Reproducible Computational based Research

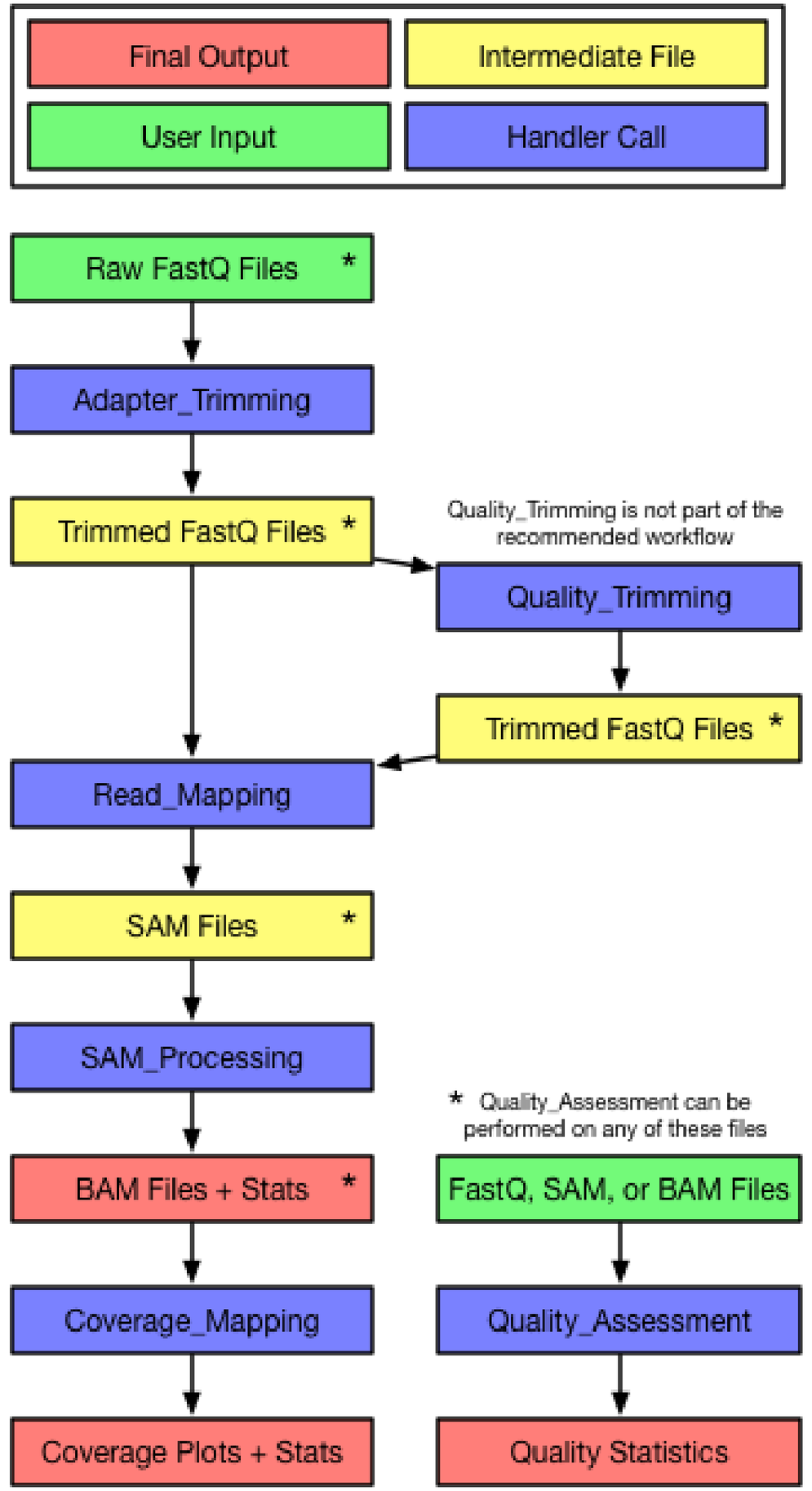
To infinity and beyond

11 September 2017

Outline - getting to reproducibility

- What does reproducibility mean?
- Should I design my data analysis as code?
- What is data & what is metadata?
- Should I keep a digital notebook?

https://github.com/MorrellLAB/sequence_handling



Inspirations for talk

- Yosef Cohen - Statistics and Data with R
- Vince Buffalo - Bioinformatics Data Skills
- Karl Broman - Initial steps toward reproducible research
- Greg Wilson, Titus Brown, et al. - Best practices for scientific computing
- Every dissertation, manuscript, or paper I read!

NSF Statement

What does reproducible mean?

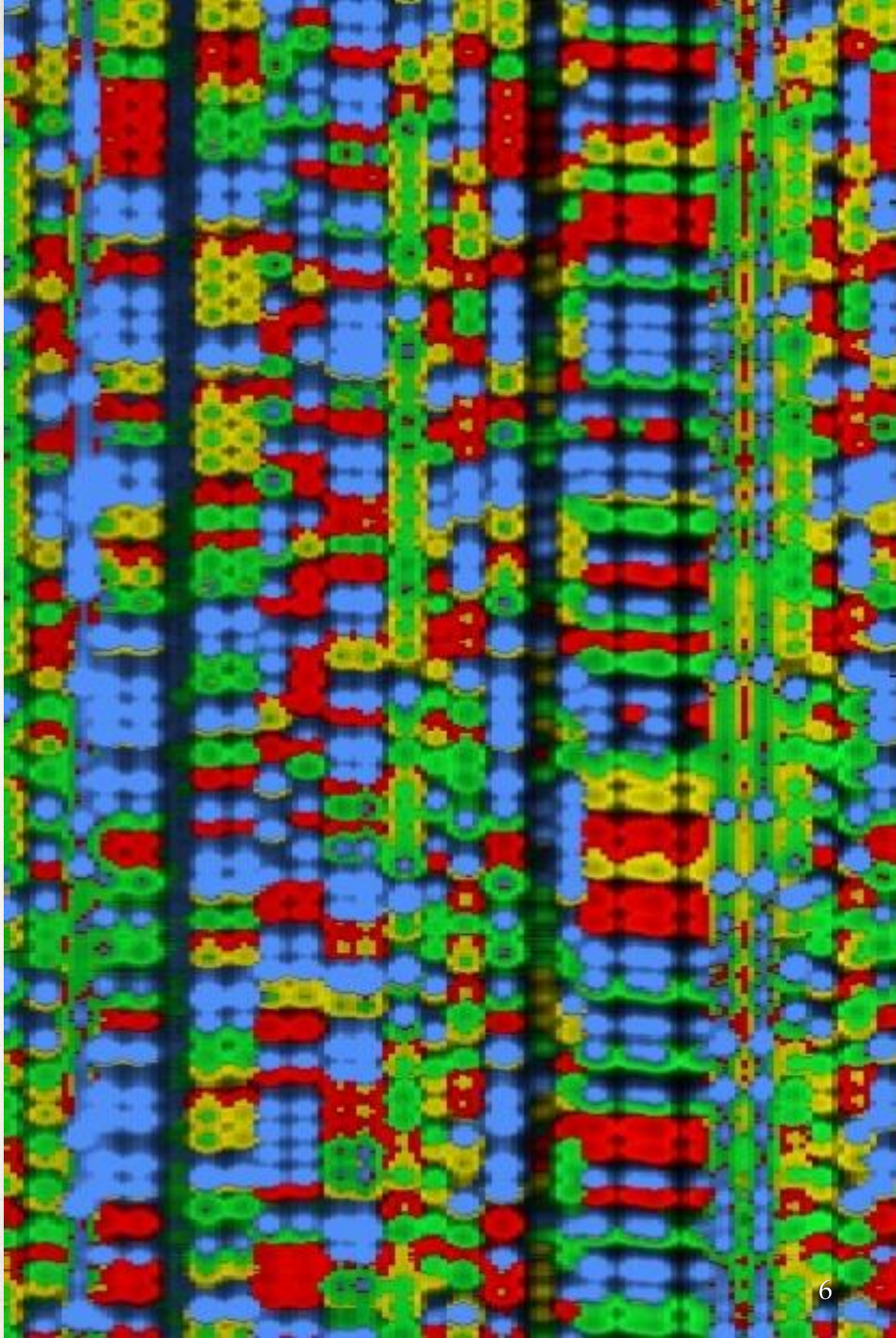
- Person “skilled in the art” can reproduce results
- Or collect new data and get to similar results

Why should research be reproducible?

- Public investment requires reliable results

- Research training is NOT intended to teach a boutique set of skills only an artisan can replicate

- Work is replicated and updated; current human genome is version 38

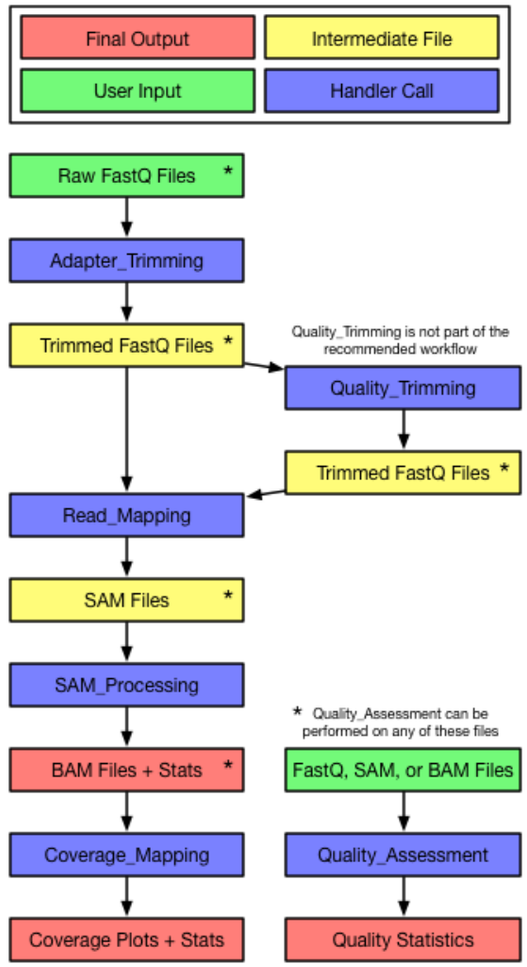


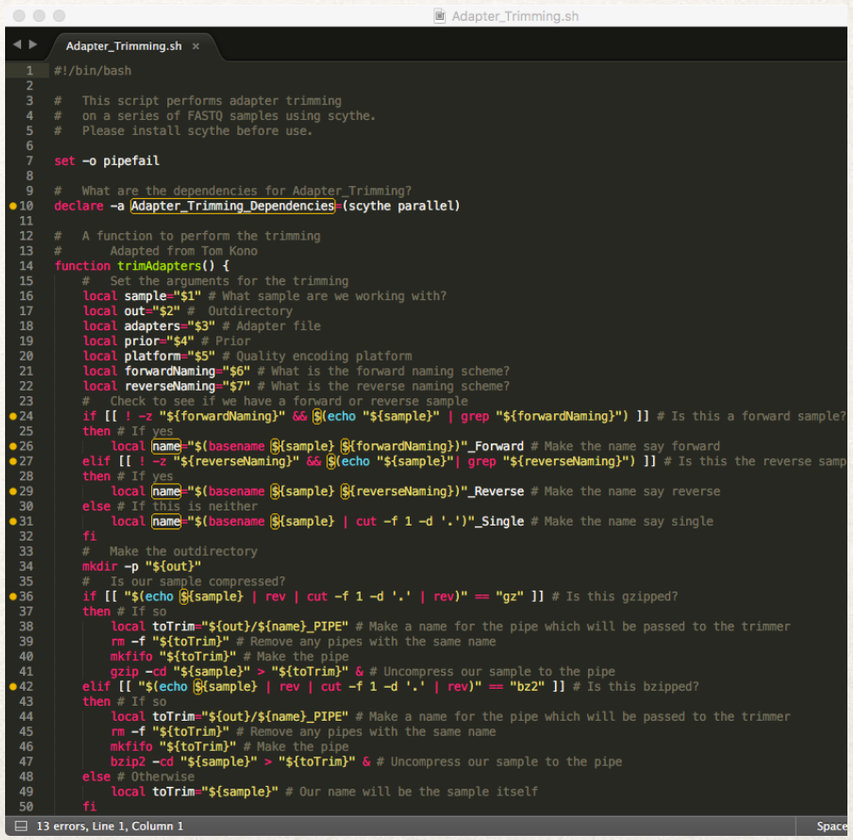
Why this does [not] apply to me?

