Nextflow:
a tutorial through examples

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CODATA

Outline

Introduction to Nextflow

Introduction to Nextflow

Introduction

Resources

- https://github.com/fpsom/CODATA-RDA-Advanced-Bioinformatics-2019/blob/phele/4.Day4.md

Workflow Languages

Many scientific applications require

- Multiple data files
- Multiple applications
- Perhaps different parameters

General purpose languages not well suited

- Too low a level of abstraction
- Does not separate workflow from application
- Not reproducible

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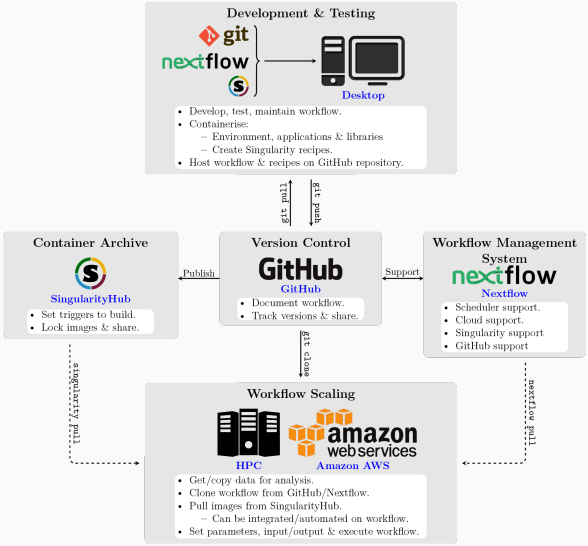

Nextflow

Groovy-based language

- Expressing workflows
- Portable
  - works on most Unix-like systems
- Very easy to install
  - NB: requires Java 7, 8
- Scalable
- Supports Docker/Singularity
- Supports a range of scheduling systems

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Key concepts of Nextflow

- Processes:
  - actual work being done (usually simple).
  - call program that does the analysis.
- Channels:
  - for communication between processes.
  - handles inputs and outputs.
- When all inputs ready, process is executed.
- Each process runs in its own directory (files are staged).
- Supports resumption of previous partial runs.

Introduction to Nextflow

Nextflow Script

Simple Example: Using BASH

Input is a file

- With 6 columns
- Column 2 is an index column
- Identify rows with identical field 2
- Remove identical rows

```
11   11:189256   0   189256   A   G
11   11:193788   0   193788   T   C
11   11:194062   0   194062   T   C
11   11:194228   0   194228   A   G
11   11:193788   0   193788   A   C
```

Using BASH:

```
cut -f 2 data/11.bim  | sort | uniq -d  > dups
grep -v -f dups data/11.bim > 11.clean
```

Simple Example: Using `nextflow`

```
1  #!/usr/bin/env nexflow
2
3  input_ch = Channel.fromPath("data/11.bim")
4
5  process getIDs {
6    input:
7    file input from input_ch
8
9    output:
10   file "ids" into id_ch
11   file "11.bim" into orig_ch
12
13   script:
14   "cut -f 2 $input | sort > ids"
15 }
16
17 process getDups {
18     input:
19     file input from id_ch
20
21     output:
22     file "dups" into dups_ch
23
24     script:
25     """
26     uniq -d $input > dups
27     touch ignore
28     """
29 }
```

Simple Example: Using `nextflow`

```
#!/usr/bin/env nexflow

input_ch = Channel.fromPath("data/11.bim")

process getIDs {
  input:
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  output:
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process getDups {
    input:
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    output:
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    script:
    """
    uniq -d $input > dups
    touch ignore
    """
}
```

```
process removeDups {
    input:
    file badids from dups_ch
    file orig from orig_ch

    output:
    file "clean.bim" into output

    script:
    "grep -v -f $badids $orig > clean.bim "
}

output.subscribe { print "Done!" }
```

Simple Example: Using `nextflow`

```
#!/usr/bin/env nexflow

input_ch = Channel.fromPath("data/11.bim")

process getIDs {
  input:
  file input from input_ch

  output:
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  file "11.bim" into orig_ch

  script:
  "cut -f 2 $input | sort > ids"
}

process getDups {
    input:
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    output:
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    """
    uniq -d $input > dups
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}
```

```
process removeDups {
    input:
    file badids from dups_ch
    file orig from orig_ch

    output:
    file "clean.bim" into output

    script:
    "grep -v -f $badids $orig > clean.bim "
}

output.subscribe { print "Done!" }
```

```
$ nextflow run cleandups.nf

N E X T F L O W  ~  version 19.04.1
Launching `cleandups.nf` [soggy_jennings] - revision: 795e2aa39d
[warm up] executor > local
executor >  local (3)
[84/7e1ad1] process > getIDs     [100%] 1 of 1
[19/cc8bf9] process > getDups    [100%] 1 of 1
[f9/ed086d] process > removeDups [100%] 1 of 1
Completed at: 31-Jul-2019 09:00:50
Duration    : 1.5s
CPU hours   : (a few seconds)
Succeeded   : 3
```

