BGGN 213

Find a Gene Assignment

Lecture 10

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http://thegrantlab.org/bggn213

[MPA Responses]

[Kevin’s StackExchange Link]

Thanks Kevin!

Find-a-Gene Project Assignment

- A total of 20% of the course grade will be assigned based on the "find-a-gene project assignment"
- The objective with this assignment is for you to demonstrate your grasp of database searching, sequence analysis, structure analysis and the R environment that we have covered to date in class.
- You may wish to consult the scoring rubric (in the linked project description) and the example report for format and content guidance.
  - ➡ Your responses to questions Q1-Q4 are due at the beginning of class Friday May 18th (05/18/18).
  - ➡ The complete assignment, including responses to all questions, is due at the beginning of class Wed June 1st (06/01/18).

Questions:

[Q1] Tell me the name of a protein you are interested in. Include the species and the accession number. This can be a human protein or a protein from any other species as long as it's function is known.

If you do not have a favorite protein, select human RBP4 or KIF11. Do not use beta globin as this is in the worked example report that I provide you with online.

[Q2] Perform a BLAST search against a DNA database, such as a database consisting of genomic DNA or ESTs. The BLAST server can be at NCBI or elsewhere. Include details of the BLAST method used, database searched and any limits applied (e.g. Organism).

Also include the output of that BLAST search in your document. If appropriate, change the font to `Courier size 10` so that the results are displayed neatly. You can also screen capture a BLAST output (e.g. alt print screen on a PC or on a MAC press ⌘-shift-4. The pointer becomes a bulls eye. Select the area you wish to capture and release. The image is saved as a file called `Screen Shot [].png` in your Desktop directory). It is **not** necessary to print out all of the blast results if there are many pages.

On the BLAST results, clearly indicate a match that represents a protein sequence, encoded from some DNA sequence, that is homologous to your query protein. I need to be able to inspect the pairwise alignment you have selected, including the E value and score. It should be labeled a "genomic clone" or "mRNA sequence", etc. - but include no functional annotation.

In general, [Q2] is the most difficult for students because it requires you to have a “feel” for how to interpret BLAST results. You need to distinguish between a perfect match to your query (i.e. a sequence that is not “novel”), a near match (something that might be “novel”, depending on the results of [Q4]), and a non-homologous result.

If you are having trouble finding a novel gene try restricting your search to an organism that is poorly annotated.

[Q3] Gather information about this “novel” **protein**. At a minimum, show me the protein sequence of the “novel” protein as displayed in your BLAST results from [Q2] as FASTA format (you can copy and paste the aligned sequence subject lines from your BLAST result page if necessary) or translate your novel DNA sequence using a tool called EMBOSS Transeq at the EBI. Don’t forget to translate all six reading frames; the ORF (open reading frame) is likely to be the longest sequence without a stop codon. It may not start with a methionine if you don’t have the complete coding region. Make sure the sequence you provide includes a header/subject line and is in traditional FASTA format.

Here, tell me the name of the novel protein, and the species from which it derives. It is very unlikely (but still definitely possible) that you will find a novel gene from an organism such as *S. cerevisiae*, human or mouse, because those genomes have already been thoroughly annotated. It is more likely that you will discover a new gene in a genome that is currently being sequenced, such as bacteria or plants or protozoa.

[Q4] Prove that this gene, and its corresponding protein, are novel. For the purposes of this project, “novel” is defined as follows. Take the protein sequence (your answer to [Q3]), and use it as a query in a blastp search of the nr database at NCBI.

- If there is a match with 100% amino acid identity to a protein in the database, from the same species, then your protein is NOT novel (even if the match is to a protein with a name such as “unknown”). Someone has already found and annotated this sequence, and assigned it an accession number.
- If the top match reported has less than 100% identity, then it is likely that your protein is novel, and you have succeeded.
- If there is a match with 100% identity, but to a different species than the one you started with, then you have likely succeeded in finding a novel gene.
- If there are no database matches to the original query from [Q1], this indicates that you have partially succeeded: yes, you may have found a new gene, but no, it is not actually homologous to the original query. You should probably start over.

[Q5] Generate a multiple sequence alignment with your novel protein, your original query protein, and a group of other members of this family from different species. A typical number of proteins to use in a multiple sequence alignment for this assignment purpose is a minimum of 5 and a maximum of 20 - although the exact number is up to you. Include the multiple sequence alignment in your report. Use Courier font with a size appropriate to fit page width.

Side-note: Indicate your sequence in the alignment by choosing an appropriate name for each sequence in the input unaligned sequence file (i.e. edit the sequence file so that the species, or short common, names (rather than accession numbers) display in the output alignment and in the subsequent answers below). The goal in this step is to create an interesting an alignment for building a phylogenetic tree that illustrates species divergence.


git

What is Git?

(1) An unpleasant or contemptible person. Often incompetent, annoying, senile, elderly or childish in character.

(2) A modern distributed version control system with an emphasis on speed and data integrity.

What is Git?

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(2) A modern distributed version control system with an emphasis on speed and data integrity.

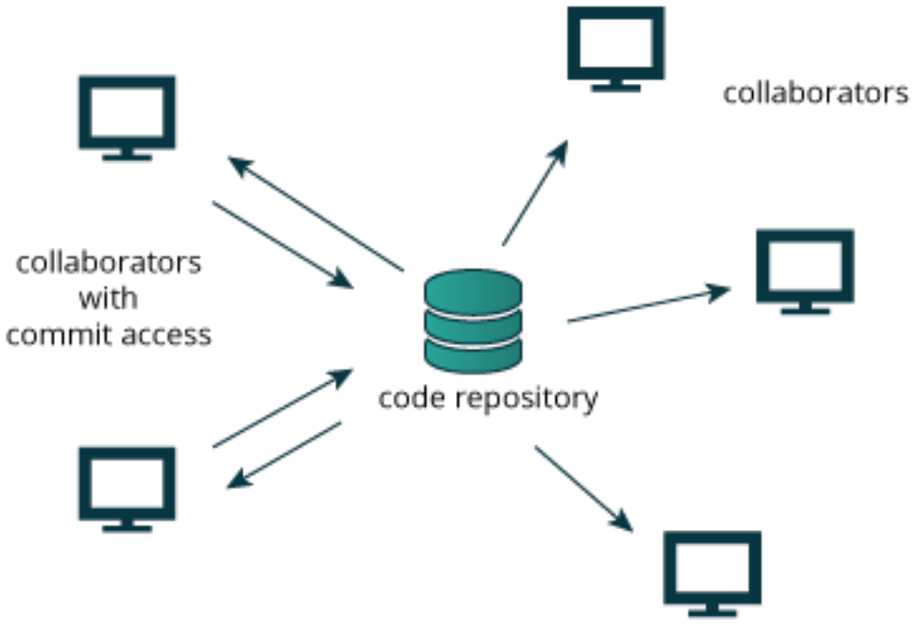
Version Control

Version control systems (VCS) record changes to a file or set of files over time so that you can recall specific versions later.

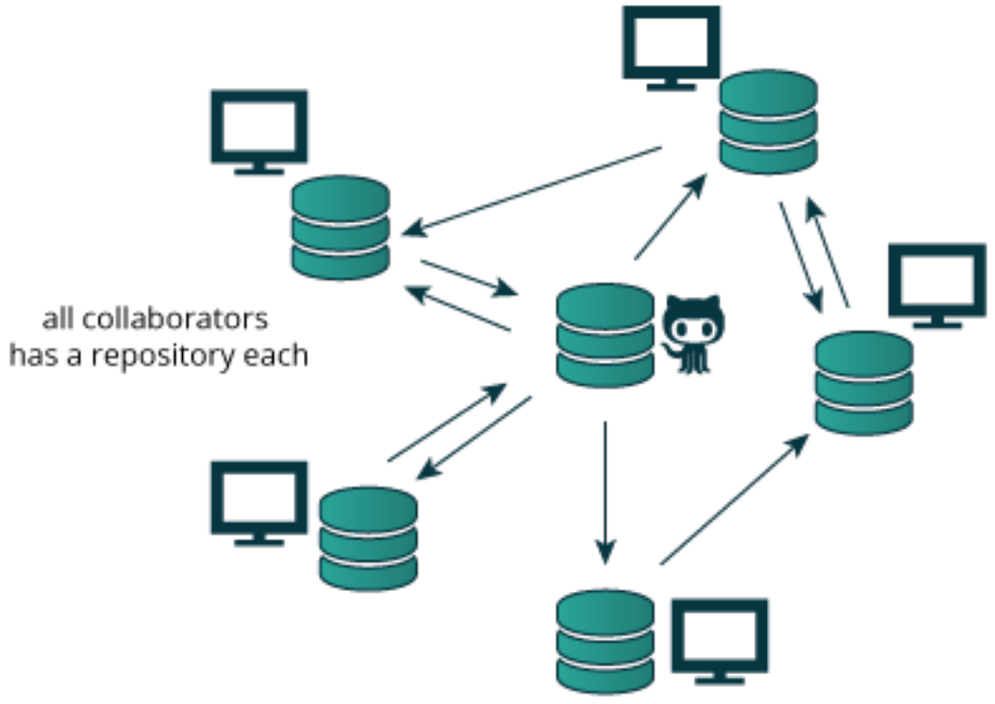
Client–server	**Free/open-source**	CVS (1986, 1990 in C) · CVSNT (1998) · QVCS Enterprise (1998) · Subversion (2000)
	Proprietary	Software Change Manager (1970s) · Panvalet (1970s) · Endevor (1980s) · Dimensions CM (1980s) · *DSEE* (1984) · Synergy (1990) · ClearCase (1992) · *CMVC* (1994) · *Visual SourceSafe* (1994) · Perforce (1995) · StarTeam (1995) · Integrity (2001) · Surround SCM (2002) · AccuRev SCM (2002) · SourceAnywhere (2003) · Vault (2003) · Team Foundation Server (2005) · Team Concert (2008)
Distributed	**Free/open-source**	*GNU arch* (2001) · Darcs (2002) · *DCVS* (2002) · *ArX* (2003) · Monotone (2003) · *SVK* (2003) · *Codeville* (2005) · Bazaar (2005) · **Git** (2005) · Mercurial (2005) · Fossil (2007) · Veracity (2010)
	Proprietary	*TeamWare* (1990s?) · Code Co-op (1997) · BitKeeper (1998) · Plastic SCM (2006)

There are many VCS available, see:
https://en.wikipedia.org/wiki/Revision_control

