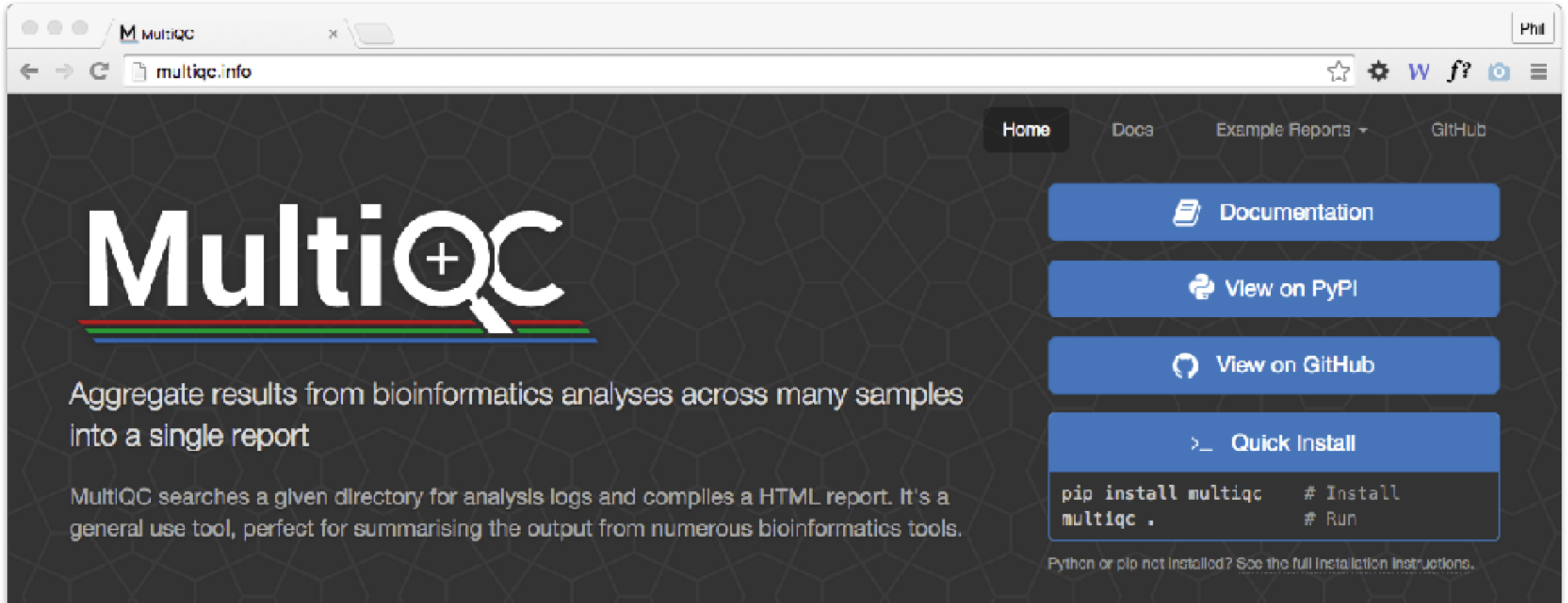
Getting MultiQC

ewels / MultiQC

BIOCONDA

http://multiqc.info

PyPI



Conclusions

If you have a project

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

https://ngisweden.scilifelab.se

support@ngisweden.se

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Find our tools

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

http://opensource.scilifelab.se

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Acknowledgements

Phil Ewels

phil.ewels@scilifelab.se

ewels

tallphil

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