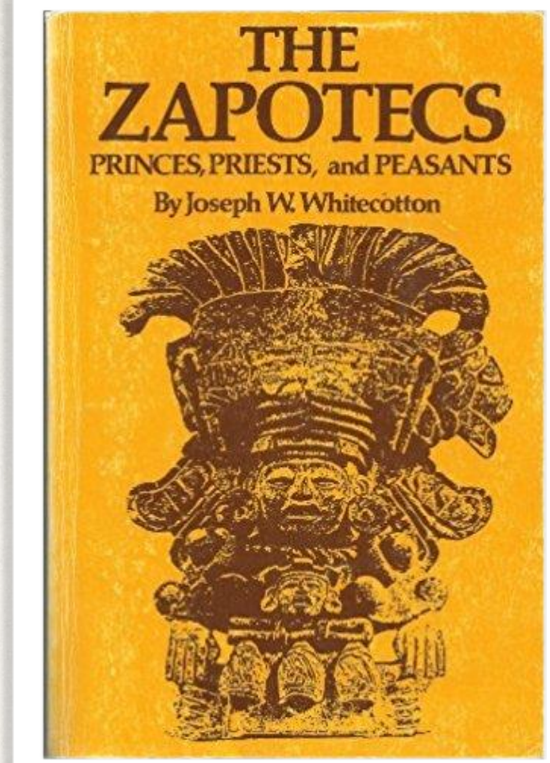
- Whenever possible approaches and methods should be "generalizable"
- "I work on a highly specialized system"
  - "Croak length in subterranean Iberian frogs"

Should I design my analysis as code?

- Easier said than done
- Goal should be self-contained code that executes analyses
- Hand-editing any file or intermediate step is a no-no
- Primarily because you will have to do it repeatedly, and could make mistakes







"You all grew up in Latin America, you just don't know it yet."

–Joseph Whitecotton - anthropologist - to U. of Oklahoma undergrads

"You are all computational biologists, you just don't know it yet."

–Peter Morrell - biologist - to seminar audience at U. of Minnesota

Transition to executable analysis

- Often includes code in Perl, Python, R, BASH, C++ or a combination of languages

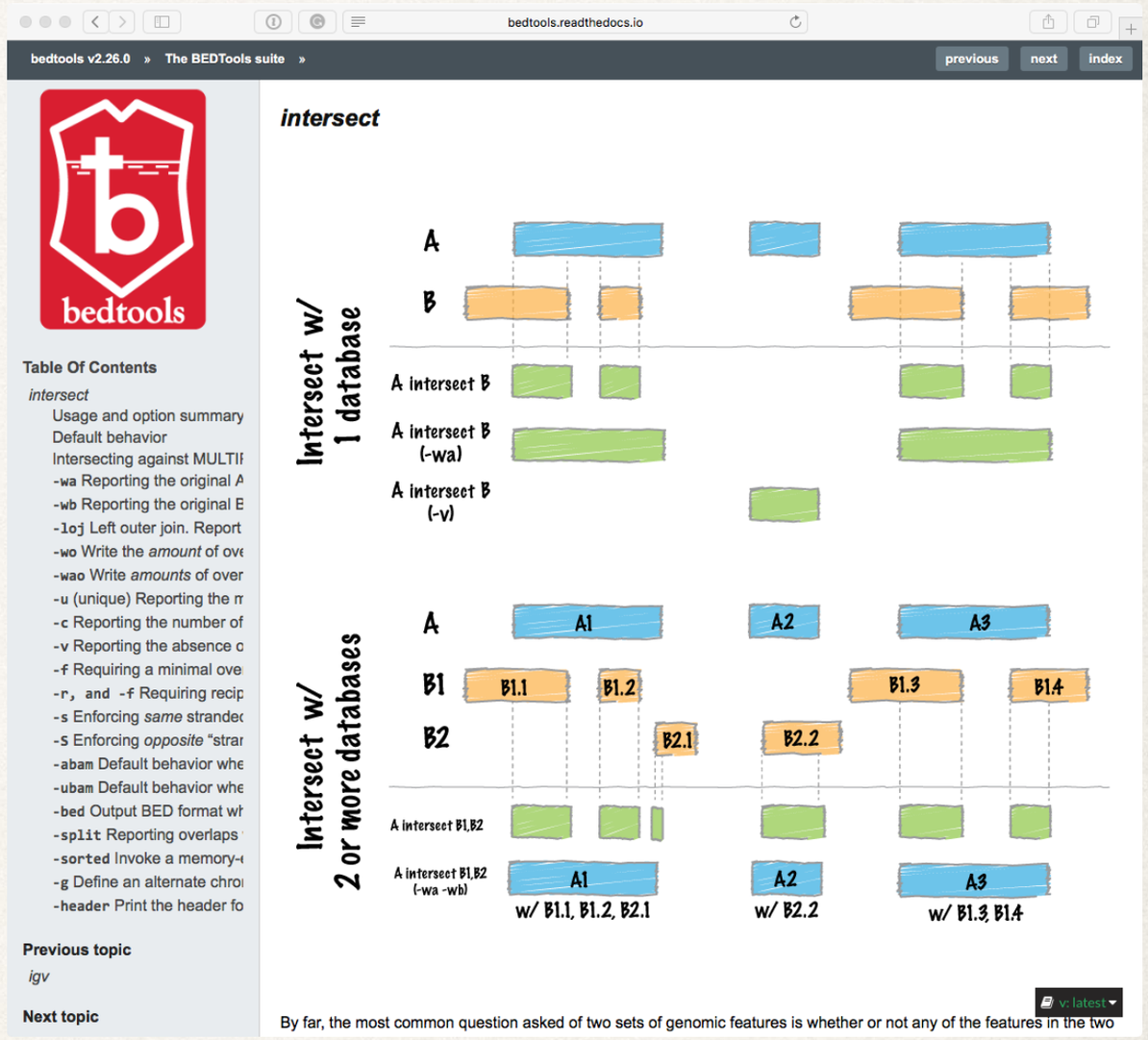
- Elegance of design and simplicity of interpretation increase with experience

Reproducibility starts with good tools that are well vetted

- "I wrote my own sequence read mapper" - bad idea
  - Unless you are a computer science grad student
  - Even then, bad idea because there are dozens already
- Testing of these tools is crowd sourced

Power tools

- Primary tools for most analyses are developed by professional programmers and statisticians
- Use them whenever possible!
  - BWA - sequence read mapping
  - R/QTL - biparental QTL mapping
  - Bedtools - for genome slicing and dicing



Good code is well commented

- Comments should explain the "why" of a step taken in code
- Should be sufficient for anyone "skilled in the art"
- Avoid "!@##$!!@#*%" in comments; code eventually becomes public!