Client-Server *vs* Distributed VCS

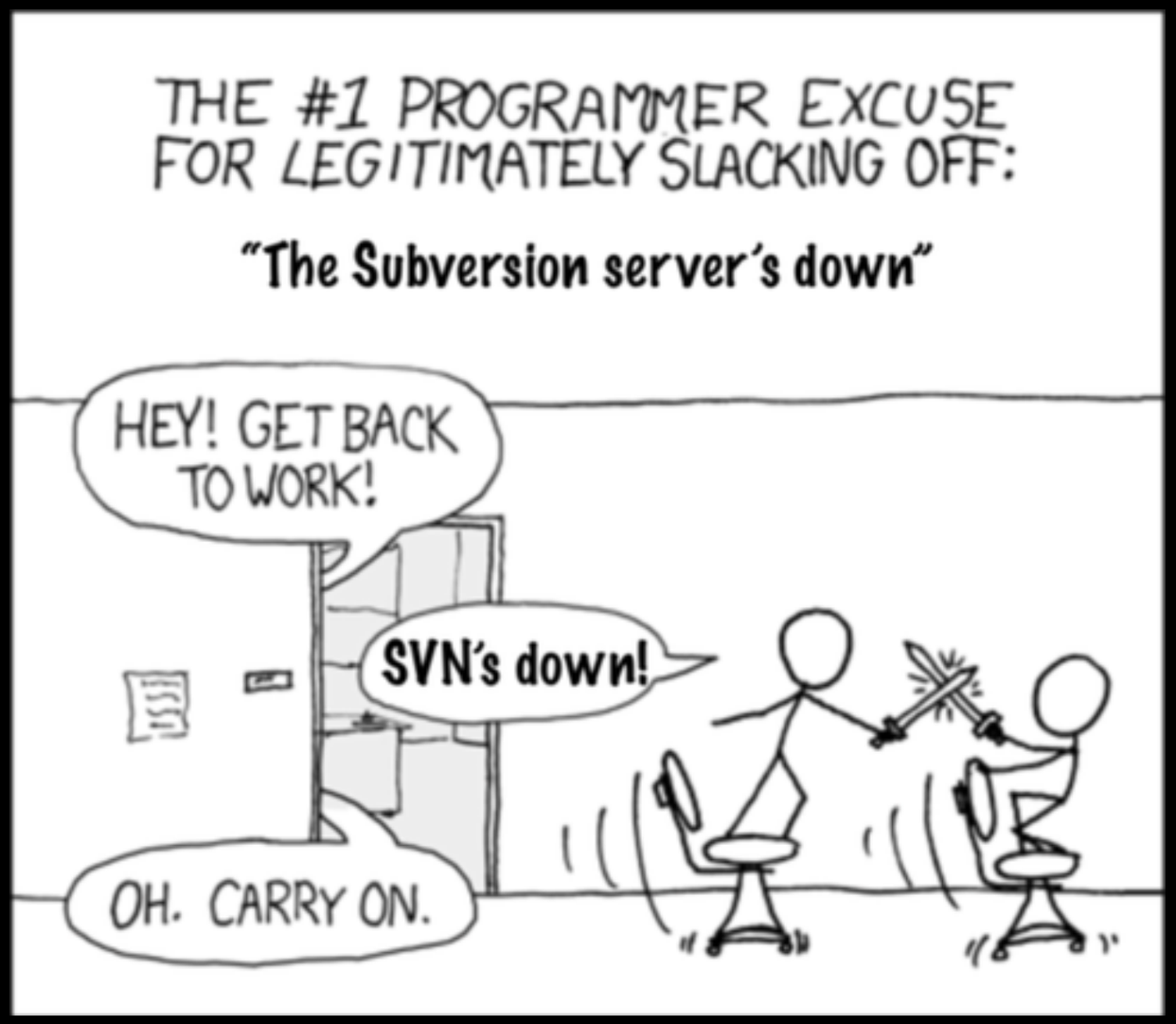


Client-server approach



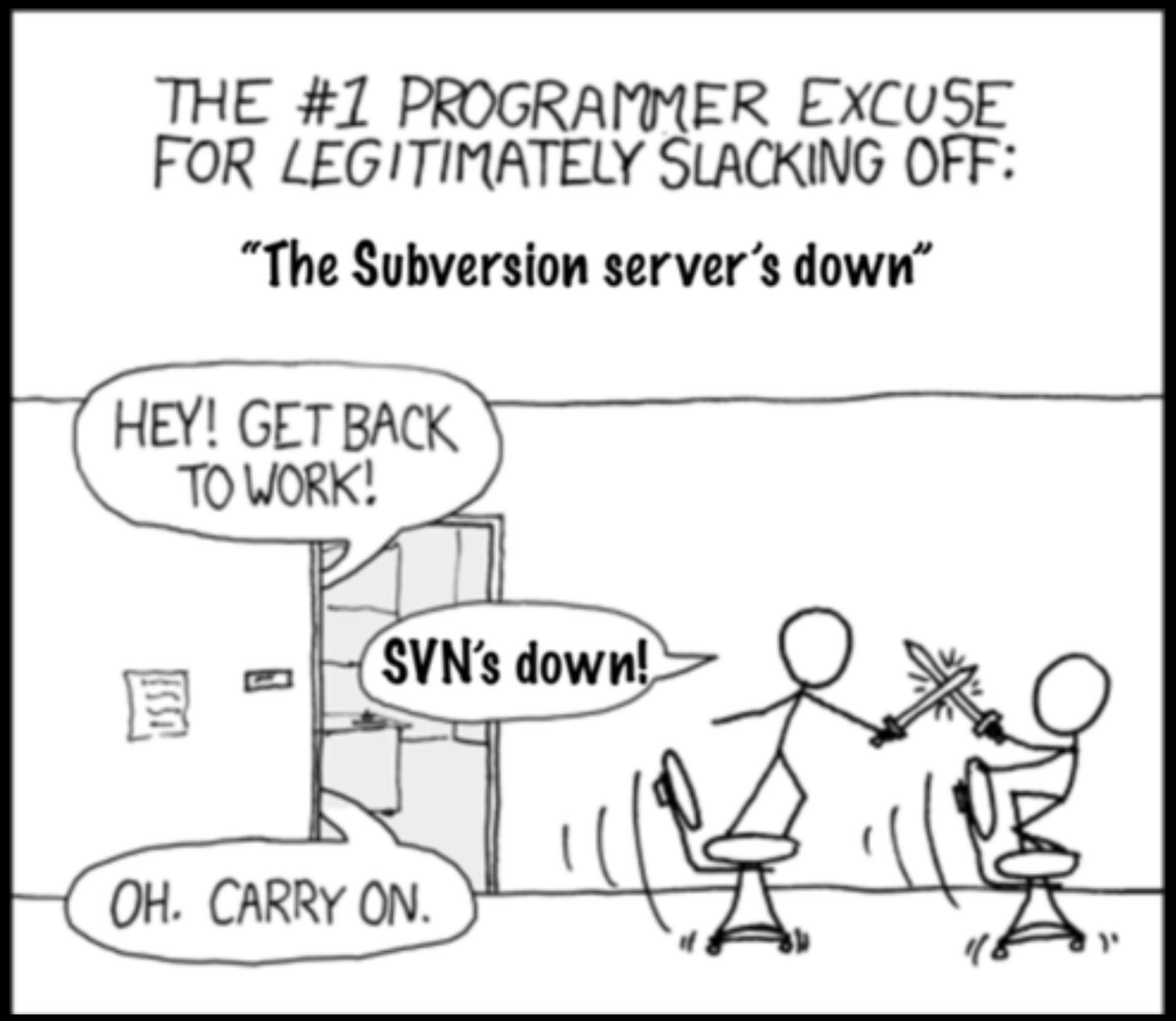
Distributed approach

Distributed version control systems (DCVS) allows multiple people to work on a given project without requiring them to share a common network.



http://tinyurl.com/distributed-advantages

Git is now the most popular free VCS!



Git offers:

- Speed
- Backups
- Off-line access
- Small footprint
- Simplicity*
- Social coding

http://tinyurl.com/distributed-advantages

Where did Git come from?

Written initially by Linus Torvalds to support Linux kernel and OS development.

Meant to be distributed, fast and more natural.

Capable of handling large projects.

Now the most popular free VCS!

Why use Git?

Q. Would you write your lab book in pencil, then erase and overwrite it every day with new content?

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Version control is the lab notebook of the digital world: it's what professionals use to keep track of what they've done and to collaborate with others.

Why use Git?

- Provides '**snapshots**' of your project during development and provides a full record of project **history**.
- Allows you to easily **reproduce** and **rollback** to past versions of analysis and compare differences. (N.B. Helps fix software regression bugs!)
- Keeps **track of changes** to code you use from others such as fixed bugs & new features
- Provides a mechanism for sharing, updating and collaborating (like a social network)
- Helps keep your work and software organized and available

Obtaining Git

Note: You might already have git installed
To check open the "**Terminal**" tab in RStudio and type:

```
which git
```

Obtaining Git

Do it Yourself!

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```
which git
```

Installing Git

Windows
Follow the GitBash instructions here:
https://bioboot.github.io/bimm143_S18/setup/

Mac & Linux
Download git directly from here:
https://git-scm.com/downloads

Configuring Git

Do it Yourself!

Configuring Git

(RStudio **Terminal** Tab)

(…or *RStudio* **>** *Tools* **>** *Shell*)