Simple Example: Using `nextflow`

The `work` directory

```
|--work
|  |--90
|  |  |--cebf3649d883f88381e32b4912b560
|  |  |  |--ids -> /Users/phele/day4/work/b3/aa0380f2a1bca447259b7ffd390083/ids
|  |  |  |--ignore
|  |--9c
|  |  |--e0cb7d8d26682d7d4a1c44392f2bb3
|  |  |  |--11.bim -> /Users/phele/day4/data/11.bim
|  |  |  |--clean.bim
|  |  |  |--dups -> /Users/phele/day4/work/90/cebf3649d883f88381e32b4912b560/dups
|  |--b3
|  |  |--aa0380f2a1bca447259b7ffd390083
|  |  |  |--11.bim -> /Users/phele/day4/data/11.bim
|  |  |  |--ids
```

Introduction to Nextflow

Partial Execution

Partial Execution

If execution of workflow is only partial

- Because of error
- Only need to resume from process that failed

```
nextflow run cleandups.nf -resume
```

Introduction to Nextflow

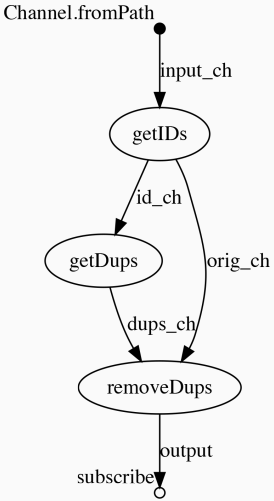
Visualising the Workflow

Visualising the Workflow

Nextflow supports several visualisation tools:

-with-dag

```
nextflow run cleandups.nf -with-dag <file-name>
```



Visualising the Workflow

Nextflow supports several visualisation tools:

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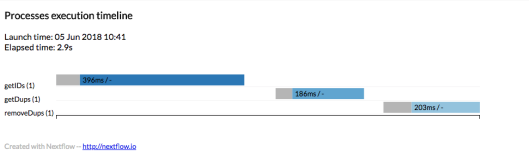
```
nextflow run cleandups.nf -with-dag <file-name>
```

-with-timeline

```
nextflow run cleandups.nf -with-timeline <file-name>
```



Visualising the Workflow

Nextflow supports several visualisation tools:

-with-dag

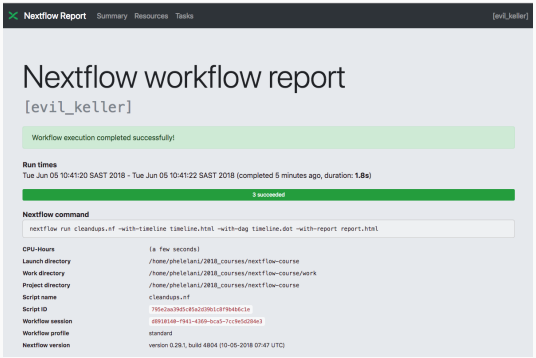
```
nextflow run cleandups.nf -with-dag <file-name>
```

-with-timeline

```
nextflow run cleandups.nf -with-timeline <file-name>
```

-with-report

```
nextflow run cleandups.nf -with-report <filename>
```



Groovy

Groovy

Nextflow is a DSL built with Groovy

- Can inter-mix Nextflow, Groovy and Java code.
- Very powerful, flexible.
- Don't need to know much (any?) Groovy but a little knowledge is a powerful thing

Groovy

Groovy Closures

Groovy: Closures

Closures are anonymous functions

- Similar to lambdas in Python
- Don't want the overhead of naming a function we only use once
- Typically use with higher-order functions
  - Functions that take other functions as arguments
- Very powerful and useful

Syntax for a closure that takes one argument:

```
{ parm -> expression }
```

Groovy: Closures

Closures are anonymous functions

- Similar to lambdas in Python
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  - Functions that take other functions as arguments
- Very powerful and useful

Syntax for a closure that takes one argument:

```
{ parm -> expression }
```

```
1  { a -> a*a } (3)
2
3  { a -> a*a+7*a - 2 } (3)
4
5  for (n in 1..5) print( {it*it} (n));
6
7  { x, y ->  Math.sqrt(x*x + y*y) } (3,4)
8
9  int doX(f, nums) {
10   sum=0;
11   for ( n in nums ) {
12     sum = sum+f(n);
13   }
14   return sum
15 }
16
17 print doX ( {a->a},   [4,5,16] );
18
19 print doX ( {a->a*a}, [4,5,16] );
20
21 print doX ( { it*it }, [4,5,16]);
22
23 m=10
24
25 print doX({a->m*a+2}, [1,2,3])
```

Generalising and Extending

Extending the Example

- Parameterise the input
- Want output to go to convenient place
- Workflow takes in multiple input files – processes are executed on each in turn.
- Complication : may need to carry the base name of the input to the final output;
- Can repeat some steps for different parameters.