Adapter_Trimming.sh

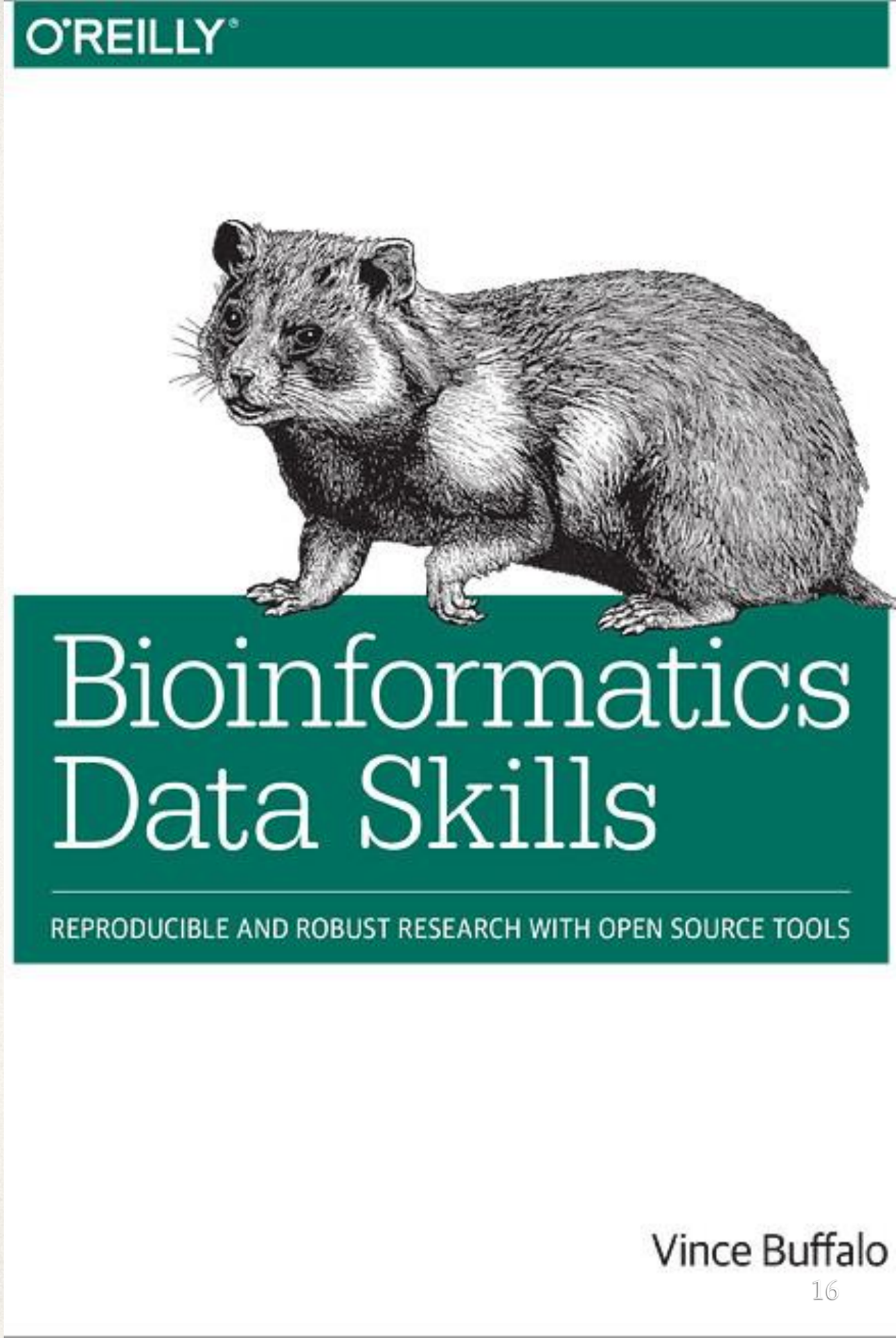
Adapter_Trimming.sh ×

```
1  #!/bin/bash
2
3  #   This script performs adapter trimming
4  #   on a series of FASTQ samples using scythe.
5  #   Please install scythe before use.
6
7  set -o pipefail
8
9  #   What are the dependencies for Adapter_Trimming?
10 declare -a Adapter_Trimming_Dependencies=(scythe parallel)
11
12 #   A function to perform the trimming
13 #       Adapted from Tom Kono
14 function trimAdapters() {
15     #   Set the arguments for the trimming
16     local sample="$1" # What sample are we working with?
17     local out="$2" #  Outdirectory
18     local adapters="$3" # Adapter file
19     local prior="$4" # Prior
20     local platform="$5" # Quality encoding platform
21     local forwardNaming="$6" # What is the forward naming scheme?
22     local reverseNaming="$7" # What is the reverse naming scheme?
23     #   Check to see if we have a forward or reverse sample
24     if [[ ! -z "${forwardNaming}" && $(echo "${sample}" | grep "${forwardNaming}") ]] # Is this a forward sample?
25     then # If yes
26         local name="$(basename ${sample} ${forwardNaming})"_Forward # Make the name say forward
27     elif [[ ! -z "${reverseNaming}" && $(echo "${sample}"| grep "${reverseNaming}") ]] # Is this the reverse samp
28     then # If yes
29         local name="$(basename ${sample} ${reverseNaming})"_Reverse # Make the name say reverse
30     else # If this is neither
31         local name="$(basename ${sample} | cut -f 1 -d '.')"_Single # Make the name say single
32     fi
33     #   Make the outdirectory
34     mkdir -p "${out}"
35     #   Is our sample compressed?
36     if [[ "$(echo ${sample} | rev | cut -f 1 -d '.' | rev)" == "gz" ]] # Is this gzipped?
37     then # If so
38         local toTrim="${out}/${name}_PIPE" # Make a name for the pipe which will be passed to the trimmer
39         rm -f "${toTrim}" # Remove any pipes with the same name
40         mkfifo "${toTrim}" # Make the pipe
41         gzip -cd "${sample}" > "${toTrim}" & # Uncompress our sample to the pipe
42     elif [[ "$(echo ${sample} | rev | cut -f 1 -d '.' | rev)" == "bz2" ]] # Is this bzipped?
43     then # If so
44         local toTrim="${out}/${name}_PIPE" # Make a name for the pipe which will be passed to the trimmer
45         rm -f "${toTrim}" # Remove any pipes with the same name
46         mkfifo "${toTrim}" # Make the pipe
47         bzip2 -cd "${sample}" > "${toTrim}" & # Uncompress our sample to the pipe
48     else # Otherwise
49         local toTrim="${sample}" # Our name will be the sample itself
50     fi
```

13 errors, Line 1, Column 1 Space

If you only learn one language - BASH

- Unix shell (BASH) scripting provides access to pro tools
- Necessary to use a supercomputer
- Bioinformatics Data Skills - Chapters 3 & 7



Example shell script

- Avoid hard coding file paths in the middle of a script
  - Hard coding nearly eliminates reproducibility
- Set paths as variable names at the top of the script
  - WORKSHOP=/panfs/roc/groups/9/morrellp/pmorrell/Workshop

```
#!/bin/sh

#PBS -l mem=128gb,nodes=1:ppn=8,walltime=72:00:00
#PBS -m abe
#PBS -M useremail@umn.edu
#PBS -q mesabi

#   Required for Java
module load java/jdk1.8.0_45

#   Path to the GATK
GATK=/panfs/roc/groups/9/morrellp/shared/Software/GATK-3.6/GenomeAnalysisTK.jar
#   Build the sample list
SAMPLE_LIST=/panfs/roc/groups/9/morrellp/shared/Projects/Barley_NAM_Parents/sequence_handling/SAM_
    Processing/Picard/Finished/180_bam.list
#   The output targets file
RTC_OUT=/panfs/roc/groups/9/morrellp/shared/Projects/Barley_NAM_Parents/SNP_calling/realign.
    intervals
#   The reference sequence
REF=/panfs/roc/groups/9/morrellp/shared/References/Reference_Sequences/Barley/Morex/barley_RefSeq_
    v1.0/barley_pseudomolecules_parts.fa

#   Put them into a format that will be accepted by the GATK command line
GATK_IN=()
for s in "${SAMPLE_LIST[@]}"
do
    GATK_IN+=("-I $s")
done

#   JAVA OPTIONS
#   -Xmx[amount]    :   use [amount] of memory.
#   -jar <file>     :   execute <file>, which is a jar file

#   GATK OPTIONS
#   -T RealignerTargetCreator
#       Create a list of regions to realign
#   -L Regions
#       Operate only in the genomic intervals specified in this regions file
#   -nct <int>
#       Use <int> CPU cores
#       NOTE: This option can make performance WORSE if the system is
#       IO-limited, and not compute-limited.
#   -R <FASTA file>
#       Where the reference sequence is stored
#   -I <BAM file>
#       The BAM file for which to create intervals
#   -known <file>
#       <file> contains known INDELs. VCF or BED format
#   -o <file>
#       Write the intervals to this file
#       NOTE: This file must end in .list, .intervals, or .interval_list

export _JAVA_OPTIONS="-Xmx127g -Djava.io.tmpdir=${HOME}/tmp"
java -jar ${GATK}\
    -T RealignerTargetCreator\
    -nt 1\
    -R ${REF}\
    ${GATK_IN[@]}\
    -o ${RTC_OUT}
```

What is a concurrent versioning system?

- Track files and changes over versions of the files
- Why is it relevant for my system?
- Remember, “I work on croak length of subterranean frogs”
- Rerunning analysis during manuscript revision, on a similar project years later, or by another group analyzing rooster crows

Git & Github are preferred options

- Git repositories can be local or shared on the internet
- Flaky WiFi and poor internet connectivity are not friendly for remote work
- Github stores repositories, can be accessed through a web browser or cloned to another computer (or server account)
- Alternatives include Bitbucket and UMN Github

What types of files belong in a repository?

- Plain text (with Markdown) is preferred format

- Code, readme files, workflow diagrams all belong in a repository – example https://github.com/MorrellLAB/sequence_handling

- Data generally belong elsewhere

README.md — UNREGISTERED

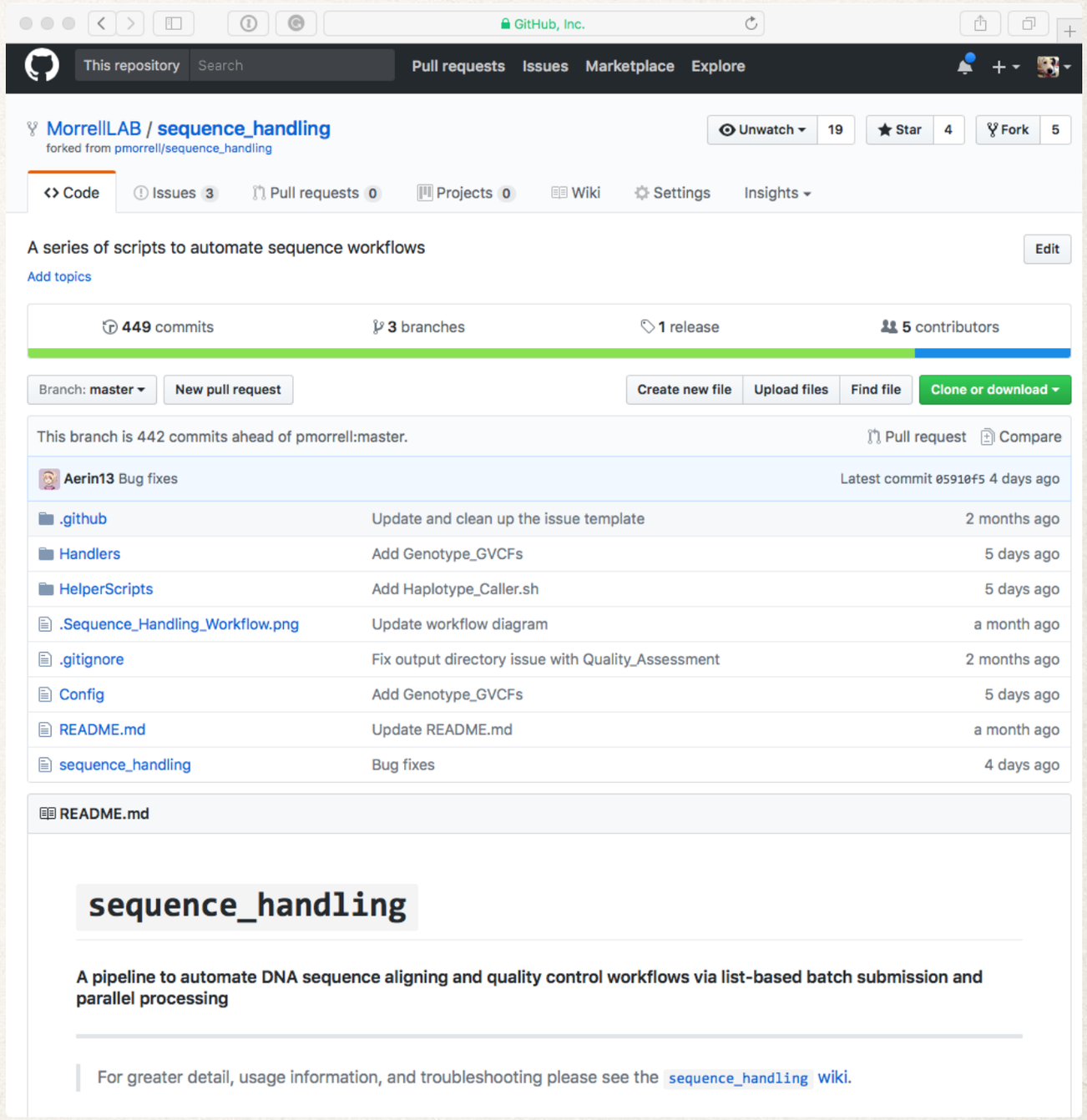
README.md

```
# `sequence_handling`
#### A pipeline to automate DNA sequence aligning and quality control workflows via list-based
batch submission and parallel processing
___

> For greater detail, usage information, and troubleshooting please see the [`sequence_handling`
wiki](https://github.com/MorrellLAB/sequence_handling/wiki).

## What is `sequence_handling` for?

`sequence_handling` is a series of scripts, called handlers, that automate and speed up DNA
sequence alignment and quality control. Currently, `sequence_handling` is designed to work with
Illumina paired-end whole-genome or exome capture sequences. Work is underway to expand `
sequence_handling` to accept GBS sequences.

The workflow is intended to be 100% reproducible provided that you have the Config file and version
of `sequence_handling` that was used. It is also intended to be easy for beginner UNIX users to
configure and run independently, given that they read the [wiki](https://github.com/MorrellLAB/
sequence_handling/wiki).

> **NOTE:** This workflow is designed to use the [Portable Batch System](http://www.pbsworks.com/)
and run on the [Minnesota Supercomputing Institute](https://www.msi.umn.edu). Heavy modifications
will need to be made if not using these systems.

## Dependencies

Due to the pseudo-modularity of this workflow, dependencies for each individual handler are listed
below. Some general dependencies for the workflow as a whole are also listed here:

 - [GNU Parallel](http://www.gnu.org/software/parallel/)
 - A quality control mechanism, such as [FastQC](http://www.bioinformatics.babraham.ac.uk/projects/
fastqc/)
 - An adapter trimmer, such as [Scythe](https://github.com/vsbuffalo/scythe)
 - A read mapper, such as [The Burrows-Wheeler Aligner](http://bio-bwa.sourceforge.net/) (BWA)
 - SAM file processing utilities, such as [SAMTools](http://www.htslib.org/) and/or [Picard](http://
broadinstitute.github.io/picard/)
 - Tools for plotting coverage, such as [R](http://cran.r-project.org/)

Please note that this is not a complete list of dependencies. Check the [dependencies wiki page](
https://github.com/MorrellLab/sequence_handling/wiki/Dependencies) for dependencies for each
handler.

## Basic Usage

A guide on how to install, set up, and run `sequence_handling` for the first time can be found on
the [Getting Started](https://github.com/MorrellLab/sequence_handling/wiki) page of the wiki.

A brief usage message can be viewed by passing no arguments to `sequence_handling`:
```