```
# First tell Git who you are
> git config --global user.name  “Barry Grant”
> git config --global user.email  “bjgrant@ucsd.edu”
```

Using Git

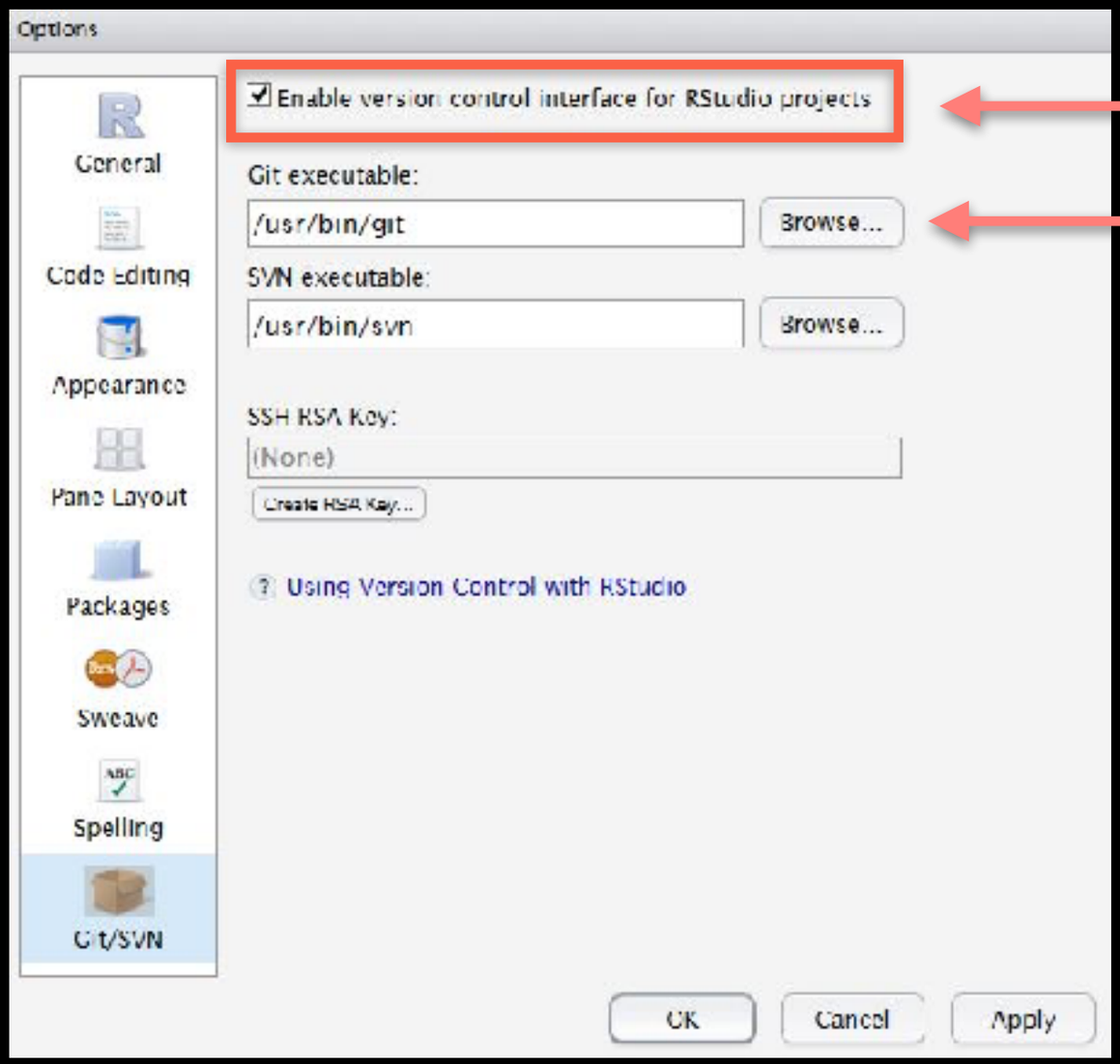
Using Git

1. Initiate a Git repository.
2. Edit content (i.e. change some files).
3. Store a 'snapshot' of the current file state.*

Using Git with RStudio

Do it Yourself!

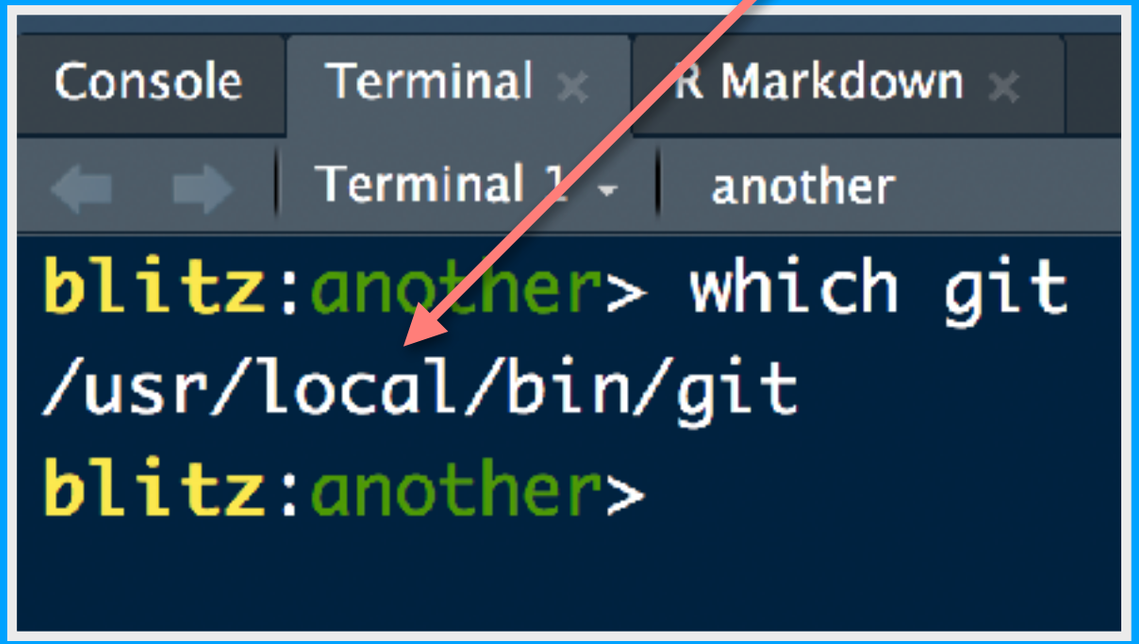
Go to: **RStudio > Tools > Global Options > Git/SVN**



1 *Make sure this is **ticked**!*

2 Make sure this is **correct**!

Checkin your RStudio "Terminal" tab:

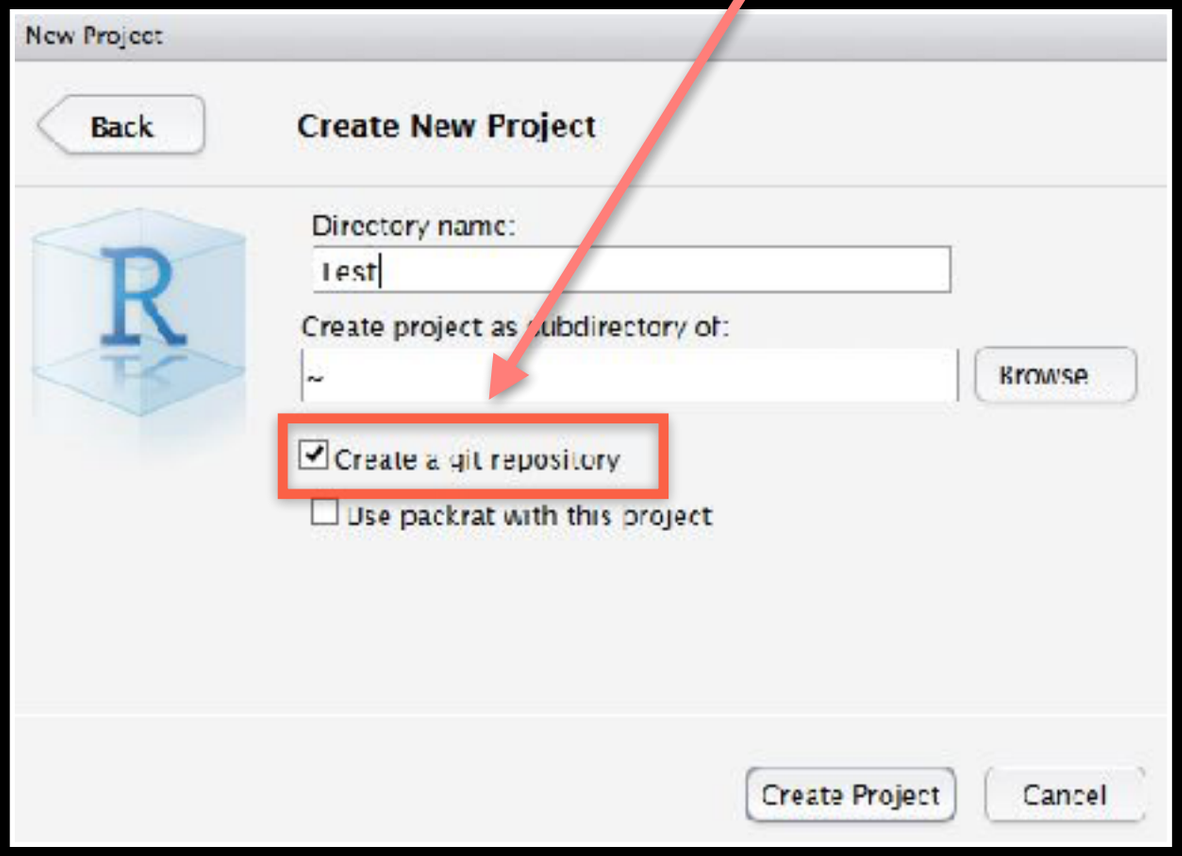


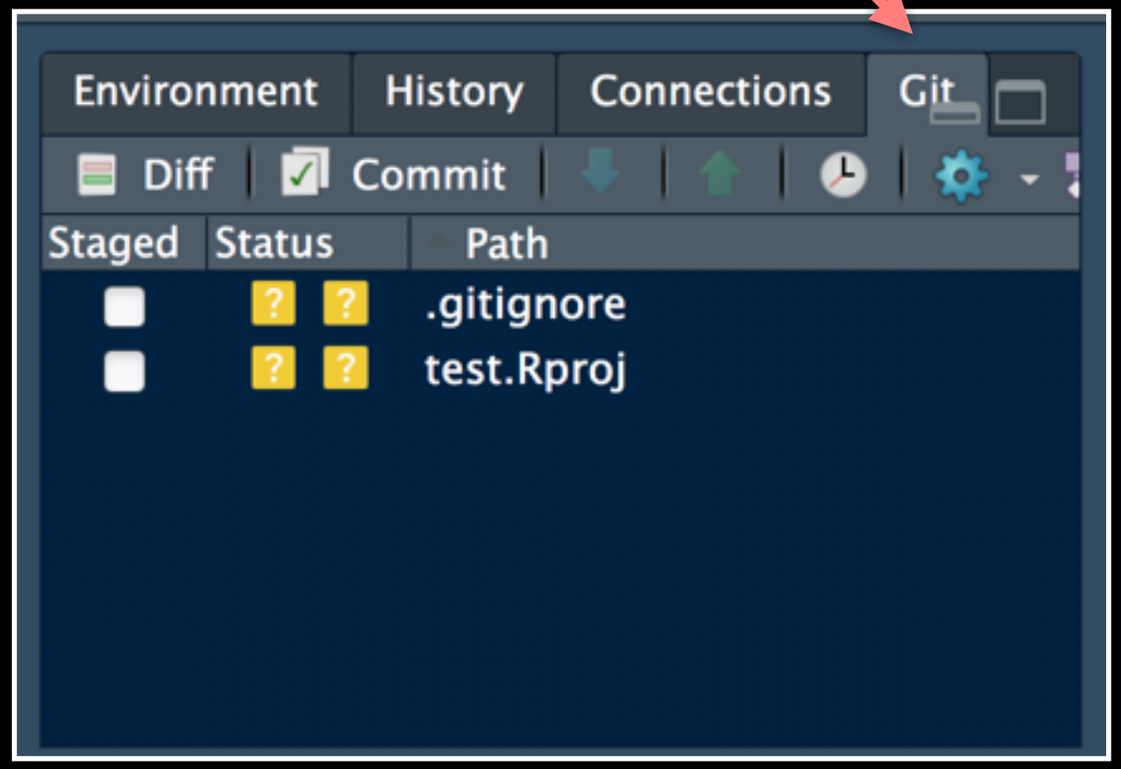
Create a new RStudio project

Do it Yourself!

1 New option to create a Git repository…

2 New Git tab…

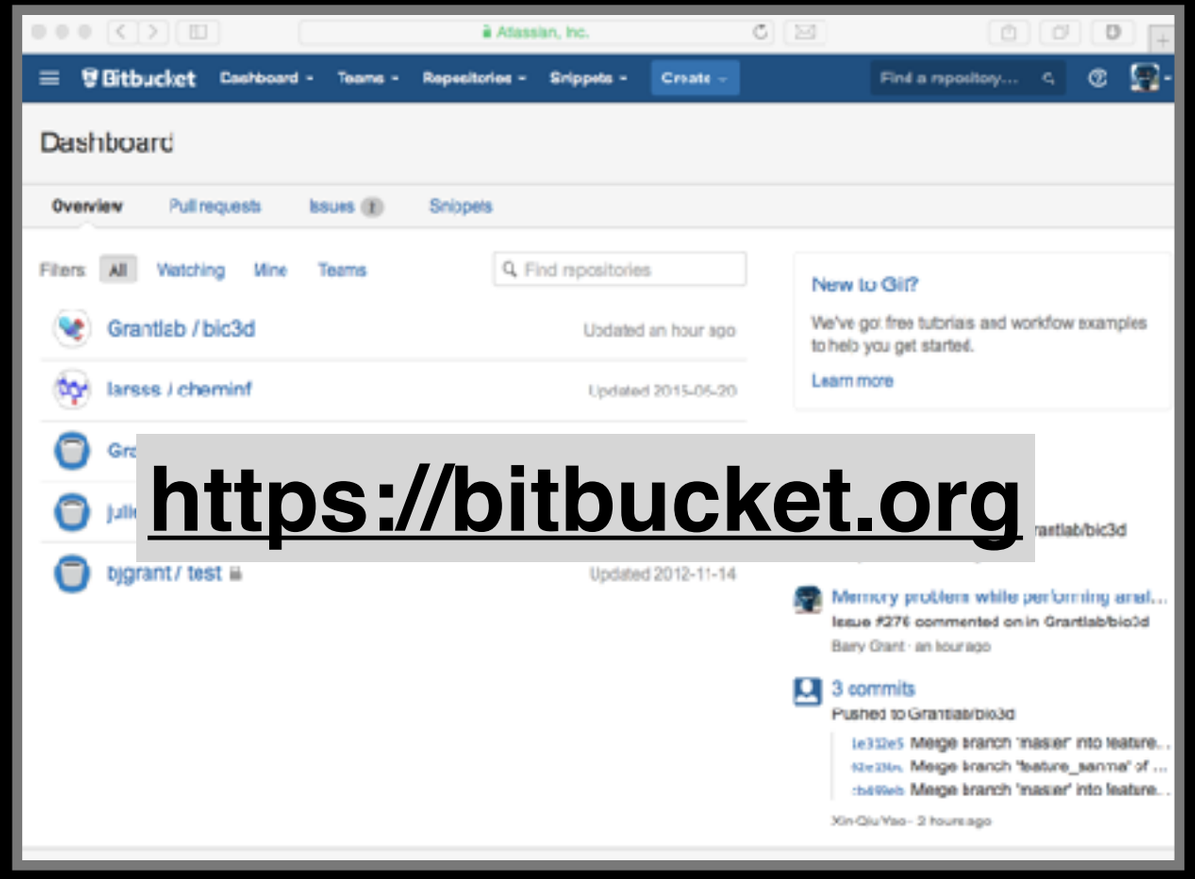




Check if new Git options appear in RStudio?

GitHub & Bitbucket

GitHub and **Bitbucket** are two popular hosting services for Git repositories. These services allow you to share your projects and collaborate with others using both '**public**' and '**private**' repositories*.



wsj.com

Nikkei 17893.73 0.49% | Hang Seng 21404.96 0.72% | U.S. 10 Yr -0/32 Yield 2.074% | Crude Oil 39.17 -0.36% | Yen 119.16 0.26% | EXPAND

THE WALL STREET JOURNAL.

Workers Get New Tools for Airing Their Gripes

Cell Carriers Battle for Wi-Fi Airwaves

Snapchat Names ex-Mattel Exec Vollero Its Finance Chief

TECH

GitHub Raises $250 Million at $2 Billion Valuation

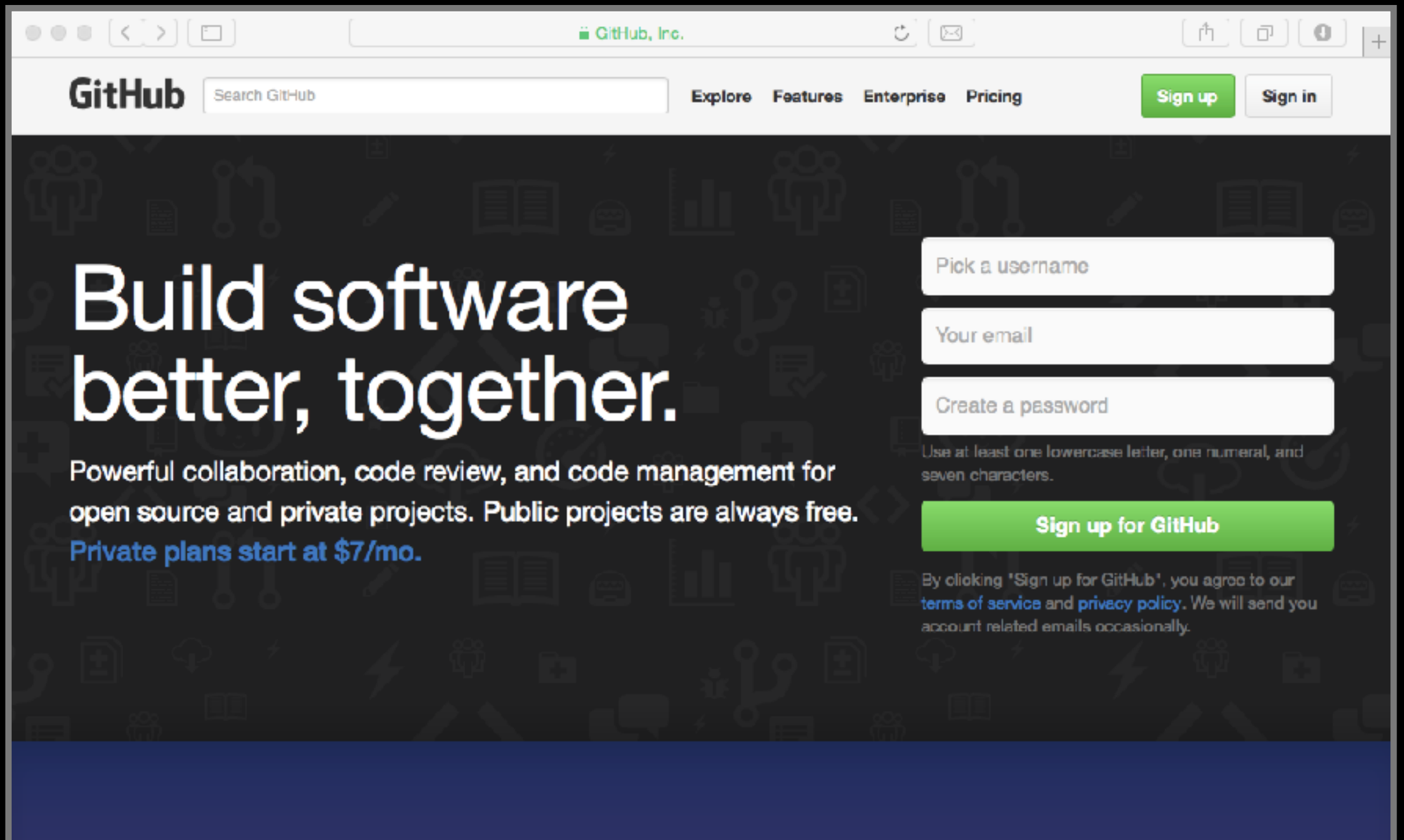
Capital raise puts company's total funding at $350 million

What is the big deal?

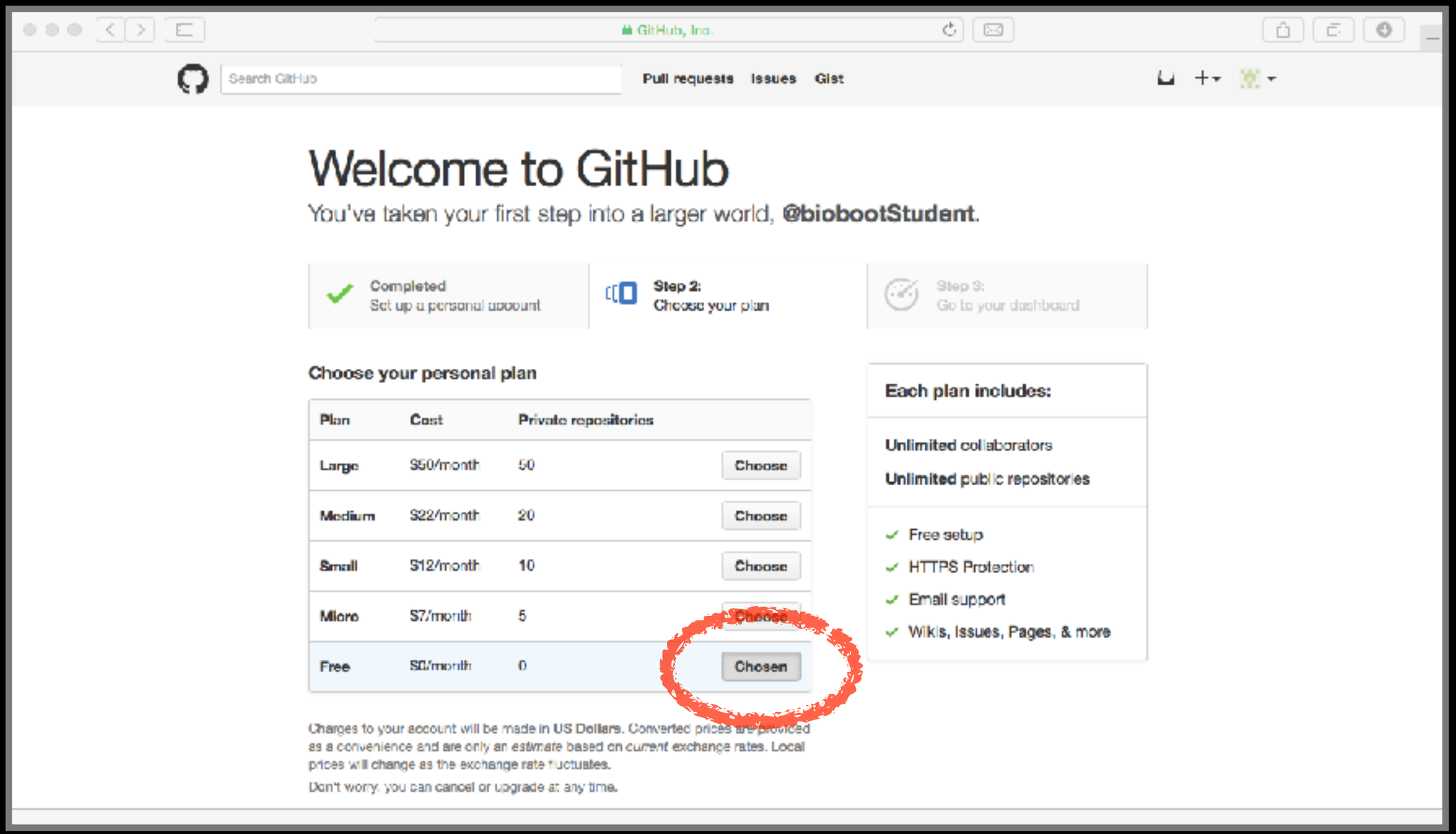
- At the simplest level GitHub and Bitbucket offer **backup** of your projects history and a centralized mechanism for **sharing** with others by putting **your Git repo online**.
