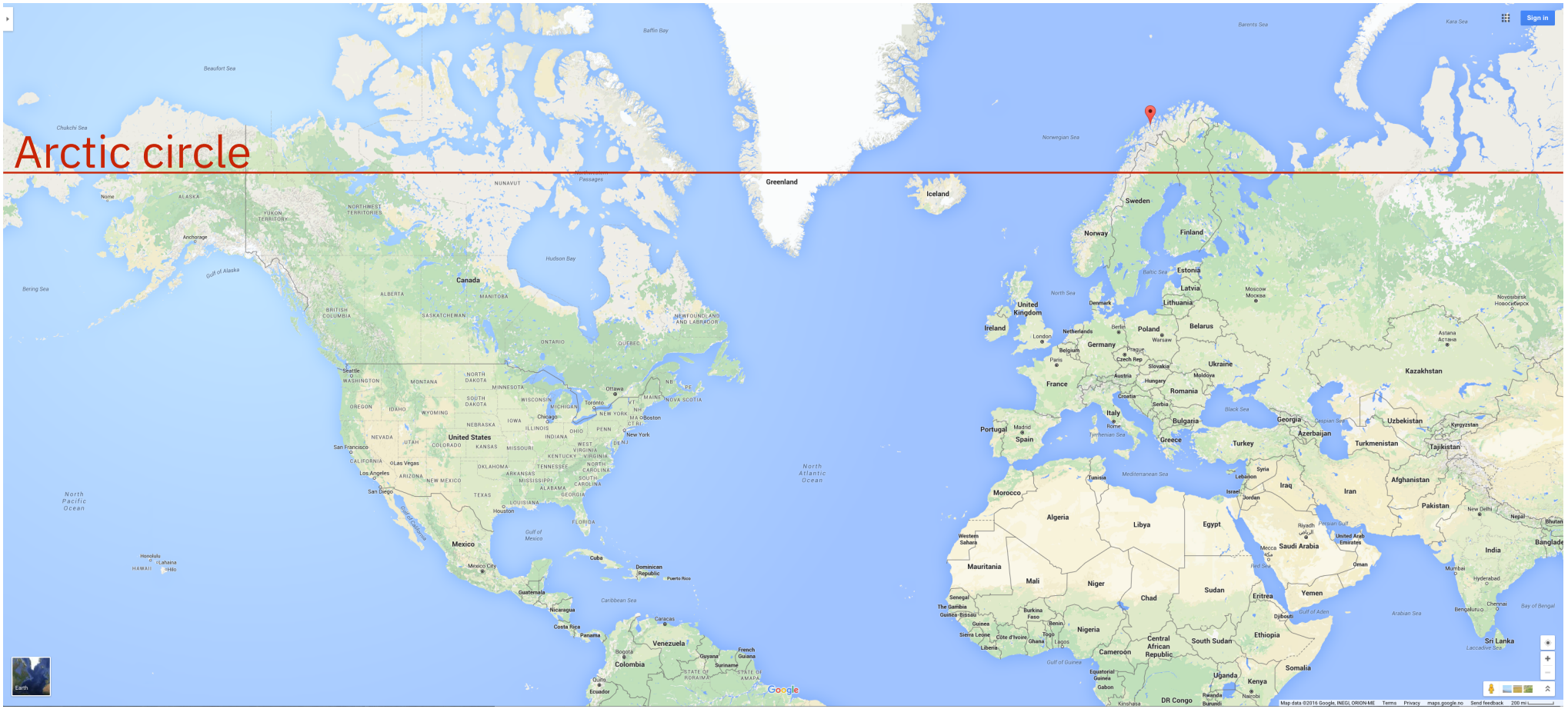
Slides at **fjukstad.github.io**
Code at **bdps.cs.uit.no**

Reproducible Data Analysis Pipelines for Precision Medicine

Bjørn Fjukstad, PhD
Department of Computer Science, UiT The Arctic University of Norway
DIPS AS

Tromsø


Arctic circle
Greenland
Iceland
Sweden
Norway
Finland
Canada
United States
Mexico
Russia
Kazakhstan
China
India
Sign in


Tromsø, 9.2.2019

Motivation

Cornerstone of Science

- The principal goal of scientific publications is to teach new concepts, show the resulting implications of those concepts in an illustration, and provide enough detail to make the work reproducible.

Great Potential

- High-throughput technologies and simpler access to datasets have revolutionized biology
- Datasets can reveal genetic basis of disease in patients, but require a collaborative effort
- Constantly ensure the quality of the data and analyses behind any interpretation
- Inaccurate results from improperly developed analyses can lead to negative consequences for patient care

Precision Medicine

- Precision medicine uses patient-specific molecular information to diagnose and categorize disease to tailor treatment to improve health outcome.
- High-throughput sequencing is currently the main technology to facilitate personalized diagnosis and treatment
- Downstream computational analysis and interpretation is the major cost. $1000 genome, $1,000,000 analysis

Genomic Analysis

- Analysis pipelines consist of many steps that transform raw data into interpretable results
- It is necessary to carefully explore different tools and parameters to answer a dedicated question
- Improperly developed analysis pipelines may generate inaccurate results, which may have negative consequences for patient care

Definitions

Different definitions

- ***Replicate***: carry out the same task as the original researcher, and get the same results
  - Same code, same compiler, same hardware and same operating system (OS), gives the same result.
- ***Reproduce*****:** carry out tasks that are similar to the original and get the same result
  - Same data, similar code, similar compiler, similar hardware, similar OS, gives the same result.

Different definitions

- **Preproducibility:** An experiment is preproducible if it has been described in adequate detail for others to undertake it.