Generalising and Extending

Parameters

Parameters

In Nextflow file:

```
input_ch = Channel.fromPath(params.data_dir)
```

And run it like this

```
nextflow run phylo1.nf --data_dir data/polyseqs.fa
```

Generalising and Extending

Channels

Data Types in Channels

Channels support different types:

- file
- val
- set

Creating Channels

```
Channel.create()
Channel.empty
Channel.from("blast","plink")
Channel.fromPath("data/*.fa")
Channel.fromFilePairs("data/{YRI,CEU,BEB}.*")
Channel.watchPath("*fa")
```

Many, many operations you can do on channels and their contents

bind	buffer	close
filter	map/reduce	group
join, merge	mix	copy
split	spread	fork
count	min/max/sum	print/view

Generalising and Extending

Generalising Our Example

Workflow: Multiple Inputs

```
params.data_dir = "data"
input_ch = Channel.fromPath("${params.data_dir}/*.bim")

process getIDs {
    input:
    file input from input_ch

    output:
    file "${input.baseName}.ids" into id_ch
    file "$input" into orig_ch

    script:
    "cut -f 2 $input | sort > ${input.baseName}.ids"
}

process getDups {
    input:
    file input from id_ch

    output:
    file "${input.baseName}.dups" into dups_ch

    script:
    out = "${input.baseName}.dups"
    """
    uniq -d $input > $out
    touch ignore
    """
}
```

Workflow: Multiple Inputs

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input_ch = Channel.fromPath("${params.data_dir}/*.bim")

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    input:
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    output:
    file "${input.baseName}.ids" into id_ch
    file "$input" into orig_ch

    script:
    "cut -f 2 $input | sort > ${input.baseName}.ids"
}

process getDups {
    input:
    file input from id_ch

    output:
    file "${input.baseName}.dups" into dups_ch

    script:
    out = "${input.baseName}.dups"
    """
    uniq -d $input > $out
    touch ignore
    """
}
```

```
process removeDups {
    publishDir "output", pattern: "${badids.baseName}_clean.bim"
      ↪ , overwrite:true, mode:'copy'

    input:
    file badids from dups_ch
    file orig from orig_ch

    output:
    file "${badids.baseName}_clean.bim" into cleaned_ch

    script:
    "grep -v -f $badids $orig > ${badids.baseName}_clean.bim "
}
```

Workflow: Multiple Inputs

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params.data_dir = "data"
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    input:
    file badids from dups_ch
    file orig from orig_ch

    output:
    file "${badids.baseName}_clean.bim" into cleaned_ch

    script:
    "grep -v -f $badids $orig > ${badids.baseName}_clean.bim "
}
```

```
$ nextflow run cleandups.nf

Launching `cleandups.nf` [distracted_hodgkin] - revision: 29
       ↪ fdb384a6
[warm up] executor > local
executor >  local (9)
[1a/431eb7] process > getIDs     [100%] 3 of 3
[cc/fc0aaa] process > getDups    [100%] 3 of 3
[03/c31154] process > removeDups [100%] 3 of 3
Completed at: 31-Jul-2019 10:26:23
Duration    : 2s
CPU hours   : (a few seconds)
Succeeded   : 9
```

Workflow: Multiple Parameters

Now try splitting the file but use different split values

```
split  -l 400 data.txt dataX
```

will produce files dataXaa, dataXab, dataXac and so on ...

Try:

```
splits = [400,500,600]

process splitIDs  {
    input:
    file bim from cleaned_ch
    each split from splits

    output:
    file ("*-$split-*") into output_ch;

    script:
    "split -l $split $bim ${bim.baseName}-$split- "
}
```

Generalising and Extending

Managing Grouped Files

Grouped Files

Use **PLINK** as an example.

```
## Short version of the command
plink --bfile /path/YRI --freq --out /tmp/YRI

## Long version of the command
plink --bed YRI.bed \
    --bim YRI.bim \
    --fam YRI.fam \
    --freq \
    --out /tmp/YRI
```

Problem:

- Pass the files on another channel(s) to be staged
- Pass the base name as value/or work it out

Pros/Cons

- Simple
- Need extra channel/some gymnastics

Grouped Files

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    --out /tmp/YRI
```

Problem:

- Pass the files on another channel(s) to be staged
- Pass the base name as value/or work it out

Pros/Cons

- Simple
- Need extra channel/some gymnastics

RECAP CLOSURES

Simply, a *closure* is an anonymous function

- Code wrapped in braces {, }
- Default argument called *it*