    - GitHub in particular is often referred to as the “nerds FaceBook and LinkedIn combined”.
- At their core both services **offer a new paradigm for open collaborative project development**, particularly for software.
    - In essence they allow <u>anybody to contribute</u> to any public project and get acknowledgment.

First sign up for a GitHub account

https://github.com

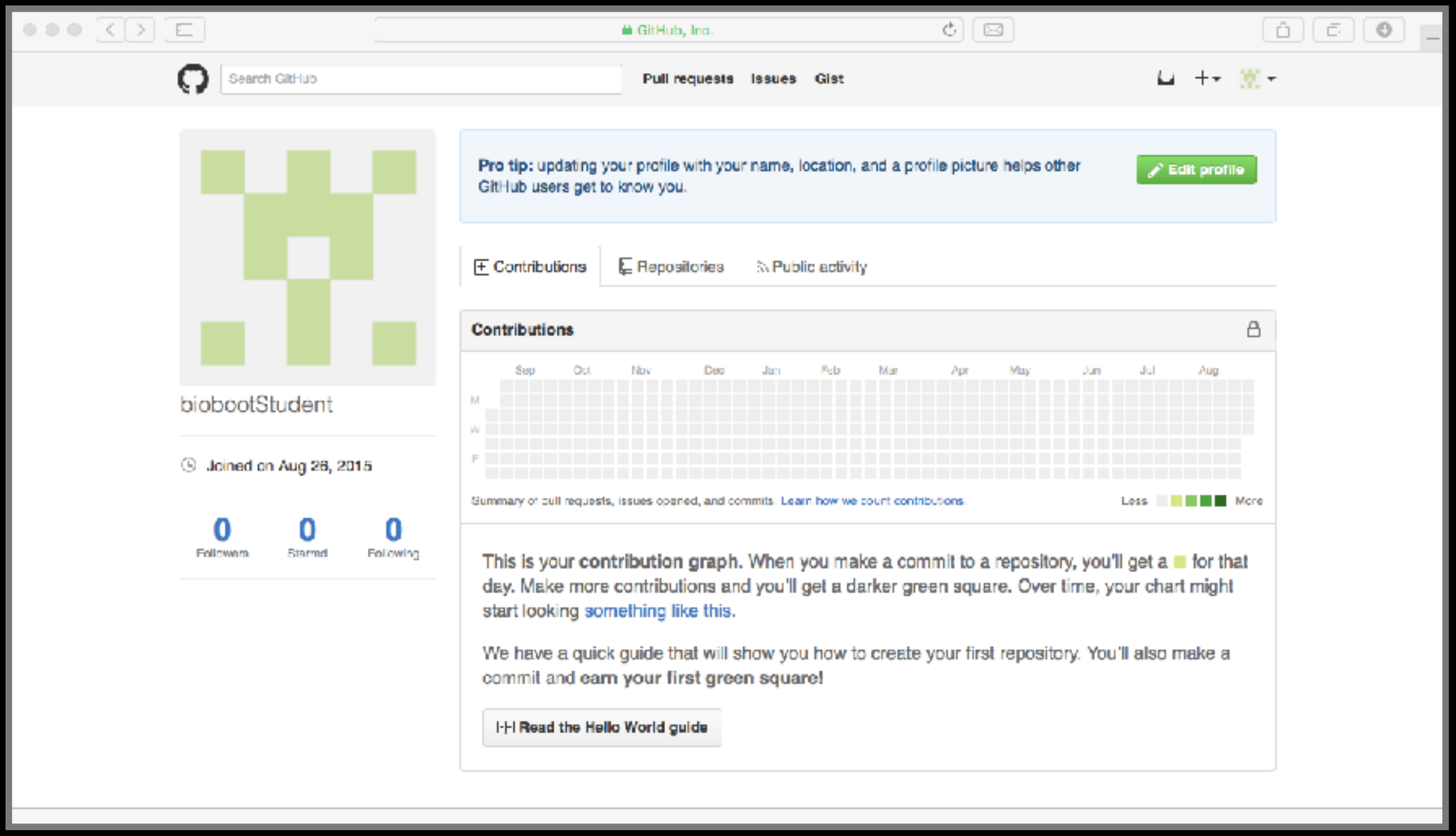


Pick the FREE plan!



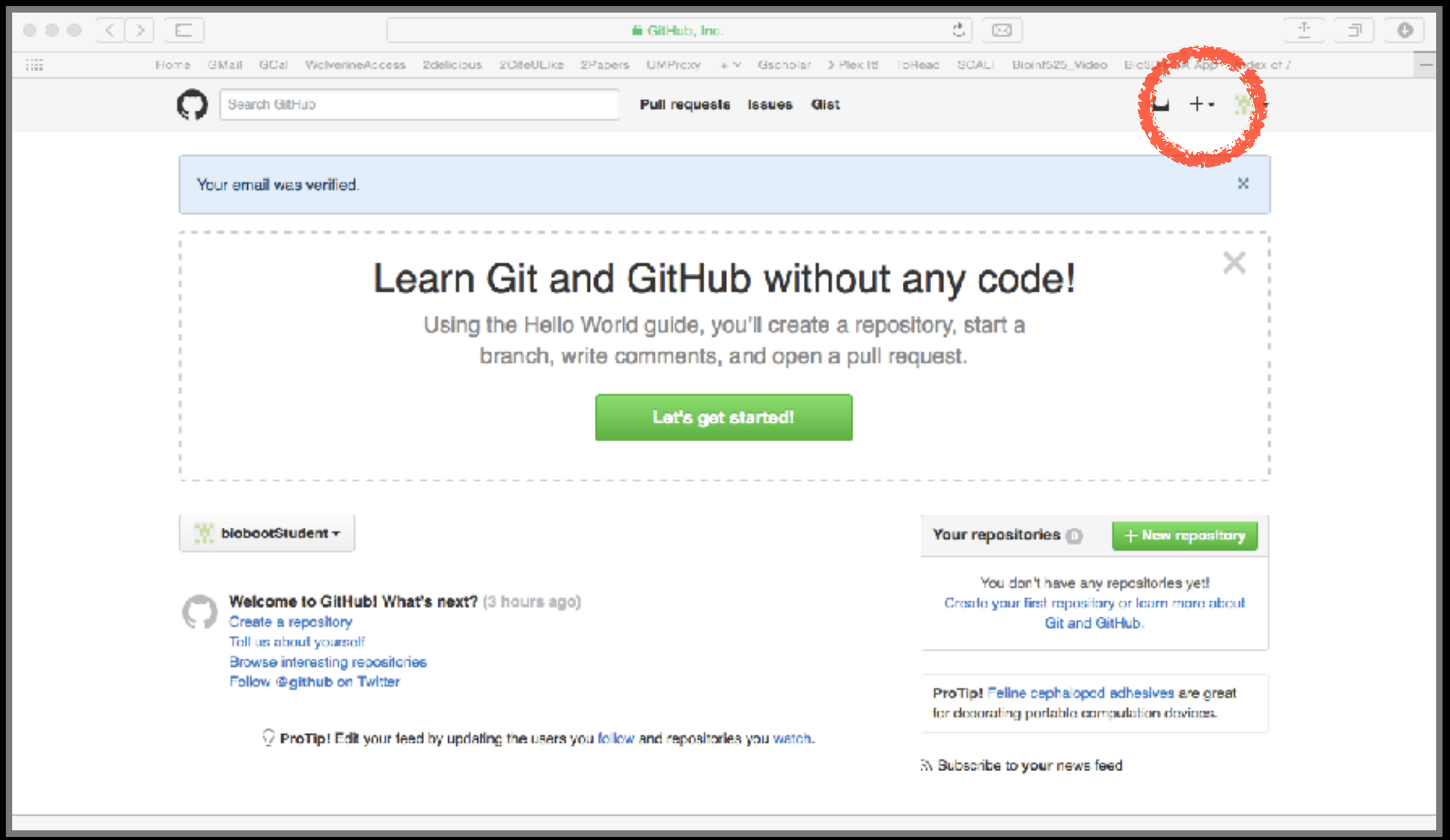
Your GitHub homepage

Check your email for verification request



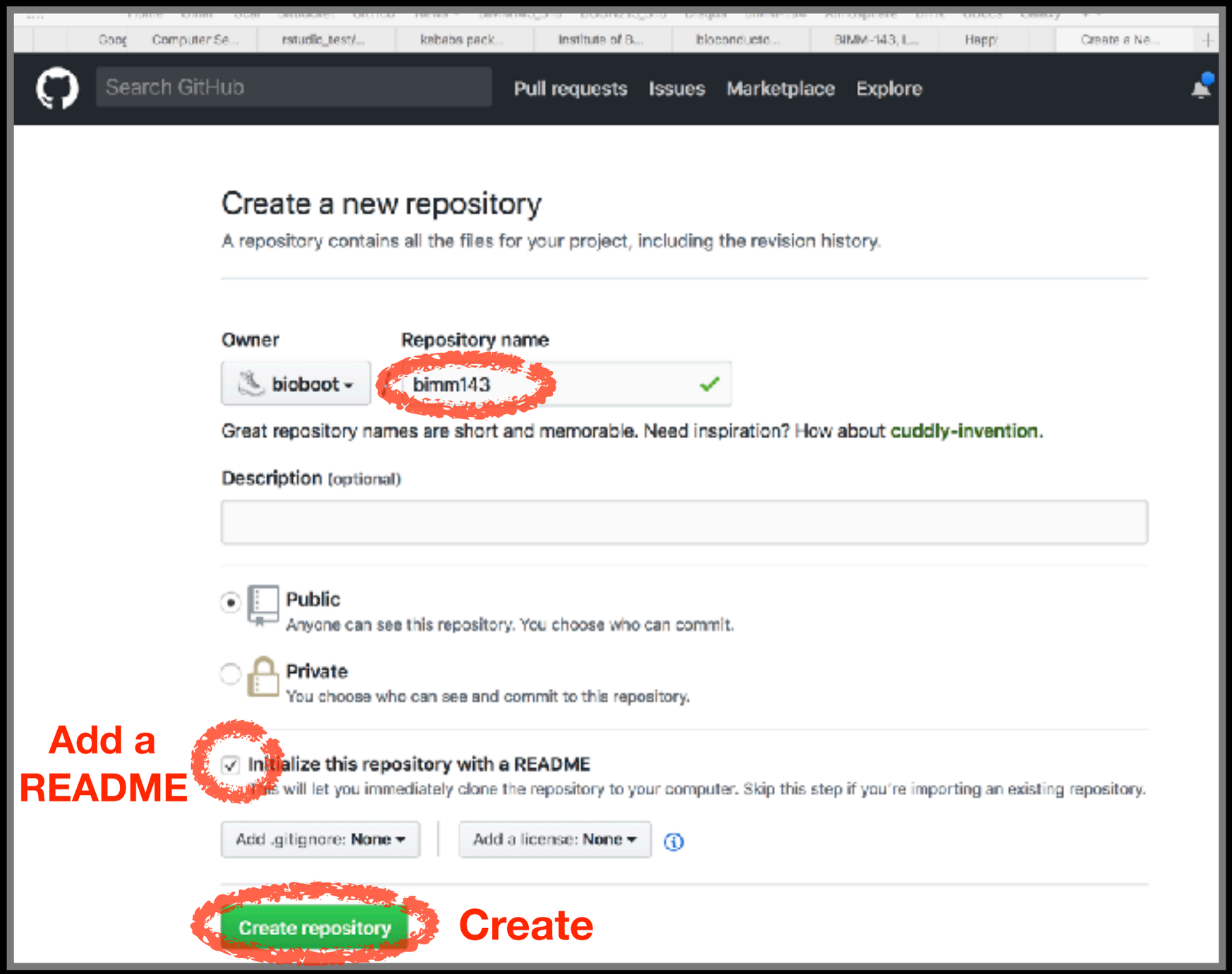
Skip the hello-world tutorial

https://guides.github.com/activities/hello-world/

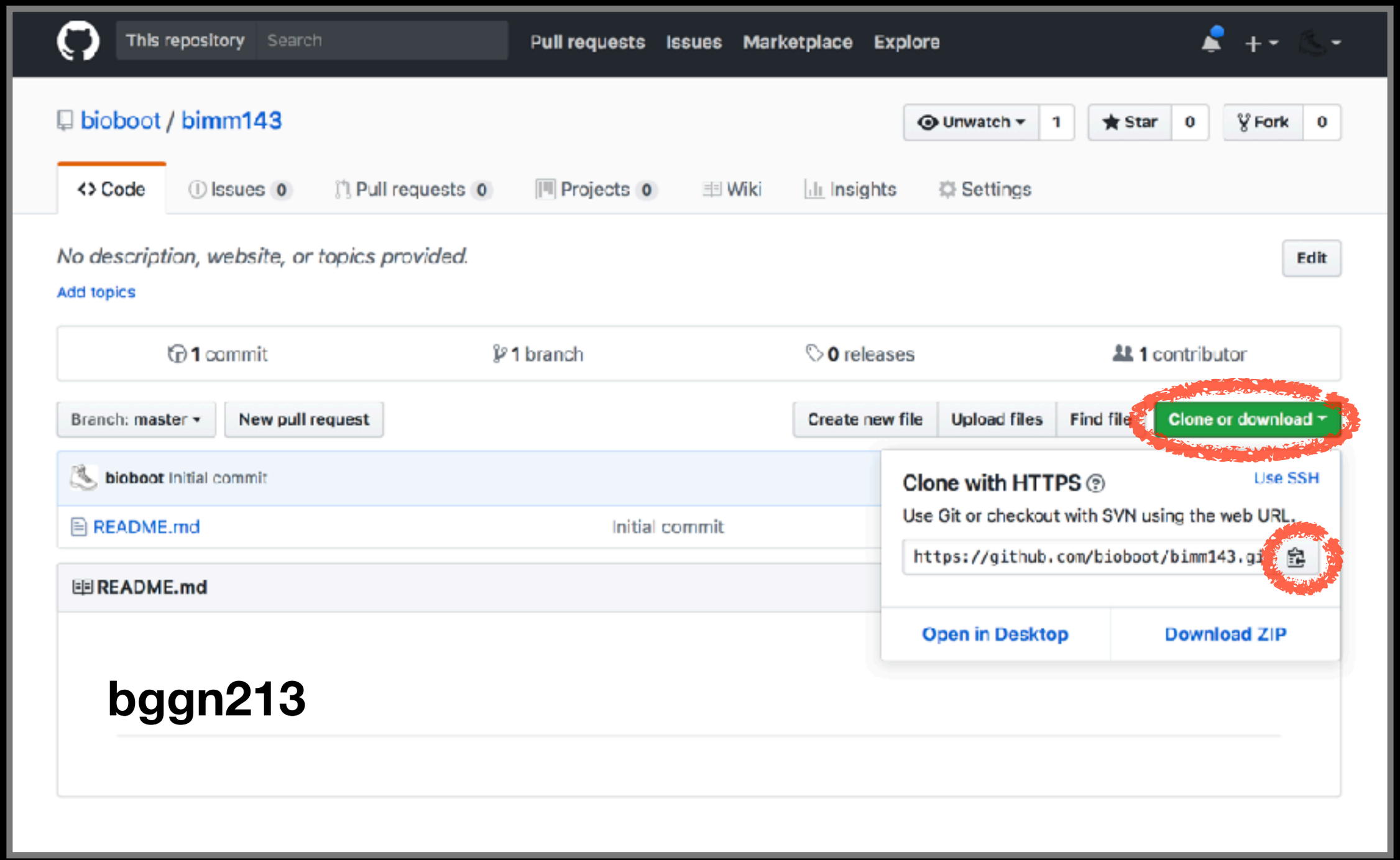


Name your repo

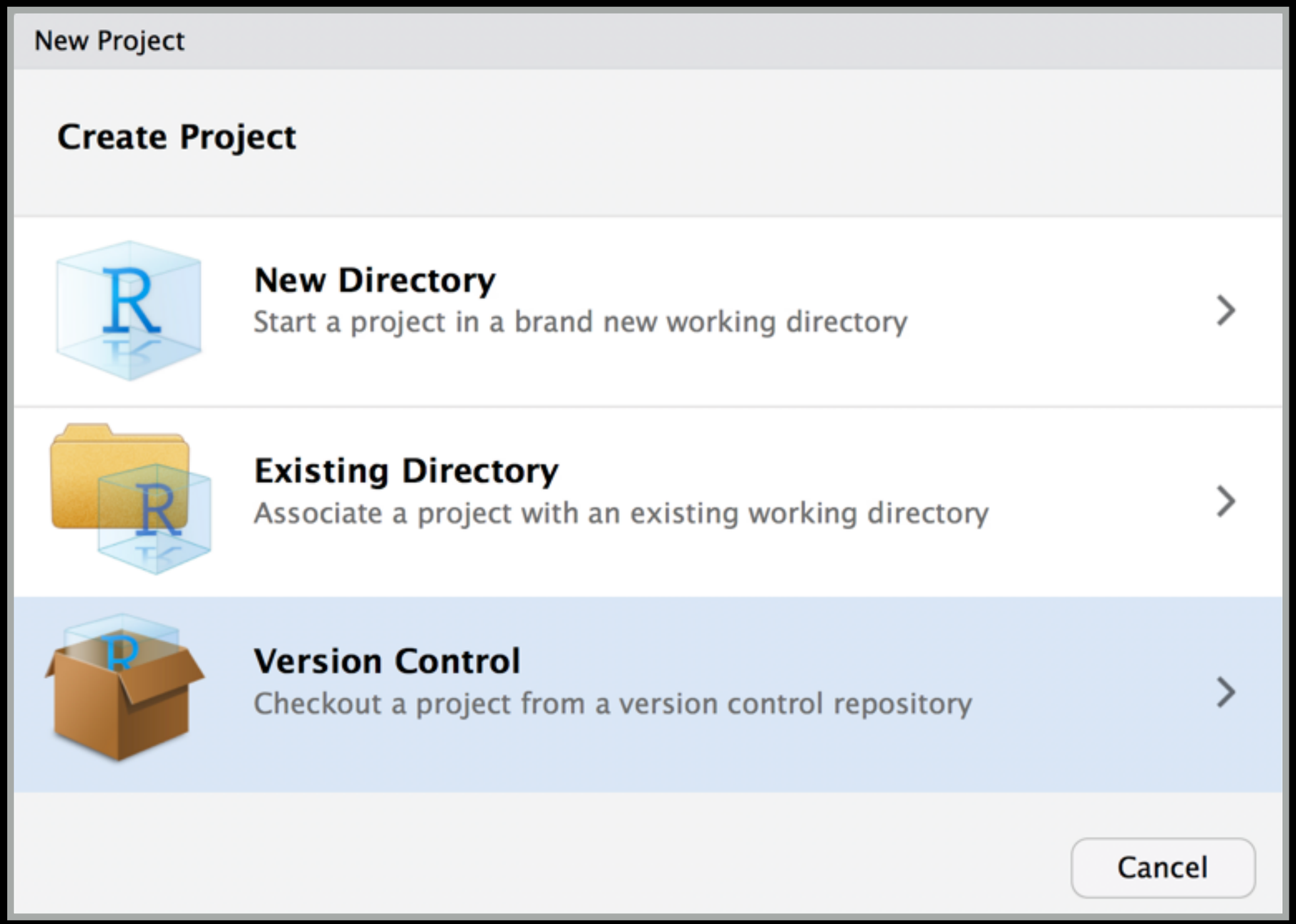
bggn213



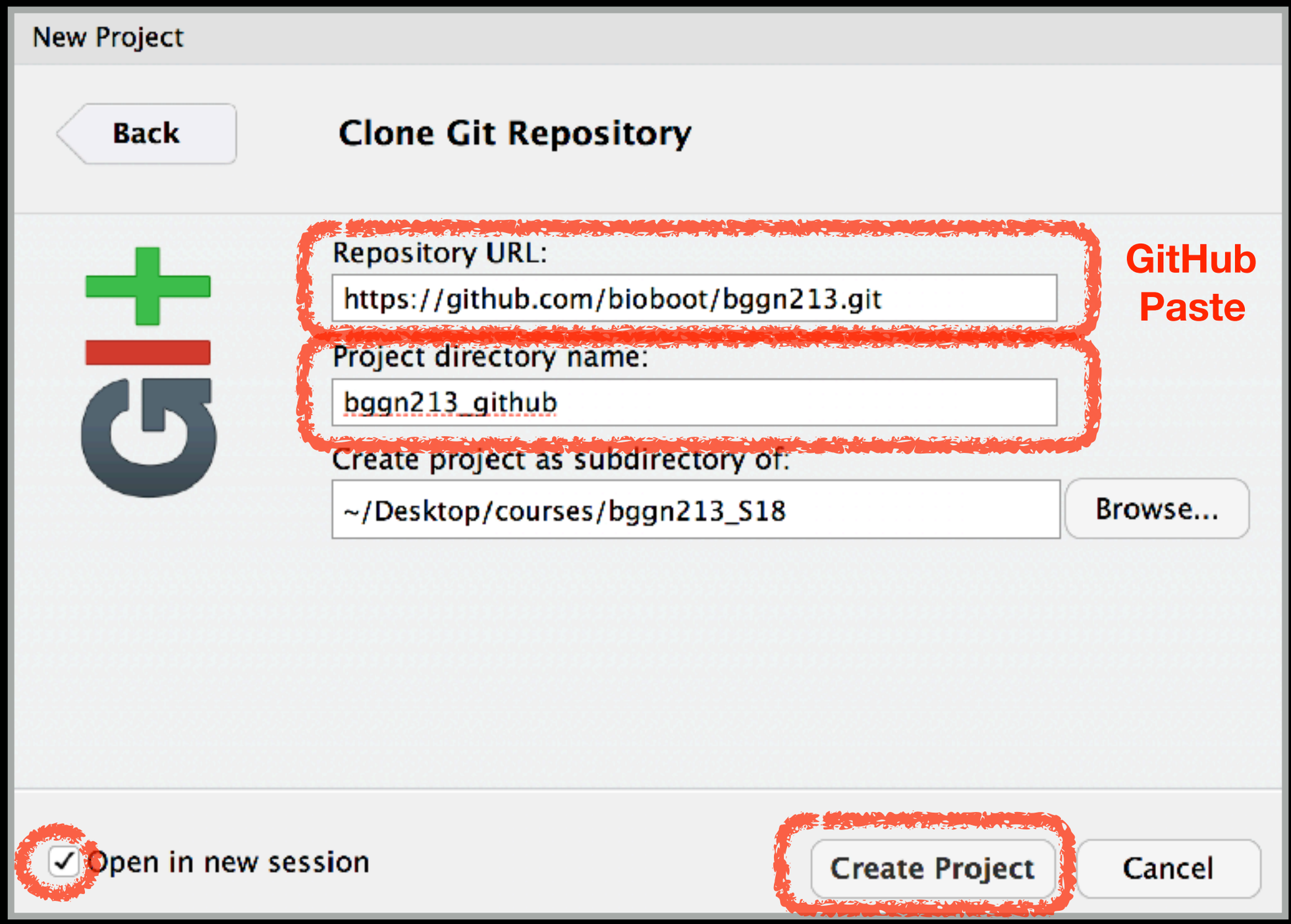
Copy the "**Clone**" HTTPS link



RStudio > New Project > Version Control

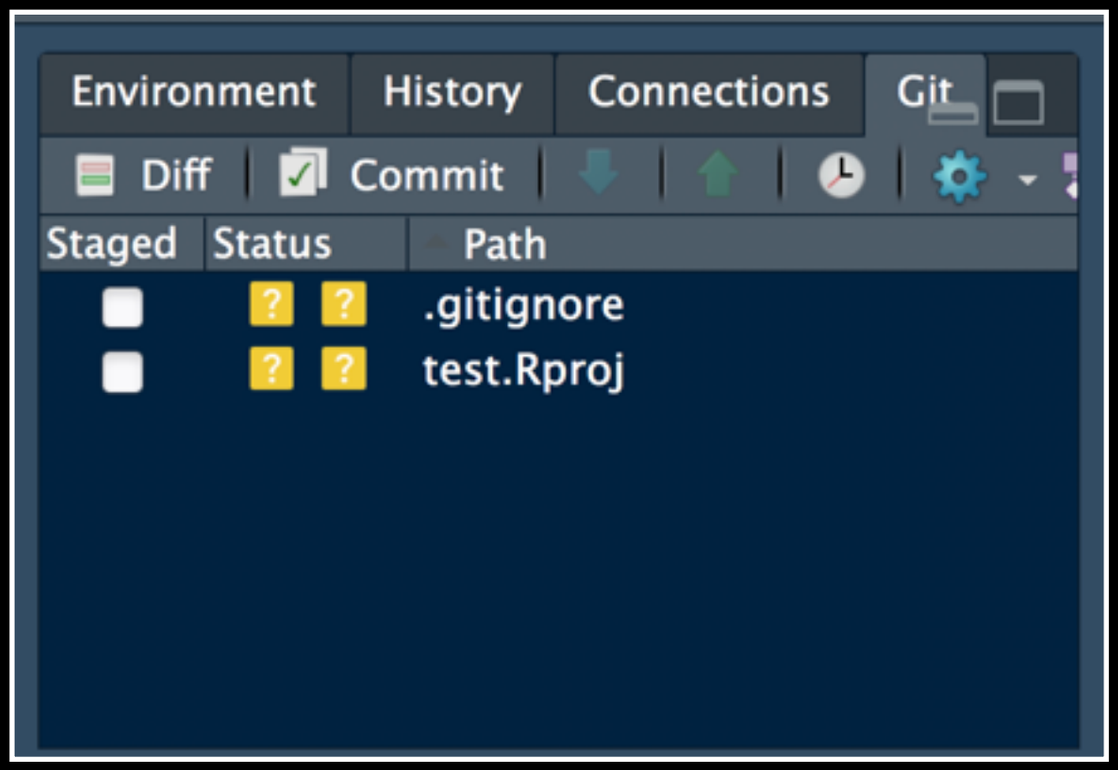


RStudio > New Project > Version Control



Demo of *editing*, *adding* *committing* and *pushing*

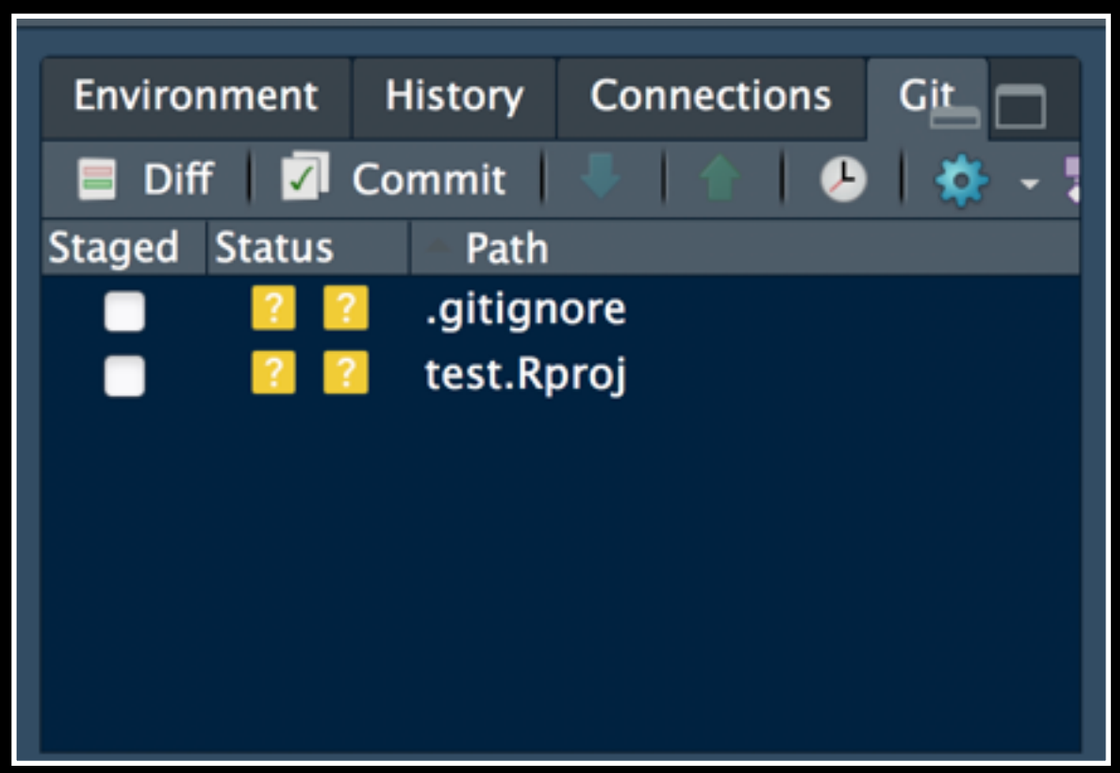
Check if new Git tab Appears in RStudio?



Now experiment editing the README.md file in RStudio and adding, committing and pushing changes to GitHub via this tab

Demo of *editing*, *adding* *committing* and *pushing*

Check if new Git tab Appears in RStudio?

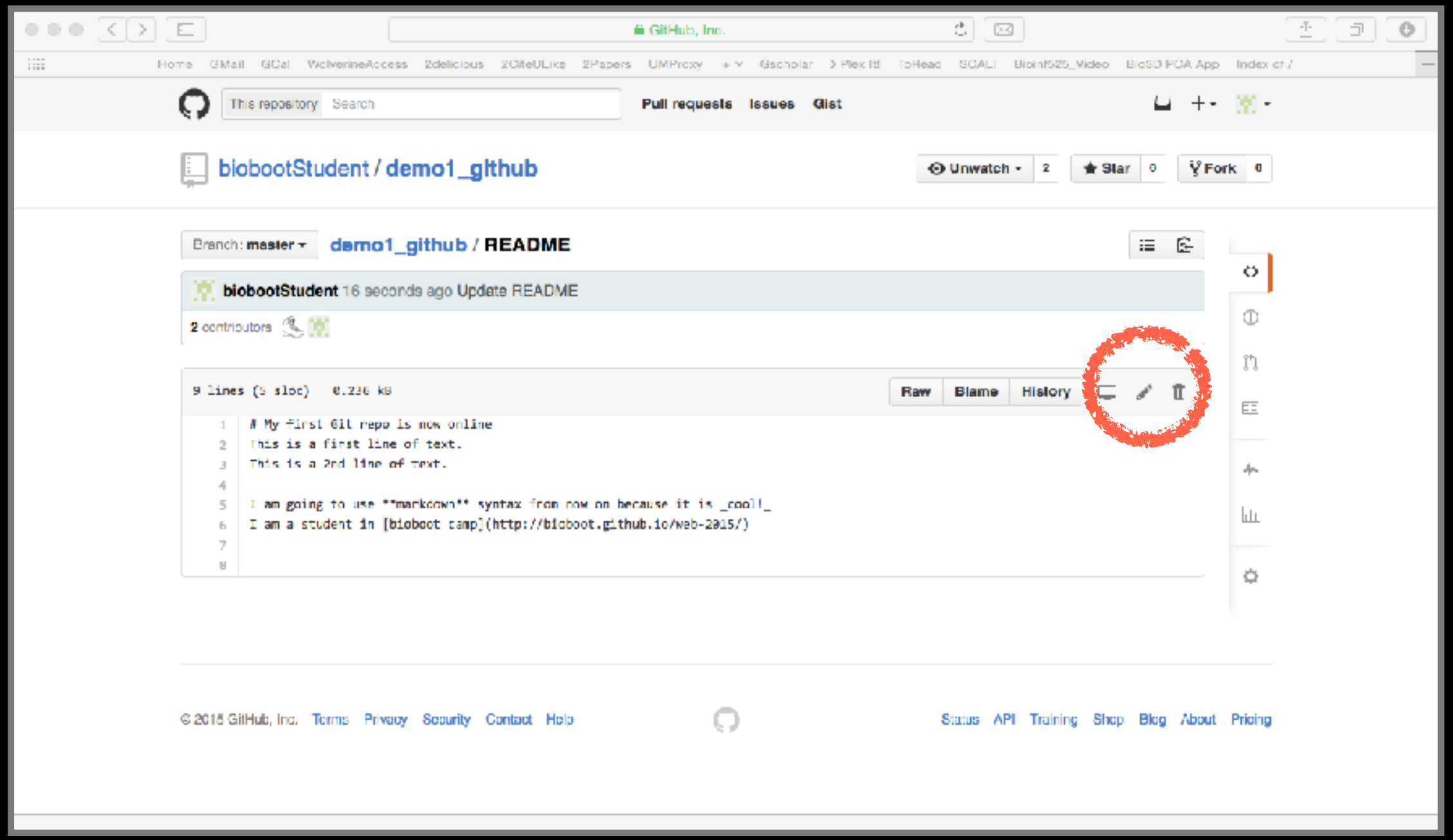


Now experiment editing the README.md file in RStudio and adding, committing and pushing changes to GitHub via this tab

When you are ready copy your different class directories/projects to this new GitHub tracked folder

Side-note: How to edit online

