NGI-ChIPseq

Processing ChIP-seq data at the National Genomics Infrastructure

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NBIS ChIP-seq tutorial
2017-11-29

SciLifeLab NGI

NATIONAL GENOMICS 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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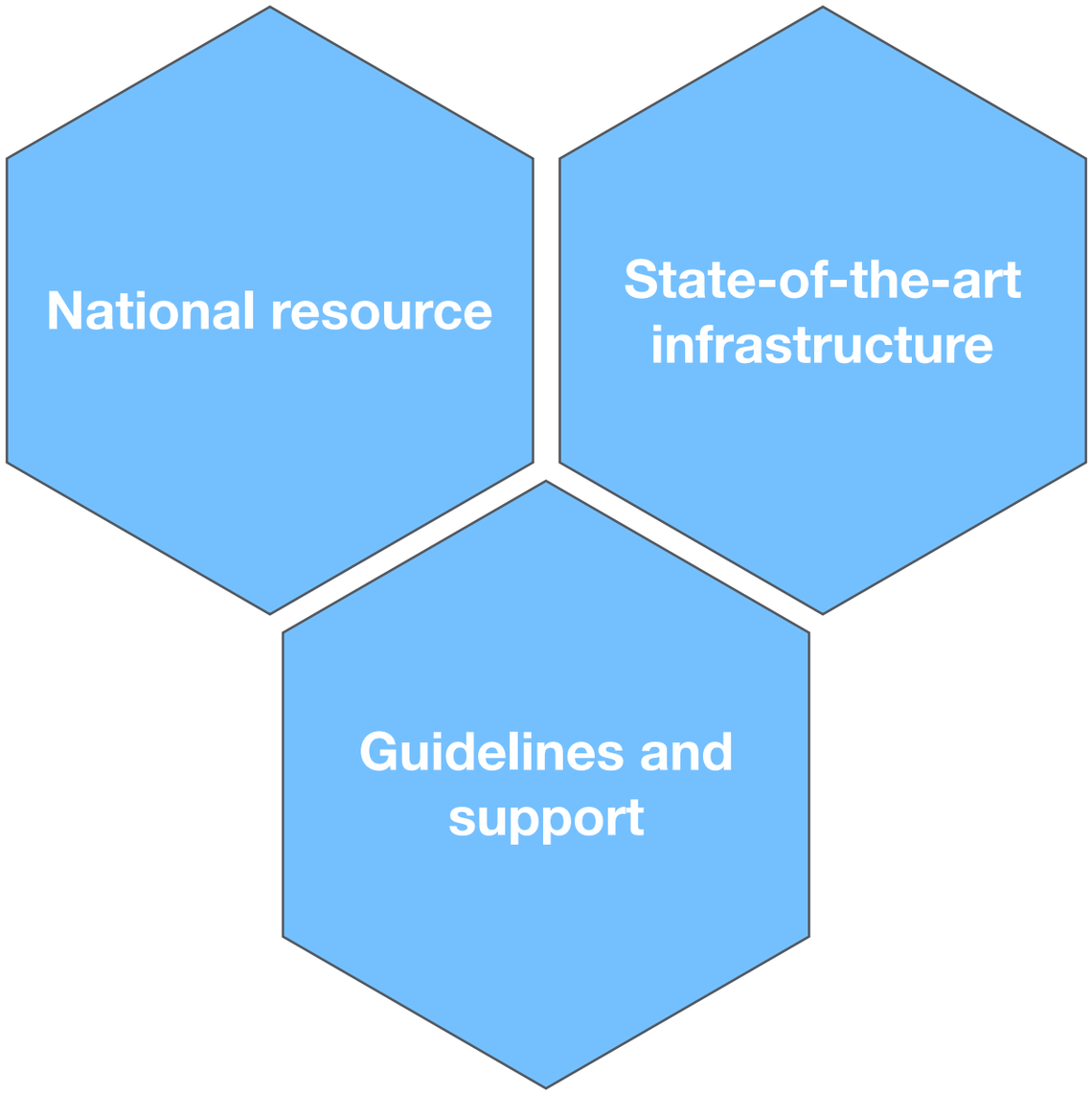