```
[1,2,3].each { print it * it }
[1,2,3].each { num -> print num * num }
```

Grouped Files - Version 1: map

```
#!/usr/bin/env nextflow
params.dir = "data/pops/"
dir = params.dir
params.pops = ["YRI","CEU","BEB"]

Channel
    .from(params.pops)
    .map { pop ->
        [ file("$dir/${pop}.bed"),
          file("$dir/${pop}.bim"),
          file("$dir/${pop}.fam")]
    }
    .set { plink_data }

plink_data.subscribe { println "$it" }
```

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```
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params.dir = "data/pops/"
dir = params.dir
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Channel
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          file("$dir/${pop}.fam")]
    }
    .set { plink_data }

plink_data.subscribe { println "$it" }
```

```
[data/pops/YRI.bed, data/pops/YRI.bim, data/pops/YRI.fam]
[data/pops/CEU.bed, data/pops/CEU.bim, data/pops/CEU.fam]
[data/pops/BEB.bed, data/pops/BEB.bim, data/pops/BEB.fam]
```

Grouped Files - Version 1: map

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dir = params.dir
params.pops = ["YRI","CEU","BEB"]

Channel
    .from(params.pops)
    .map { pop ->
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          file("$dir/${pop}.bim"),
          file("$dir/${pop}.fam")]
    }
    .set { plink_data }

plink_data.subscribe { println "$it" }
```

```
process getFreq {
  input:
    set file(bed), file(bim), file(fam) from plink_data
  output:
    file "${bed.baseName}.frq" into result

  """
  plink --bed $bed \
    --bim $bim \
    --fam $fam \
    --freq \
    --out ${bed.baseName}"
  """
}
```

```
[data/pops/YRI.bed, data/pops/YRI.bim, data/pops/YRI.fam]
[data/pops/CEU.bed, data/pops/CEU.bim, data/pops/CEU.fam]
[data/pops/BEB.bed, data/pops/BEB.bim, data/pops/BEB.fam]
```

Grouped Files - Version 2: `fromFilePairs`

Use `fromFilePairs`.

- Takes a closure used to gather files together with the same key

```
x_ch = Channel.fromFilePairs( files ) { closure }
```

- Specify the files as a glob
- Closure associates each file with a key
- `fromPairs` puts all files with same key together
- Returns a list of pairs (key, list)

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- Specify the files as a glob
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- `fromPairs` puts all files with same key together
- Returns a list of pairs (key, list)

```
1  #!/usr/bin/env nextflow
2
3  commands = Channel.fromFilePairs("/usr/bin/*", size:-1) {
4                  it.baseName[0]
5              }
6
7  commands.subscribe { k= it[0];
8    n=it[1].size();
9    println "There are $n files starting with $k";
10 }
```

A more complex example – default closure

```
1  Channel
2      .fromFilePairs
3          ("${params.dir}/*.{bed,fam,bim}",size:3, flat : true)
4      .ifEmpty { error "No matching plink files" }
5      .set { plink_data }
6
7  plink_data.subscribe { println "$it" }
```

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- Takes a closure used to gather files together with the same key

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A more complex example – default closure

```
1  Channel
2      .fromFilePairs
3          ("${params.dir}/*.{bed,fam,bim}",size:3, flat : true)
4      .ifEmpty { error "No matching plink files" }
5      .set { plink_data }
6
7  plink_data.subscribe { println "$it" }
```

```
[CEU, [data/pops/CEU.bed, data/pops/CEU.bim, data/pops/CEU.fam]]
[YRI, [data/pops/YRI.bed, data/pops/YRI.bim, data/pops/YRI.fam]]
[BEB, [data/pops/BEB.bed, data/pops/BEB.bim, data/pops/BEB.fam]]
```

Grouped Files - Version 2: `fromFilePairs`

```
process checkData {
    input:
    set pop, file(pl_files) from plink_data

    output:
    file "${pl_files[0]}.frq" into result

    script:
    base = pl_files[0].baseName
    "plink --bfile $base --freq --out ${base}"
}
```

Grouped Files - Version 2: `fromFilePairs`

```
process checkData {
    input:
    set pop, file(pl_files) from plink_data

    output:
    file "${pl_files[0]}.frq" into result

    script:
    base = pl_files[0].baseName
    "plink --bfile $base --freq --out ${base}"
}
```

```
process checkData {
    input:
    set pop, file(pl_files) from plink_data

    output:
    file "${pop}.frq" into result

    script:
    "plink --bfile $pop --freq  --out $pop"
}
```

Grouped Files - Version 2: `fromFilePairs`

