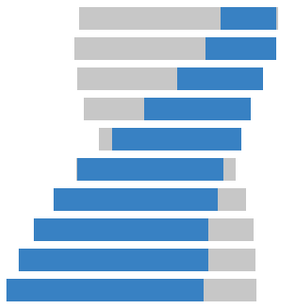

NGI-RNAseq

Processing RNA-seq data at the National Genomics Infrastructure

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NGI stockholm

Phil Ewels
phil.ewels@scilifelab.se

NBIS RNA-seq tutorial
2017-11-09

SciLifeLab NGI

GANATIONALCTAC
ATCAGENOMICSGT
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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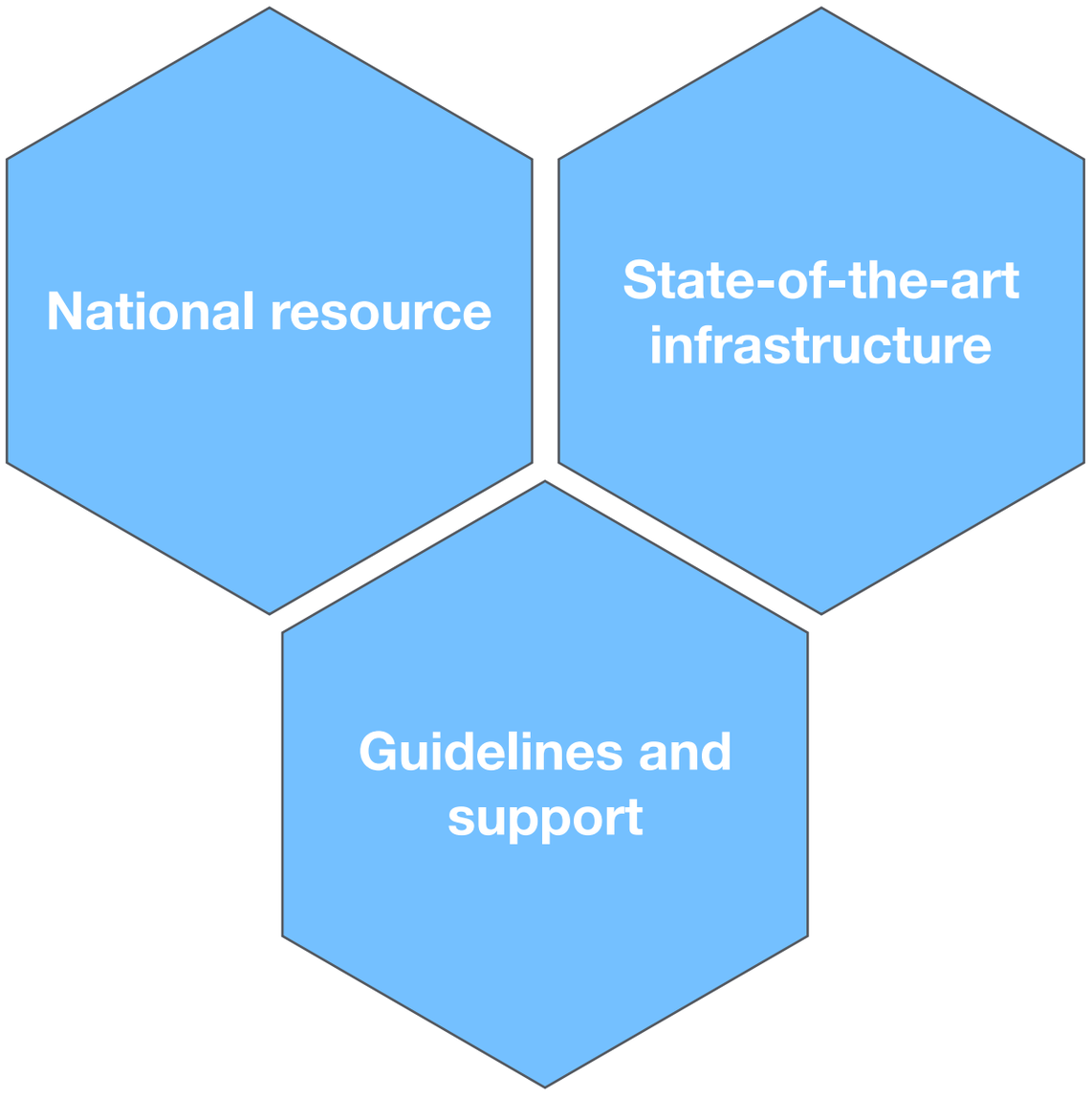
SciLifeLab NGI