NATIONAL GENOMICS INFRASTRUCTURE

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

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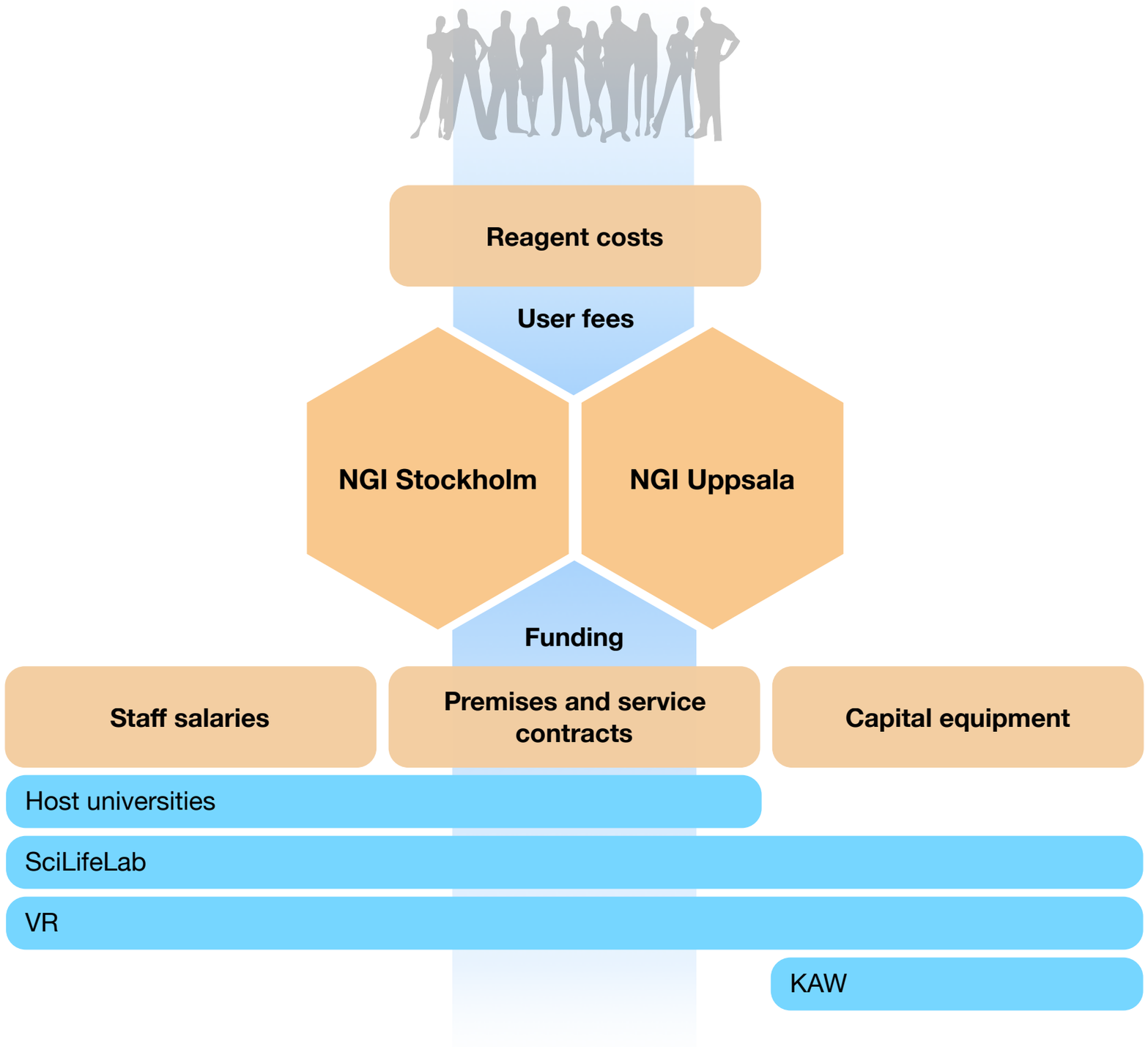

