Programming and Data Science for Biology (PDSB)

Session 12
Spring 2018

Today’s topics

1. Conda environments Py2/3
2. Parallelization
3. RAD-seq, ipyrad, and the API
4. Genomic analyses with API wrappers for parallel execution, and writing wrappers.

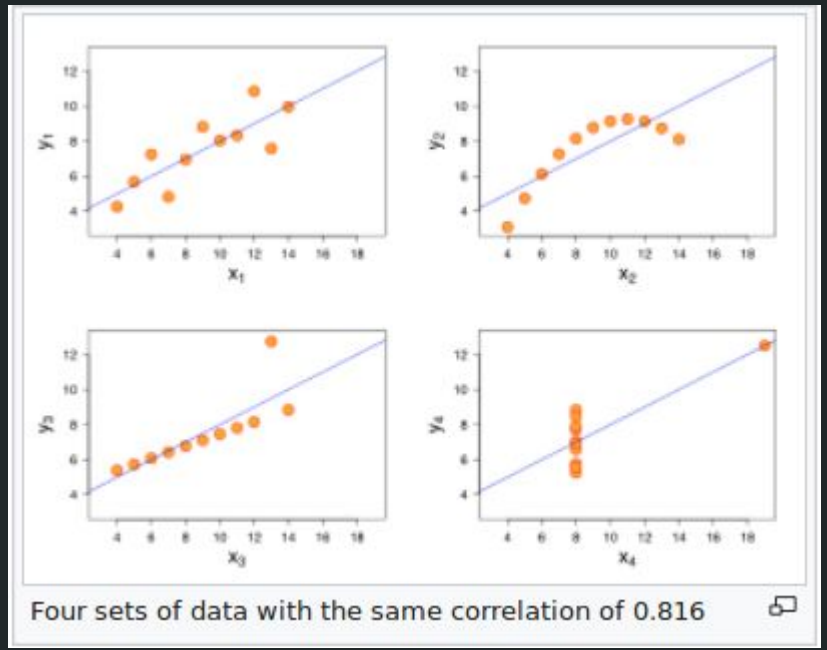
Upcoming topics

1. **Today:** Parallel code, presentation example, and genomics part I.
2. **Next week:** Presentations part I. And genomics part II.
3. **Two weeks:** Presentations part II. And genomics part III.

Last week's assignment:
Plotting tools.

Graph types

Plotting helps to visualize data, and thus avoid errors and motivate hypotheses to test.



Wikipedia

Conda environments:

Isolating tools for when different requirements are needed, or for temporary use.

Conda environments

Managing conda environments: https://conda.io/docs/user-guide/tasks/manage-environments.html

Conda environments allow you to keep different versions of your software separate.

The main thing you likely want is to have a py2 and py3 environment. Using ipykernel you can start a notebook and access both.

```
# set conda in your path (if not already there)
export PATH=~/miniconda3/bin/:$PATH
```