GANATIONALCTAC
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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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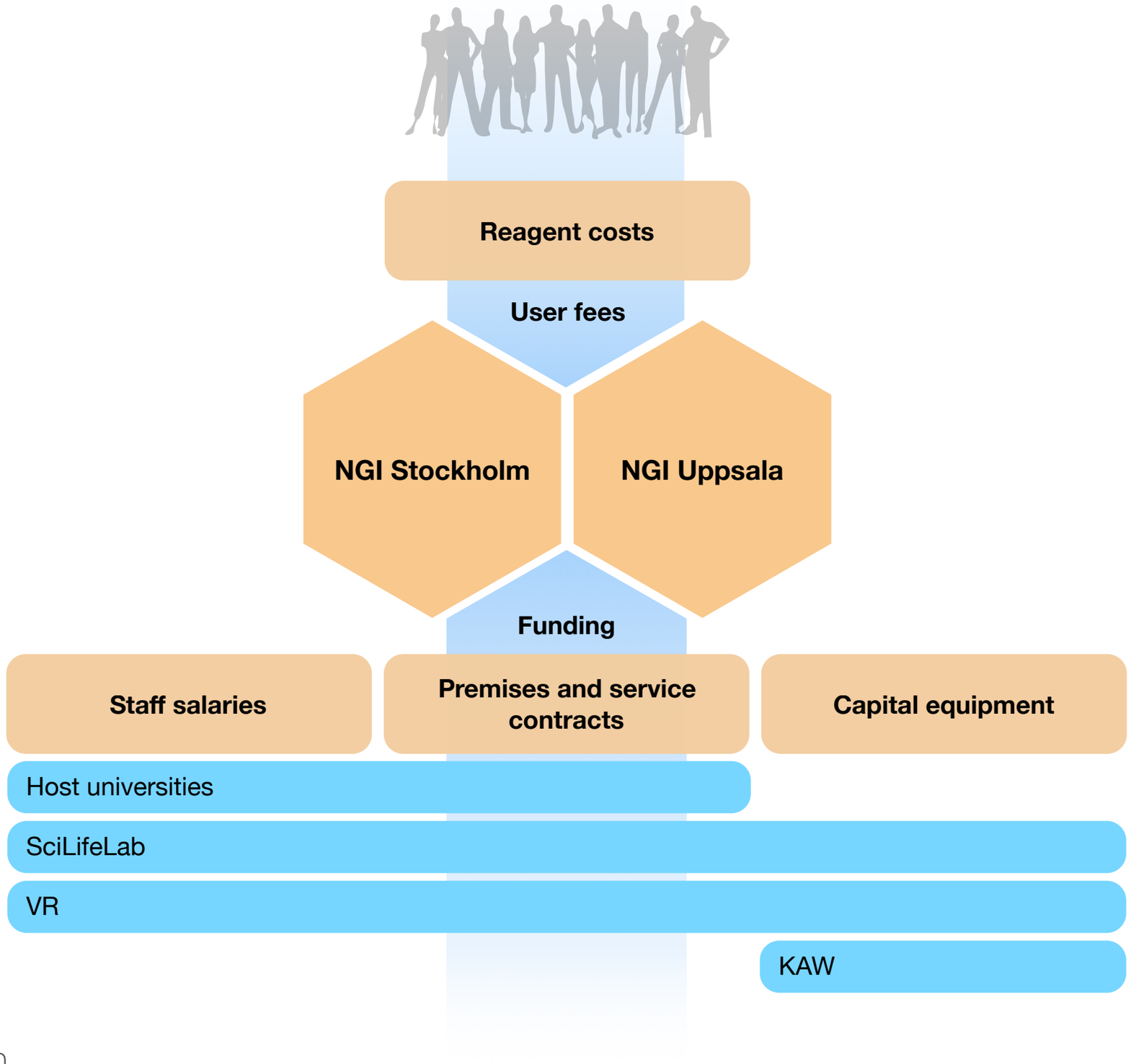