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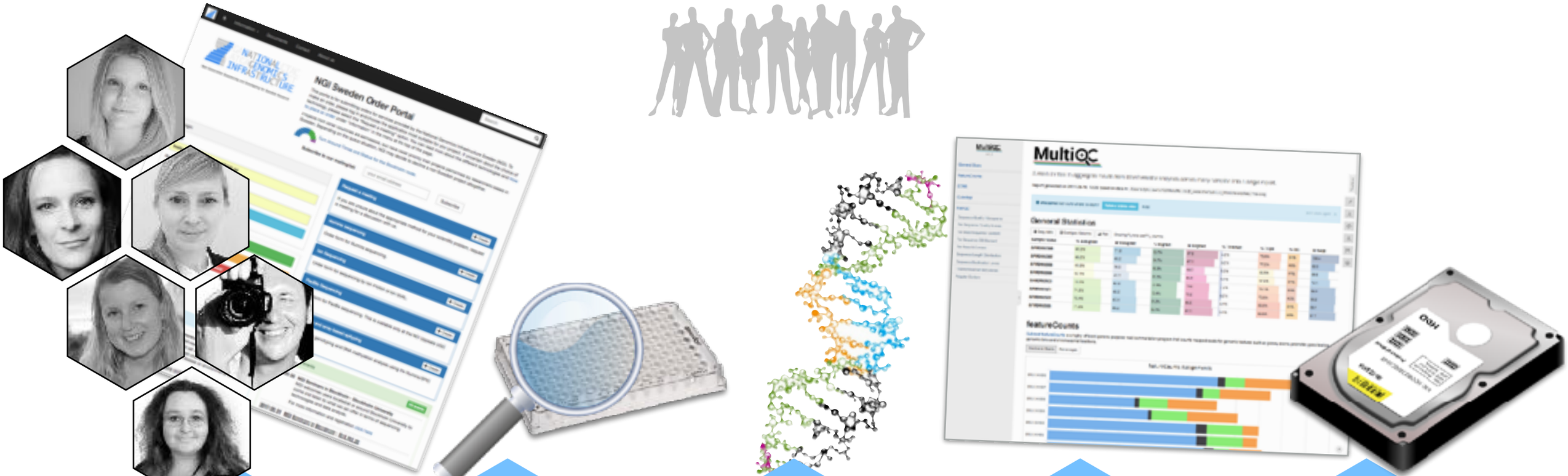
Project timeline



Scientific support and project consultation

Sample QC

Library preparation, Sequencing, Genotyping

Data processing and primary analysis

Data delivery

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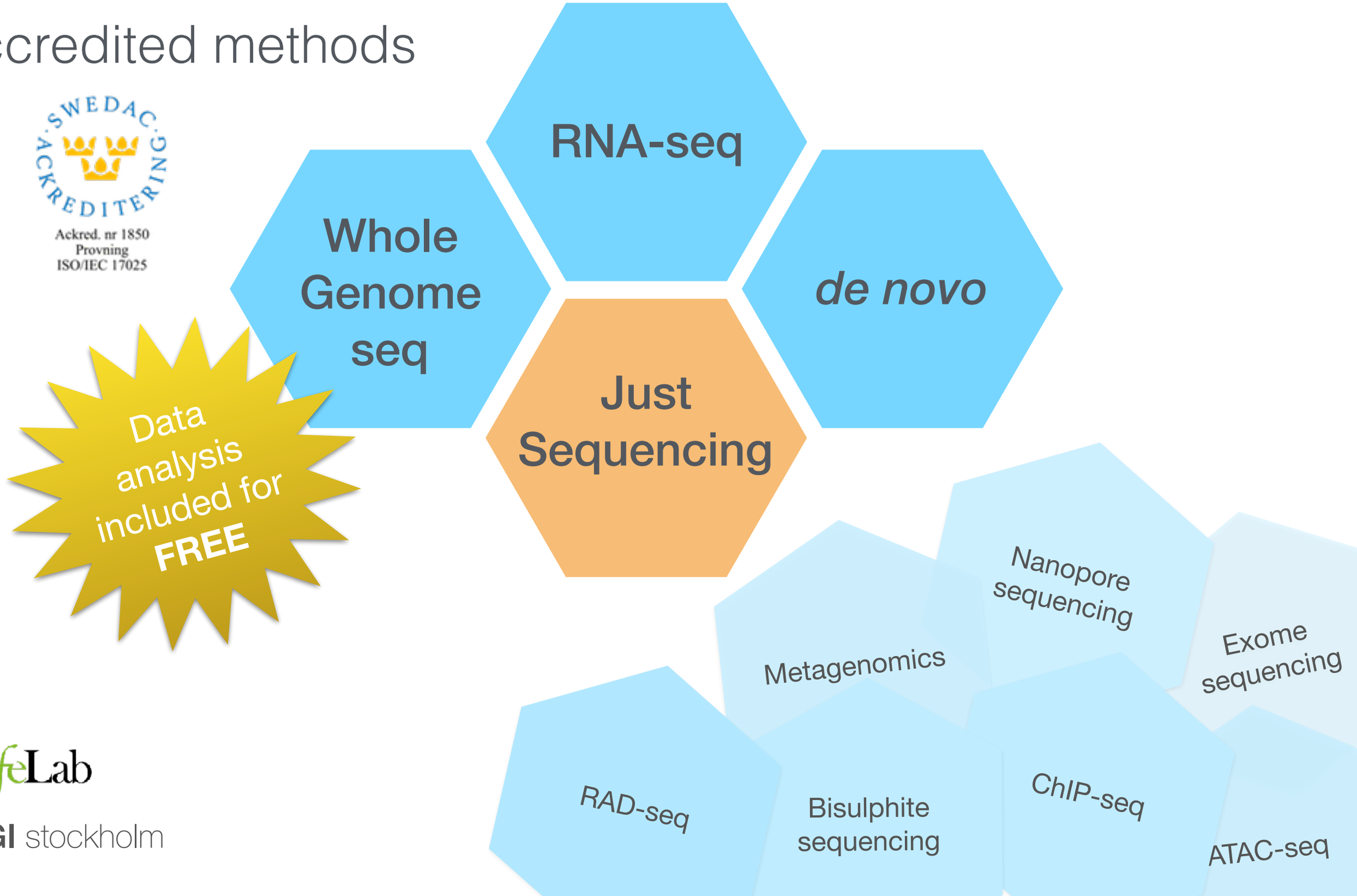
Methods offered at NGI

