Gmail **Katie Lennard <katieviljoen@gmail.com>**

# NGI-RNAseq igenomes setup

11 messages

**Katie Lennard** <katieviljoen@gmail.com> Wed, Mar 28, 2018 at 9:46 AM
To: phil.ewels@scilifelab.se

Hi Phil,
Thanks for making your pipeline available to the community. I'm currently trying to setup the pipeline on our PBS server at the University of Cape Town. If you could give me some adivvvice on how to setup the iGenomes part that would be very helpful. I've downloaded the GRCh37 iGenome from https://support.illumina.com/sequencing/sequencing_software/igenome.html but it does not contain all the files you specify in your igenomes.config i.e. I found `Homo_sapiens/Ensembl/GRCh37/Sequence/WholeGenomeFasta/genome.fa` but not `Homo_sapiens/Ensembl/GRCh37/Annotation/Genes/genes.bed` or `/Homo_sapiens/Ensembl/GRCh37/Annotation/Genes/genes.gtf` or `/Homo_sapiens/Ensembl/GRCh37/Sequence/STARIndex/`

Can you advise me on how to set this up correctly?

Regards,
--

Katie

Bioinformatician
Division of Computational Biology
Institute of Infectious Diseases & Molecular Medicine
University of Cape Town
South Africa
+27 21 406 6176

**Phil Ewels** <phil.ewels@scilifelab.se> Wed, Mar 28, 2018 at 10:06 AM
To: Katie Lennard <katieviljoen@gmail.com>

Hi Katie,

Sounds great! The original iGenomes resource is lacking several indexes as you say - the ones referenced in the pipeline are from my modified version. This is hosted on AWS and I set it up with the intention of making it easier to run these pipelines in the cloud - I had hoped that I could get someone from illumina to take ownership of the iGenomes resource (no-one seems to know who made it), or Amazon to make it a proper public resource, but nothing has happened with either of these yet.

You should hopefully be able to download my version of the iGenomes resource here: https://ewels.github.io/AWS-iGenomes/
Note that there was recently some changes to the hosting setup though, the command line script is still working for me but sometimes there are weird authentication problems for other people. Please let me know if it doesn't work for you.

You can see the details of how I generated the extra references here: https://github.com/ewels/AWS-iGenomes#data-origin

Finally, regarding the pipeline - since the v1.4 release a few days ago, I've started work on porting the pipeline to my new pipeline-sharing initiative called nf-core: https://nf-core.github.io/ - the reason for this is to get away from the SciLifeLab branding to encourage other groups to collaborate on the same pipeline instead of forking their own version.

In practice, this really just means that future releases will be called nf-core/RNAseq instead of SciLifeLab/NGI-RNAseq. The latter will remain in place for future reproducibility though, as a read-only archive repository. If you would like to get involved, say hi at https://github.com/nf-core/nf-core.github.io/issues/1 and maybe pop into the gitter channel :)

Phil

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--
Phil Ewels, Ph.D.
Head of Facility, NGI Applications Development
National Genomics Infrastructure (NGI), SciLifeLab Sweden
phil.ewels@scilifelab.se - Twitter: @tallphil
NGI order portal: https://ngisweden.scilifelab.se

**Katie Lennard** <katieviljoen@gmail.com> Wed, Mar 28, 2018 at 10:09 AM
To: Phil Ewels <phil.ewels@scilifelab.se>

Fantastic! Thanks so much Phil, very helpful, will give that a go.

Regards,
Katie.
[Quoted text hidden]

**Katie Lennard** <katieviljoen@gmail.com> Wed, Apr 4, 2018 a
To: Phil Ewels <phil.ewels@scilifelab.se>

Hi Phil,

Getting the data from AWS is proving to be trickier than I thought. I'm not familiar with AWS but have setup an account and used the 'aws configure' command to setup access keys. How still can't access the S3 bucket you specify at https://ewels.github.io/AWS-iGenomes/ If I try use the CLI

```
curl -fsSL https://ewels.github.io/AWS-iGenomes/aws-igenomes.sh | bash
```