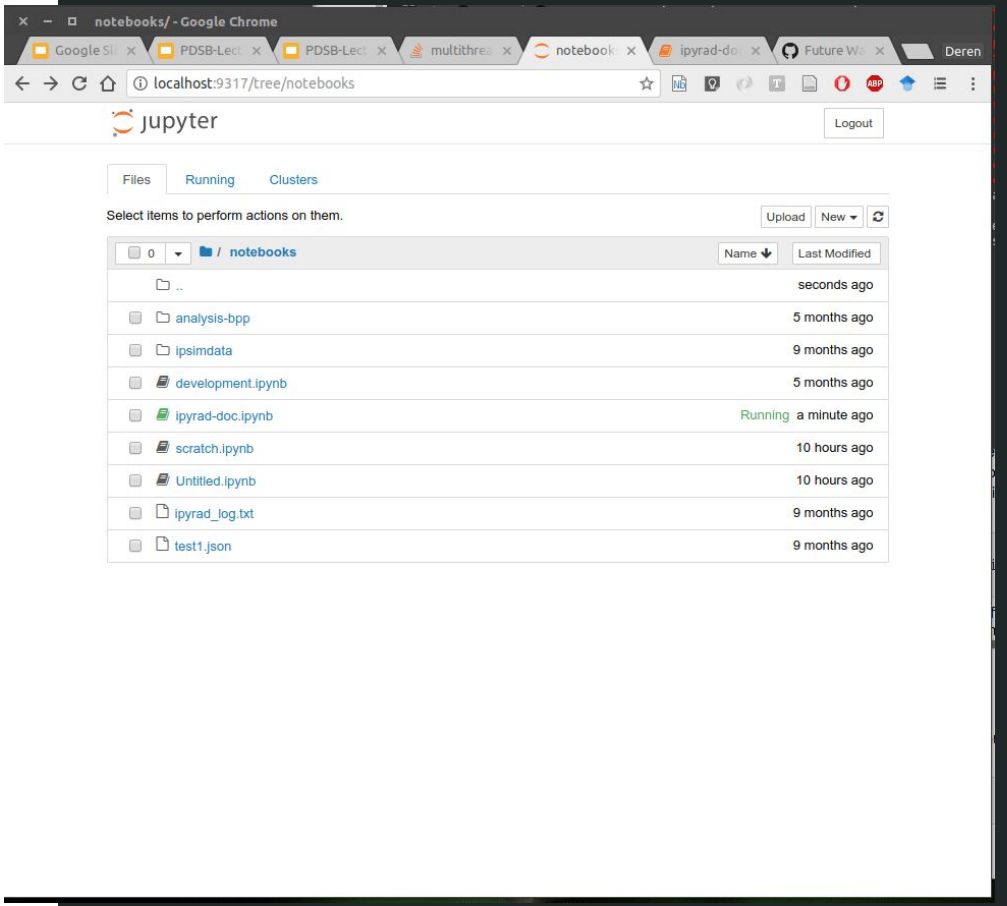
```
# create a py27 env and register kernel
conda create -n py27 python=2.7
source activate py27
conda install notebook ipykernel
ipython kernel install --user
```

```
# create a py36 env and register kernel
conda create -n py36 python=3.6
source activate py36
conda install notebook ipykernel
ipython kernel install --user
```

```
# show your conda environments
conda env list
```

Conda environments

Access multiple **kernels** from jupyter. You can additionally install kernels to access other languages like *bash, R, and julia.*



Presentations

An example and guide

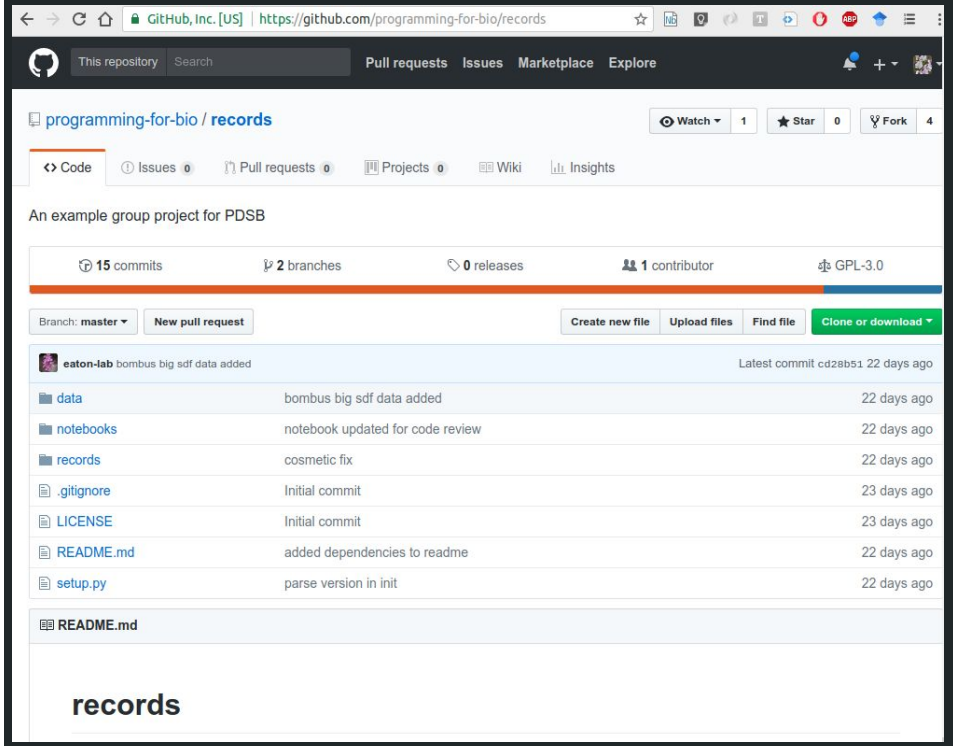
Project presentations

Main Points --

1. Describe your proposal.
2. Demonstrate your progress.

Topics to include:

1. How are you are representing your data (e.g., as an array, a network, a dictionary).
2. Where are you stuck or unsure?
3. What do you plan to explore next to solve the problem?