Line 1, Column 1 | Tab Size: 4 | Markdown

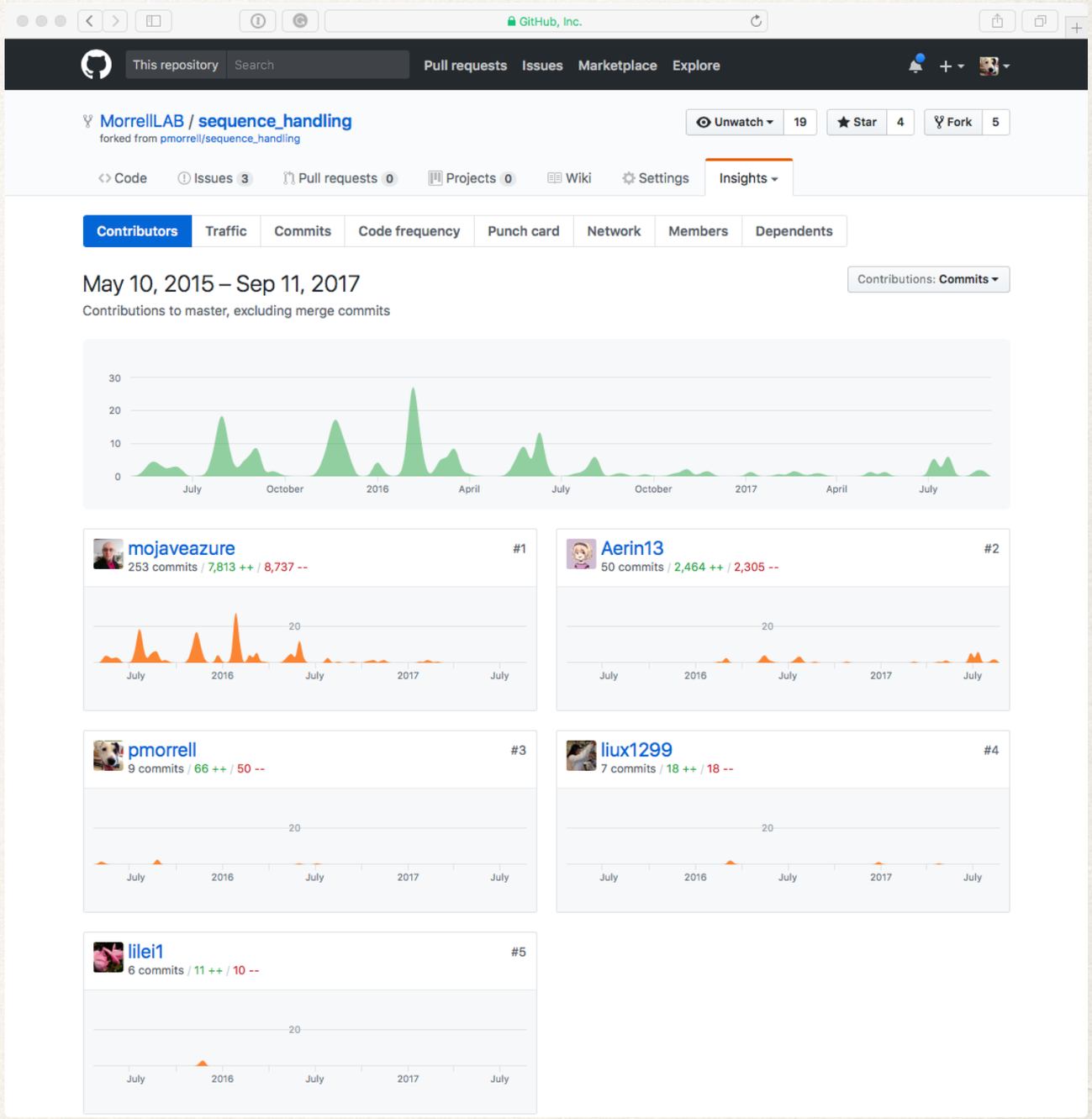
“Code base” repository

- A Git/Github repository used across multiple projects
- Should be generalizable and extensible
- Changes relatively slowly
- Are often public during development