```
process checkData {
    input:
    set pop, file(pl_files) from plink_data

    output:
    file "${pl_files[0]}.frq" into result

    script:
    base = pl_files[0].baseName
    "plink --bfile $base --freq --out ${base}"
}
```

```
process checkData {
    input:
    set pop, file(pl_files) from plink_data

    output:
    file "${pop}.frq" into result

    script:
    "plink --bfile $pop --freq  --out $pop"
}
```

Grouped Files - Final Version

```groovy
Channel.fromPath("data/*.dat").set { data }

process P1 {
    echo true
    input:
    file(data)

    output:
    set val(data.baseName), file("${fbase}.pre") into channelA
    set val(data.baseName), file(data) into channelB

    script:
    fbase=data.baseName
    "echo dummy > ${fbase}.pre"
}

process P2 {
    echo true
    input:
    set name, file(pre) from channelA

    output:
    set name, file(pre) into channelC

    script:
    if (pre.baseName = /.*TMP.*/)
        "sleep 4"
    else
        "sleep 1"
}
```

```groovy
process P3 {
    echo true

    input:
    set name, file(data), file(pre) from channelB.join(channelC)

    """
    echo "${data} - ${pre}"
    """
}
```

Generalising and Extending

On absolute paths

Absolute paths

```
input = Channel.fromPath("/data/batch1/myfile.fa")

process show {
    input:
    file data from input

    output:
    file 'see.out'

    script:
    cp $data /home/scott/answer
    ...
```

Nextflow and Docker

Nextflow and Docker

Docker & Singularity Containers

Docker & Singularity Containers

Light-weight virtualisation abstraction layer

- Currently runs on Unix like systems
  - Linux
  - macOS
- Windows support coming

Can create images locally or get from repositories

```
## Docker
docker pull ubuntu
docker pull quay.io/banshee1221/h3agwas-plink

## Singularity
singularity pull docker://ubuntu
singularity pull docker://quay.io/banshee1221/h3agwas-plink
```

Running images

```
## Docker
docker run <some-image-name>

## Singularity
singularity exec <some-image-name>
```

- Docker/Singularity often run images in background
- Can also run interactively

```
## Running Docker interactively
sudo docker run -t -i quay.io/banshee1221/h3agwas-plink

## Running Singularity interactively
singularity shell docker://quay.io/banshee1221/h3agwas-plink
```

Nextflow supports Docker & Singularity

- Well designed script should be highly portable
- Each process gets run as a separate image call
  - Under the hood, a `docker run` or a `singularity exec` is called
- Can use the same or different images for each process
  - Parameterisable

Assuming all processes use the same image:

```
## For Docker
nextflow run plink2.nf -with-docker quay.io/banshee1221/h3agwas-plink

## For Singularity
nextflow run plink.nf -with-singularity docker://quay.io/banshee1221/h3agwas-plink
```

Nextflow and Docker

Directory & File Access

Directory & File access

Nextflow Docker/Singularity support highly transparent – but pay attention to good practice

- For each process Docker/Singularity mounts the work directory for **that** process on the Docker/Singularity image.
- Files can be staged in and out using Nextflow mechanisms.
- Other files available: directories mounted through Docker/Singularity run time options or on the Docker image
- No other files on the host machine including the current directory
- Process executes in the Docker/Singularity environment

Directory & File access

```
1  data = Channel.fromPath("/popdata/1k-2014/pops/YRI.bim")
2
3  process see {
4      echo true
5      publishDir params.publish, overwrite:true, mode:'move'
6
7      input:
8      file bim from data
9
10     output:
11     file count
12
13     """
14     hostname
15     echo "Path is \$( pwd )\n "
16     echo "Parent directory has \$( ls .. )\n"
17     echo "My home directory has \$( ls /home/scott )\n"
18     wc -l $bim > count
19     ls
20     """
21 }
```

```
N E X T F L O W  ~  version 0.21.2
Launching show_env.nf
[warm up] executor > local
[94/597f09] Submitted process > see (1)
89ad448ae0b2
Path is /home/scott/witsGWAS/dockerized/work/94/597f09ca6cc01c7be
Parent directory has 597f09ca6cc01c7be
My home directory has witsGWAS

YRI.bim
count
```

Directory & File access

Note that although the script's pwd shows:
`/home/scott/witsGWAS/dockerized/work/94/597f09ca6cc01c7be`

- Only these specific directories are mounted
- Only the files in the innermost directory are available

Any absolute paths (other than those used in staging) will result in error.

Profiles

In nextflow.config

```
profiles {
  ...
  docker {
    process.container = 'quay.io/banshee1221/h3agwas-plink:latest'
    docker.enabled = true
  }
}
```

Now can run as:

```
nextflow run gwas.nf -profile docker
```

This can be extended in many ways

- Different processes can use different containers
- Can mount other host directories
- Can pass arbitrary Docker parameters

Executors

Executors

Executors

Executors

A Nextflow *executor* is the mechanism which Nexflow runs the code in each of the processes

- Default is `local`: process is run as a script

Many others

- PBS/Torque
- SLURM
- Amazon (AWS Batch)
- SGE (Sun Grid Engine)

Selecting an executor Annotating each process

- `executor` directive, e.g. `executor 'pbs'`
- resource constraints

Or, `nextflow.config` file

- either global or per-process

Executors

Nextflow on a cluster (HPC)

Running Nextflow on a cluster (HPC)

Script runs on the *head* node

- Nextflow uses the `executor` information to decide how the job should run
- Each process can be handled differently
- Nextflow submits each process to the job scheduler on your behalf (e.g, if using PBS/Torque, `qsub` is done)

Example

```
process {
  executor = 'pbs'
  queue = 'batch'
  scratch = true
  cpus = 5
  memory = '2GB'
}
```

Executors

Scheduler + Docker

Scheduler + Docker

```
process.container = 'quay.io/banshee1221/h3agwas-plink:latest'
docker.enabled = false

process {
  executor = 'pbs'
  queue = 'batch'
  scratch = true
  cpus = 5
  memory = '2GB'
}
```

Executors

Amazon EC2

Amazon EC2

Netflow has native support for EC2

- You need an account on EC2
- Image (AMI) with the appropriate support

Launch your code:

```
nextflow cloud create GenomeCloud -c 5
```

If successful, Nextflow will give you the name of the headnode of your cluster

- `ssh` into into it
- run Nextflow on it.

Afterwards shut down:

```
nextflow shutdown GenomeCloud
```

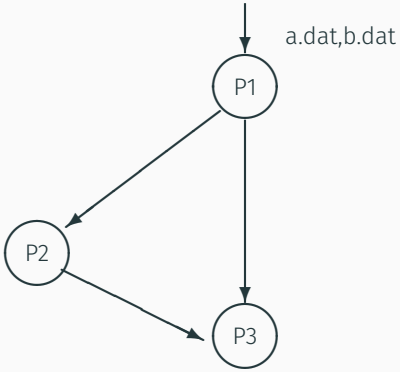
Channel Operations

Channel operations

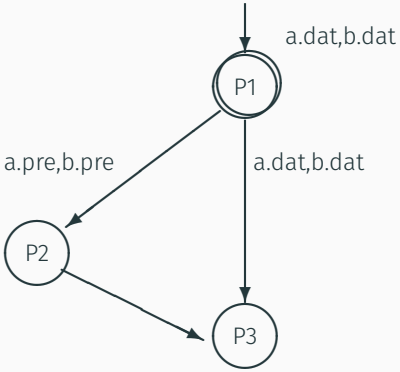
Nextflow tries to maximise concurrency

- processes are by default synchronised by channels
- when data arrives on all input channels, process executes

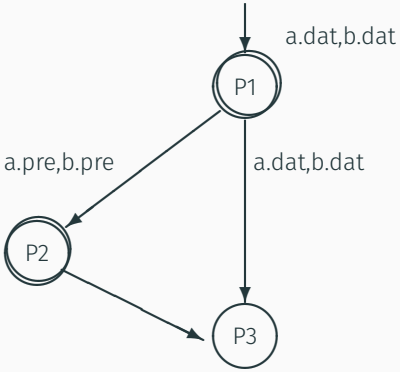
Channel operations



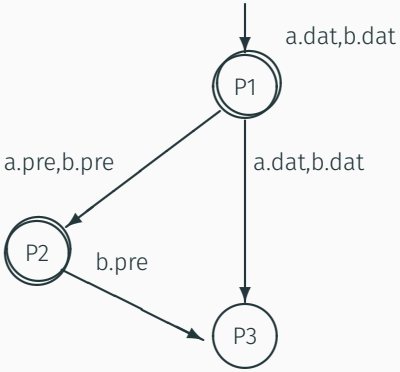
Channel operations



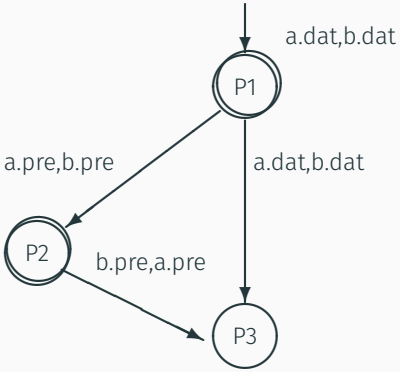
Channel operations



Channel operations



Channel operations



Channel operations