I get

```
Error: could not contact S3 bucket. Is AWS authentication set up?
```

I've tried to follow the instructions here https://docs.aws.amazon.com/quicksight/latest/user/troubleshoot-connect-S3.html but don't know how to add an S3 bucket. Am I on the right track

Also tried the sync command

```
aws s3 --region eu-west-1 sync s3://ngi-igenomes/igenomes/Homo_sapiens/Ensembl/GRCh37/
Sequence/WholeGenomeFasta/ ./references/Homo_sapiens/Ensembl/GRCh37/Sequence/
WholeGenomeFasta/
```

but that also didn't work:

```
fatal error: An error occurred (AccessDenied) when calling the ListObjects operation: Access Denied
```

Is there something obviously wrong with my setup or is this perhaps the authentication problems you were referring to previously?

Thanks for your help.

Katie.
[Quoted text hidden]

---

**Phil Ewels** <phil.ewels@scilifelab.se> Wed, Apr 4, 2018 at 6:15 PM
To: Katie Lennard <katieviljoen@gmail.com>

Hi Katie,

Sorry, you're right - I get the same error if I remove my credentials. I think I must have deleted something that I shouldn't have when I was working with this the other day.

Anyway, I *think* that I've now successfully made everything fully public now. I've also added `--no-sign-request` to the commands in the bash script and on the website. With this parameter, the syncing should even work without any aws credentials at all.

Let me know how you get on this time..

Phil
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---

**Katie Lennard** <katieviljoen@gmail.com> Thu, Apr 5, 2018 at 3:06 PM
To: Phil Ewels <phil.ewels@scilifelab.se>

Sorted, it works now, thanks Phil!

Katie.
[Quoted text hidden]

---

**Phil Ewels** <phil.ewels@scilifelab.se> Thu, Apr 5, 2018 at 3:07 PM
To: Katie Lennard <katieviljoen@gmail.com>

Great! Thanks for letting me know.. :)

Phil
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---

**Katie Lennard** <katieviljoen@gmail.com> Fri, Apr 6, 2018 at 11:09 AM
To: Phil Ewels <phil.ewels@scilifelab.se>

Hi Phil,

I'm wondering if I can ask you a couple more questions on how to configure the pipeline to run on our cluster. I'm just a bit uncertain as to which config files need to be changed and whether or not we need to wget the pipeline in addition to the singularity image (as it seems to be looking for the conf/base.config file in the pwd). The test command I have currently is:

```
nextflow run /researchdata/fhgfs/katie/NGI-RNAseq-test/NGI-RNAseq-master -with-singularity /scratch/DB/bio/singularity-containers/ngi-rnaseq.img --reads '/researchdata/fhgfs/katie/NGI-RNAseq-test/*_R{1,2}.fastq.gz' --genome GRCh37 --outdir /researchdata/fhgfs/katie/NGI-RNAseq-test/nextflow-output -profile hex –c /researchdata/fhgfs/katie/NGI-RNAseq-test/nextflow.config
```