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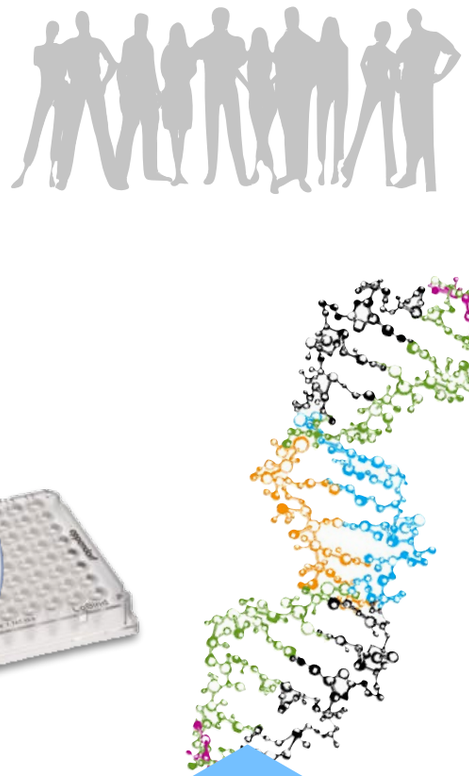
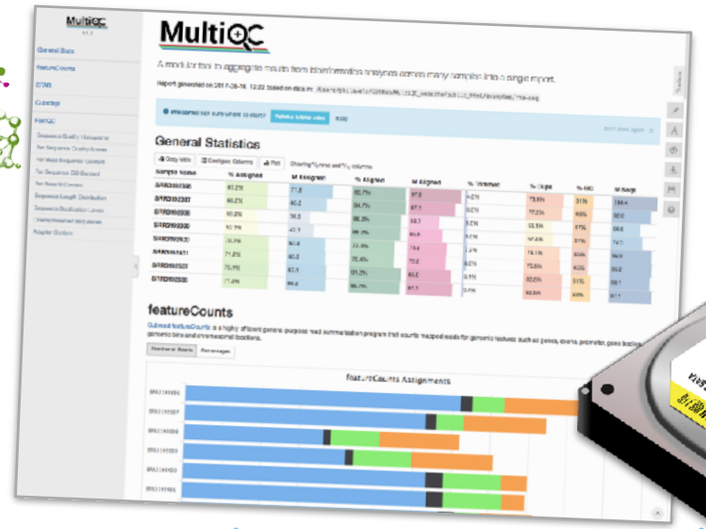
Project timeline





Scientific support and project consultation

Sample QC

Library preparation, Sequencing, Genotyping

Data processing and primary analysis

Data delivery

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Methods offered at NGI

Accredited methods

SWEDAC ACKREDITERING
Ackred. nr 1850
Provning
ISO/IEC 17025

Whole Genome seq

RNA-seq

de novo

Just Sequencing

Data analysis included for **FREE**

Metagenomics

Nanopore sequencing

Exome sequencing

RAD-seq

Bisulphite sequencing

ChIP-seq

ATAC-seq

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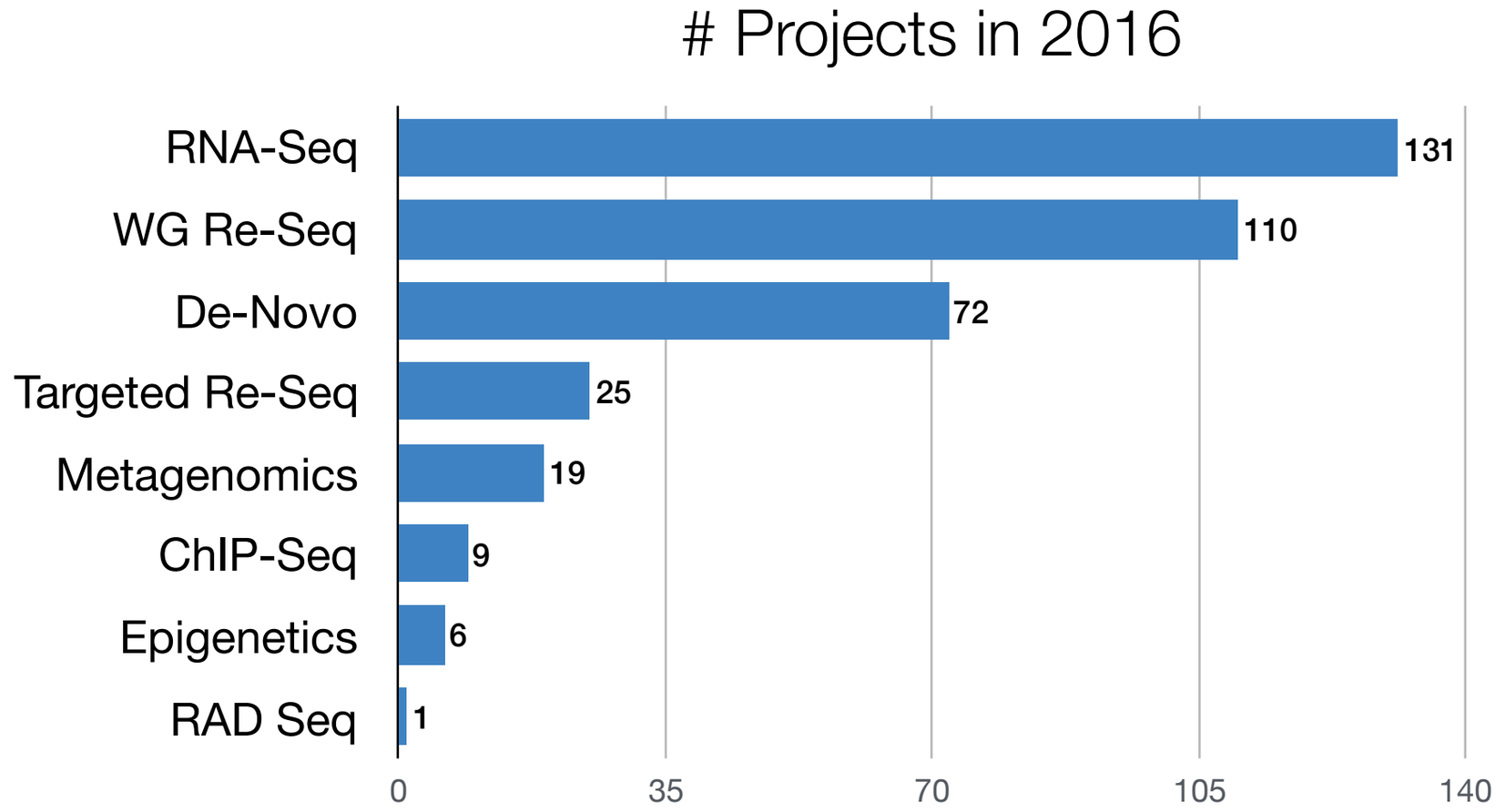
RNA-Seq: NGI Stockholm