Accredited methods



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ChIP-seq: NGI Stockholm

- You do the ChIP, we do the seq
- Rubicon ThruPlex DNA (NGI Production)
  - Min 1 ng input
  - Min 10 µl
  - 0.2-10 ng/µl
  - Ins. size 200-800 bp
  - 963 kr / prep

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ChIP-seq: NGI Stockholm

- You do the ChIP, we do the seq
- Rubicon ThruPlex DNA (NGI Production)
- Typically run SE 50bp
  - Illumina HiSeq High Output mode v4, SR 1x50bp
  - 1226 kr / sample (40M reads)

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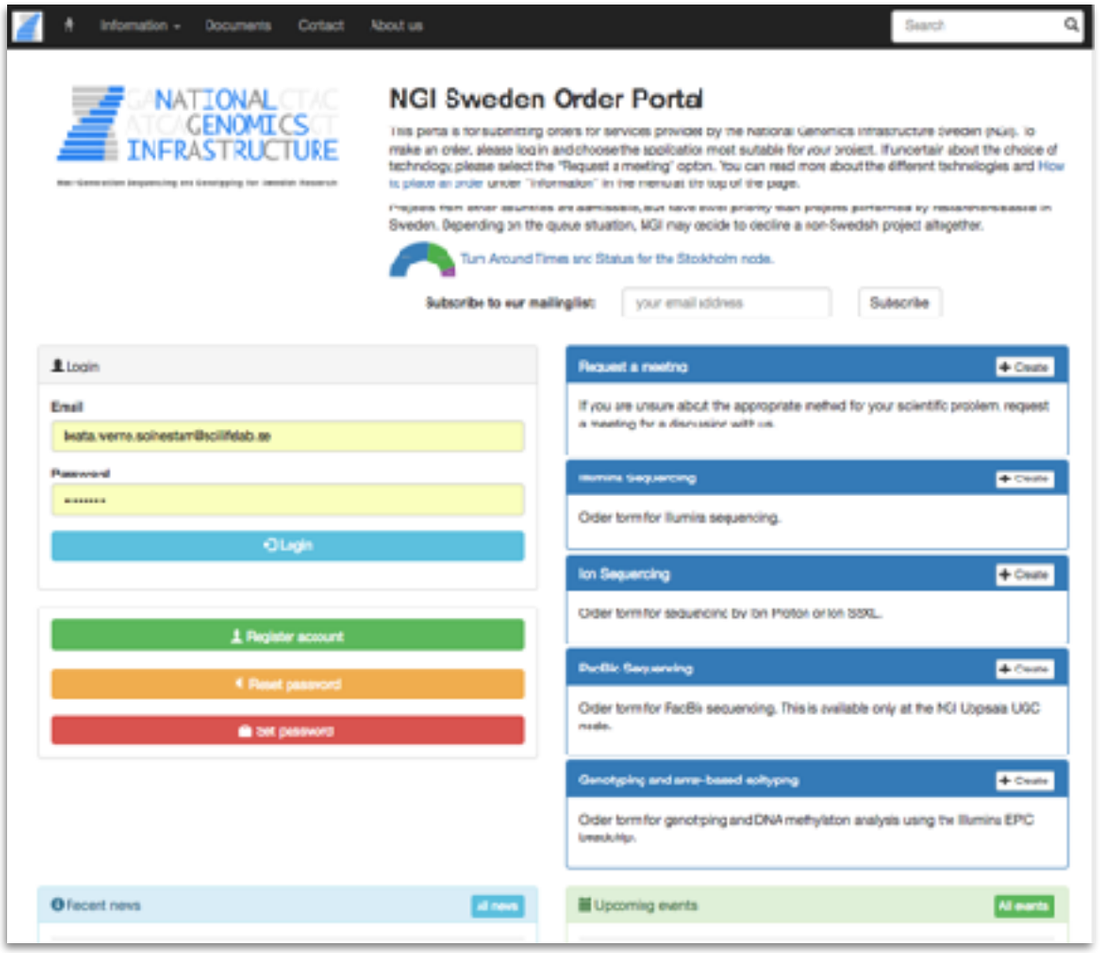
ChIP-seq: NGI Stockholm