Specifically lets add some Markdown content



Summary

- Git is a popular ‘distributed’ version control system that is lightweight and free
- GitHub and BitBucket are popular hosting services for git repositories that have changed the way people contribute to open source projects
- Introduced basic git and GitHub usage within RStudio and encouraged you to adopt these ‘best practices’ for your future projects.

Learning Resources

- **Set up Git**. If you will be using Git mostly or entirely via **GitHub**, look at these how-tos.
  < https://help.github.com/categories/bootcamp/ >

- **Getting Git Right**. Excellent **Bitbucket** git tutorials
  < https://www.atlassian.com/git/ >

- **Pro Git**. A complete, book-length guide and reference to Git, by Scott Chacon and Ben Straub.
  < http://git-scm.com/book/en/v2 >

- **StackOverflow**. Excellent programming and developer Q&A.
  < http://stackoverflow.com/questions/tagged/git >

Learning git can be painful!

However in practice it is not nearly as crazy-making as the alternatives:

- Documents as email attachments
- Hair-raising ZIP archives containing file salad
- Am I working with the most recent data?
- Archaelogical “digs” on old email threads and uncertainty about how/if certain changes have been made or issues solved

Finally Please remember that **GitHub** and **BitBucket** are **PUBLIC** and that you should cultivate your professional and scholarly profile with intention!

[Muddy Point Assessment]

Reference Slides

Using Command Line Git

1. Initiate a Git repository.
2. Edit content (i.e. change some files).
3. Store a 'snapshot' of the current file state.*

Initiate a Git repository

Do it Yourself!

Initiate a Git repository