- RNA-seq is the most common project type



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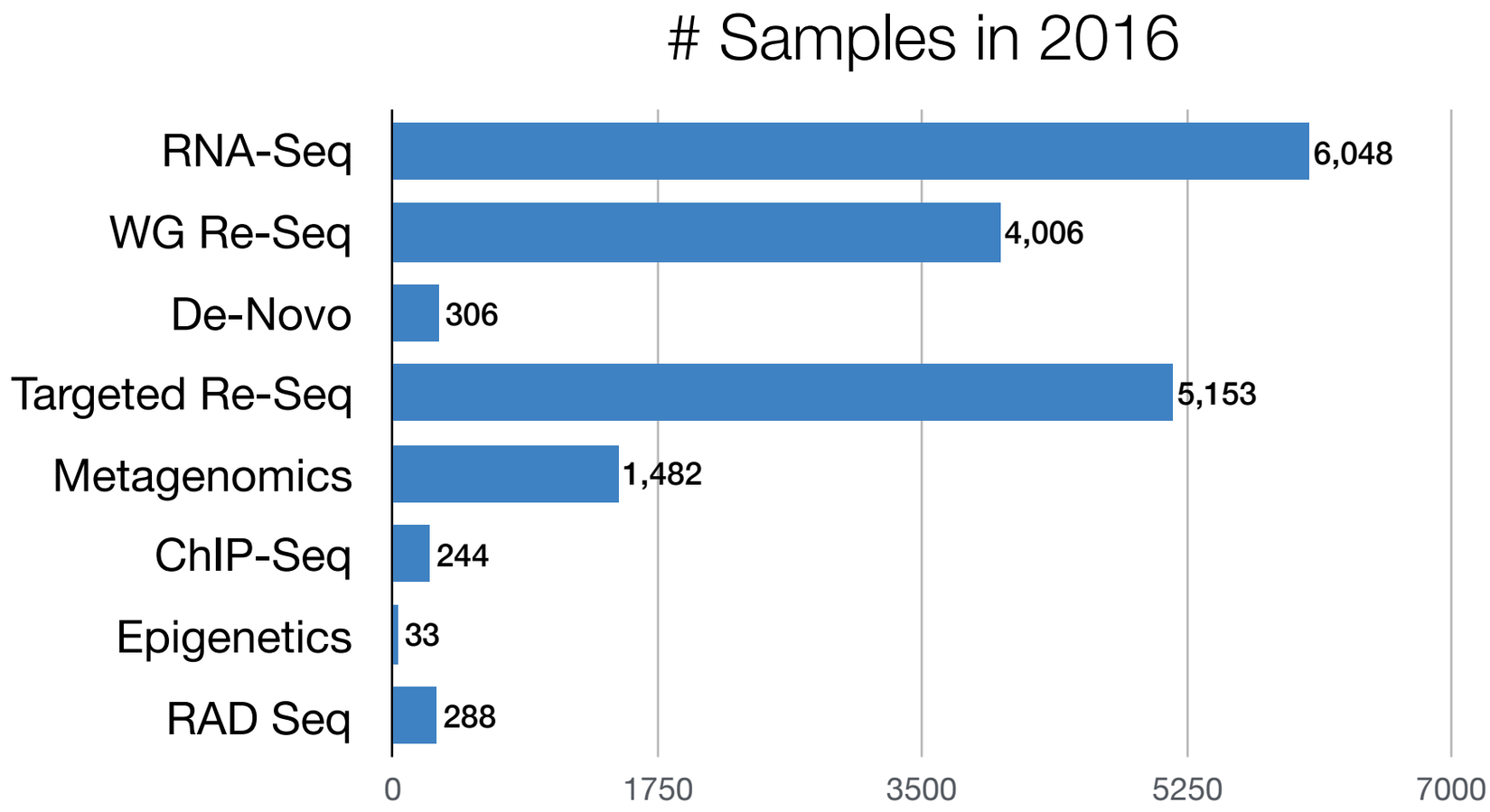
RNA-Seq: NGI Stockholm