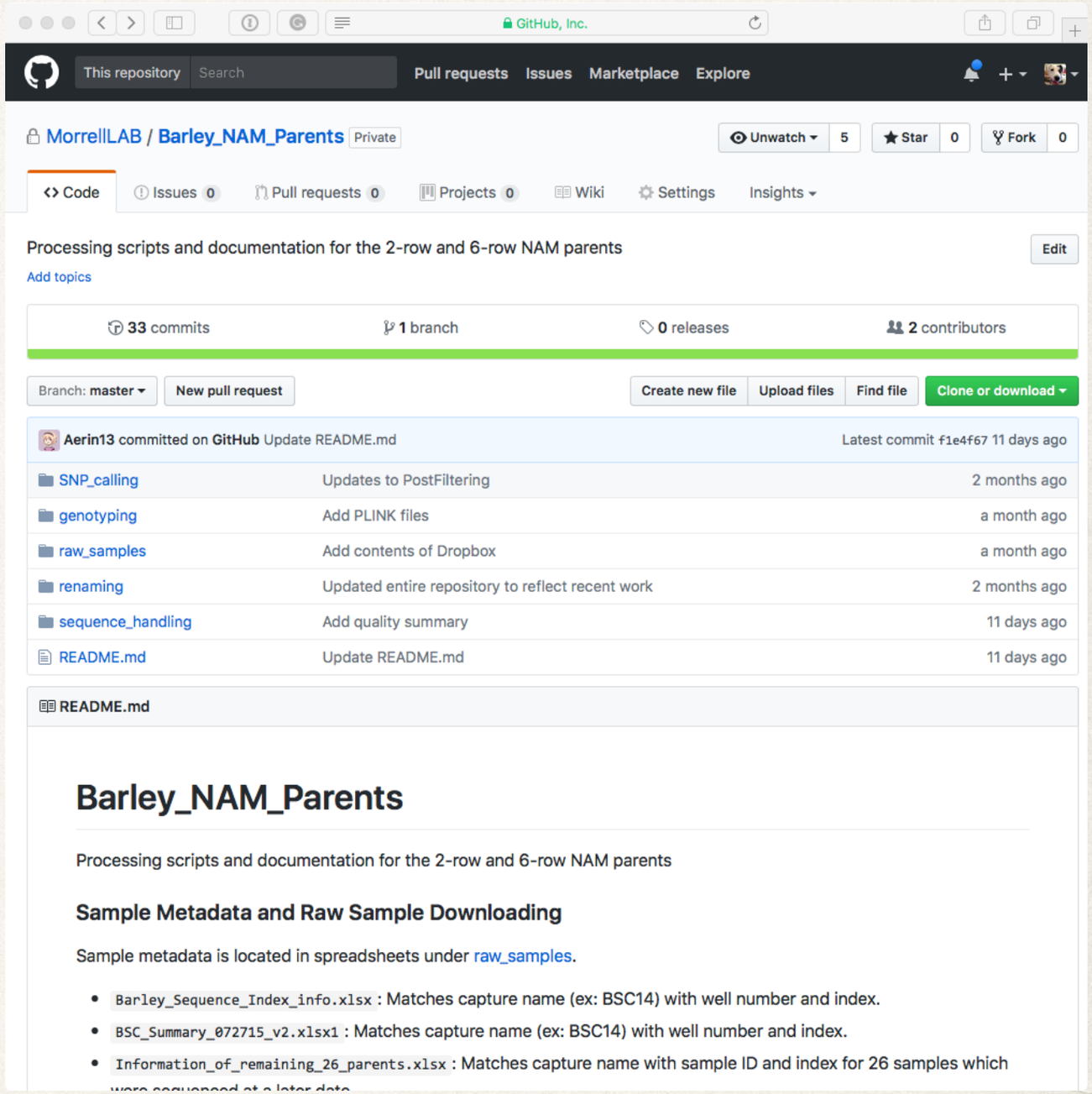
“Code base” repository

- Multiple users contribute
- Active contributors change over time



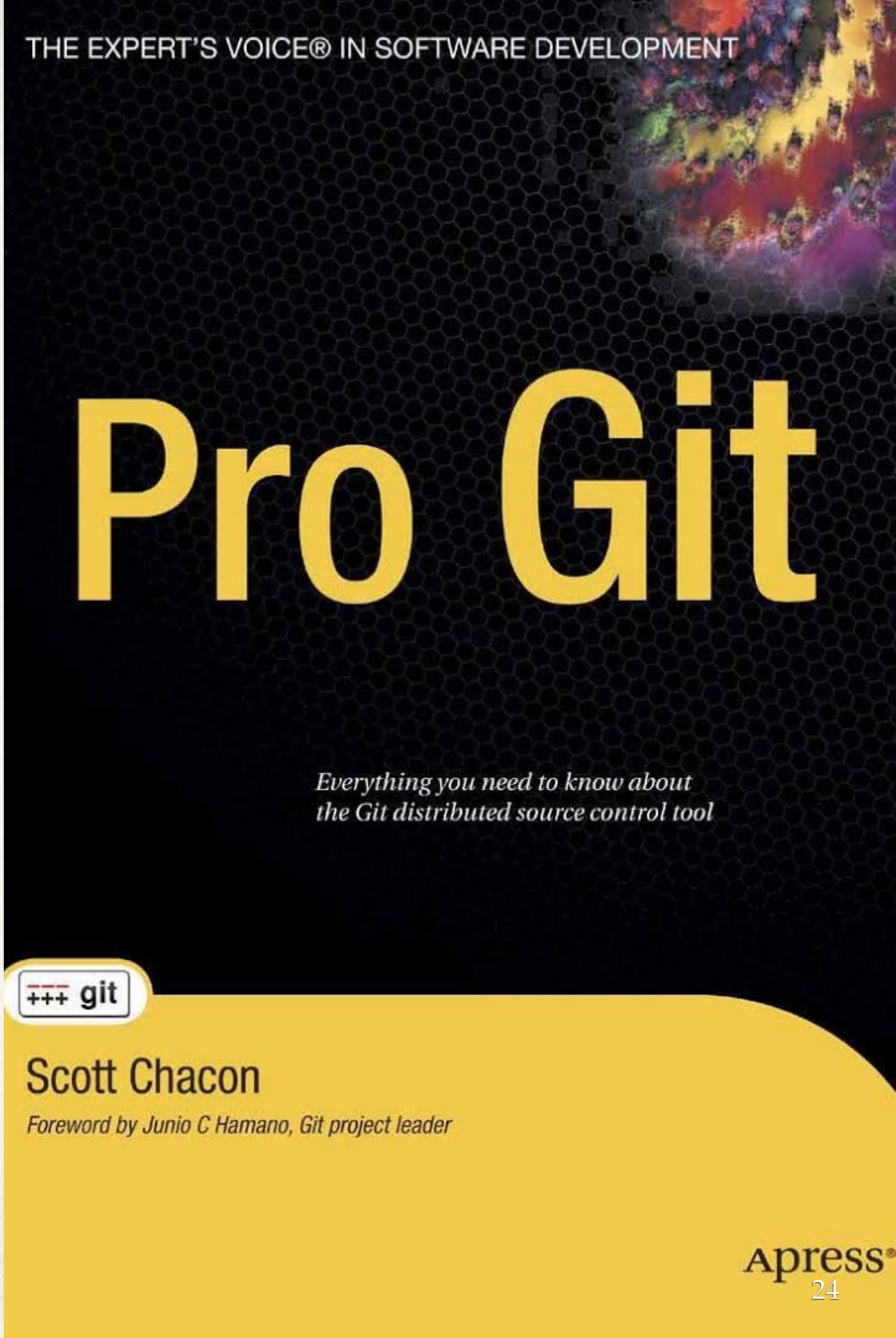
Project repository

- Git/Github repository with files for a specific project
- Can be specific challenges in a data set
- Changes often and quickly as a project develops
- Are often private until project is published



Getting started in Git/Github

- Bioinformatics Data Skills - Chapter 5
  - Slides are available from Does[0]compute?
- Pro Git, a free book - 1st 3 chapters and chapter 6 are really useful



Example from my maize research

- Began research using maize reference genome V2 , at completion of research V4 was released.
- Original analysis of gene ontologies used V2 of genome with AgriGO. AgriGO updated to V2 in 2017.
- Coauthors want to submit paper in 2 days, and my computer is being serviced so no access to data files and notes.
- What would have been a better strategy?

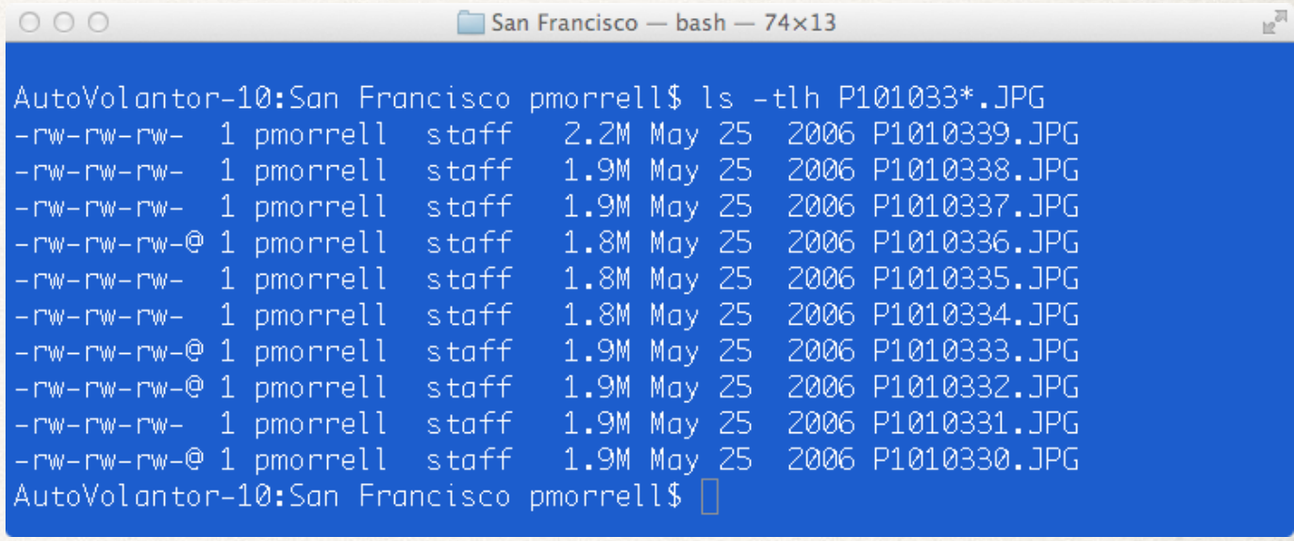
When to make the first commit?

- Create a repository as the first step to starting a project
- Create a README and jot down goals of project and expectations for analysis
- Commit code as soon as you have a few lines that are executable; even if only “Hello World!”

What is data, what is metadata?

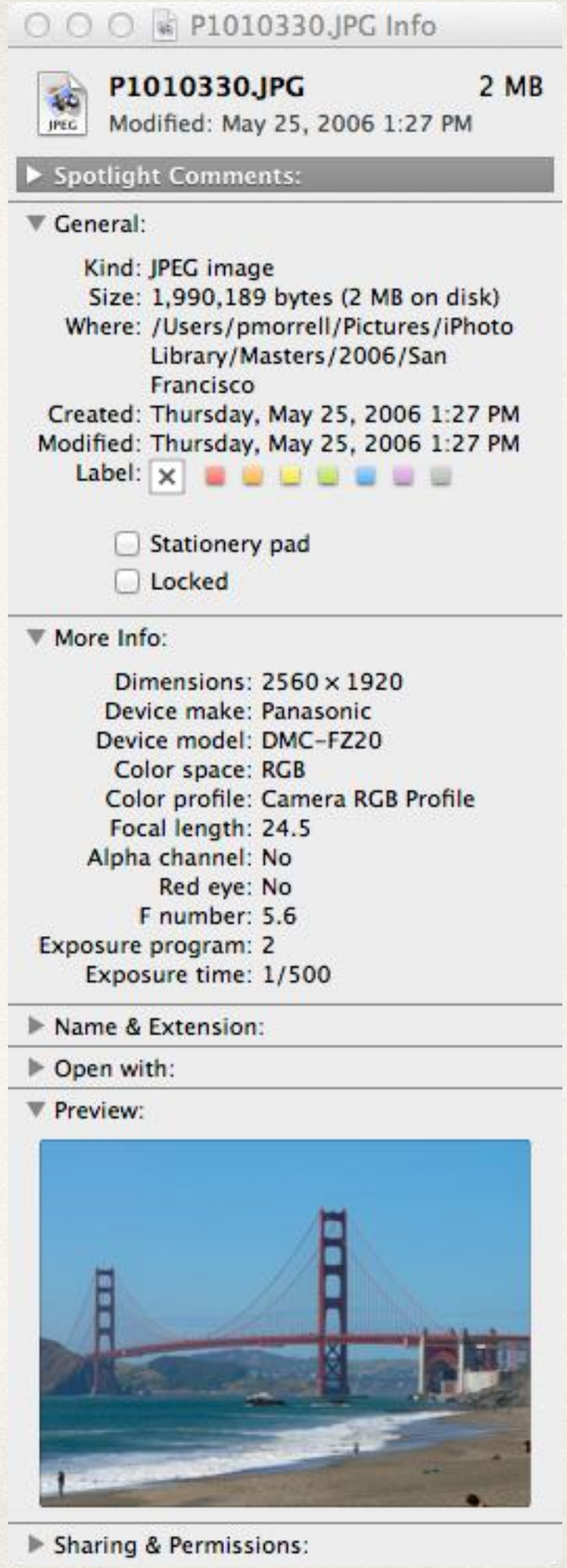
- Actual information; e.g., the contents of a photo

- Metadata includes description of the file containing the photo



File Metadata

- All files have a physical location, size, dates
- Photos have extended attributes - EXIF data
  - Geolocation - spatial metadata
  - Doesn't include - accelerometer or inertial navigation info



San Francisco — bash — 139×55

```
AutoVolantor-10:San Francisco pmorrell$ mdls P1010330.JPG
kMDItemAcquisitionMake          = "Panasonic"
kMDItemAcquisitionModel         = "DMC-FZ20"
kMDItemBitsPerSample            = 32
kMDItemColorSpace               = "RGB"
kMDItemContentCreationDate      = 2006-05-25 18:27:12 +0000
kMDItemContentModificationDate  = 2006-05-25 18:27:12 +0000
kMDItemContentType              = "public.jpeg"
kMDItemContentTypeTree          = (
    "public.jpeg",
    "public.image",
    "public.data",
    "public.item",
    "public.content"
)
kMDItemCreator                  = "Ver1.0 "
kMDItemDisplayName              = "P1010330.JPG"
kMDItemEXIFVersion              = "2.2"
kMDItemExposureMode             = 0
kMDItemExposureProgram          = 2
kMDItemExposureTimeSeconds      = 0.002
kMDItemFlashOnOff               = 0
kMDItemFNumber                  = 5.599999904632568
kMDItemFocalLength              = 24.5
kMDItemFSContentChangeDate      = 2006-05-25 18:27:12 +0000
kMDItemFSCreationDate           = 2006-05-25 18:27:12 +0000
kMDItemFSCreatorCode            = ""
kMDItemFSFinderFlags            = 0
kMDItemFSHasCustomIcon          = 0
kMDItemFSInvisible              = 0
kMDItemFSIsExtensionHidden      = 0
kMDItemFSIsStationery           = 0
kMDItemFSLabel                  = 0
kMDItemFSName                   = "P1010330.JPG"
kMDItemFSNodeCount              = 1990189
kMDItemFSOwnerGroupID           = 20
kMDItemFSOwnerUserID            = 502
kMDItemFSSize                   = 1990189
kMDItemFSTypeCode               = ""
kMDItemHasAlphaChannel          = 0
kMDItemIsApplicationManaged     = 1
kMDItemISOSpeed                 = 80
kMDItemKind                     = "JPEG image"
kMDItemLogicalSize              = 1990189
kMDItemOrientation              = 0
kMDItemPhysicalSize             = 1990656
kMDItemPixelCount               = 4915200
kMDItemPixelHeight              = 1920
kMDItemPixelWidth               = 2560
kMDItemProfileName              = "Camera RGB Profile"
kMDItemRedEyeOnOff              = 0
kMDItemResolutionHeightDPI      = 72
kMDItemResolutionWidthDPI       = 72
kMDItemSupportFileType          = (
    iPhotoPreservedOriginal
```