```
> cd ~/Desktop
> mkdir git_class   # Make a new directory
> cd git_class      # Change to this directory
> git init          # Our first Git command!
> ls -a             # what happened?
```

Side-Note: The .git/ directory

- Git created a ‘hidden’ **.git/** directory inside your current working directory.
- You can use the ‘**ls -a**’ command to list (*i.e.* see) this directory and its contents.
- This is where Git stores all its goodies - **this is Git!**
- You should not need to edit the contents of the **.git** directory for now but do feel free to poke around.

Important Git commands

```
> git status      # report on content changes
```

```
> git add <filename>     # stage/track a file
> git commit  -m “message”    # snapshot
```

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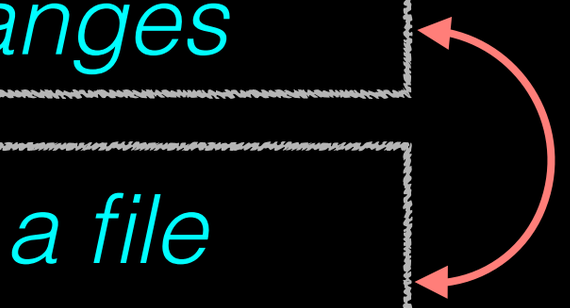


You will use these three commands again and again in your Git workflow!

Git TRACKS your directory content

- To get a report of changes (since last commit) use:

  > **git status**

- You tell Git which files to track with:

  > **git add <filename>**

  This adds files to a so called **STAGING AREA** (akin to a “shopping cart” before purchasing).

- You tell Git when to take an historical **SNAPSHOT** of your staged files (*i.e.* record their current state) with:

  > **git commit -m ‘Your message about changes’**

Example Git workflow

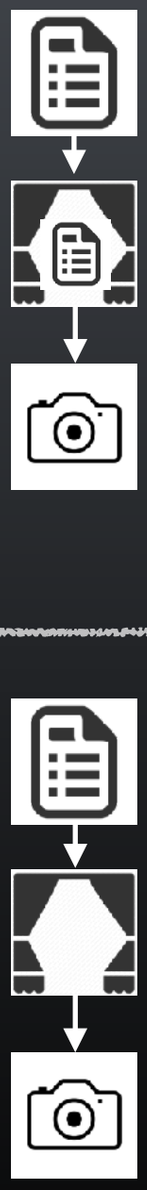
Eva creates a README text file
(this starts as untracked)

Adds file to STAGING AREA*
(tracked and ready to take a snapshot)

Commit changes*
(records snapshot of staged files!)

Example Git workflow

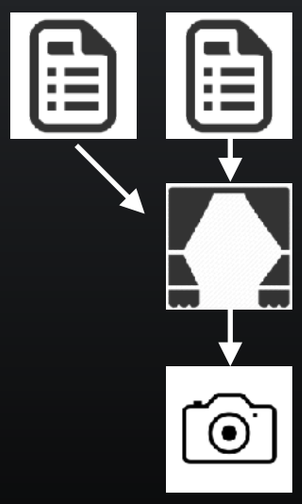
Hands on example!



Eva creates a README text file

Adds file to STAGING AREA*

Commit changes*



Eva modifies README and adds a ToDo text file

Adds both to STAGING AREA*

Commit changes*

1. Eva creates a README file

Do it Yourself!