Different definitions

- ***Verify***: The task of replicating an experiment to see if it yields the same results

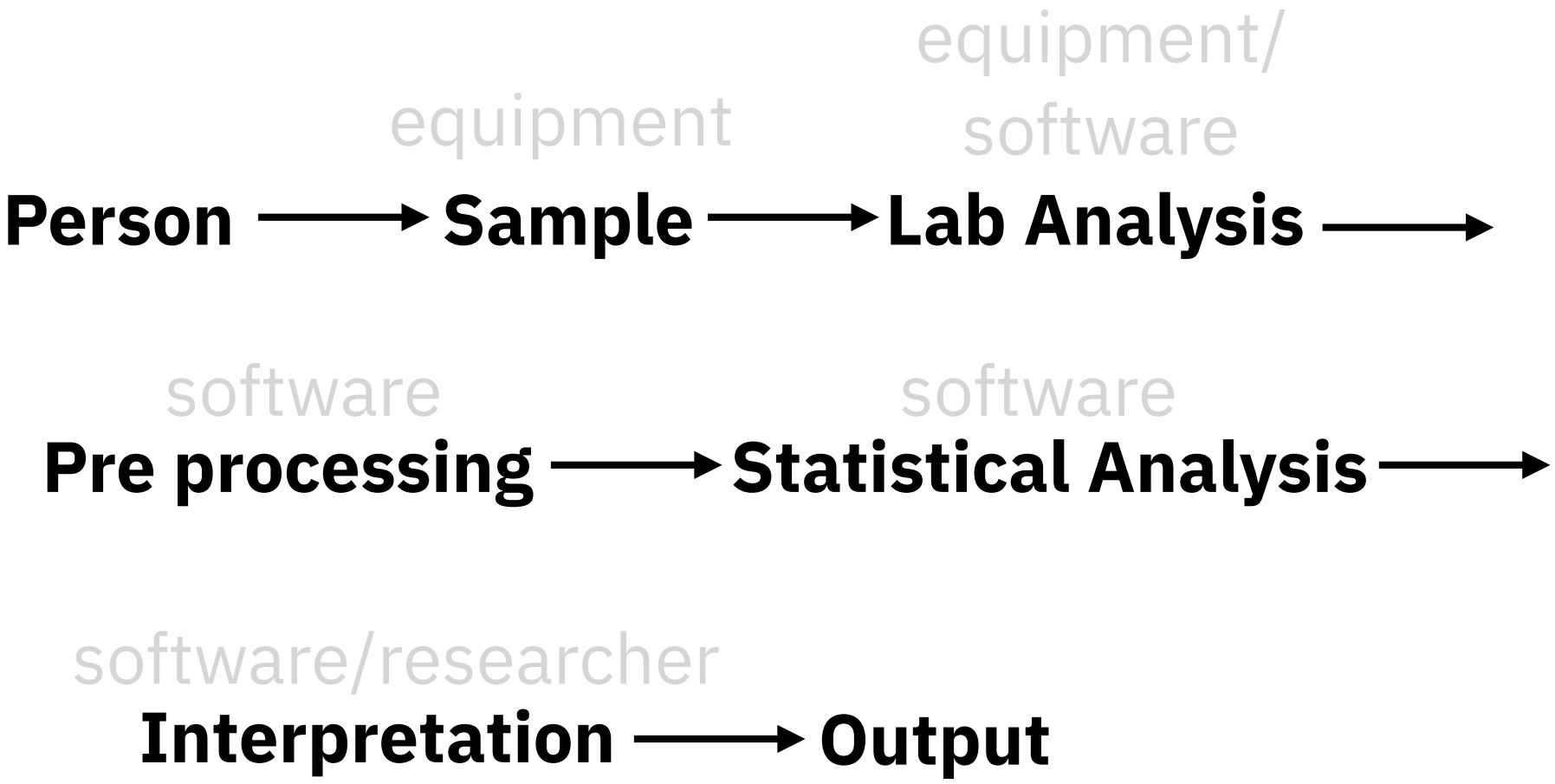
- ***Validate****: the task of evaluating a result to see if the researcher's conclusions are warranted.*

Consequences

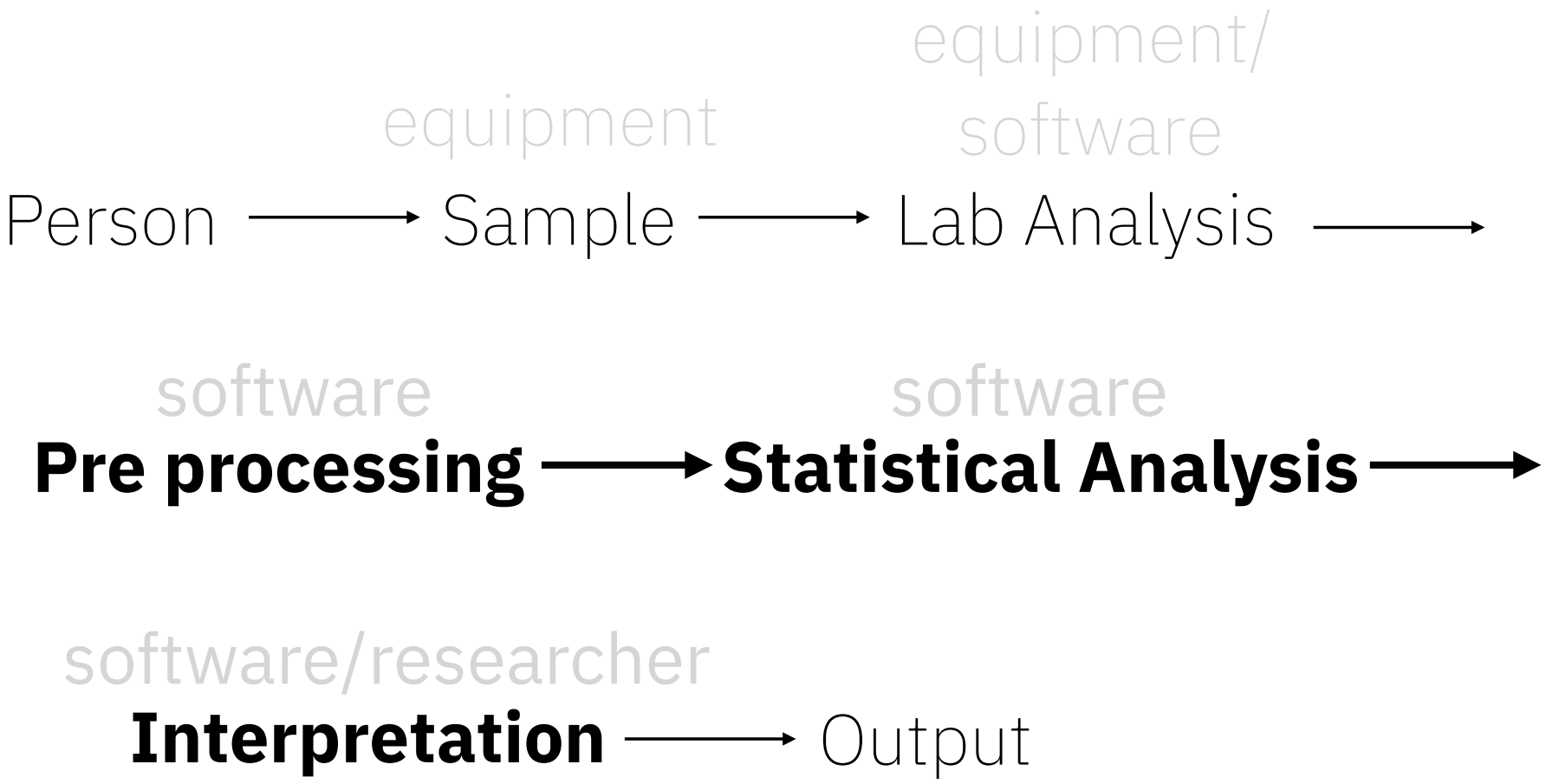
- Science moves forward when researchers verify other's results. Science advances faster when people waste less time pursuing false leads
- The society and companies can potentially waste hundreds of millions on failed drug development programs
- If science is not useful, why should we fund or support it? Why should people donate blood to a study that can't be reproduced?