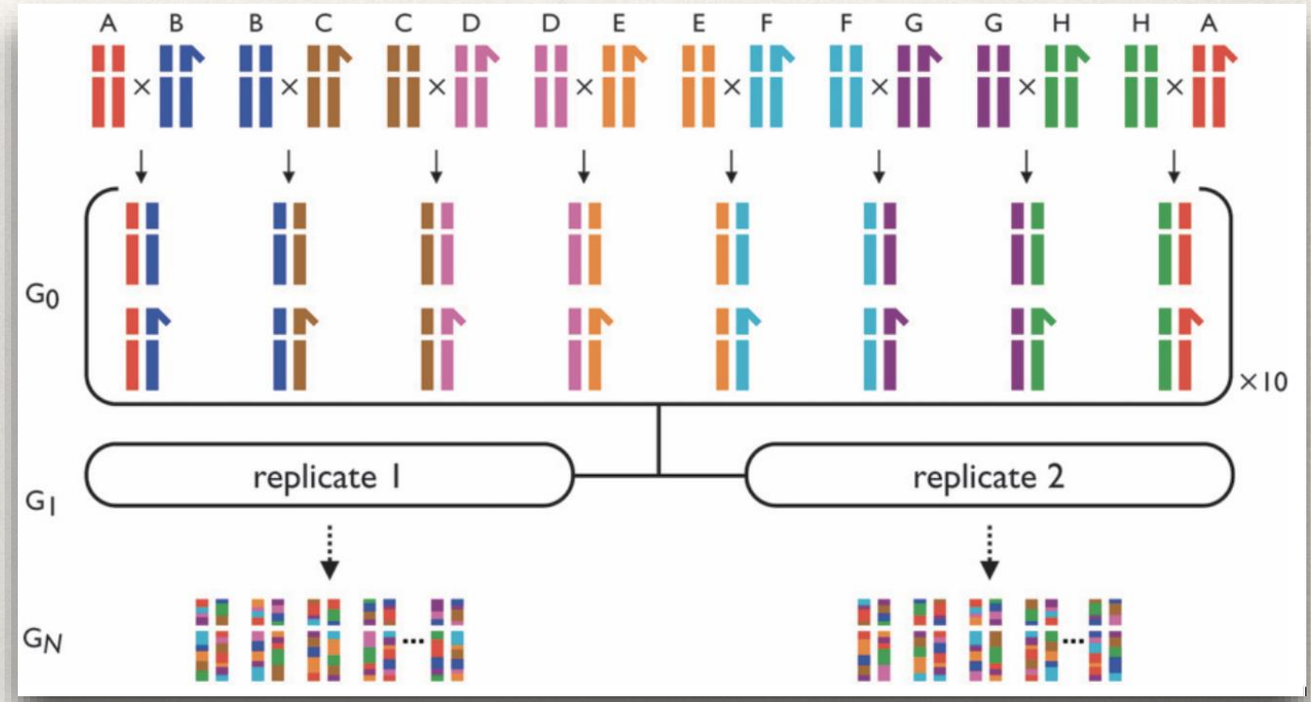

iPhoto
LIBRARY
Events
Photos
Faces
Places
RECENT
Jun 7, 2009
Last Import
Last 12 Months
Photo Stream
Flagged
Printing
Trash
SUBSCRIPTIONS
Nova Scotia
Alaska, 6/05
ALBUMS
Santa Monica...
Nova Scotia
San Francisco
Spring in Palm...
Wheat Diversity
Sayulita
Early Photos
Belo & Rio
WEB
Peter Morrell
Peter Morrell
Peter Morrell
Photos
1,699 of 3,912
DMC-FZ20
AWB
2560 × 1920
2.0 MB
JPEG
ISO 80
24.5 mm
0 EV
f/5.6
1/500
Baker Beach
May 23, 2006 4:09:00 AM
Golden Gate
Faces
Add a face...
Baker Beach
Mt Tamalpais State Park
Marin City
Golden Gate National Recreation Area
San Francisco
Davidson, Mount
Google
Map data ©2012 Google - Terms of Use
Zoom
Info
Edit
Create
Add To
Share

Metadata for RNASeq

- Tissue, growth stage, genotype, time of day, light intensity

Hypothetical Study

- Barley 8 parent MAGIC population
- Reporting on population creation, sequence and genetic mapping
- Recombination rate variation as a phenotype
- Genetics or PLoS Genetics manuscript



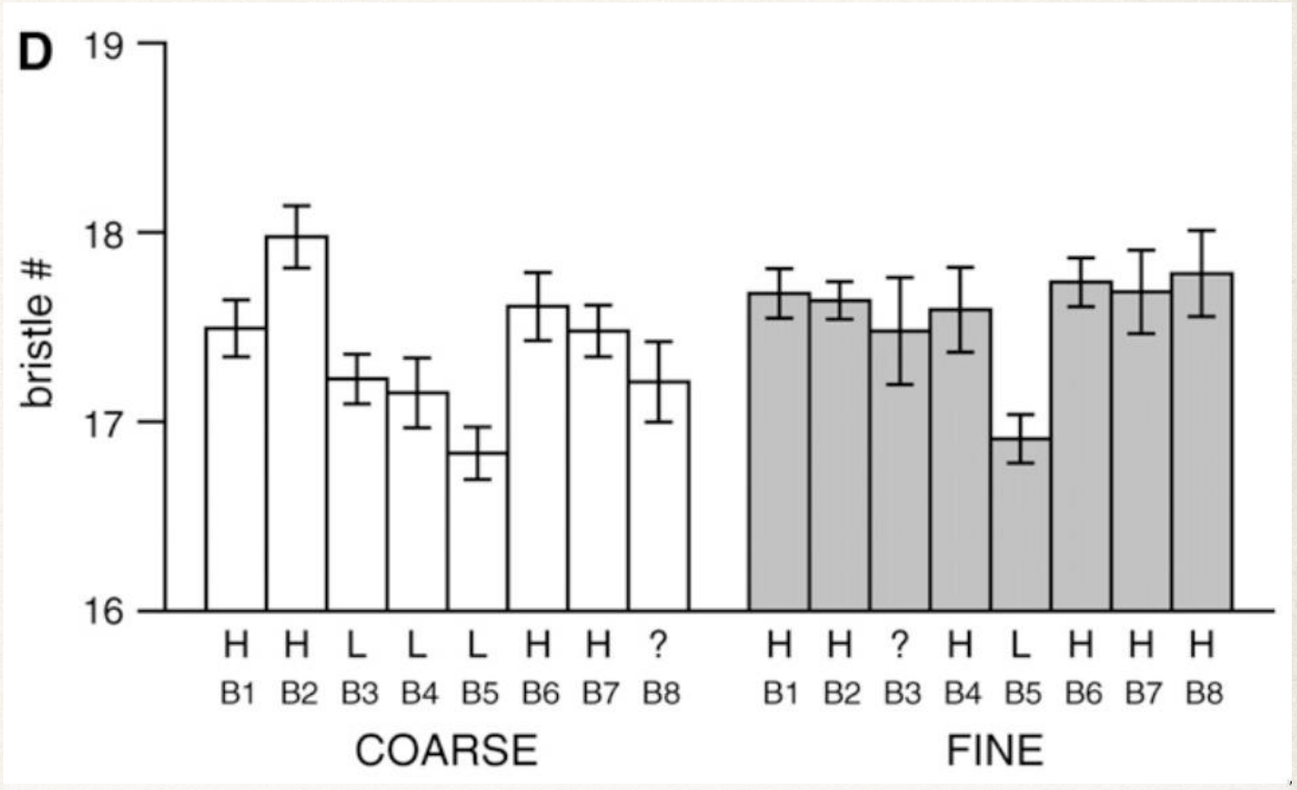
What data & metadata should we collect?

What data & metadata should we collect?

- Nanopore / Illumina sequencing of each parent
- Skim sequencing of progeny
- Recombination events per chromosomal segment for progeny

Primary manuscript

- Figures & tables
  - Names of parental lines
- Sequence depth
- Crossover frequency
- Location, number, & effect of QTL discovered



Macdonald & Long 2007 - Genetics

Supplementary material

- Pedigree of parental lines
- Number of variants from barley Morex reference for each parent
- Proportion of variants that are coding, noncoding, deleterious, etc.

Table 1. Mean Numbers of SNPs in Various Classes.