```
> # cd ~/Desktop/git_class
> # git init

> echo "This is a first line of text." > README
> git status          # Report on changes
# On branch master
#
# Initial commit
#
# Untracked files:
#   (use "git add <file>..." to include in what will be committed)
#
#     README
#
# nothing added to commit but untracked files present (use "git add" to track)
```

2. Adds to ‘staging area’

```
> git add README      # Add README file to staging area
> git status          # Report on changes
# On branch master
#
# Initial commit
#
# Changes to be committed:
#   (use "git rm --cached <file>..." to unstage)
#
#     new file:   README
#
```

3. Commit changes

```
> git commit -m “Create a README file”     # Take snapshot
# [master (root-commit) 8676840] Create a README file
#  1 file changed, 1 insertion(+)
#  create mode 100644 README
```

```
> git status                  # Report on changes
# On branch master
# nothing to commit, working directory clean
```

4. Eva modifies README file and adds a ToDo file

```
> echo "This is a 2nd line of text." >> README
> echo "Learn git basics" >> ToDo

> git status          # Report on changes
# On branch master
#
# Changes not staged for commit:
#   (use "git add <file>..." to update what will be committed)
#   (use "git checkout -- <file>..." to discard changes in working directory)
#
#     modified:   README
#
# Untracked files:
#   (use "git add <file>..." to include in what will be committed)
#
#     ToDo
#
# no changes added to commit (use "git add" and/or "git commit -a")
```

5. Adds both files to ‘staging area’

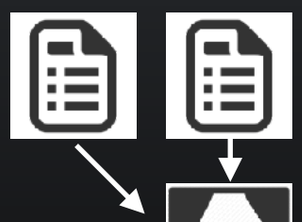
```
> git add README ToDo     # Add both files to ‘staging area’
> git status              # Report on changes
# On branch master
# Changes to be committed:
#   (use "git reset HEAD <file>..." to unstage)
#
#    modified:   README
#      new file:   ToDo
#
```

6. Commits changes

```
> git commit -m "Add ToDo and modify README"
# [master 7b679fa] Add ToDo and modify README
#  2 files changed, 2 insertions(+)
#  create mode 100644 ToDo
```

```
> git status
# On branch master
# nothing to commit, working directory clean
```

Example Git workflow

1.  Eva creates a README text file
2.  Adds file to STAGING AREA*
3.  Commit changes*
4.  Eva modifies README and adds a ToDo text file
5.  Adds both to STAGING AREA*
6.  Commit changes*

…But, how do we see the history of our project changes?

git log: Timeline history of snapshots (*i.e.* commits)

```
> git log
# commit 7b679fa747e8640918fcaad7e4c3f9c70c87b170
# Author: Barry Grant <bjgrant@umich.edu>
# Date:   Thu Jul 30 11:43:40 2015 -0400
#
#    Add ToDo and finished README
#
# commit 867684016107 70ae32e2fd4faee07d1d5c68619c
# Author: Barry Grant <bjgrant@umich.edu>
# Date:   Thu Jul 30 11:26:40 2015 -0400
#
#    Create a README file
#
```

git log: Timeline history of snapshots (*i.e.* commits)

```
> git log
# commit 7b679fa747e8640918fcaad7e4c3f9c70c87b170
# Author: Barry Grant <bjgrant@umich.edu>
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# Author: Barry Grant <bjgrant@umich.edu>
# Date:   Thu Jul 30 11:26:40 2015 -0400
#
#    Create a README file
#
```
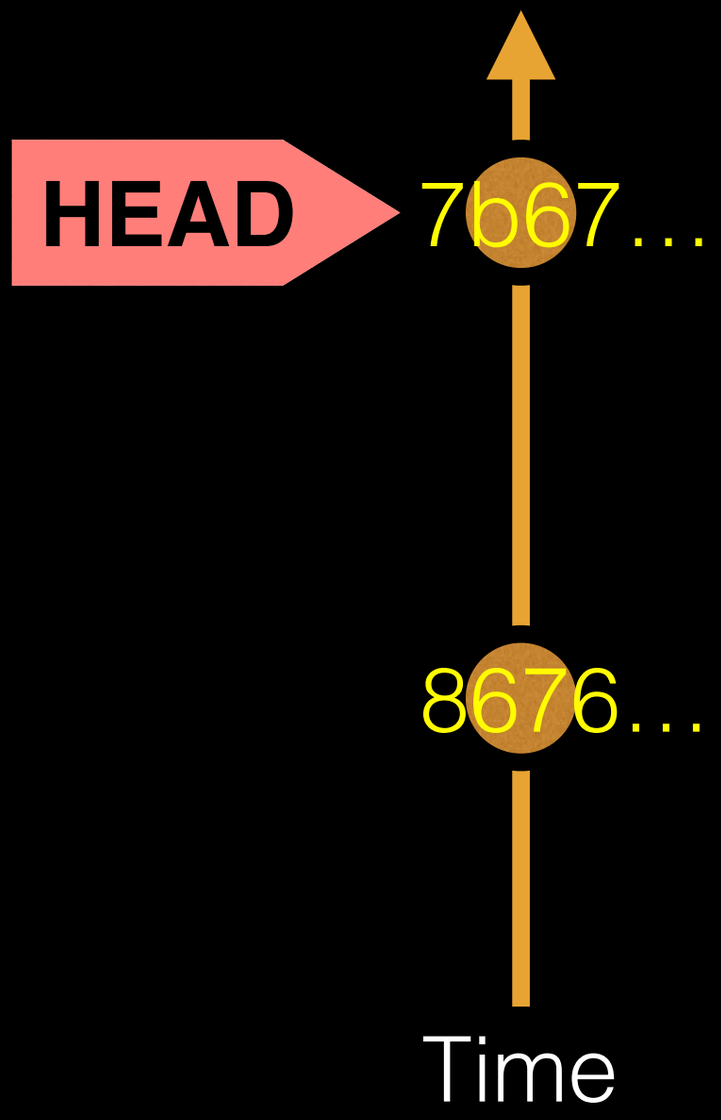

Side-Note: Git history is akin to a graph

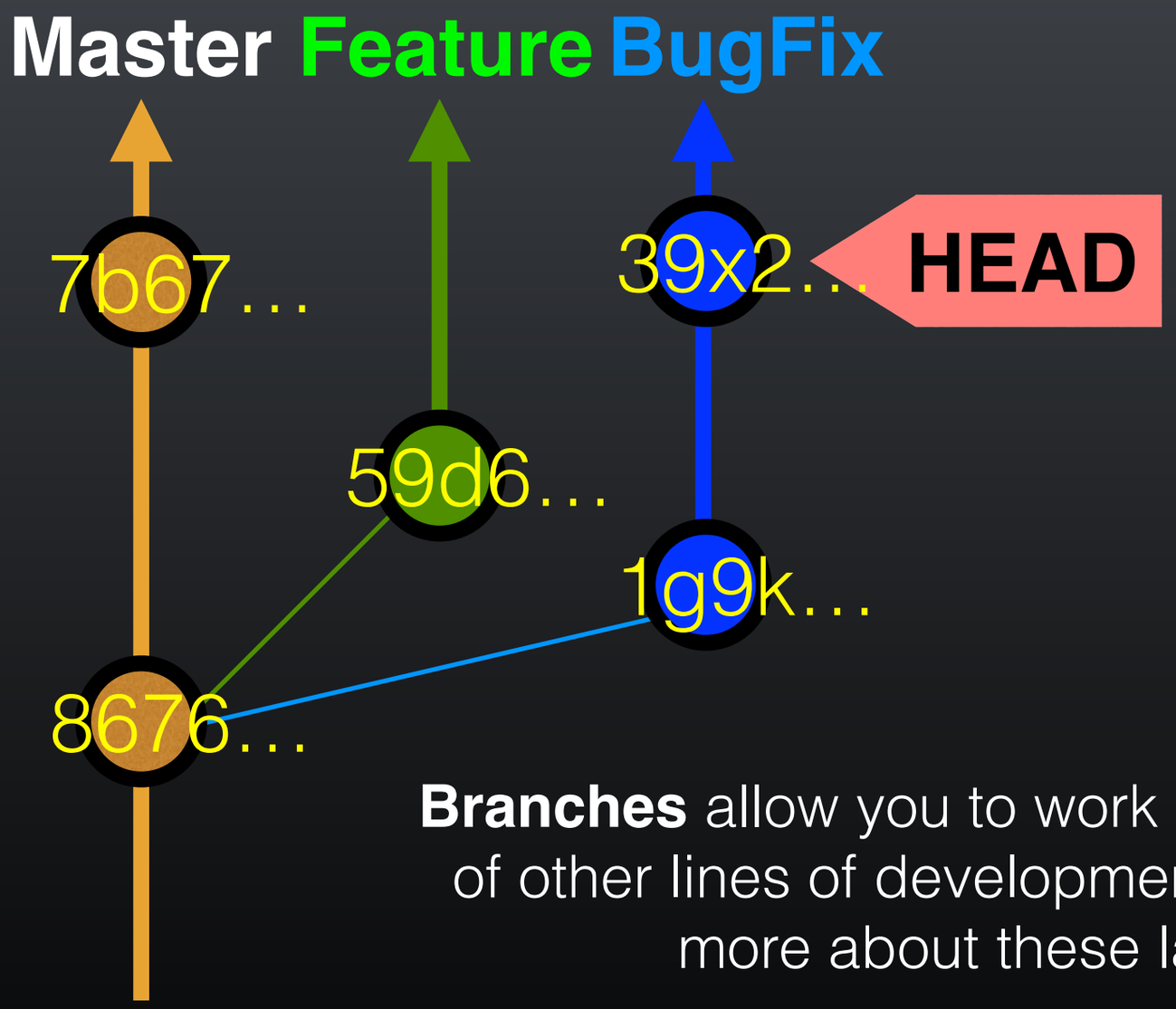


Nodes are **commits** labeled by their unique '**commit ID**'.

(This is a CHECKSUM of the commits author, time, commit msg, commit content and previous commit ID).

HEAD is a reference (or '**pointer**') to the currently checked out commit (typically the most recent commit).

Projects can have complicated graphs due to **branching**



Branches allow you to work independently of other lines of development we will talk more about these later!

Key Points:

You explicitly and iteratively tell git what files to track ("**git add**") and snapshot ("**git commit**").

Git keeps an historical log "(**git log**") of the content changes (and your comments on these changes) at each past commit.

It is good practice to regularly check the status of your working directory, staging arena repo ("**git status**")

Break

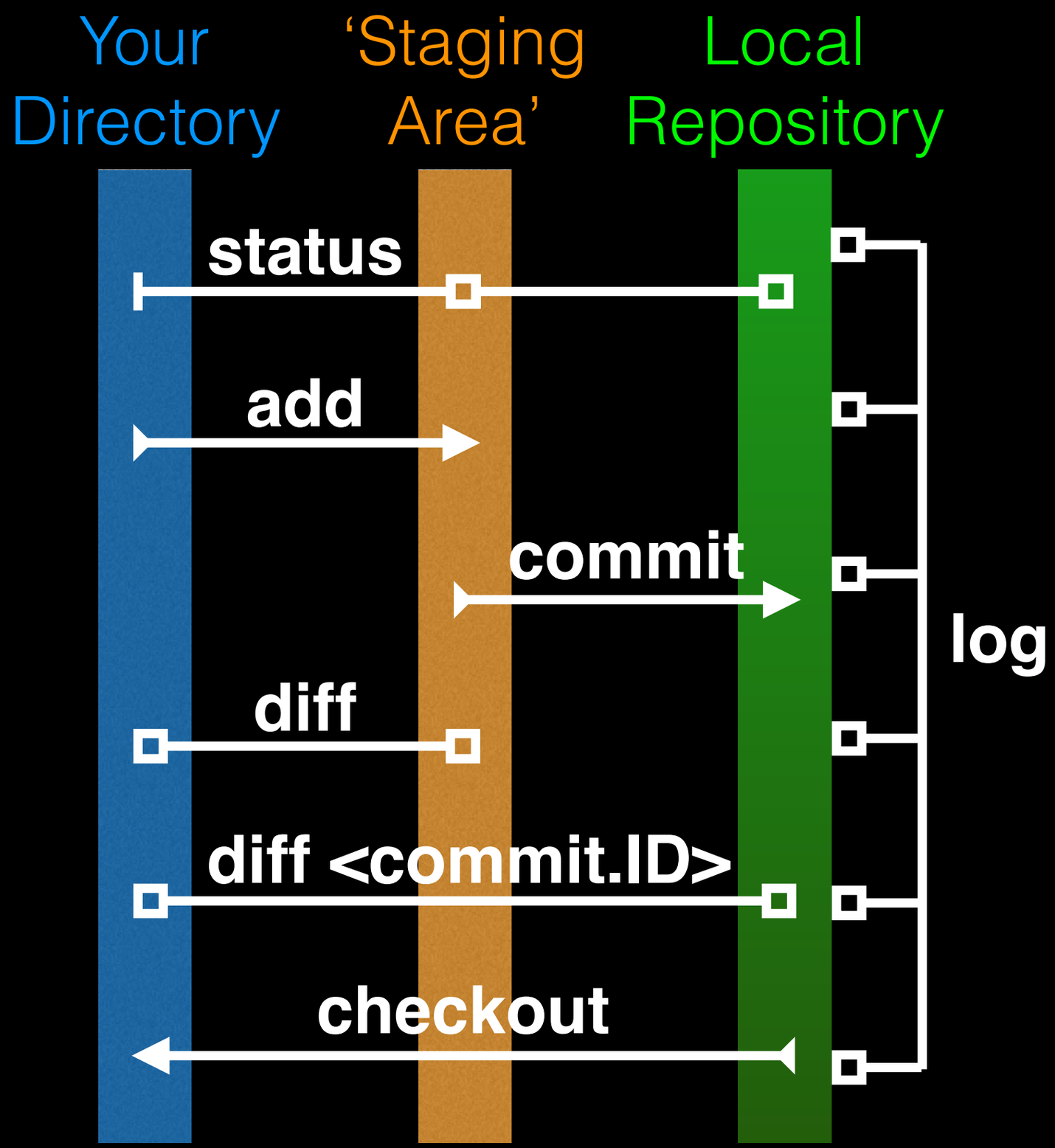
Summary of key Git commands:

```
> git status          # Get a status report of changes since last commit
```

```
> git add <filename>          # Tell Git which files to track/stage

> git commit -m ‘Your message’     # Take a content snapshot!
```

```
> git log                                  # Review your commit history
```

```
> git diff <commit.ID>  <commit.ID> # Inspect content differences

> git checkout  <commit.ID>  # Navigate through the commit history
```


Your Directory
'Staging Area'
Local Repository
status
add
commit
log
diff
diff <commit.ID>
checkout

git diff: Show changes between commits

> **git diff 8676 7b67**

```
# diff --git a/README b/README
# index 73bc85a..67bd82c 100644
# --- a/README
# +++ b/README
# @@ -1 +1,2 @@
#  This is a first line of text.
# +This is a 2nd line of text.