- You do the ChIP, we do the seq
- Rubicon ThruPlex DNA (NGI Production)
- Typically run SE 50bp
- Start by organising a planning meeting

https://ngisweden.scilifelab.se



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ChIP-seq Pipeline

- Takes raw FastQ sequencing data as input
- Provides range of results
  - Alignments (BAM)
  - Peaks (optionally filtered)
  - Quality Control
- Pipeline in use since early 2017 (on request)

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NGI-ChIPseq

ChIP-seq Pipeline

NGI-ChIPseq

FastQ	FastQC	*Sequence QC*
	TrimGalore!	*Read trimming*
BAM	BWA	*Alignment*
	Samtools, Picard	*Sort, index, mark duplicates*
	Phantompeakqualtools	*Strand cross-correlation QC*
	deepTools	*Fingerprint, sample correlation*
	NGSPlot	*TSS / Gene profile plots*
BED	MACS2	*Peak calling*
	Bedtools	*Filtering blacklisted regions*
HTML	MultiQC	*Reporting*

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

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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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
  """
}
```

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-ChIPseq

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SciLifeLab / NGI-ChIPseq
forked from chuan-wang/NGI-ChIPseq

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Nextflow ChIP-seq data analysis pipeline, National Genomics Infrastructure, Science for Life Laboratory in Stockholm Edit

nextflow bioinformatics bioinformatics-pipeline chip-seq pipeline Manage topics

178 commits 1 branch 0 releases 2 contributors

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

This branch is 2 commits ahead of chuan-wang:master. Pull request Compare

ewels Merge pull request #49 from chuan-wang/master ··· Latest commit 51b7c2d 6 days ago

assets	Fix errors in scripts for reporting software versions; Add scripts fo...	7 days ago
bin	Fixing bugs	6 days ago
blacklists	v1.4 Updates: Adding post peak calling filtering and annotations; Gen...	14 days ago

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

NGI-ChIPseq

README.md

NGI-ChIPseq Results

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

- `installation.md`
  - Pipeline installation and configuration instructions
- `usage.md`
  - Instructions on how to run the NGI-ChIPseq pipeline
- `output.md`
  - Document describing all of the results produced by the pipeline, and how to interpret them.

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

Running NGI-ChIPseq

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-ChIPseq pipeline

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

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Running NGI-ChIPseq

Step 3: Choose your reference

- Common organism - use iGenomes