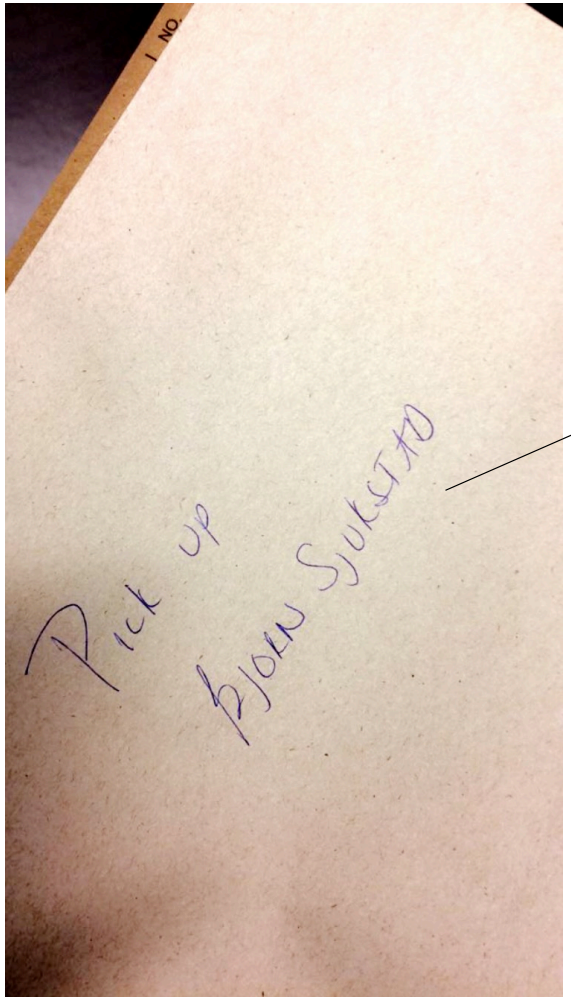
Where things (can) go wrong

The Trajectory of a Biomedical Research Project



The Trajectory of a biomedical research project





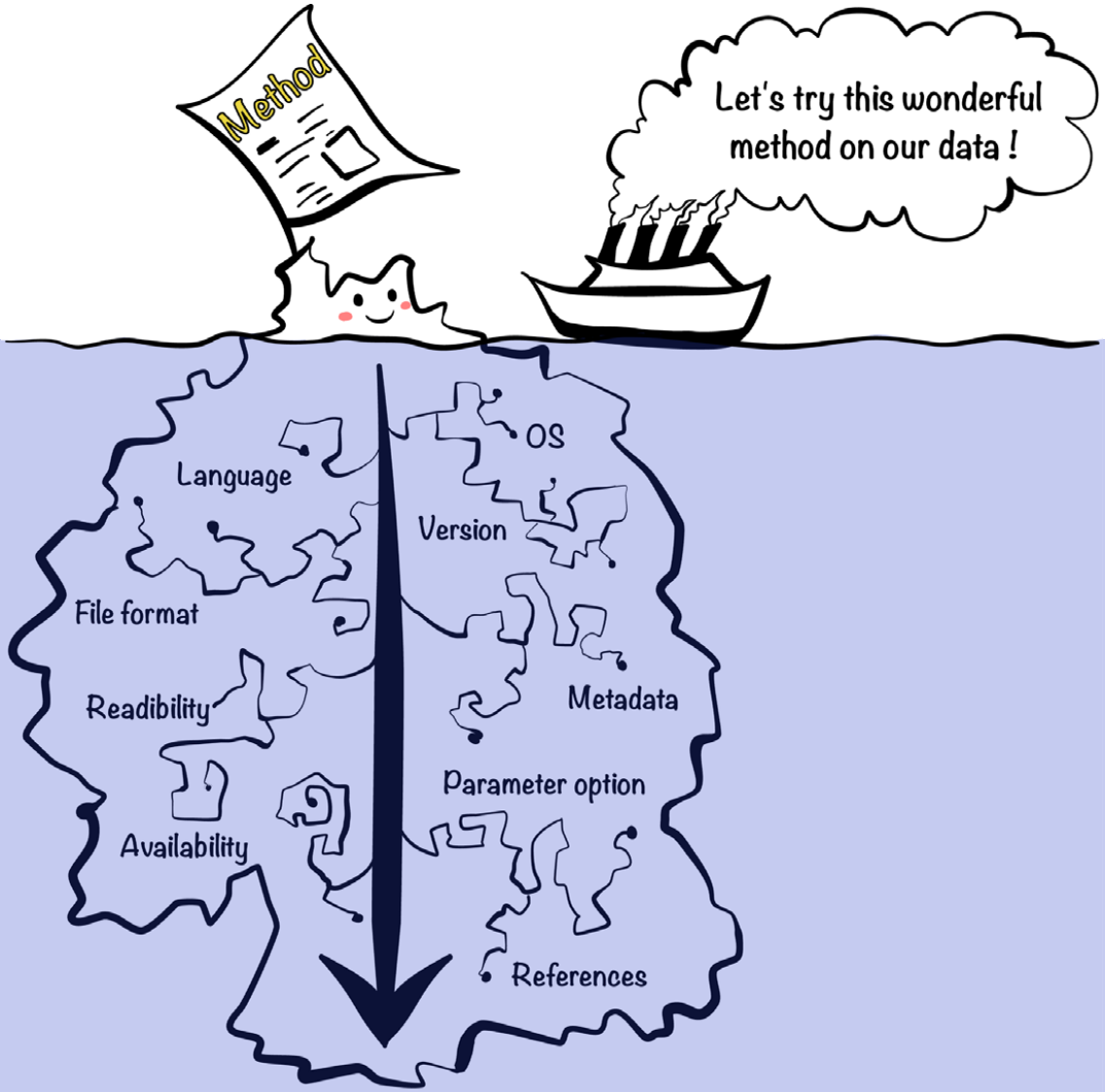
Bjørn ~~Fjukstad~~

Sjukstad ≈ Sicktown

14 October 2016, pick up of biological sample at McGill University Health Centre

The Problem


Method
Let's try this wonderful method on our data !