- RNA-seq is the most common project type
- Production protocols:
  - TruSeq (poly-A)
  - RiboZero
- In development:
  - SMARTer Pico
  - RNA Access



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RNA-Seq: NGI Stockholm

- RNA-seq is the most common project type
- Production protocols:
  - TruSeq (poly-A)
  - RiboZero
- In development:
  - SMARTer Pico
  - RNA Access

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RNA-Seq Pipeline

- Takes raw FastQ sequencing data as input
- Provides range of results
  - Alignments (BAM)
  - Gene counts (Counts, FPKM)
  - Quality Control
- First RNA Pipeline running since 2012
- Second RNA Pipeline in use since April 2017

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NGI-RNAseq

RNA-Seq Pipeline

NGI-RNAseq

FastQC	*Sequence QC*
TrimGalore!	*Read trimming*
STAR	*Alignment*
dupRadar	*Duplication QC*
featureCounts	*Gene counts*
StringTie	*Normalised FPKM*
RSeQC	*Alignments QC*
Preseq	*Library complexity*
edgeR	*Heatmap, clustering*
MultiQC	*Reporting*

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RNA-Seq Pipeline

NGI-RNAseq

FastQ

BAM

TSV

HTML

FastQC	*Sequence QC*
TrimGalore!	*Read trimming*
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MultiQC	*Reporting*

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Nextflow

- Tool to manage computational pipelines
- Handles interaction with compute infrastructure
- Easy to learn how to run, minimal oversight required

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Nextflow

nextflow

```
#!/usr/bin/env nextflow

cheers=Channel.from "Bonjour","Ciao","Hello","Hola"

process sayHello {
  input:
  val x from cheers

  """
  echo $x world!
  """
}
```

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https://www.nextflow.io/

Nextflow


```
#!/usr/bin/env nextflow

input = Channel.fromFilePairs( params.reads )
process fastqc {
  input:
  file reads from input

  output:
  file "*_fastqc.{zip,html}" into results

  script:
  """
  fastqc -q $reads
  """
}
```

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Nextflow

```
#!/usr/bin/env nextflow

input = Channel.fromFilePairs( params.reads )
process fastqc {
  input:
  file reads from input

  output:
  file "*_fastqc.{zip,html}" into results

  script:
  """
  fastqc -q $reads
  """
}
```

Default: Run locally, assume software is installed

```
process {

  executor = 'slurm'
  clusterOptions = { "-A b2017123" }

  cpus = 1
  memory = 8.GB
  time = 2.h

  $fastqc {
    module = ['bioinfo-tools', ‘FastQC']
  }

}
```

Submit jobs to SLURM queue
Use environment modules

UPPNEX

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Nextflow

```
#!/usr/bin/env nextflow

input = Channel.fromFilePairs( params.reads )
process fastqc {
  input:
  file reads from input

  output:
  file "*_fastqc.{zip,html}" into results

  script:
  """
  fastqc -q $reads
  """
}
```

```
process {

  executor = 'slurm'
  clusterOptions = { "

  cpus = 1
  memory = 8.GB
  time = 2.h

  $fastqc {
    module = ['bioinfo-tools', ‘FastQC']
  }

}
```

```
docker {
 enabled = true
}

process {
  container = 'biocontainers/fastqc'

  cpus = 1
  memory = 8.GB
  time = 2.h
}
```