The nextflow.config specified, I've edited as follows (I'm new to nextflow so excuse me ;):

```
/*
vim: syntax=groovy
-*- mode: groovy;-*-
 * ------------------------------------------------
 *  NGI-RNAseq Nextflow config file - KL incoporated relevant lines from https://github.com/SciLifeLab/NGI-RNAseq/blob/master/nextflow.config
 * with the rest being from our 16S nextflow.config
 * ------------------------------------------------
 * Default config options for all environments.
 * Cluster-specific config options should be saved
 * in the conf folder and imported under a profile
 * name here.
 */

docker.enabled = false
singularity.enabled = true
singularity.cacheDir = "/scratch/DB/bio/singularity-containers"

process {

    cache = true

    stageInMode='symlink'
    stageOutMode='rsync'

}

executor{
    jobName = { "$task.tag" }
}

params {

    igenomes_base = '/scratch/DB/bio/rna-seq/references'
    clusterOptions = false
    outDir = "/researchdata/fhgfs/katie/NGI-RNAseq-test/nextflow-output"

}

profiles{
    standard {
        process.executor = 'local'
    }
```

```
    hex {
        //The next 3 lines includeConfig from the NGI-RNAseq nextflow.config not sure if they need to be changed
        includeConfig 'conf/base.config'
        includeConfig 'conf/igenomes.config'
        //The remaining lines are from Gerrit's nextflow.config for 16S pipeline
        process.executor = 'pbs'
        process.queue = 'UCTlong'
        process.clusterOptions = '-M katie.viljoen@uct.ac.za -m abe -l nodes=1:ppn=1:series600'
    }
}

// KL: the remainder of the code was copy pasted from https://github.com/SciLifeLab/NGI-RNAseq/blob/master/nextflow.config
// Capture exit codes from upstream processes when piping
process.shell = ['/bin/bash', '-euo', 'pipefail']

timeline {
  enabled = true
  file = "${params.outdir}/pipeline_info/NGI-RNAseq_timeline.html"
}
report {
  enabled = true
  file = "${params.outdir}/pipeline_info/NGI-RNAseq_report.html"
}
trace {
  enabled = true
  file = "${params.outdir}/pipeline_info/NGI-RNAseq_trace.txt"
}

manifest {
  homePage = 'https://github.com/SciLifeLab/NGI-RNAseq'
  description = 'Nextflow RNA-Seq Best Practice analysis pipeline, used at the SciLifeLab National Genomics
Infrastructure.'
}

// Function to ensure that resource requirements don't go beyond
// a maximum limit
def check_max(obj, type) {
  if(type == 'memory'){
    try {
      if(obj.compareTo(params.max_memory as nextflow.util.MemoryUnit) == 1)
        return params.max_memory as nextflow.util.MemoryUnit
      else
        return obj
    } catch (all) {
      println "   ### ERROR ###   Max memory '${params.max_memory}' is not valid! Using default value: $obj"
      return obj
    }
  } else if(type == 'time'){
    try {
      if(obj.compareTo(params.max_time as nextflow.util.Duration) == 1)
        return params.max_time as nextflow.util.Duration
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        return obj
    } catch (all) {
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  } else if(type == 'cpus'){
    try {
      return Math.min( obj, params.max_cpus as int )
    } catch (all) {
      println "   ### ERROR ###   Max cpus '${params.max_cpus}' is not valid! Using default value: $obj"
      return obj
    }
  }
}
```

So at the moment the -hex profile is not recognised (error is 'unknown profile hex') Should I rather not specify -profile hex and just include the relevant process specifications (executor, queue, clusterOptions) in the nextflow.config file?

Regards,
Katie.

[Quoted text hidden]

---

**Phil Ewels** <phil.ewels@scilifelab.se>
To: Katie Lennard <katieviljoen@gmail.com>

Hi Katie,

No problem - a few observations and questions..

1) Typically, I'd recommend not altering the pipeline code. By using the exact code and container that's in a release on the GitHub URL, it makes your results more reproducible and reus

If you need to have a custom configuration you can supply this in a separate file on the command line using -c (this is what we do in our production environment). You can combine this v only specify the stuff that you want to change.
You're also welcome to contribute a config profile to the public GitHub version of the pipeline so that it ends up in a release.

The downside of just editing the pipeline files as you have here is that you can't easily update the pipeline when new releases come out, as they will overwrite your changes.

2) Related to the above - are you running on a system without an internet connection? If you have an internet connection, it is probably easier and cleaner to just run "nextflow run SciLife cache the pipeline files.

3) A lot of the stuff in the config file is a bit confused and sometimes not needed. I'm not sure if you're allowed to embed config statements inside the profile {} scope for example. Oh crik -c option as well. This isn't needed, as a file called nextflow.config alongside the main nextflow pipeline is automatically loaded. So you're double loading the config here, which may do s

I've attached your nextflow.config file here with a few comments prefixed with // PAE - to try to explain stuff that I think is not needed or could be improved.