Method
Let's try this wonderful method on our data !
Language
OS
Version
File format
Readibility
Metadata
Parameter option
Availability
References

2016 Nature Survey

- Nature asked **1,576** researchers to respond to a brief online questionnaire on reproducibility in research
- **70%** responded that they have tried and failed to reproduce another scientist’s experiment.
- More than **50%** have failed to reproduce their own experiments
- **52%** said it's a significant reproducibility crisis, **38%** said it's a slight crisis

Cancer Research

- Biotechnology company Amgen tried to reproduce 53 “landmark” papers in cancer research : could only reproduce 11% of the findings
- Bayer HealthCare surveyed 67 in-house projects, but could only reproduce 21% of the projects

"Reproducing" Computer Science Papers

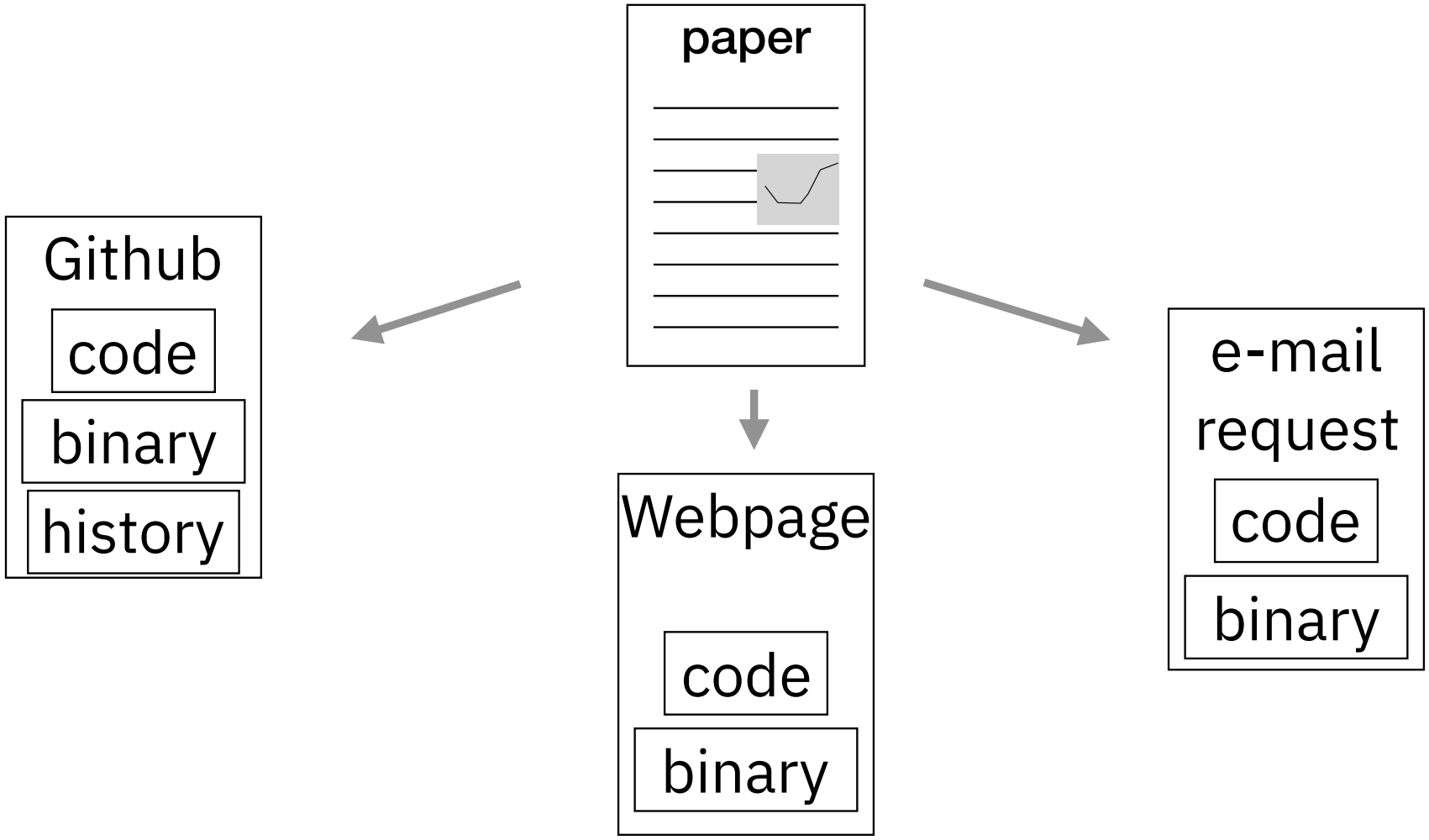
- Study from University of Arizona downloaded **601** papers from eight ACM conferences (including SOSP, SIGMOD, OSDI) and five journals.
- Investigated if the code was available, and if they could build it
  - Excluded **199** papers
  - Did not find, or receive, code for **176** papers.
  - **226** papers backed by code: **130** built within 30 minutes; **64** after more than 30 minutes; **23** not built, but authors claim it will; **9** cannot be built by team or original authors