Run locally, use docker container for all software dependencies

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NGI-RNAseq

This repository Search Pull requests Issues Marketplace Explore

SciLifeLab / NGI-RNAseq
forked from ewels/NGI-RNAseq

Unwatch 13 Unstar 23 Fork 19

Code Issues 13 Pull requests 1 Insights Settings

Nextflow RNA-Seq Best Practice analysis pipeline, used at the SciLifeLab National Genomics Infrastructure.
https://ngisweden.scilifelab.se/

Edit

bioinformatics rna-seq rnaseq rna-sequencing pipeline nextflow Manage topics

598 commits 3 branches 12 releases 8 contributors MIT

Branch: master New pull request Create new file Upload files Find file Clone or download

This branch is even with ewels:master. Pull request Compare

ewels Merge pull request #162 from na399/na399-patch-1 ... Latest commit 6dbb58a 5 days ago

assets	Got software version stuff working.	4 months ago
bin	Dynamically generate base64 images for pipeline report. New 'assets' ...	6 months ago
conf	Merge branch 'master' of github.com:SciLifeLab/NGI-RNAseq	26 days ago

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https://github.com/SciLifeLab/NGI-RNAseq

NGI-RNAseq

README.md

NGI-RNAseq Documentation

The NGI-RNAseq documentation is split into a few different files:

- `installation.md`
  - Pipeline installation and configuration instructions
- `usage.md`
  - Instructions on how to run the NGI-RNAseq pipeline
- `output.md`
  - Document describing all of the results produced by the pipeline, and how to interpret them.
- `amazon_web_services.md`
  - Docs about running the pipeline in the cloud with AWS.

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https://github.com/SciLifeLab/NGI-RNAseq

Running NGI-RNAseq

Step 1: Install Nextflow

- Uppmax - load the Nextflow module
  `module load nextflow`
- Anywhere (including Uppmax) - install Nextflow

```
curl -s https://get.nextflow.io | bash
```

Step 2: Try running NGI-RNAseq pipeline

```
nextflow run SciLifeLab/NGI-RNAseq --help
```

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Running NGI-RNAseq

Step 3: Choose your reference

- Common organism - use iGenomes

  `--genome GRCh37`

- Custom genome - Fasta + GTF (minimum)

  `--fasta genome.fa --gtf genes.gtf`

Step 4: Organise your data

- One (if single-end) or two (if paired-end) FastQ per sample
- Everything in one directory, simple filenames help!

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Running NGI-RNAseq

Step 5: Run the pipeline on your data

- Remember to run detached from your terminal
  `screen / tmux / nohup`

Step 6: Check your results

- Read the Nextflow log and check the MultiQC report

Step 7: Delete temporary files

- Delete the `./work` directory, which holds all intermediates

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Typical pipeline output

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Using UPPMAX

```
nextflow run SciLifeLab/NGI-RNAseq
    --project b2017123
    --genome GRCh37
    --reads "data/*_R{1,2}.fastq.gz"
```

- Default config is for UPPMAX
  - Knows about central iGenomes references
  - Uses centrally installed software

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Using other clusters

```
nextflow run SciLifeLab/NGI-RNAseq
    -profile hebbe
    --fasta genome.fa --gtf genes.gtf
    --reads "data/*_R{1,2}.fastq.gz"
```

C3SE

BIOCONDA

- Can run just about anywhere
  - Supports local, SGE, LSF, SLURM, PBS/Torque, HTCondor, DRMAA, DNAnexus, Ignite, Kubernetes

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Using Docker

```
nextflow run SciLifeLab/NGI-RNAseq
    -profile docker
    --fasta genome.fa --gtf genes.gtf
    --reads "data/*_R{1,2}.fastq.gz"
```

- Can run anywhere with Docker
  - Downloads required software and runs in a container
  - Portable and reproducible.

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Using AWS

```
nextflow run SciLifeLab/NGI-RNAseq
    -profile aws
    --genome GRCh37
    --reads "s3://my-bucket/*_{1,2}.fq.gz"
    --outdir "s3://my-bucket/results/"
```

- Runs on the AWS cloud with Docker
  - Pay-as-you go, flexible computing
  - Can launch from anywhere with minimal configuration

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Input data

```
ERROR ~ Cannot find any reads matching: XXXX
NB: Path needs to be enclosed in quotes!
NB: Path requires at least one * wildcard!
If this is single-end data, please specify
--singleEnd on the command line.
```

```
--reads '*_R{1,2}.fastq.gz'
--reads '*.fastq.gz' --singleEnd
```

```
--reads sample.fastq.gz
--reads *_R{1,2}.fastq.gz
--reads '*.fastq.gz'
```