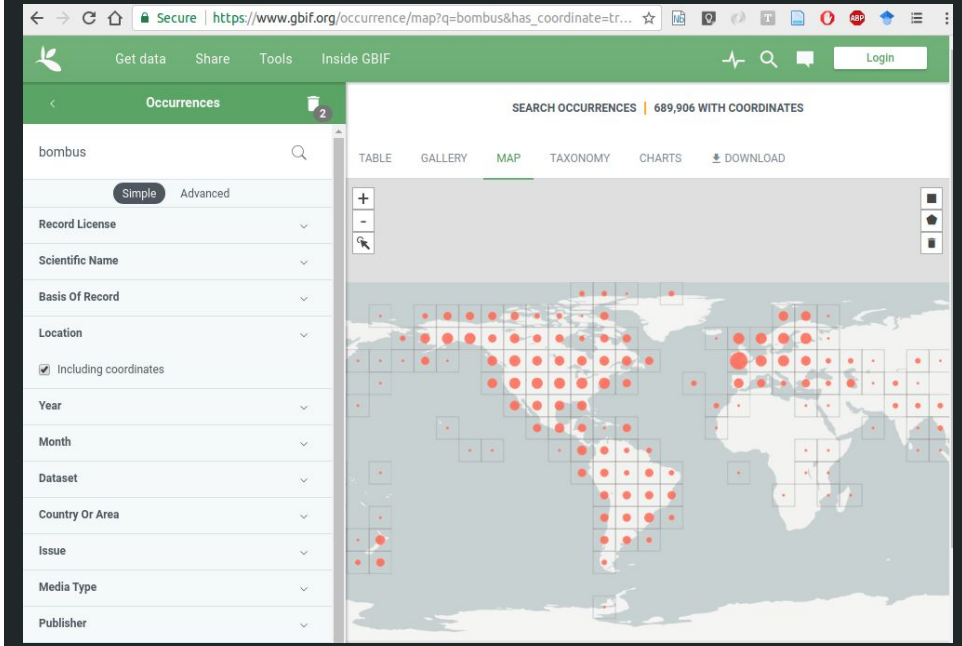


The **records** library

The problem: extracting and analyzing spatial data from GBIF using the API is not easy, I'd like to develop a simple tool for doing this. I want to ask, has bumblebee diversity changed through time?

The data: GBIF occurrence records, in particular my example dataset is of Bombus (bumblebees).

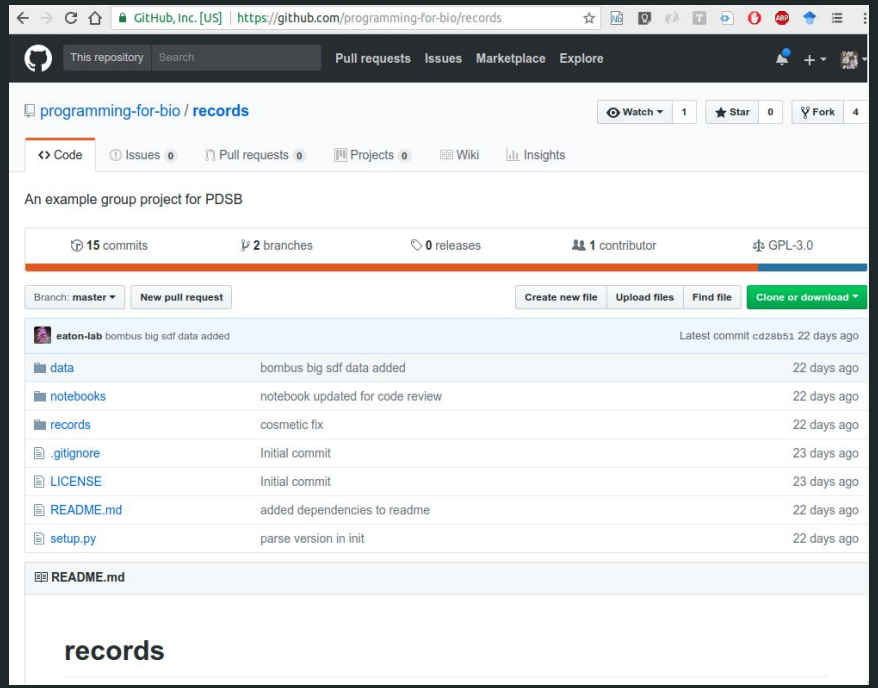
The tools: I am using the `requests` package to access data from GBIF using the REST API. Then I'm using `pandas` to organize it as a dataframe, filter and clean the data, and finally `toyplot` for plotting and `pymc3` for statistical analyses.