BONUS: Why researchers can’t/won’t share code

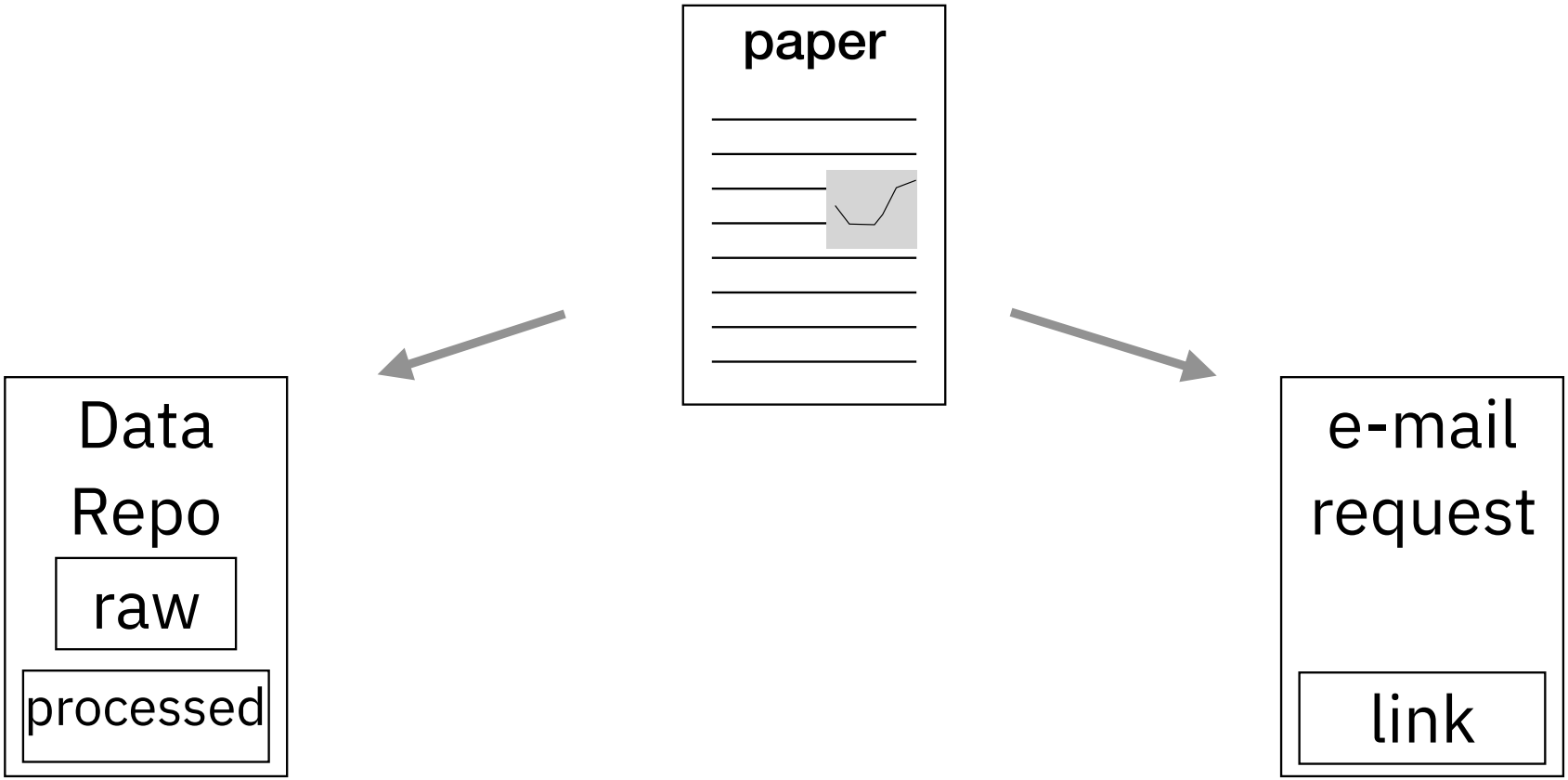
- *Versioning: Don’t know if this version is the final one*
- *Available soon*
- *We don’t intend to make the code available, ever*
- *The programmer left*
- *The system physically ceased to exist, got stolen or crashed*
- *The code is commercial or proprietary, and won’t be open-sourced or given a permissive license*
- *Too busy to help out*

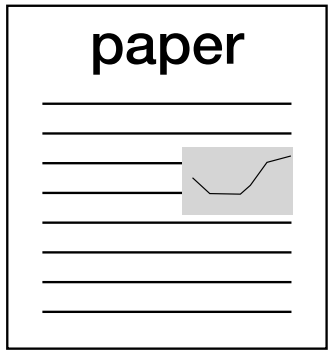
The technical and practical difficulties of reproducing results

Simply getting the required program



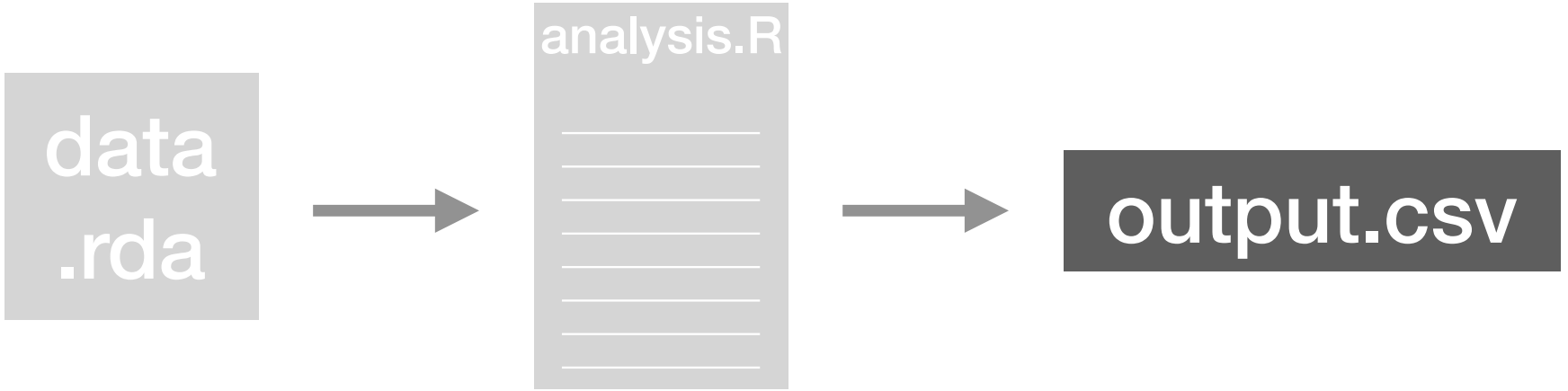
Get a hold of the data





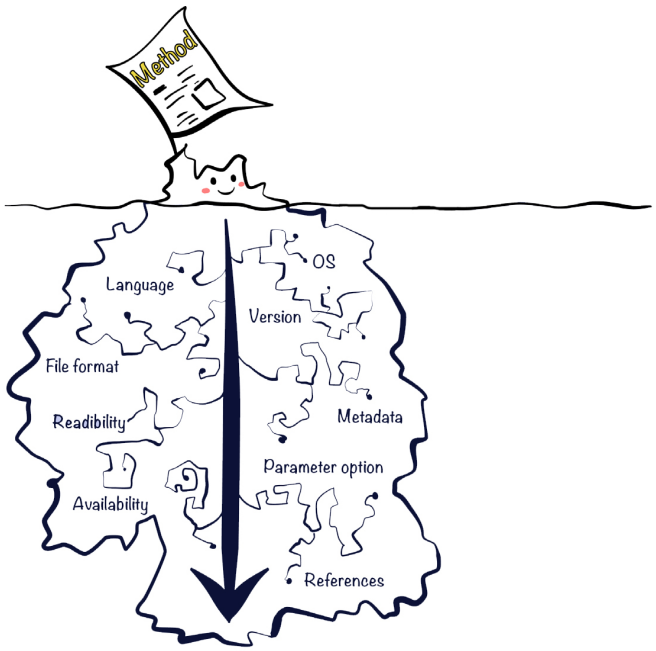
Example

```
> Rscript analysis.R --method="a" --input=data.rda --output=output.csv
```