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Read trimming

- Pipeline runs TrimGalore! to remove adapter contamination and low quality bases automatically

- Some library preps also include additional adapters
  - Will get poor alignment rates without additional trimming

```
--clip_r1 [int]
--clip_r2 [int]
--three_prime_clip_r1 [int]
--three_prime_clip_r2 [int]
```

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Library strandedness

- Most RNA-seq data is strand-specific now
  - Can be "forward-stranded" (same as transcript) or "reverse-stranded" (opposite to transcript)

- UPPMAX config runs as reverse stranded by default

- If wrong, QC will say most reads don't fall within genes

```
--forward_stranded
--reverse_stranded
--unstranded
```

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Lib-prep presets

- There are some presets for common kits
- Clontech SMARTer PICO
  - Forward stranded, needs R1 5' 3bp and R2 3' 3bp trimming

```
--pico
```

- Please suggest others!

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Saving intermediates

- By default, the pipeline doesn't save some intermediate files to your final results directory
  - Reference genome indices that have been built
  - FastQ files from TrimGalore!
  - BAM files from STAR (we have BAMs from Picard)

```
--saveReference
--saveTrimmed
--saveAlignedIntermediates
```

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Resuming pipelines

- If something goes wrong, you can resume a stopped pipeline
  - Will use cached versions of completed processes
  - NB: Only one hyphen!

```
-resume
```

- Can resume specific past runs
  - Use `nextflow log` to find job names

```
-resume job_name
```

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Customising output

`-name`	Give a name to your run. Used in logs and reports
`--outdir`	Specify the directory for saved results
`--aligner hisat2`	Use HiSAT2 instead of STAR for alignment
`--email`	Get e-mailed a summary report when the pipeline finishes

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Nextflow config files

- Can save a config file with defaults
  - Anything with two hyphens is a params

```
./nextflow.config

~/.nextflow/config

-c /path/to/my.config
```

```
params {

  email = 'phil.ewels@scilifelab.se'
  project = "b2017123"

}

process.$multiqc.module = []
```