The **records** library

The novelty: By combining data extraction and analysis into a single pipeline my tool can be reused on many other types of data sets. The statistical method I will apply is also novel in that I have not seen it applied to GBIF data before. And this question about Bombus diversity is relevant to conservation.

The goal: A Python library with an easy to use API that can be easily imported and used by other users. I will have an example notebook demonstrating its use with plots and statistics as well for the Bombus example.



The **records** library

How am I developing it?

I've been writing code in sublimetext and testing it in a jupyter notebook.

I've written two main Class objects, one is the **Records** class, used to make queries to GBIF. The other is the **Epochs** Class, used to organize Records results into a dataframe and provide functions for users to calculate statistics.

```
import records
import pandas as pd
import pymc3 as pm
import toyplot
```

Query database to get records of Bombus

```
# retrieve records from GBIF database
rec = records.Epochs("Bombus", 1900, 2015, 5)
```

```
# save dataframe to disk
rec.df.to_csv("/home/deren/PDSB/records/data/Bombus-1900-2015.csv")
```

The **records** library

See the code on GitHub:

http://github.com/programming-for-bio/records/

```
class Records:
    """
    Returns a Records class instance with GBIF occurrence records stored
    in a pandas DataFrame for a queried taxon between a range of years.

    Parameters:
    -----------
    q: str
        Query taxonomic name.
    interval: tuple
        Range of years to return results for. Should be (min, max) tuple.

    Attributes:
    -----------
    baseurl: The REST API URL for GBIF.org.
    params: The parameter dictionary to filter GBIF search.
    df: Pandas DataFrame with returned records.
    sdf: A view of the 'df' DataFrame selecting only three relevant columns.
    """
    def __init__(self, q, interval, **kwargs):
        # the API url for searching GBIF occurrences
        self.baseurl = "http://api.gbif.org/v1/occurrence/search?"

        # the default REST API options plus user entered args
        self.params = {
            'q': q,
            'year': ",".join([str(i) for i in interval]),
            'basisOfRecord': "PRESERVED_SPECIMEN",
            'hasCoordinate': "true",
            'hasGeospatialIssue': "false",
            "country": "US",
            "offset": "0",
            "limit": "300",
        }

        # allow users to enter or modify other params using kwargs
        self.params.update(kwargs)

        # run the request query until all records are obtained
        self.df = pd.DataFrame(self._get_all_records())
```

The **records** library

The .df and .sdf attributes return a full view and shortened view of the dataframe, respectively.

Access dataframe of results

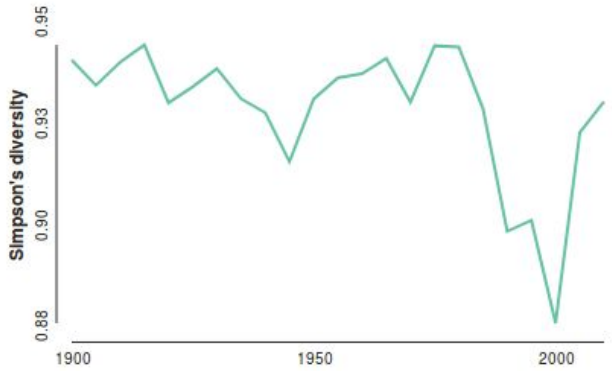
```
rec.sdf.head(10)
```

	species	year	epoch	country	stateProvince
0	Bombus fervidus	1900	1900	United States	Kansas
1	Bombus fervidus	1900	1900	United States	Kansas
2	Bombus vosnesenskii	1900	1900	United States	California
3	Bombus affinis	1900	1900	United States	New York
4	Bombus fervidus	1900	1900	United States	Kansas
5	Bombus pensylvanicus	1900	1900	United States	Nebraska
6	Bombus fervidus	1900	1900	United States	Kansas
7	Bombus variabilis	1900	1900	United States	Illinois
8	Bombus fervidus	1900	1900	United States	Kansas
9	Bombus sylvicola	1900	1900	United States	Alaska

The **records** library

The .df and .sdf attributes return a full view and shortened view of the dataframe, respectively.

From Epochs class objects users can calculate Simpson's diversity (probability two individuals are the same species).