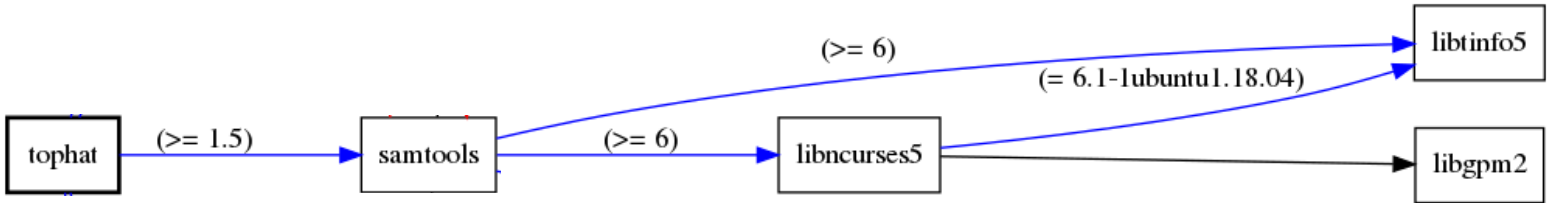
Difficulties all the way down

```
> Rscript analysis.R --method="a" --input=data.rda --output=output.csv
```

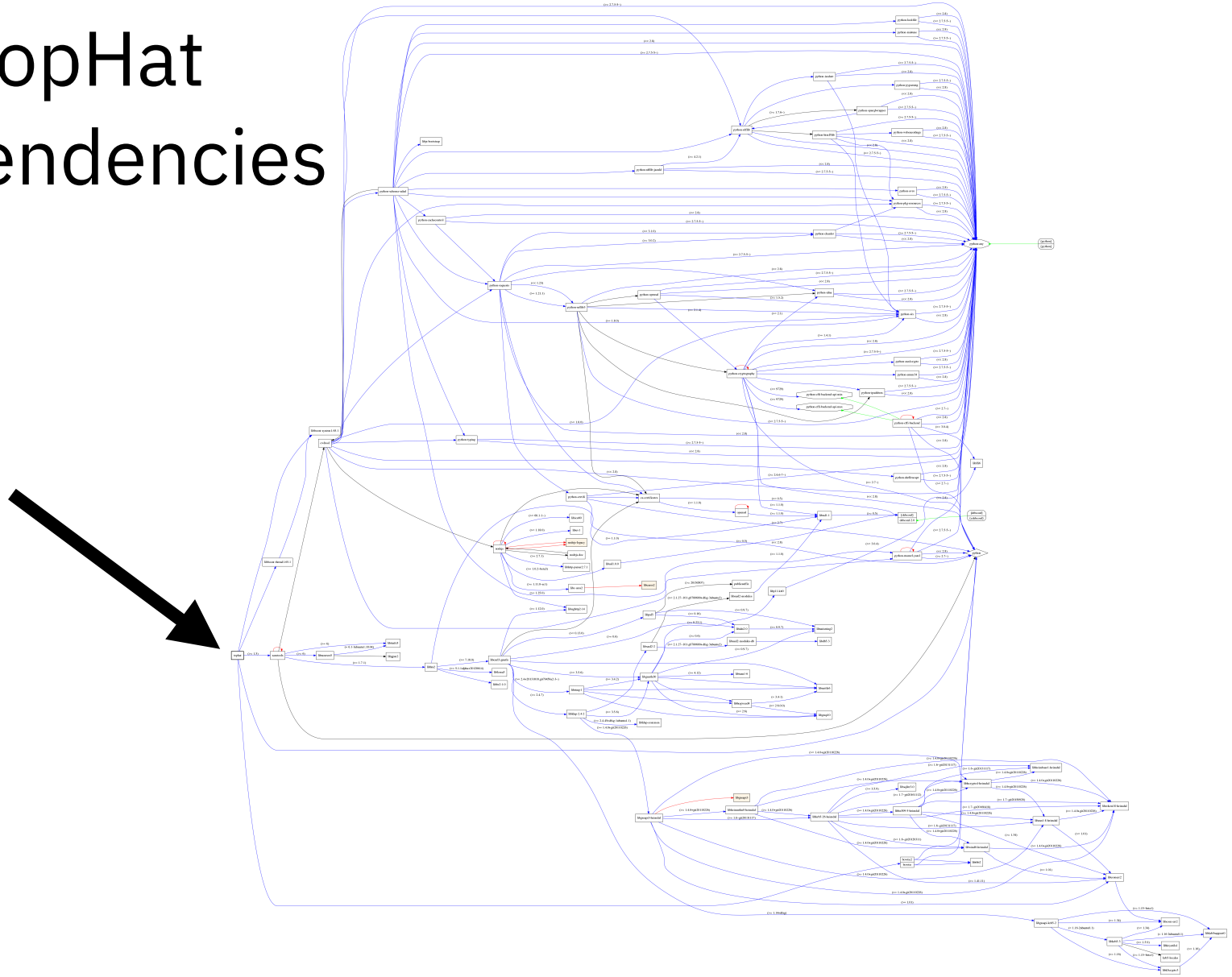


Command, data, and parameters	`a, data.rda, output.csv`
Software	`R/Rscript 3.40, plotly 3.4.1, ...`
Operating System	`OS X High Sierra 10.13.6`
Kernel	`Darwin Kernel Version 17.7.0 xnu-4570.71.2~1/RELEASE_X86_64 x86_64`
Hardware	`Macbook Pro (Retina, 13-inch, Mid 2014), 3 GHz Intel Core i7, 16 GB RAM, 250 GB SSD`