# diff --git a/ToDo b/ToDo
# new file mode 100644
# index 0000000..14fbd56
# --- /dev/null
# +++ b/ToDo
# @@ -0,0 +1 @@
# +Learn git basics
```

git diff: Show changes between commits

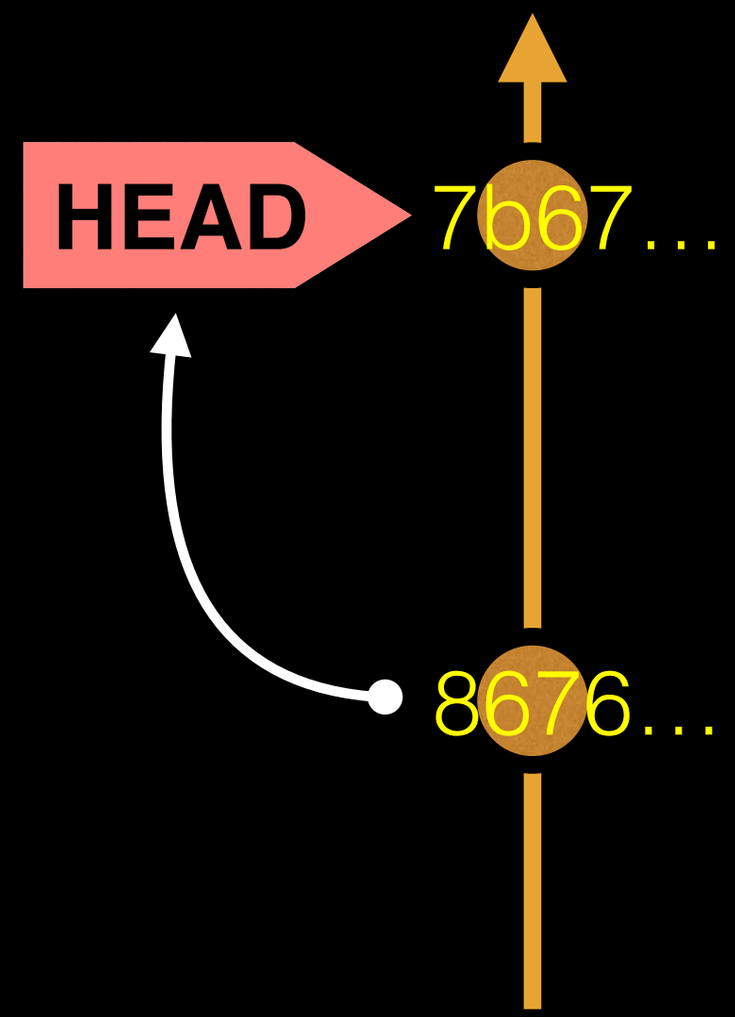
> **git diff 7b67 8676**

```
# diff --git a/README b/README
# index 67bd82c..73bc85a 100644
# --- a/README
# +++ b/README
# @@ -1,2 +1 @@
#  This is a first line of text.
# -This is a 2nd line of text.

# diff --git a/ToDo b/ToDo
# deleted file mode 100644
# index 14fbd56..0000000
# --- a/ToDo
# +++ /dev/null
# @@ 1 +0,0 @@
# -Learn git basics
```

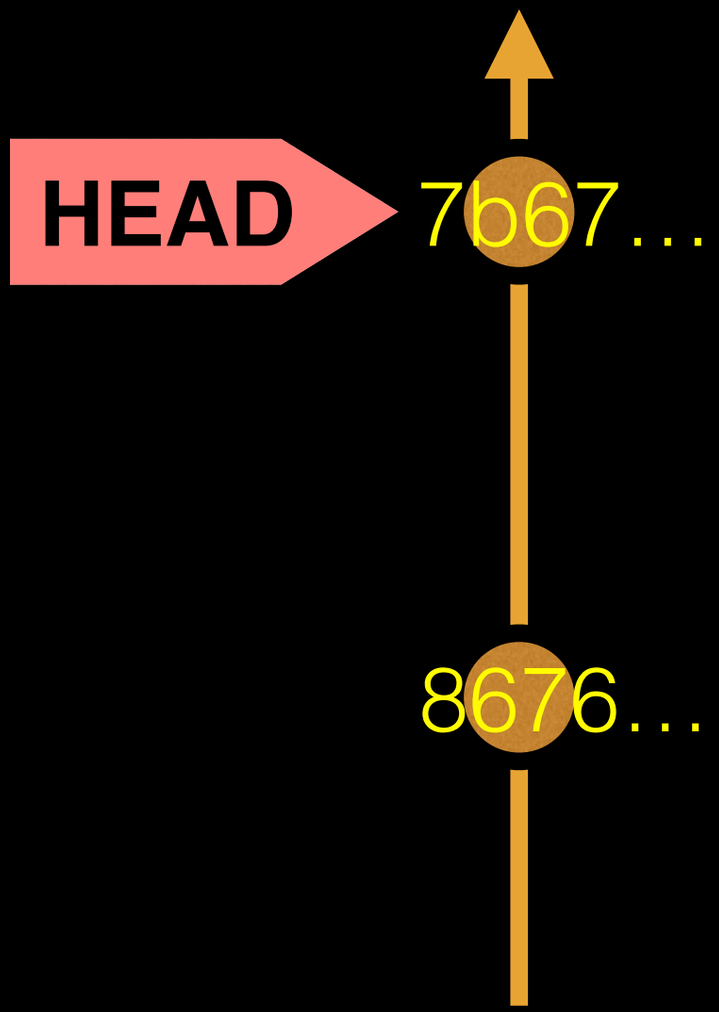
git diff: Show changes between commits

> **git diff 8676** **## Difference to current HEAD position!**

```
# diff --git a/README b/README
# index 73bc85a..67bd82c 100644
# --- a/README
# +++ b/README
# @@ -1 +1,2 @@
#  This is a first line of text.
# +This is a 2nd line of text.

# diff --git a/ToDo b/ToDo
# new file mode 100644
# index 0000000..14fbd56
# --- /dev/null
# +++ b/ToDo
# @@ -0,0 +1 @@
# +Learn git basics
```



HEAD advances automatically with each new commit



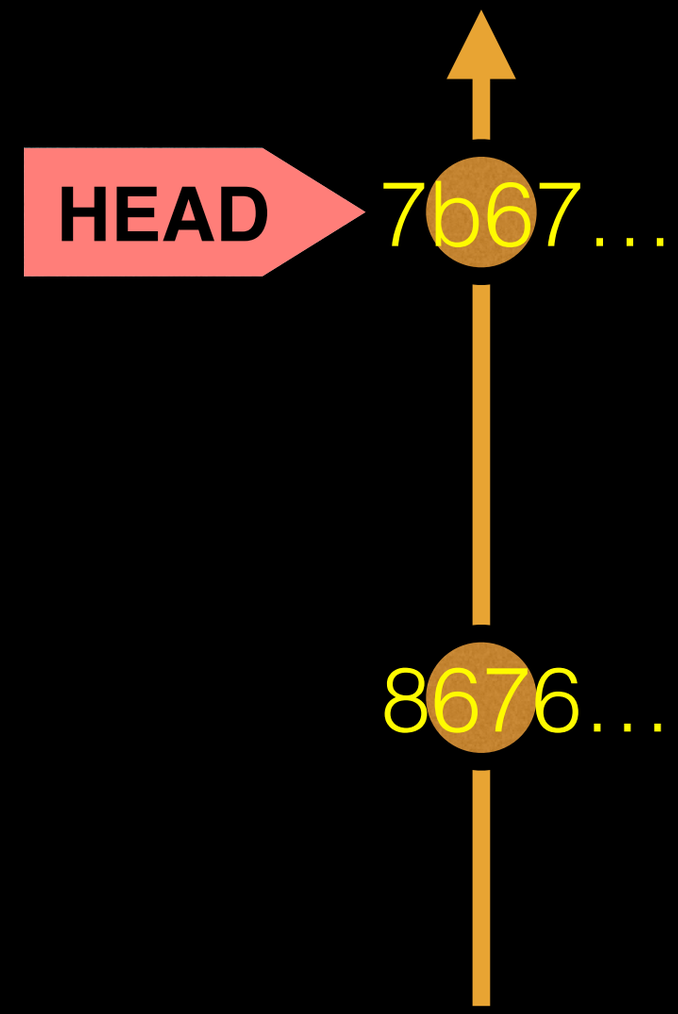
To move **HEAD** (back or forward) on the Git graph (and retrieve the associated snapshot content) we can use the command:

> **git checkout <commit.ID>**

git checkout: Moves HEAD

> **more README**
This is a first line of text.
This is a 2nd line of text.

> **git log --oneline**
7b679fa Add ToDo and finished README
8676840 Create a README file



git checkout: Moves HEAD (e.g. back in time)

Do it Yourself!

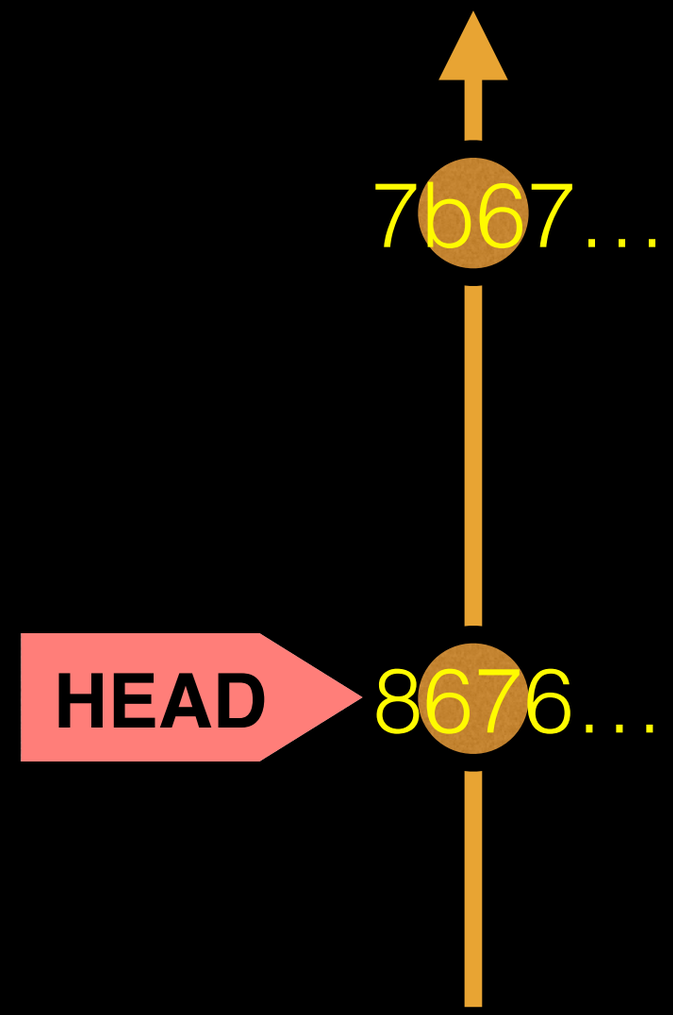
> **more README**
This is a first line of text.
This is a 2nd line of text.

> **git log --oneline**
7b679fa Add ToDo and finished README
8676840 Create a README file

> **git checkout 86768**
You are in 'detached HEAD' state…<cut>…
HEAD is now at 8676840... Create a README file

> **more README**
This is a first line of text.

> **git log --oneline**
8676840 Create a README file

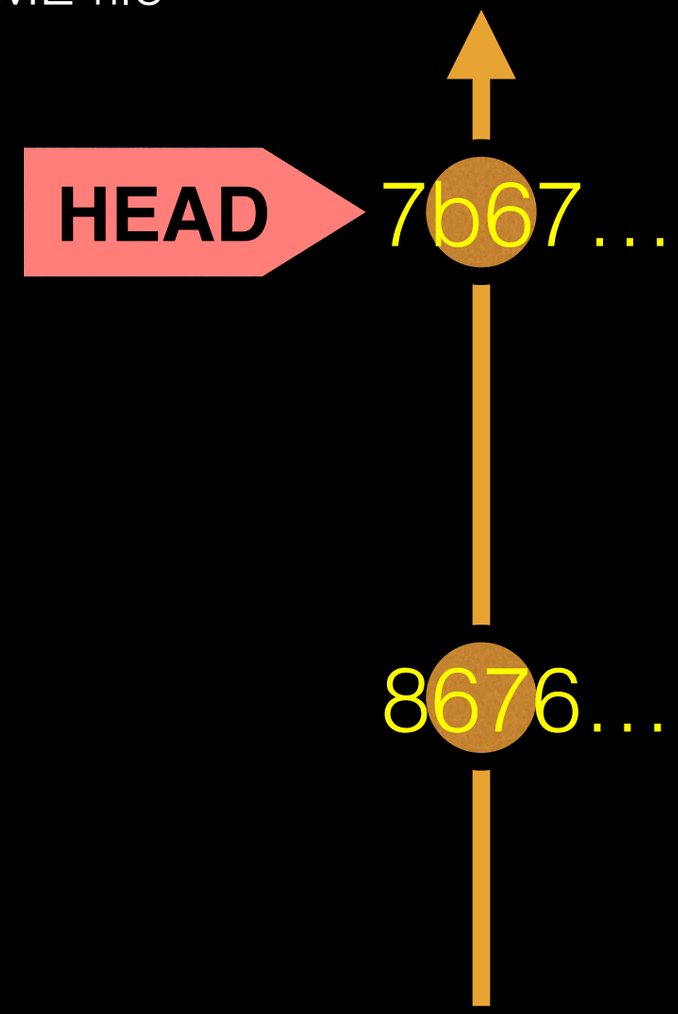


git checkout: Moves HEAD (e.g. back to the future!)

> **git checkout master**
Previous HEAD position was 8676840... Create a README file
Switched to branch 'master'

> **git log --oneline**
7b679fa Add ToDo and finished README
8676840 Create a README file

> **more README**
This is a first line of text.
This is a 2nd line of text.



Side-Note: There are two* main ways to use **git checkout**

- Checking out a **commit** makes the entire working directory match that commit. This can be used to view an old state of your project.

```
> git checkout <commit.ID>
```

- Checking out a **specific file** lets you see an old version of that particular file, leaving the rest of your working directory untouched.

```
> git checkout <commit.ID> <filename>
```

You can discard revisions with **git revert**

- The **git revert** command undoes a committed snapshot.
- But, instead of removing the commit from the project history, it figures out how to **undo the changes** introduced by the commit and **appends a new commit** with the resulting content.

```
> git revert <commit.ID>
```

- This prevents Git from losing history!

Removing untracked files with **git clean**

- The **git clean** command removes untracked files from your working directory.
- Like an ordinary **rm** command, **git clean** is not undoable, so make sure you really want to delete the untracked files before you run it.

```
> git clean -n # dry run display of files to be ‘cleaned’

> git clean -f   # remove untracked files
```

Demo Tower

GUIs

Tower (Mac only)
GitHub_Desktop (Mac, Windows)
SourceTree (Mac, Windows)
SmartGit (Linux)
RStudio

https://git-scm.com/downloads/guis