Calculate diversity indices

```
# diversity across all state by epoch
rec.simpsons_diversity("epoch")
```

```
epoch
1900    0.940813
1905    0.934551
1910    0.940228
1915    0.944503
1920    0.930227
1925    0.934184
1930    0.938666
1935    0.931209
1940    0.927794
1945    0.915737
1950    0.931189
1955    0.936433
1960    0.937387
1965    0.941193
1970    0.930395
1975    0.944311
1980    0.944033
1985    0.928776
1990    0.898571
1995    0.901256
2000    0.875938
2005    0.922909
2010    0.930507
Name: species, dtype: float64
```

The **records** library

What's next?

- Groupby state and epoch
- Filter bad state names
- Apply switchpoint model in pymc3 for each state using a hierarchical model.

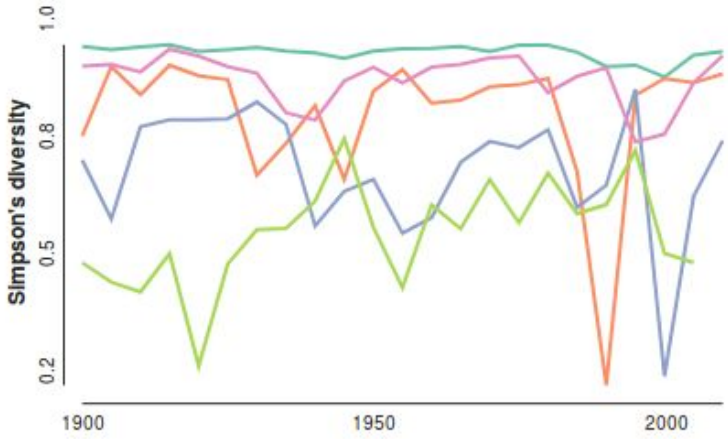
```
sdf = df.unstack()
mask = (
    (sdf.index == '[California]') |
    (sdf.index == '[Not Stated]') |
    (sdf.index == 'Not Stated') |
    (sdf.index == 'Colorado - New Mexico - Oklahoma') |
    (sdf.index == 'District Of Columbia') |
    (sdf.index == 'Washington, D. C.') |
    (sdf.index == 'Mass') |
    (sdf.index == 'District of Columbia')
)
sdf = sdf[~mask].T

# state with more than 5 NaNs are removed
mask = np.sum(sdf.isna(), axis=0) <= 5
sdf.loc[:, mask].head()
```

stateProvince	Alaska	Arizona	California	Colorado	Connecticut	Florida	Georgia	Idal
epoch								
1900	0.750000	0.584933	0.699000	0.899221	0.743802	0.480000	0.565089	0.8163
1905	0.898438	0.145957	0.573519	0.902970	0.507545	0.438776	0.269231	0.7551
1910	0.838296	NaN	0.769859	0.886856	0.574827	0.417778	0.675900	0.7636
1915	0.901213	0.909970	0.785077	0.935441	0.757847	0.499241	0.691358	0.8476
1920	0.878903	NaN	0.784623	0.920467	0.331066	0.259969	0.647500	0.8593

The **records** library

There is a lot of variability by state, so my analyses should probably take this into account.




```
# diversity across all state by epoch
c, a, m = toyplot.plot(
    div.epoch, div.species,
    width=450, height=300,
    ylabel="Simpson's diversity");

a.plot(sdf.index, sdf.Alaska)
a.plot(sdf.index, sdf.California)
a.plot(sdf.index, sdf.Colorado)
a.plot(sdf.index, sdf.Florida)
```

```
<toyplot.mark.Plot at 0x7f2e2b197828>
```

The **records** library

What's next?

- Groupby state and epoch
- Filter bad state names
- Apply switchpoint model in pymc3 for each state using a hierarchical model.

The switchpoint model is still in progress. I'm trying to figure out how to apply a hierarchical model and switchpoint models together. I'm reading more on the pymc3 docs and running tests to figure it out.

```
with pm.Model() as model:

    # sample switchpoint from within the bounds of our data timescale
    switchpoint = pm.DiscreteUniform(
        'switchpoint',
        lower=div.epoch.min(),
        upper=div.epoch.max(),
        testval=1980)

    # Priors for pre- and post-switch rates for number of disasters
    early_rate = pm.Exponential('early_rate', 1.)
    late_rate = pm.Exponential('late_rate', 1.)

    # Allocate appropriate Poisson rates to years before and after current
    rate = pm.math.switch(switchpoint >= div.epoch, early_rate, late_rate)
    diversity = pm.Poisson('disasters', rate, observed=div.species)
```

```
with model:
    trace = pm.sample(10000)
```

```
Assigned Metropolis to switchpoint
Assigned NUTS to early_rate_log__
Assigned NUTS to late_rate_log__
 90%|[redacted] | 9473/10500 [00:08<00:00, 1177.62it/s]
```