TopHat Dependencies

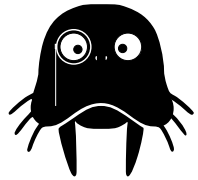


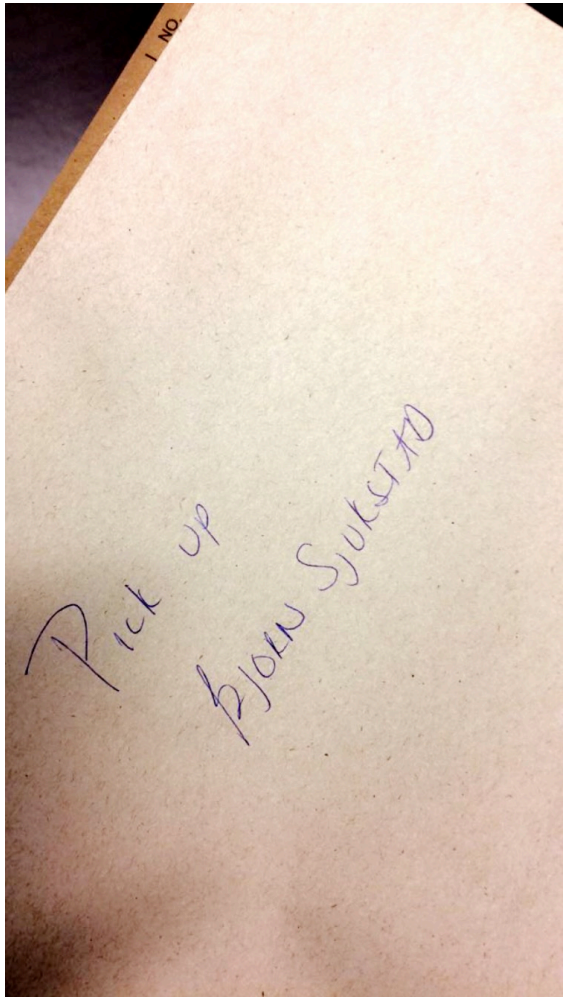
TopHat Dependencies



walrus

github.com/uit-bdps/walrus





Use Case

- Previous: Identify the molecular signature of the patient's tumor and germline with DNA sequence data from the patient's primary tumor and adjacent normal cells

- **Provide an extensive comparison of the metastasis against the primary, and to identify the molecular drivers of the tumor**

14 October 2016, pick up of biological sample at McGill University Health Centre

Lessons from the initial analysis pipeline

- The pipeline tools should be kept for later use, either as binaries or code
- Datasets and databases should be stored along with the pipeline description
- It should be easy to install and include new tools to a new or existing pipeline

