Tools for reproducible research

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Devon Ryan, Björn Grüning, and Johannes Köster

Agenda

- Introduction to conda ←
    - Packages & channels
    - Environments
    - Writing recipes
- Introduction to Snakemake
    - Workflow definition
    - Workflow execution
    - Live demo

A “typical” irreproducible analysis

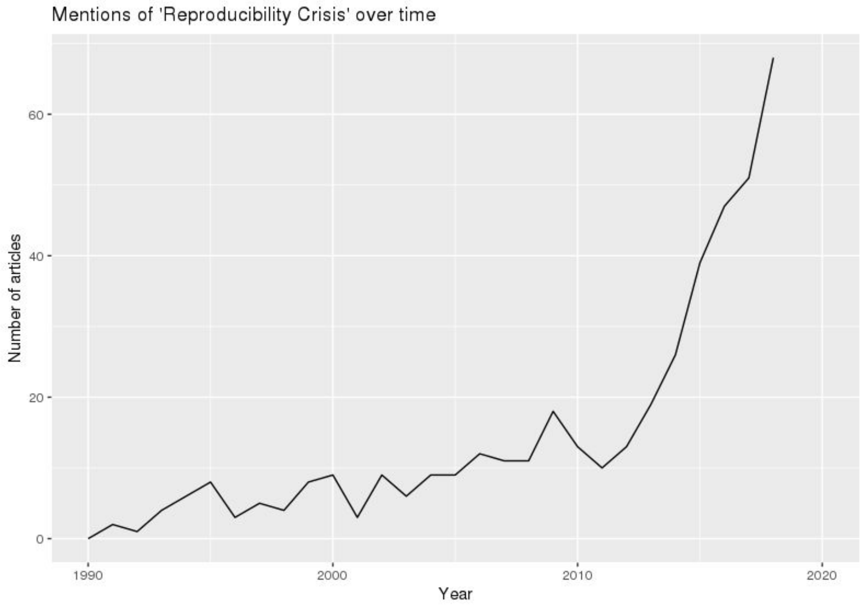
```
$ tree
 .
├── analysis.2.sh
├── analysis.B.sh
├── analysis.sh
├── final_results.really_final.txt
├── final_results.txt
├── final_results.version2.txt
├── results
│       └── alignments.bam
├── results2
│       └── alignments.bam
├── results2b
│       └── alignments.bam
└── results3
        └── alignments.bam
```

```
...
for sample in `cut -f 1 sample_sheet.txt | sed
"s#_1#_R1#" | uniq` ; do
    bowtie2 -x $GENOME \
                 -U $sample \
                 -p 10 \
                 $BT2PARAMS \
        | samtools view -o tmp/$sample.bam
done
...
```

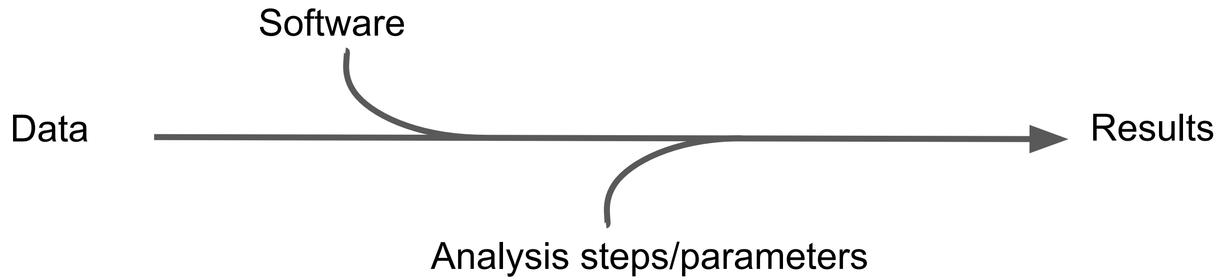
- Which result is the “correct” one?
- How was it produced?
- What bowtie 2 version was used?
- What were the parameters?
- What does the analysis flow look like?

Reproducible research?

- The idea that all code and software needed to produce a given set of results are available and usable by others.
- "Crisis of reproducibility"



What’s needed for “reproducible research”?



Component	Requirement
Software	- List of all required packages/versions - Recreate environments on demand
Analysis	- Tools for each step - Parameters in each step - Documentation of order, input, and output

Why is this hard?

- Packages are hard to install
  - Dependency hell
  - Need root?
- Multiple versions
- Incompatible packages
- How do we recreate an analysis environment elsewhere?
- Automatic cloud/cluster deployment?
- Non-hack workflows
- Workflow portability?
- Workflow management of packages?
- Transparent scaling to cluster/cloud

How do Conda & Snakemake fit in?