Species	Diff. from Ref.	Noncoding	Syn.	Nonsyn.	Nonsense
Barley	162,954 (51,231.34)	115,456 (41,065.22)	15,591 (5,691.81)	12,351 (4,492.53)	77 (33.13)
Soybean	82,840 (56,780.03)	44,704 (29,477.65)	14,167 (8,161.21)	18,695 (11,289.72)	540 (345.05)

Syn., Synonymous; Nonsyn., Nonsynonymous. Numbers are mean (SD)

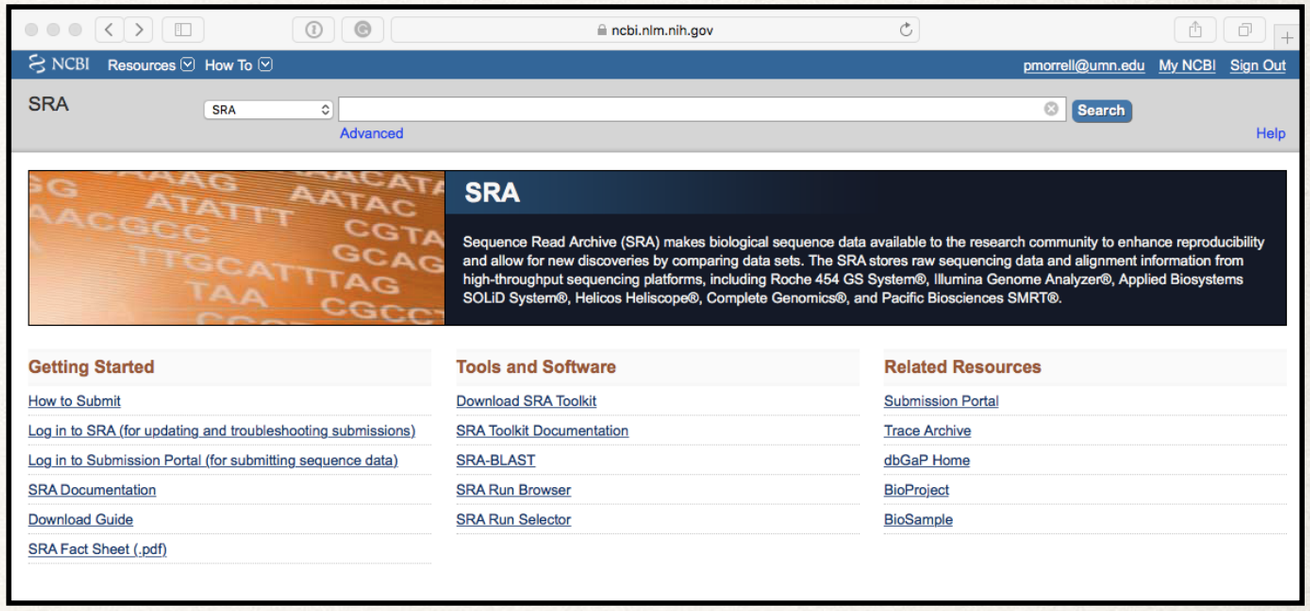
Kono et al. 2016 - Mol Bio Evol

Data Storage

- Git/Github not a location for data storage

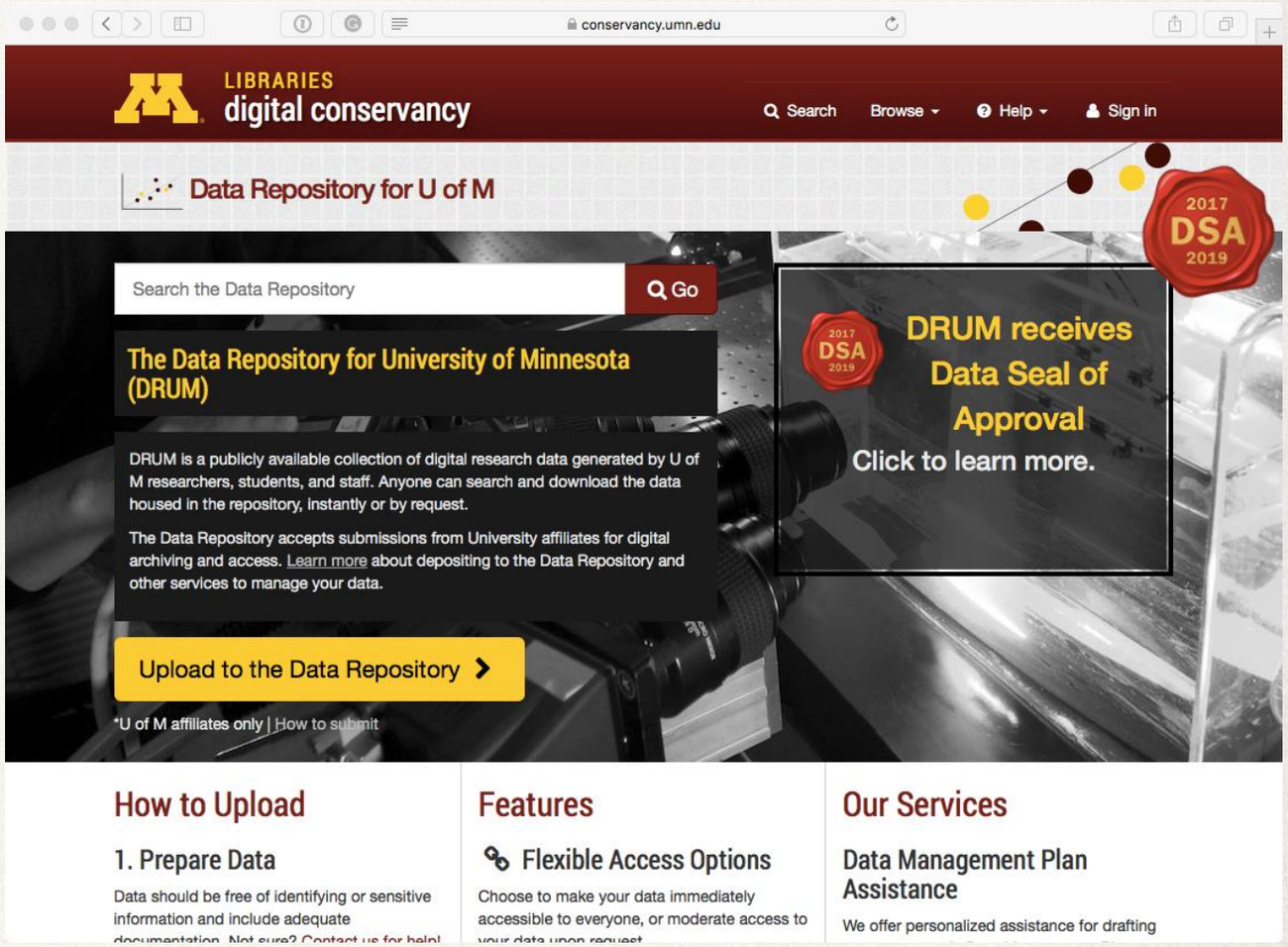
Public archive

- Sequence Read Archive (SRA)
- Stores nucleotide sequence & quality values (FASTQ files)
- FASTQ for skim sequencing of progeny



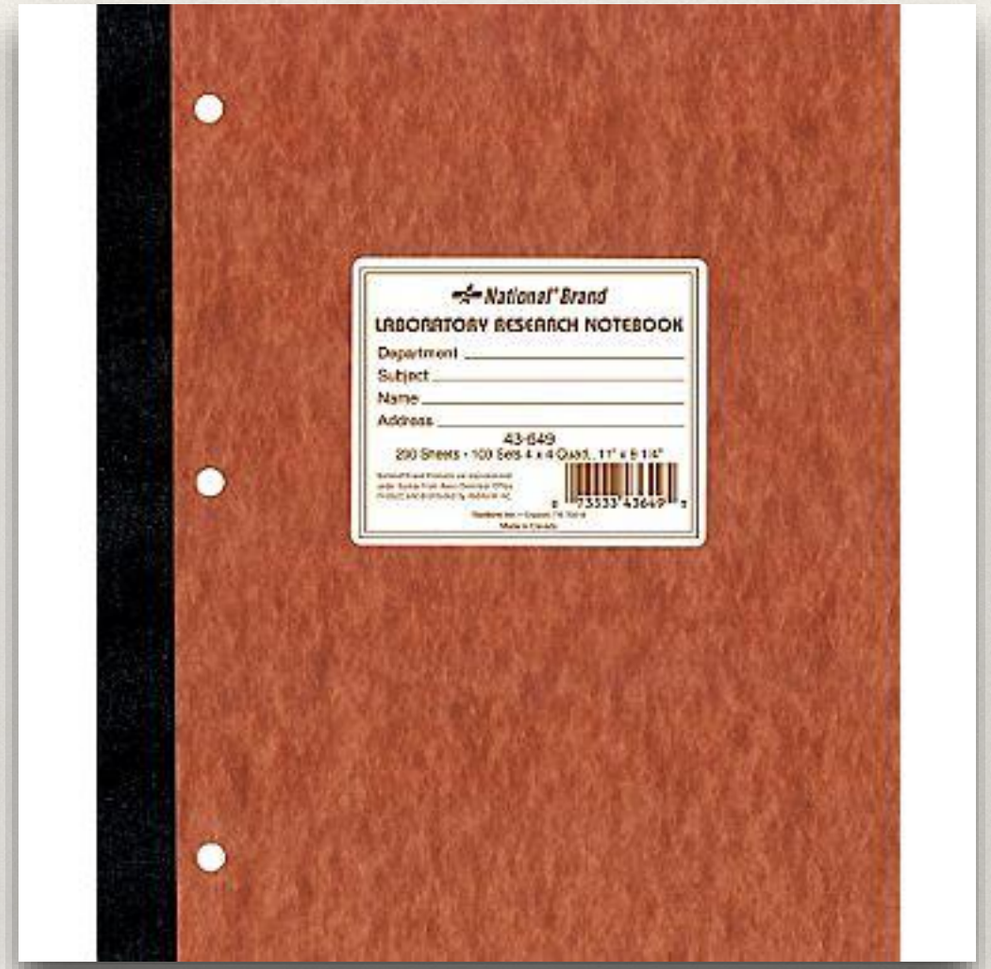
DRUM

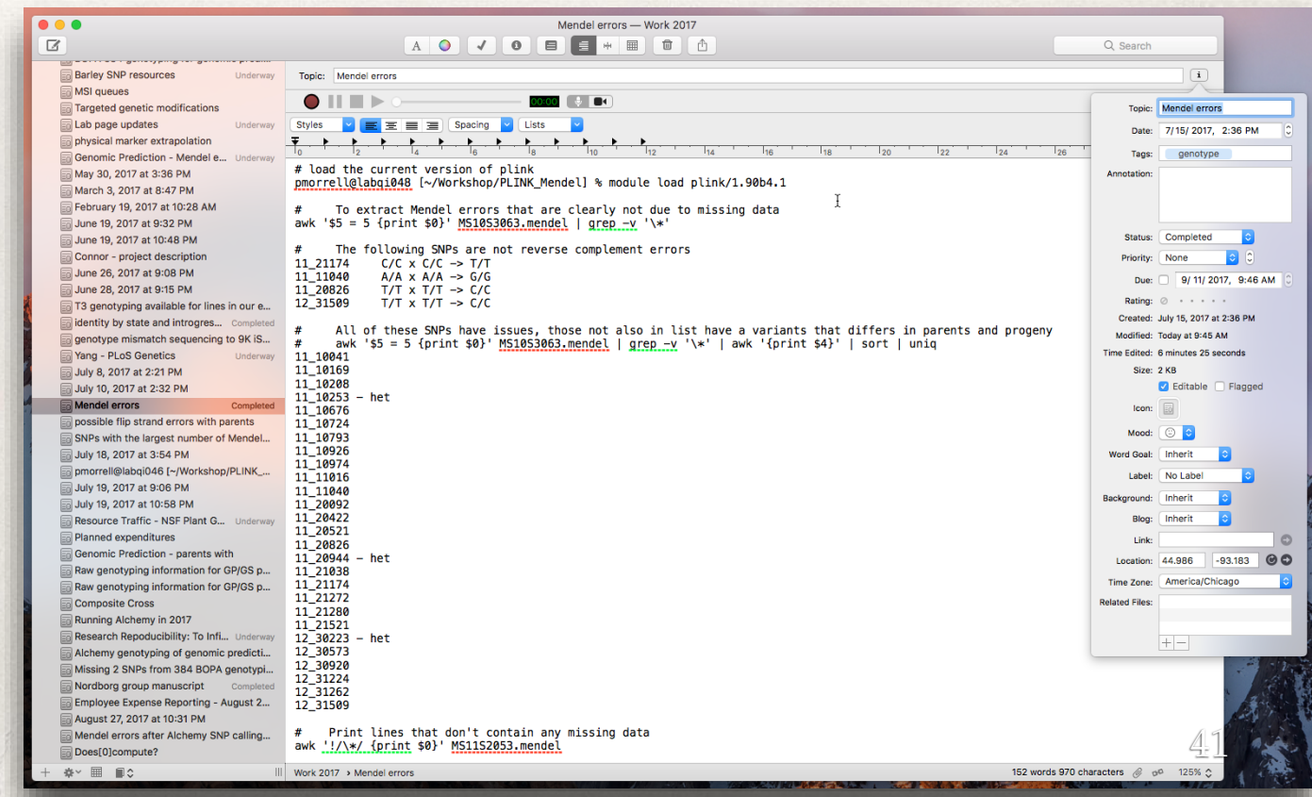
- Data needed to reproduce research
  - Variant Call Format (VCF) - millions of SNPs from 8 barley parents
  - Detailed variant annotation for each parent
- Get DOI with permanent URL to data