But - better still is to add your config profile to the main pipeline. I've just done this in a pull request here: https://github.com/nf-core/RNAseq/pull/3

Note that we are moving the pipeline from SciLifeLab/NGI-RNAseq to nf-core/RNAseq (see https://nf-core.github.io/), hence the name and some stuff in the pipeline there will look differe

If you're happy, we can merge that change and you can pull that latest version of the pipeline code and run with:

nextflow run nf-core/RNAseq -r dev -profile uct_hex --outdir ... --email ... --reads ... -with-singularity ...

Note that if the singularity container is always in the same place for all users, we can add that to the config file and not have any need for the -with-singularity command line bit.

Phil

On 6 Apr 2018, at 11:09, Katie Lennard <katieviljoen@gmail.com> wrote:

Hi Phil,

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in addition to the singularity image (as it seems to be looking for the conf/base.config file in the pwd). The test command I have currently is:

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Regards,
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> >>> On 4 Apr 2018, at 10:57, Katie Lennard <katieviljoen@gmail.com> wrote:
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> >>> ```
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> >>> ```
> >>>
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> >>> Error: could not contact S3 bucket. Is AWS authentication set up?
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> >>> Also tried the sync command
> >>>
> >>> ```
> >>> aws s3 --region eu-west-1 sync s3://ngi-igenomes/igenomes/Homo_sapiens/Ensembl/GRCh37/Seque
> >>> nce/WholeGenomeFasta/ ./references/Homo_sapiens/Ensembl/GRCh37/Sequence/WholeGenomeFasta/
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> >>>
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> >>> ```
> >>> fatal error: An error occurred (AccessDenied) when calling the ListObjects operation: Access Denied
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Is there something obviously wrong with my setup or is this perhaps the authentication problems you were referring to previously?

Thanks for your help.

Katie.

On Wed, Mar 28, 2018 at 10:09 AM, Katie Lennard <katieviljoen@gmail.com> wrote:

> Fantastic! Thanks so much Phil, very helpful, will give that a go.
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> > > but it does not contain all the files you specify in your igenomes.config i.e. I found `Homo_sapiens/Ensembl/GRCh37/Sequence/WholeGenomeFasta/gen`
> > > `Homo_sapiens/Ensembl/GRCh37/Annotation/Genes/genes.bed`
> > > or `/Homo_sapiens/Ensembl/GRCh37/Annotation/Genes/genes.gtf or /Homo_sapiens/Ensembl/GRCh37/Sequence/STARIndex/`
> > >
> > > Can you advise me on how to set this up correctly?
> > >
> > > Regards,
> > > --
> > >
> > > Katie
> > >
> > > Bioinformatician
> > > Division of Computational Biology
> > > Institute of Infectious Diseases & Molecular Medicine
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> >
> > --
> > Phil Ewels, Ph.D.
> > Head of Facility, NGI Applications Development
> > National Genomics Infrastructure (NGI), SciLifeLab Sweden
> > phil.ewels@scilifelab.se - Twitter: @tallphil
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**uct_config_commented**
5K

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**Katie Lennard** <katieviljoen@gmail.com> Fri, Apr 6, 2018 at 12:33 PM
To: Phil Ewels <phil.ewels@scilifelab.se>

Hi Phil,

Thanks for being so helpful!

1) Yes, absolutely, agreed
2) We do have an internet connection - I initially ran with 'nextflow run SciLifeLab/NGI-RNAseq ..' but then the conf/base.config file was not found (probably because I messed up the config files)
3) I have a lot to learn with Nextflow yes, thanks for the comments on the config file, it helps!
'I've just done this in a pull request here: https://github.com/nf-core/RNAseq/pull/3 ' - Fantastic, thanks so much! So if we need to change anything to the uct_hex profile we can fork on GitHub, edit and do pull request?

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**Phil Ewels** <phil.ewels@scilifelab.se> Fri, Apr 6, 2018 at 12:35 PM
To: Katie Lennard <katieviljoen@gmail.com>

3) Yes, exactly. I'll merge the PR in that case - but note that this is only on the dev branch (not the default master) and that this isn't really production ready code just yet.

If you're interested in these pipelines and the new nf-core project, you can come and join our chat channel here: https://gitter.im/nf-core/Lobby

Phil
[Quoted text hidden]