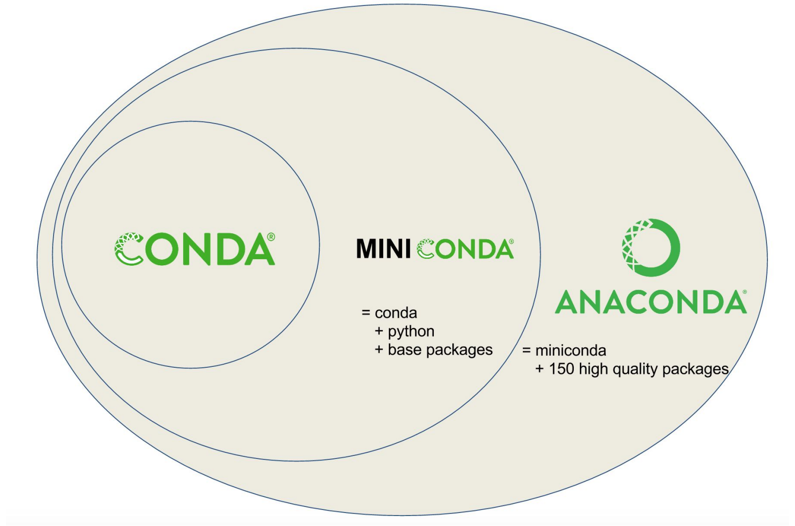
Component	Requirement
Software	- List of all required packages - Recreate environments on demand - Multiple versions/environments simultaneously
Analysis	- Tools for each step - Parameters in each step - Documentation of order, input, and output - Archive environments - Direct interaction with conda!

CONDA

Snakemake
a Python workflow manager

What exactly is Conda?

- A package and environment manager
  - Like apt/yum, but MUCH better
  - Environments are isolated from each other
- User-contributed package recipes
  - Different “channels”, can create your own
  - Updated constantly
- Prebuilt binaries
  - Linked to libraries in the same environment



Conda packages

- Specific versions
- Various sources (“channels”)
- Defined requirements
  - Usually from the same or predefined other channels

```
.
├── info
│   └── recipe
│       ├── conda_build_config.yaml
│       ├── meta.yaml
│       └── meta.yaml.template
├── python-scripts
└── site-packages
    ├── deeptools
    └── deepTools-3.3.0.dist-info
        ├── LICENSE.txt
        └── zip-safe
```

Conda channels

Channel 1

package-1.2.3
package-1.2.2
package-1.2.1
package-1.2.0
package-1.0.0alpha2
package-1.0.0alpha1

Channel 2

dependency-1.1.2
package-1.2.3
package-1.2.2
package-1.2.1
package-1.2.0
salmon-0.14.0
samtools-1.9
snakemake-5.5.2

Channel 3

gcc-4.7
libcurl-7.6.41
zlib-1.2.11
numpy-1.16.4
scipy-1.3.0

Conda channels

- Conda-forge: Most dependencies (numpy, scipy, zlib, CRAN packages, etc.)
- Bioconda: Most bioinfo packages (salmon, STAR, samtools, DESeq2, etc.)
- Defaults: Packages built by Anaconda Inc.

```
$ conda config --show channels
channels:
  - conda-forge
  - bioconda
  - defaults

$ conda config --add channels bioconda
```

- Order matters, use this one!

Finding packages

- Search on http://anaconda.org
- Use `conda search`

```
$ conda search deepTools
Loading channels: done
# Name                         Version            Build  Channel
deeptools                        3.1.3  py36h14c3975_1  bioconda
deeptools                        3.1.3  py36h470a237_0  bioconda
deeptools                        3.1.3  py37h14c3975_1  bioconda
deeptools                        3.2.0            py_0  bioconda
deeptools                        3.2.1            py_0  bioconda
deeptools                        3.3.0            py_0  bioconda
```

- Packages have versions, build numbers and build hashes
  - Build hashes include dependency information

Practical 1

What are the most recent versions of STAR and Salmon?

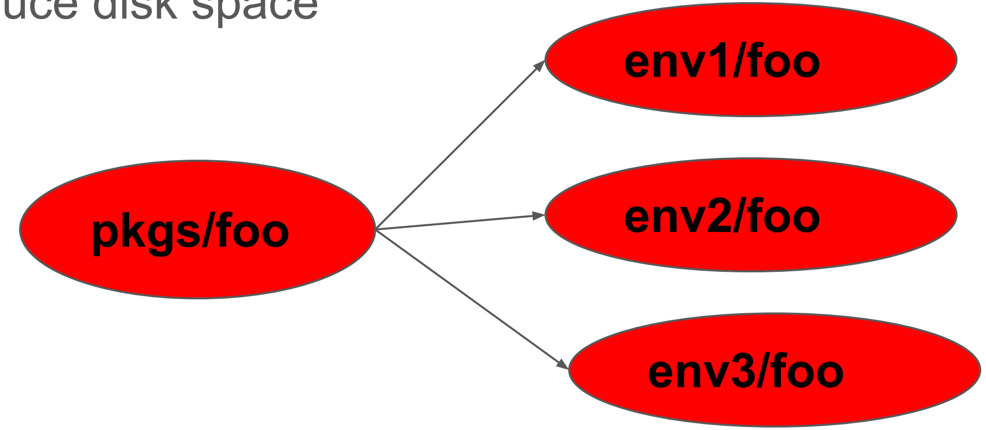
```
$ conda search STAR | tail -n 1
star                 2.7.1a                  0  bioconda

$ conda search salmon | tail -n 1
salmon               0.14.1       h86b0361_1  bioconda
```