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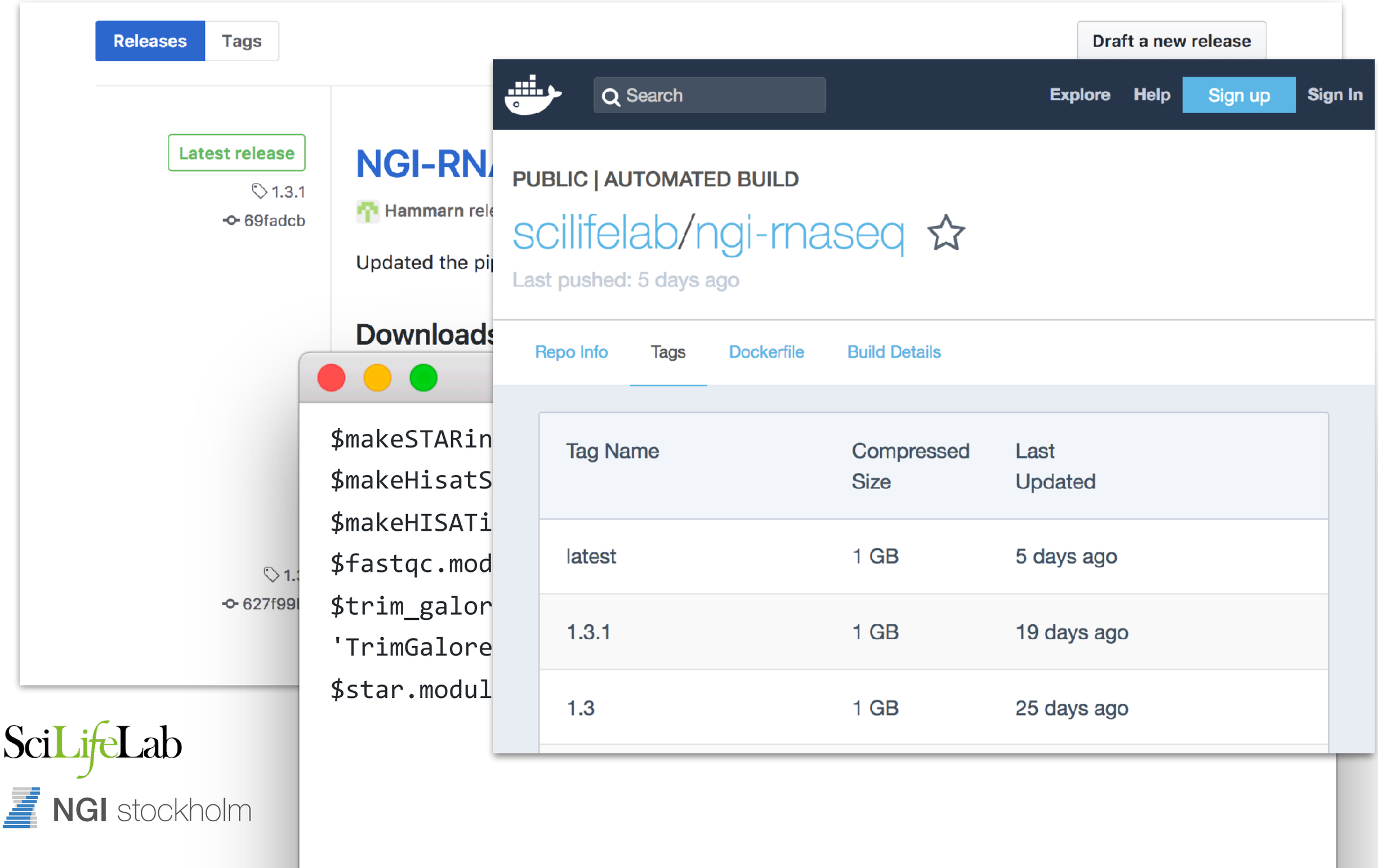
NGI-RNAseq config

```
N E X T F L O W  ~  version 0.25.5
Launching `/home/phil/GitHub/NGI-RNAseq/main.nf` [amazing_laplace] - revision: 8b9f416d01
=========================================
 NGI-RNAseq : RNA-Seq Best Practice v1.3.1
=========================================
Run Name        : amazing_laplace
Reads           : data/7_111116_AD0341ACXX_137_*_{1,2}.fastq.gz
Data Type       : Paired-End
Genome          : GRCh37
Strandedness    : Reverse
Trim R1         : 0
Trim R2         : 0
Trim 3' R1      : 0
Trim 3' R2      : 0
Aligner         : STAR
STAR Index      : /sw/data/uppnex/igenomes//Homo_sapiens/Ensembl/GRCh37/Sequence/STARIndex/
GTF Annotation : /sw/data/uppnex/igenomes//Homo_sapiens/Ensembl/GRCh37/Annotation/Genes/genes.gtf
BED Annotation : /sw/data/uppnex/igenomes//Homo_sapiens/Ensembl/GRCh37/Annotation/Genes/genes.bed
Save Reference : Yes
Save Trimmed    : No
Save Intermeds : No
Output dir      : ./results
Working dir     : /pica/h1/phil/nbis_rnaseq/work
Current home    : /home/phil
Current user    : phil
Current path    : /home/phil/nbis_rnaseq
R libraries     : /home/phil/R/nxtflow_libs/
Script dir      : /home/phil/GitHub/NGI-RNAseq
Config Profile : UPPMAX
UPPMAX Project : b2017123
E-mail Address : phil.ewels@scilifelab.se
=========================================
```

Version control



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Version control

- Pipeline is always released under a stable version tag
- Software versions and code reproducible
- For full reproducibility, specify version revision when running the pipeline

```
nextflow run SciLifeLab/NGI-RNAseq -r v1.3.1
```

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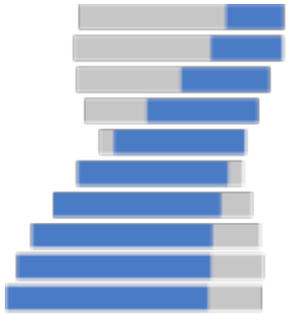
Conclusion

- **Use NGI-RNAseq to prepare your data if you want:**
  - To not have to remember every parameter for STAR
  - Extreme reproducibility
  - Ability to run on virtually any environment

- Now running for all RNA projects at NGI-Stockholm



NGI-RNAseq

Conclusion

GitHub https://github.com/

Open Source Initiative

NGI-RNAseq SciLifeLab/NGI-RNAseq

NGI-smRNAseq SciLifeLab/NGI-smRNAseq

NGI-MethylSeq SciLifeLab/NGI-MethylSeq

NGI-ChIPseq SciLifeLab/NGI-ChIPseq

MIT Licence

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Conclusion

GitHub

https://github.com/

NGI-RNAseq

SciLifeLab/NGI-RNAseq

NGI-smRNAseq

SciLifeLab/NGI-smRNAseq

NGI-MethylSeq

SciLifeLab/NGI-MethylSeq

NGI-ChIPseq

SciLifeLab/NGI-ChIPseq

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http://opensource.scilifelab.se

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