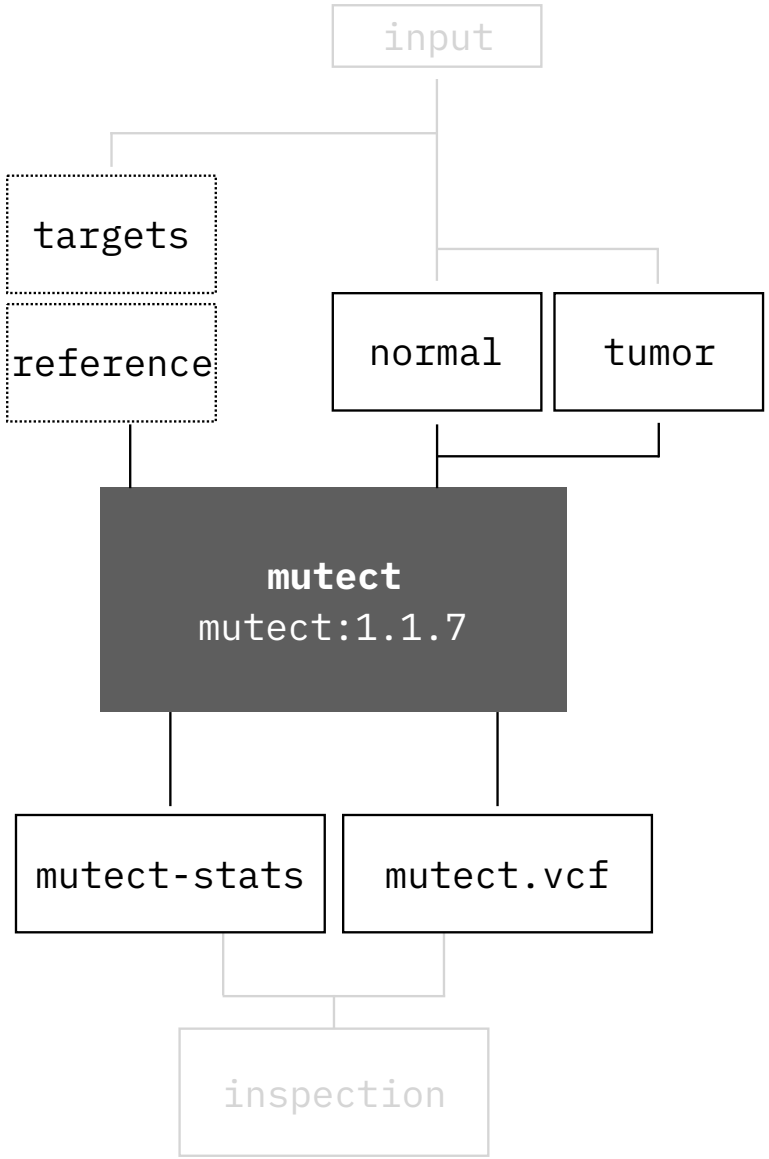
Related Work

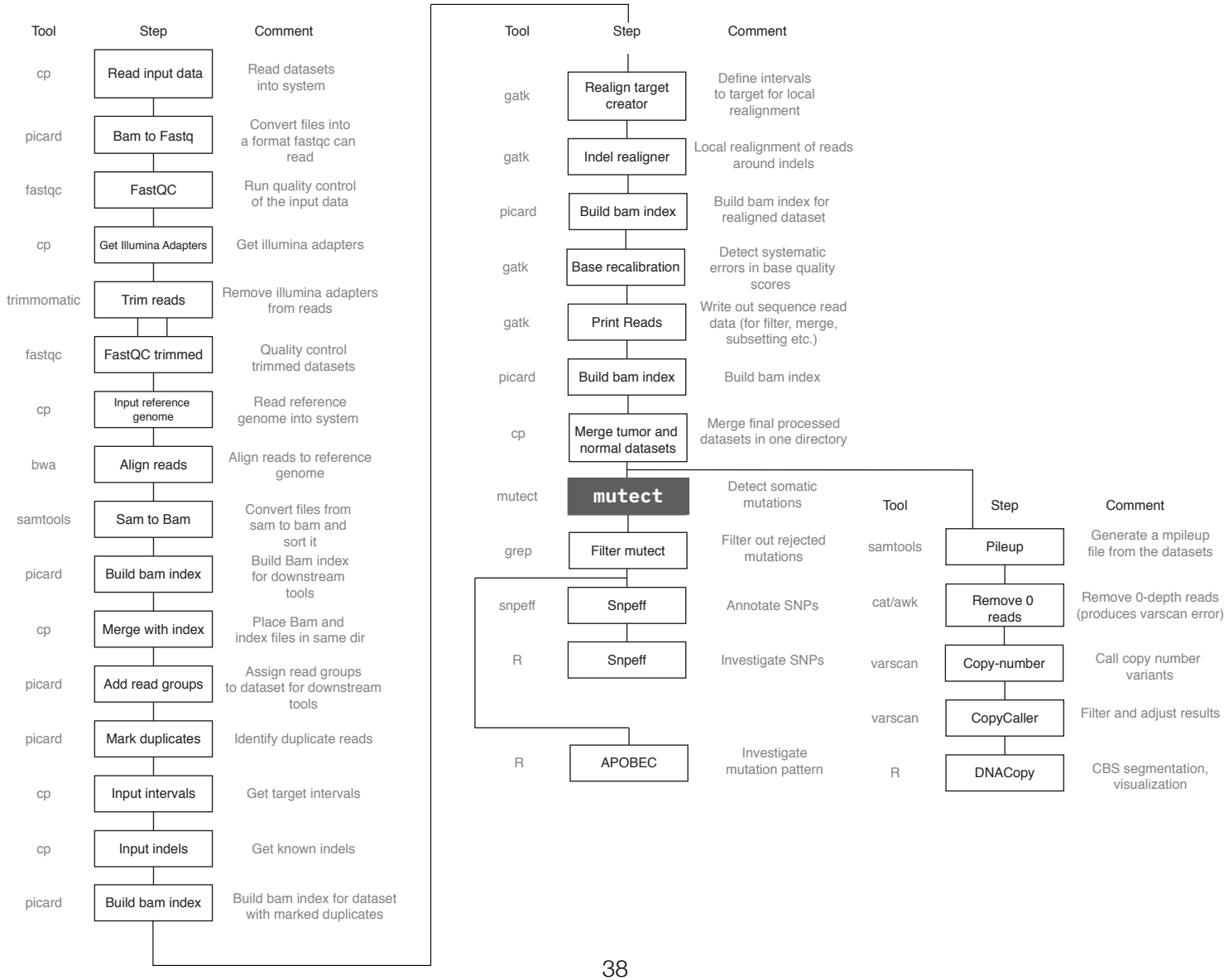
- A wealth of pipeline description formats and workflow managers available
- Common Workflow Language (CWL) is a popular specification formation that is supported by many workflow managers, e.g. Galaxy, Rabix, Toil, and AWE. Implementation specific data management.
- Pachyderm provides version control for data, and reproducible data analysis pipelines. Runs on top of Kubernetes.
- Snakemake is a popular tool that has lately got support software containers

walrus

- Tool for developing and executing data analysis pipelines
- Stores: tool versions, tool parameters, input data, intermediate data, output data, along with the execution environment
- Users write pipeline descriptions in JSON/YAML files
- Users use the commandline interface which executes the pipeline

```
{
    "Name": "mutect",
    "Image": "fjukstad/mutect:1.1.7",
    "Cmd": [
        "--analysis_type","MuTect",
        "--reference_sequence","/walrus/input/reference.fasta",
        "--input_file:normal","/walrus/input/normal.bam",
        "--input_file:tumor","/walrus/input/tumor.bam",
        "-L", "/walrus/input/targets.bed",
        "--out", "/walrus/mutect/mutect-stats-txt",
        "--vcf","/walrus/mutect/mutect.vcf"
    ],
    "Inputs":[
      "input"
    ]
}
```




Tool
Step
Comment
cp
Read input data
Read datasets into system
picard
Bam to Fastq
Convert files into a format fastqc can read
fastqc
FastQC
Run quality control of the input data
cp
Get Illumina Adapters
Get illumina adapters
trimmomatic
Trim reads
Remove illumina adapters from reads
fastqc
FastQC trimmed
Quality control trimmed datasets
cp
Input reference genome
Read reference genome into system
bwa
Align reads
Align reads to reference genome
samtools
Sam to Bam
Convert files from sam to bam and sort it
picard
Build bam index
Build Bam index for downstream tools
cp
Merge with index
Place Bam and index files in same dir
picard
Add read groups
Assign read groups to dataset for downstream tools
picard
Mark duplicates
Identify duplicate reads
cp
Input intervals
Get target intervals
cp
Input indels
Get known indels
picard
Build bam index
Build bam index for dataset with marked duplicates
Tool
Step
Comment
gatk
Realign target creator
Define intervals to target for local realignment
gatk
Indel realigner
Local realignment of reads around indels
picard
Build bam index
Build bam index for realigned dataset
gatk
Base recalibration
Detect systematic errors in base quality scores
gatk
Print Reads
Write out sequence read data (for filter, merge, subsetting etc.)
picard
Build bam index
Build bam index
cp
Merge tumor and normal datasets
Merge final processed datasets in one directory
mutect
mutect
Detect somatic mutations
grep
Filter mutect
Filter out rejected mutations
snpeff
Snpeff
Annotate SNPs
R
Snpeff
Investigate SNPs
R
APOBEC
Investigate mutation pattern
Tool
Step
Comment
samtools
Pileup
Generate a mpileup file from the datasets
cat/awk
Remove 0 reads
Remove 0-depth reads (produces varscan error)
varscan
Copy-number
Call copy number variants
varscan
CopyCaller
Filter and adjust results
R
DNACopy
CBS segmentation, visualization

Pipeline Execution

- All tools are Docker images, and each pipeline step is run within a Docker container. Simplifies dependency management
- Users can create these images themselves, or use existing images from e.g. BioContainers
- A pipeline is run on a single server, but supports data and task parallelism

Data Management

- We version control all data: input, intermediate, and output
- We use `git` for the pipeline description, and `git-lfs` for the datasets
- The commandline interface takes care of tracking, and optionally restoring previous datasets

Results

- Has shown its usability in a clinical setting. We analyzed a patient’s metastasis to discover Single Nucleotide Polymorphisms(SNPs), genomic variants and somatic mutations

- Implemented a variant calling pipeline for a public dataset to evaluate performance and resource usage.

Discussion

- Still no standard for representing or sharing workflows. CWL is gaining popularity and more systems now support it
- In walrus we propose a solution where researchers can share pipeline descriptions *and* datasets
- Forcing users to package tools in Docker images can help ensure reproducibility

Summary

- A system for reproducible analysis of genomic data in a clinical setting
- Analyzed a patient’s metastasis to discover SNPs, genomic variants and somatic mutations
- Performance evaluation and example pipelines to get started

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