Conda environments

- A (mostly) self-contained directory with a set of compatible packages
- Often use links to reduce disk space

```
.
├── bin
├── condabin
├── envs
│   ├── build
│   ├── deepTools
│   ├── foo
│   ├── salmon
│   └── twine
├── etc
├── include
├── lib
├── libexec
├── pkgs
└── share
```



```
$ ls -i envs/*/bin/salmon pkgs/*/bin/salmon
20844181 envs/foo/bin/salmon
20844181 envs/salmon/bin/salmon
20844181 pkgs/salmon-0.14.1-h86b0361_1/bin/salmon
```

Conda environments

- Linking is relative to packages!
- No more conflicting dependencies between versions!

```
~/m3/envs/t/lib/python3.7/site-packages$ ldd -r pyBigWig.*.so
    linux-vdso.so.1
    libm.so.6 => /lib/x86_64-linux-gnu/libm.so.6
    libz.so.1 => ~/m3/envs/t/lib/python3.7/site-packages/./../../libz.so.1
    libcurl.so.4 => ~/m3/envs/t/lib/python3.7/site-packages/./../../libcurl.so.4
```

Conda environments - common commands

- conda env list
  - Lists available environments

```
$ conda env list
# conda environments:
#
                         ~/Task160/.snakemake/conda/4c4d318f
base                  *  /home/dpryan/miniconda3
bioconda                 /home/dpryan/miniconda3/envs/bioconda
build                    /home/dpryan/miniconda3/envs/build
deepTools                /home/dpryan/miniconda3/envs/deepTools
foo                      /home/dpryan/miniconda3/envs/foo
salmon                   /home/dpryan/miniconda3/envs/salmon
twine                    /home/dpryan/miniconda3/envs/twine
```

- You start in “base”, a * indicates an active env

Conda environments - common commands

- conda create/conda env remove
  - Create/remove environments

```
$ conda create -n myenv python=3.7 numpy deepTools>=3.3.0
$ conda env remove -n myenv
```

- Packages can have versions specified
- Min/max versions can be specified

Tip: Specifying versions makes env creation faster!

Conda environments - common commands

- conda activate/deactivate
    - Activates/deactivates an environment

```
$ which deeptools
$ conda activate myenv
$ which deeptools
/home/dpryan/miniconda3/envs/myenv/bin/deeptools
$ conda deactivate
$ which deeptools
```

- You can “stack” environments with `--stack`

Conda environments - common commands

- conda install/uninstall
- conda list

```
$ conda activate myenv
$ conda install snakemake
… a lot of status output …
$ conda list
… many packages …
$ conda uninstall snakemake
```

Tip: Keep your “base” env clean, it will prevent headaches!

Practical 2

Create a new environment named “fondue” with hisat2, samtools and deepTools. What version of numpy got installed in it?

```
$ conda create -n fondue hisat2 samtools deepTools
$ conda activate fondue  # Yum!
$ conda list | grep numpy
numpy                 1.16.4          py27h95a1406_0    conda-forge
```

Conda environments - common commands

- conda env export/create
  - Exports an env to or creates an env from a YAML file

```
$ conda activate fondue
$ conda env export > environment.yaml
$ conda env create -f environment.yaml -n moreFondue
$ head environment.yaml
name: "fondue"
channels:
  - conda-forge
  - bioconda
  - defaults
dependencies:
  - asn1crypto=0.24.0=py27_1003
  - attrs=19.1.0=py_0
  - backports=1.0=py_2
  - backports.functools_lru_cache=1.5=py_1
  - backports_abc=0.5=py_1
```

Common pitfalls

- Wrong channel order
- Installing packages in your base env
- Manually manipulating $PYTHONPATH
- Avoid manually installed packages

Conda package recipes

```
# Bioconda example
$ tree recipes/methydackel
recipes/methyldackel/
├── build.sh
└── meta.yaml
```