```
1  Channel.fromPath("data/*.dat").set { data }
2
3  process P1 {
4      input:
5      file(data)
6
7      output:
8      file  "${fbase}.pre" into channelA
9      file  data           into channelB
10
11     script:
12     fbase=data.baseName
13     "echo dummy > ${fbase}.pre"
14 }
15
16 process P2 {
17     input:
18     file pre from channelA
19
20     output:
21     file pre into channelC
22
23     script:
24     if (pre.baseName == "a")
25       "sleep 4"
26     else
27       "sleep 1"
28 }
```

Channel operations

```
Channel.fromPath("data/*.dat").set { data }

process P1 {
    input:
    file(data)

    output:
    file  "${fbase}.pre" into channelA
    file  data           into channelB

    script:
    fbase=data.baseName
    "echo dummy > ${fbase}.pre"
}

process P2 {
    input:
    file pre from channelA

    output:
    file pre into channelC

    script:
    if (pre.baseName == "a")
      "sleep 4"
    else
      "sleep 1"
}
```

Try

```
process P3 {
    echo true

    input:
    file(data) from channelB
    file(pre)  from channelC

    script:
    """
    echo "${data} - $pre"
    """
}
```

Channel operations

Solution: `join`/`merge` channels

- `x.merge(y)`
  Items emmited by the channels *x* and *y* are combined into a new channel.
- `x.join(y)`
  Items emmited by the channels *x* and *y* are joined together into one channel based on existing matching key. Default: first element in each item.

Channel Operations

Using `join`

Using join

```
1 ch1 = Channel.from( "a","b","c" )
2 ch2 = Channel.from( "a","d","e","a","c","b" )
3 ch1.join(ch2).subscribe { println it }
```

```
a
b
c
```

```
1 ch1 = Channel.from( ["a",1], ["b",4], ["c",5] )
2 ch2 = Channel.from( ["a",10], ["d",8], ["e",7], ["a",9], ["c",1], ["b",10] )
3 ch1.merge(ch2).subscribe { println it }
```

```
[a, 1, 10]
[b, 4, 10]
[c, 5, 1]
```

Channel Operations

Using `merge`

Using merge

```
1 ch1 = Channel.from( "a","b","c" )
2 ch2 = Channel.from( "a","d","e","a","c","b" )
3 ch1.merge(ch2).subscribe { println it }
```

```
[a, a]
[b, d]
[c, e]
```

```
1 ch1 = Channel.from( ["a",1], ["b",4], ["c",5] )
2 ch2 = Channel.from( ["a",10], ["d",8], ["e",7], ["a",9], ["c",1], ["b",10] )
3 ch1.merge(ch2).subscribe { println it }
```

```
[a, 1, a, 10]
[b, 4, d, 8]
[c, 5, e, 7]
```

Channel Operations

`join` vs `merge`

join vs merge

join

- If values are singletons, then the values must be the same
- If value is tuple if the, then the first element of the tuple must be the same

merge

- Merges everything into a channel, no matching.

Channel Operations

Working version of the example

Working version of the example

```
1  Channel.fromPath("data/*.dat").set { data }
2
3  process P1 {
4      echo true
5
6      input:
7      file(data)
8
9      output:
10     set val(data.baseName), file("${fbase}.pre") into channelA
11     set val(data.baseName), file(data) into channelB
12
13     script:
14     fbase=data.baseName
15     "echo dummy > ${fbase}.pre"
16
17 process P2 {
18     echo true
19
20     input:
21     set name, file(pre) from channelA
22
23     output:
24     set name, file(pre) into channelC
```

```
25     script:
26     if (pre.baseName = /.*TMP.*/)
27       "sleep 4"
28     else
29       "sleep 1"
30 }
31
32 process P3 {
33     echo true
34
35     input:
36     set name, file(data), file(pre) from channelB.join(channelC)
37
38     script:
39     """
40     echo "${data} - ${pre}"
41     """
42 }
```

Channel Operations

Copying channels

Copying channels

You often need to copy a channel

```
process do {
    ..

    output:
    file ("x.*") into out_ch

    ..
}

out_ch.separate(a_ch, b_ch, c_ch)
```

Copying channels

You often need to copy a channel

```
process do {
    ..

    output:
    file ("x.*") into out_ch

    ..
}

out_ch.separate(a_ch, b_ch, c_ch)
```

Alternatively

```
process do {
    ..

    output:
    file ("x.*") into (a_ch, b_ch, c_ch)

    ..
}
```

H3AVarCall

H3AVarCall: Hands-on Variant Calling Practical

Prepare your workspace for the variant calling workflow!