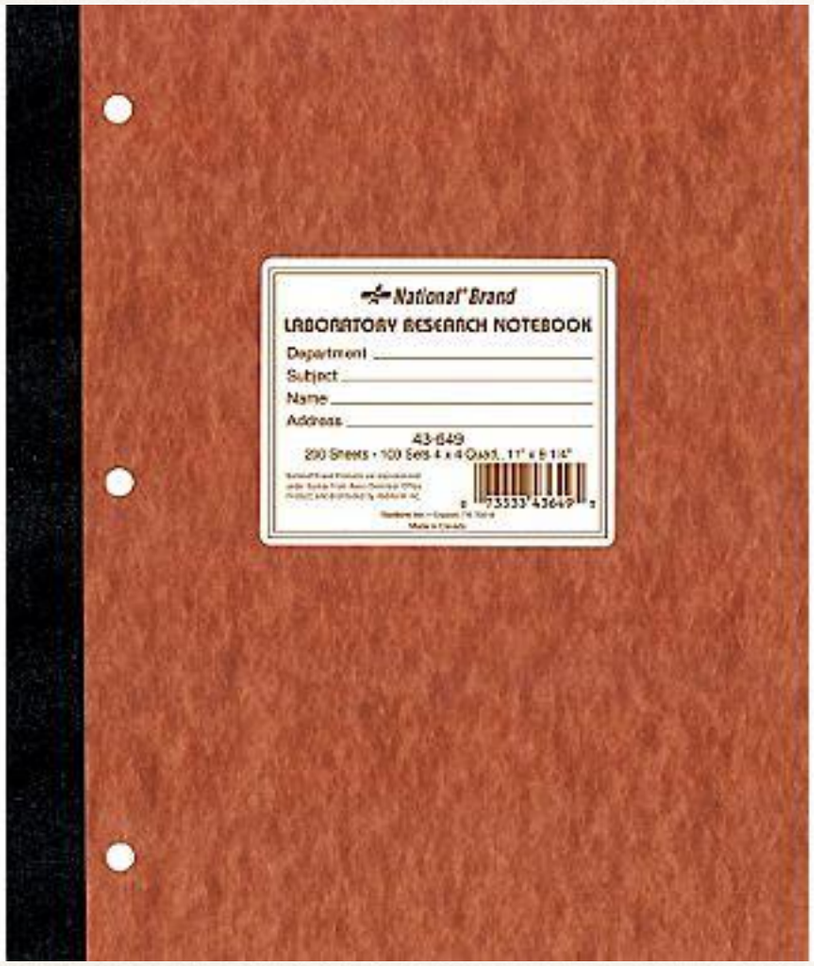


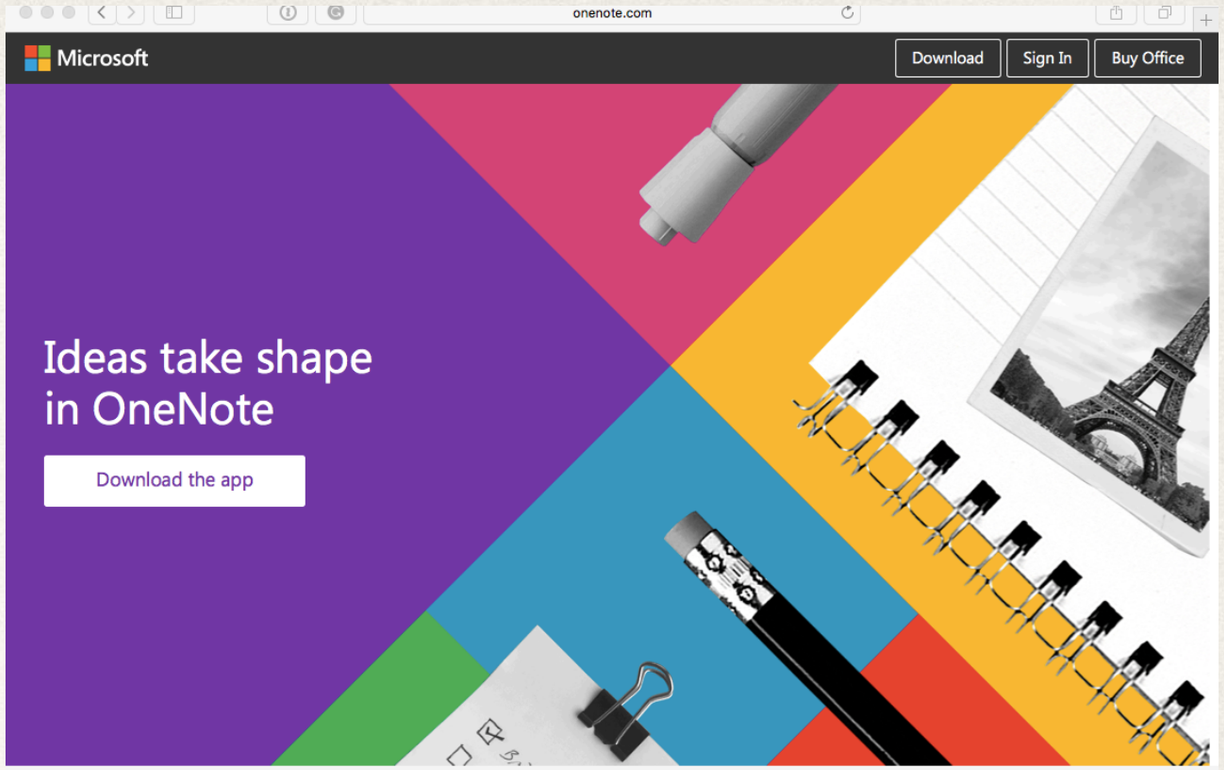
Digital notebook

- Store goals for analysis, output, code snippets
- Entries should contain as much metadata as possible









What is MacJournal?

It's journal keeping for the 21st century. Instead of paper and pen, it is your journal on your Mac. Unlike other journal applications, MacJournal is packed with features, setting it apart. You can create as many journals as you'd like: for work, home, family, or friends. You can upload your entries to your blog. Record a message or a child's first word. It is one place where you can put everything. With MacJournal you can document any life event with all the sounds, images – even video – that comes with it.

You can't do that with a notebook.

Mendel errors — Work 2017

Search

Barley SNP resources — Underway
MSI queues
Targeted genetic modifications
Lab page updates — Underway
physical marker extrapolation
Genomic Prediction - Mendel e... — Underway
May 30, 2017 at 3:36 PM
March 3, 2017 at 8:47 PM
February 19, 2017 at 10:28 AM
June 19, 2017 at 9:32 PM
June 19, 2017 at 10:48 PM
Connor - project description
June 26, 2017 at 9:08 PM
June 28, 2017 at 9:15 PM
T3 genotyping available for lines in our e...
identity by state and introgres... — Completed
genotype mismatch sequencing to 9K iS...
Yang - PLoS Genetics — Underway
July 8, 2017 at 2:21 PM
July 10, 2017 at 2:32 PM
Mendel errors — Completed
possible flip strand errors with parents
SNPs with the largest number of Mendel...
July 18, 2017 at 3:54 PM
pmorrell@labqi046 [~/Workshop/PLINK_...
July 19, 2017 at 9:06 PM
July 19, 2017 at 10:58 PM
Resource Traffic - NSF Plant G... — Underway
Planned expenditures
Genomic Prediction - parents with
Raw genotyping information for GP/GS p...
Raw genotyping information for GP/GS p...
Composite Cross
Running Alchemy in 2017
Research Repoducibility: To Infi... — Underway
Alchemy genotyping of genomic predicti...
Missing 2 SNPs from 384 BOPA genotypi...
Nordborg group manuscript — Completed
Employee Expense Reporting - August 2...
August 27, 2017 at 10:31 PM
Mendel errors after Alchemy SNP calling...
Does[0]compute?

Topic: Mendel errors

00:00

Styles | Spacing | Lists

```
# load the current version of plink
pmorrell@labqi048 [~/Workshop/PLINK_Mendel] % module load plink/1.90b4.1

#     To extract Mendel errors that are clearly not due to missing data
awk '$5 = 5 {print $0}' MS10S3063.mendel | grep -v '\*'

#     The following SNPs are not reverse complement errors
11_21174     C/C x C/C -> T/T
11_11040     A/A x A/A -> G/G
11_20826     T/T x T/T -> C/C
12_31509     T/T x T/T -> C/C

#     All of these SNPs have issues, those not also in list have a variants that differs in parents and progeny
#     awk '$5 = 5 {print $0}' MS10S3063.mendel | grep -v '\*' | awk '{print $4}' | sort | uniq
11_10041
11_10169
11_10208
11_10253 - het
11_10676
11_10724
11_10793
11_10926
11_10974
11_11016
11_11040
11_20092
11_20422
11_20521
11_20826
11_20944 - het
11_21038
11_21174
11_21272
11_21280
11_21521
12_30223 - het
12_30573
12_30920
12_31224
12_31262
12_31509

#    Print lines that don't contain any missing data
awk '!/\*/ {print $0}' MS11S2053.mendel
```

Topic: Mendel errors
Date: 7/15/ 2017, 2:36 PM
Tags: genotype
Annotation:
Status: Completed
Priority: None
Due: 9/ 11/ 2017, 9:46 AM
Rating:
Created: July 15, 2017 at 2:36 PM
Modified: Today at 9:45 AM
Time Edited: 6 minutes 25 seconds
Size: 2 KB
Editable Flagged
Icon:
Mood:
Word Goal: Inherit
Label: No Label
Background: Inherit
Blog: Inherit
Link:
Location: 44.986 -93.183
Time Zone: America/Chicago
Related Files:

Work 2017 › Mendel errors — 152 words 970 characters — 125%

Take home message

With disorder it is difficult to replicate your results, whereas order and documentation allows you and others to confirm your conclusions

Broader Impacts