- meta.yaml is required
- optional:
  - build.sh
  - (small) test files
  - license

meta.yaml sections

- package: name and version
- source: url and sha256/md5
- build: build number, platforms to skip, “noarch” information
- requirements: packages for building, linking, running
- test: commands/imports
- about: Webpage, license, summary of what it does
- extras: Comments, maintainers, etc.

meta.yaml - package

```
package:
  name: methyldackel
  version: "0.4.0"
```

```
Can use jinja variables:

{% set name = “MethylDackel” %}
{% set version = “0.4.0” %}

Package:
  Name: {{ name| lower }}
  Version: {{ version }}
```

meta.yaml - package

```
package:
  name: methyldackel
  version: 0.4.0
```

Can also use jinja2 variables:

```
{% set name = "MethylDackel" %}
{% set version = "0.4.0" %}

package:
  name: {{ name|lower }}
  version: {{ version}}
```

meta.yaml - source

```
{% set name = "MethylDackel" %}
{% set version = "0.4.0" %}

package:
  name: {{ name|lower }}
  version: {{ version }}

source:
  url: https://github.com/dpryan79/{{ name }}/archive/ {{ version }}.tar.gz
  sha256: eea3fa5167609ca5a293a2be7c4ad29566aad84a99e7d14d2991685071cfed2e
```

- Avoid `git_url` or `svn_url`
- Ony actual releases, no alpha/beta!

meta.yaml - build

```
build:
  number: 0
```

- Reset to 0 with new releases
- Increment with each change
- Can skip conditions:

```
build:
  number: 0
  skip: True  # [osx or py != 37]
```

meta.yaml - build

- "noarch" is useful, but confusing

```
build:
  noarch: generic
  number: 0
```

- generic: No platform-specific code (java, pure perl, pure R, etc.)

```
build:
  noarch: python
  number: 0
```

- python: Pure python packages, one build -> all versions

meta.yaml - build

- You can include the entire build command

```
build:
  number: 0
  noarch: python
  script: "{{ PYTHON }} -m pip install . --no-deps --ignore-installed -vvv"
```

- Alternatively, use `build.sh`

```
$ cat build.sh
#!/bin/bash
$PYTHON -m pip install . --no-deps --ignore-installed -vvv
```

- build.sh is useful for "more involved" installs
- There are many environment variables: https://bit.ly/2KUiDpd

meta.yaml - requirements

```
requirements:
  build:
    - {{ compiler('c') }}
  host:
    - htslib
    - zlib
  run:
    - htslib
    - zlib
```

- build: Compilers, preprocessors, etc.
- host: Anything linked against
- run: All other dependencies
- Use compiler functions (`{{ compiler('cxx') }}` and such)

A note on "pinnings"

- Packages need to be compatible
  - Same compiler per-platform
  - Same htslib/numpy range/zlib/libcurl/etc. versions
  - Above are "pinned"

```
$ conda search methyldackel | tail -n 1
methyldackel                   0.4.0      hc0aa232_0  bioconda
```

- Wondered about the hash? It's the pinnings' sha256.
- Bioconda uses conda-forge-pinning (https://bit.ly/2Jdz4JT)
- Version ranges are great!

```
    - python
    - pybigwig >=0.2.3
    - numpy >=1.9.0
    - scipy >=0.17.0
    - matplotlib >=2.1.1
```

meta.yaml - test

```
test:
  imports:
    - deeptools
  commands:
    - bamCompare --version
```

- Keep it simple/quick but functional
- No large test files
- "imports" works for python/perl

meta.yaml - about/extra

```
about:
  home: https://github.com/awesome/awesomeTools
  license: GPL3
  license_file: LICENSE
  summary: Awesome tools

extra:
  identifiers:
    - doi:10.1093/nar/gkw257
  recipe-maintainer:
    - your github handle
```

- Do try to package the license!

Don't fear skeletons

- Making recipes manually takes time
- Many common sources are automated

```
$ mkdir foo
$ cd foo
$ conda skeleton pypi deeptools
```

- Skeletons for: pypi, cpan, CRAN
  - For CRAN: https://github.com/bgruening/conda_r_skeleton_helper
  - CRAN packages belong on conda-forge if possible!
- We (bioconda) already make ALL bioconductor package
- On bioconda, recipes are in `recipes/`

Practical 3

Make a new cutadapt recipe

```
$ mkdir foo
$ cd foo
$ conda skeleton pypi cutadapt
$ vi cutadapt/meta.yaml  # edit noarch, host requirements, license_file
```

Common problems

- Compiling C/C++ packages are hard
- The compiler is NOT called "gcc"
- Installed into $PREFIX
- Dependencies are in $PREFIX/lib

```
$ cat build.sh
#!/bin/bash
make install CC=$CC \
             CXX=$CXX \
             CFLAGS="$CFLAGS" \
             CXXFLAGS="$CXXFLAGS" \
             prefix=$PREFIX
```