```
--genome GRCh37
```

- MACS peak calling config file

```
--macsconfig config.csv
```

Step 4: Organise your data

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

Running NGI-ChIPseq

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

Using UPPMAX

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

UPPNEX

- 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-ChIPseq
    -profile hebbe
    --bwaindex ./ref --macsconfig p.txt
    --reads "data/*_R{1,2}.fastq.gz"
```

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

Using Docker

```
nextflow run SciLifeLab/NGI-ChIPseq
    -profile docker
    --fasta genome.fa --macsconfig p.txt
    --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-ChIPseq
    -profile aws
    --genome GRCh37 --macsconfig p.txt
    --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
  - Use `--notrim` to disable this

- Some library preps also include additional adapters

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

Blacklist filtering

- Some parts of the reference genome collect incorrectly mapped reads
  - Good practice to remove these peaks
- Pipeline has ENCODE regions for Human & Mouse
- Can pass own BED file of custom regions

```
--blacklist_filtering
--blacklist regions.bed
```

Broad Peaks

- Some chromatin profiles don't have narrow, sharp peaks
  - For example, H3K9me3 & H3K27me3
- MACS2 can call peaks in "broad peak" mode
  - Pipeline uses default qvalue cutoff of 0.1

```
--broad
```

Extending Read Length

- When using single-end data, sequenced read length is shorter than the sequence fragment length
- For DeepTools, need to "extend" the read length
  - Set to 100bp by default. Use this parameter to customise this value.
  - Expected fragment length - sequence read length

```
--extendReadsLen [int]
```

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
`--saturation`	Run saturation analysis, subsampling reads from 10% - 100%
`--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-ChIPseq config

```
N E X T F L O W  ~  version 0.26.1
Launching `SciLifeLab/NGI-ChIPseq` [deadly_bose] - revision: 28e24c2a2a
=========================================
 NGI-ChIPseq: ChIP-Seq Best Practice v1.4
=========================================
Run Name             : deadly_bose
Reads                : data/*fastq.gz
Data Type            : Single-End
Genome               : GRCh37
BWA Index            : /sw/data/uppnex/igenomes//Homo_sapiens/Ensembl/GRCh37/Sequence/BWAIndex/
MACS Config          : data/macsconfig.txt
Saturation analysis  : false
MACS broad peaks     : false
Blacklist filtering  : false
Extend Reads         : 100 bp
Current home         : /home/phil
Current user         : phil
Current path         : /home/phil/demo_data/ChIP/Human/test
Working dir          : /home/phil/demo_data/ChIP/Human/test/work
Output dir           : ./results
R libraries          : /home/phil/R/nxtflow_libs/
Script dir           : /home/phil/GitHub/NGI-ChIPseq
Save Reference       : false
Save Trimmed         : false
Save Intermeds       : false
Trim R1              : 0
Trim R2              : 0
Trim 3' R1           : 0
Trim 3' R2           : 0
Config Profile       : UPPMAX
UPPMAX Project       : b2017001
E-mail Address       : phil.ewels@scilifelab.se
=====================================
```

Version control

Releases | Tags

Pre-release

v1.3

9d8b6b5

NGI-

ewels

Version v

```
$fastqc.
$trim_ga
$bw.modu
$samtool
$picard.
$phantom
$deepToo
$ngsplot
$macs.mo
$saturat
$saturat
```

PUBLIC | AUTOMATED BUILD

scilifelab/ngi-chipseq

Last pushed: a day ago

Repo Info | Tags | Dockerfile | Build Details | Build Settings | Collaborators | Webhooks

Status	Actions	Tag	Created	Last Updated
Building	Cance	v1.3	2 minutes ago	a minute ago
Canceled		v1.4	a day ago	a day ago
Success		latest	a day ago	a day ago

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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-ChIPseq -r v1.3
```

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Conclusion

- Use NGI-ChIPseq to prepare your data if you want:
  - To not have to remember every parameter for every tool
  - Extreme reproducibility
  - Ability to run on virtually any environment
- Now running for all ChIPseq projects at NGI-Stockholm

NGI-ChIPseq

Conclusion

GitHub https://github.com/

Open Source Initiative

NGI-ChIPseq SciLifeLab/NGI-ChIPseq

NGI-RNAseq SciLifeLab/NGI-RNAseq

NGI-smRNAseq SciLifeLab/NGI-smRNAseq

NGI-MethylSeq SciLifeLab/NGI-MethylSeq

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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