```
## Change directory to your day4 working folder:
cd ~/Documents/day4

## Clone the H3AVarCall repository from GitHub:
git clone https://github.com/h3abionet/h3avarcall.git

## Change directory to the repository:
cd h3avarcall

## Create symbolic links to the Singularity images with applications:
ln -s /home/nfs3/h3avarcall/containers/* containers/

## Make a temorary folder in the 'scratch directory' for your 'work' folder:
mkdir -p /scratch/<USERNAME>/work
```

Lets look at some important files:

- `main.nf`
- `main.config`
- `nextflow.config`

DONE!! Now we are ready to start with the variant calling analysis!

H3AVarCall

Quality Checks - FastQC

Quality Checks - FastQC

Time allocated for this step: **10 minutes** Run:

```
nextflow run main.nf -profile local -w /scratch/<USERNAME>/work --mode do.QC
```

Results:

```
h3avarcall
  |--variant_calling_results
  |  |--1_QC
  |  |  |--workflow_report
  |  |  |  |--h3avarcall_report.html
  |  |  |  |--h3avarcall_timeline.html
  |  |  |  |--h3avarcall_workflow.dot
  |  |  |  |--h3avarcall_trace.txt
  |  |  |--<sample_1>_R1.fastqc.html .. <sample_N>_R1.fastqc.html
  |  |  |--<sample_1>_R2.fastqc.html .. <sample_N>_R1.fastqc.html
```

H3AVarCall

Read Trimming - Trimmomatic

Read Trimming - Trimmomatic

Time allocated for this step: **10 minutes**

Run:

```
nextflow run main.nf -profile local -w /scratch/<USERNAME>/work --mode do.ReadTrimming
```

Results:

```
h3avarcall
  |--variant_calling_results
  |  |--2_Read_Trimming
  |  |  |--workflow_report
  |  |  |  |--h3avarcall_report.html
  |  |  |  |--h3avarcall_timeline.html
  |  |  |  |--h3avarcall_workflow.dot
  |  |  |  |--h3avarcall_trace.txt
  |  |  |--<sample_1>.1P.fastq.gz .. <sample_N>.1P.fastq.gz
  |  |  |--<sample_1>.2P.fastq.gz .. <sample_N>.2P.fastq.gz
```

H3AVarCall

Read Alignment - **BWA**, **GATK** and **Samtools**

Read Alignment - BWA, GATK and Samtools

Time allocated for this step: **10 minutes**

Run:

```
nextflow run main.nf -profile local -w /scratch/<USERNAME>/work --mode do.ReadAlignment
```

Results:

```
h3avarcall
  |--variant_calling_results
  |  |--3_Read_Alignment
  |  |  |--workflow_report
  |  |  |  |--h3avarcall_report.html
  |  |  |  |--h3avarcall_timeline.html
  |  |  |  |--h3avarcall_workflow.dot
  |  |  |  |--h3avarcall_trace.txt
  |  |  |--<sample_1>_md.recal.bam .. <sample_N>_md.recal.bam
  |  |  |--<sample_1>_md.recal.bai .. <sample_N>_md.recal.bai
```

H3AVarCall

Variant Calling - **GATK**

Variant Calling - GATK

Time allocated for this step: **60 minutes**

Run:

```
nextflow run main.nf -profile local -w /scratch/<USERNAME>/work --mode do.VariantCalling
```

Results:

```
h3avarcall
  |--variant_calling_results
  |  |--4_Variant_Calling
  |  |  |--workflow_report
  |  |  |  |--h3avarcall_report.html
  |  |  |  |--h3avarcall_timeline.html
  |  |  |  |--h3avarcall_workflow.dot
  |  |  |  |--h3avarcall_trace.txt
  |  |  |--chr_1_genotyped.vcf.gz .. chr_22_genotyped.vcf.gz
  |  |  |--chr_1_genotyped.vcf.gz.tbi .. chr_22_genotyped.vcf.gz.tbi
```

H3AVarCall

Variant Filtering - GATK

Variant Filtering - GATK

Time allocated for this step: **10 minutes**

Run:

```
nextflow run main.nf -profile local -w /scratch/<USERNAME>/work --mode do.VariantFiltering
```

Results:

```
h3avarcall
  |--variant_calling_results
  |  |--5_Variant_Filtering
  |  |  |--workflow_report
  |  |  |  |--h3avarcall_report.html
  |  |  |  |--h3avarcall_timeline.html
  |  |  |  |--h3avarcall_workflow.dot
  |  |  |  |--h3avarcall_trace.txt
  |  |  |--genome.SNP-recal.vcf.gz
  |  |  |--genome.SNP-recal.vcf.gz.tbi
```

H3AVarCall

Quality Checks - MultiQC

Quality Checks - MultiQC

Time allocated for this step: **10 minutes**

Run:

```
nextflow run main.nf -profile local -w /scratch/<USERNAME>/work --mode do.MultiQC
```

Results:

```
h3avarcall
  |--variant_calling_results
  |  |--MultiQC
  |  |  |--multiqc_data
  |  |  |--multiqc_report.html
```