The **records** library

Questions, recommendations, ideas?

```
with pm.Model() as model:

    # sample switchpoint from within the bounds of our data timescale
    switchpoint = pm.DiscreteUniform(
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    rate = pm.math.switch(switchpoint >= div.epoch, early_rate, late_rate)
    diversity = pm.Poisson('disasters', rate, observed=div.species)
```

```
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```

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Assigned Metropolis to switchpoint
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Assigned NUTS to late_rate_log__
 90%|[redacted] | 9473/10500 [00:08<00:00, 1177.62it/s]
```

Parallelization

Using it and writing code for it

Running parallel code

Ideally, running parallel code should be easy, as simple as designating the number of processors you want your analysis to use.

Example, in pymc3 we could provide `njobs` as an argument to run mcmc chains on separate processors in parallel. Easy!

However, knowing more about the architecture of parallelization can help us to use it more efficiently.

```
with model:
    trace2 = pm.sample(njobs=4, chain=4)
```

```
Auto-assigning NUTS sampler...
Initializing NUTS using jitter+adapt_diag...
 18%|          | 185/1000 [00:33<02:26,  5.58it/s]
```

The Global Interpreter Lock (GIL) in Python

If you have multiple functions running at the same time, then one might change the state of an object in memory when another one is trying to access it, and this could cause problems.

To avoid this, Python **locks** processes from operating on the same memory space. This is great and allows you to write simple code without worrying about complicated problems. However, to write parallel code we then need to *work around the GIL*.

```
# run one function
res1 = func1()

# progress is 'blocked' until func1 finishes,
# then the next code block can run.
res2 = func2()
```

Forms of parallel code

Concurrency is when two or more tasks can start, run, and complete in overlapping time periods. It doesn't necessarily mean they'll ever both be running at the same instant. Eg. multitasking on a single-core machine.

Parallelism is when two or more tasks are executed simultaneously.

Forms of parallel code

A **thread** is a sequence of instructions within a process. It can be thought of as a lightweight process. Threads share the same memory space.

A **process** is an instance of a program running in a computer which can contain one or more threads. A process has its independent memory space.

In Py3 concurrency is easier to use, and is often used in place of multithreading.

Concurrency

Here the *memory-space* is shared between all running functions. The code is mostly running in parallel, but it is not using more than one processor.

This is best used for functions that take time to complete, but are not doing much work during that time. This would include I/O limited tasks, or network limited (e.g., web requests).

Notebooks/nb-12.1-parallel-threading.ipynb

```
with ThreadPoolExecutor(max_workers=4) as executor:

    # submit jobs to threads
    jobs = [executor.submit(gsearch, q) for q in queries]

    # collect results
    results = [i.result() for i in jobs]
```

Multi-processing

The general workflow is to open a **client** (pool, executor) that can distribute jobs to workers.

Then send jobs to workers (submit, apply_async).

The collect the results from the jobs once they finish.

Notebooks/nb-12.2-multiprocess-ipyparallel.ipynb

```
with ProcessPoolExecutor(max_workers=4) as executor:

    # submit queries to threads
    jobs = [executor.submit(gsearch, q) for q in queries]

    # collect results
    results = [i.result() for i in jobs]
```

```
with mp.Pool(processes=4) as pool:

    # submit queries to threads (with args as tuples)
    jobs = [pool.apply_async(who, (1,)) for q in queries]

    # collect results
    results = [i.get() for i in jobs]
```

Blocking vs. Async

The normal way in which we execute code is that when we execute a function call our progress is **blocked** until that function finishes.

Asynchronous execution allows us to send a job to run remotely and to continue working locally, to send more jobs or to query whether the remote job is finished.

Notebooks/nb-12.2-multiprocess-ipyparallel.ipynb

```
# start a pool
with mp.Pool(processes=4) as pool:

    # submit a job that will run successfully
    async0 = pool.apply_async(who, [2])

    # submit a job that will fail
    async1 = pool.apply_async(who, ['apple'])

    # join jobs into an iterable
    jobs = [async0, async1]

    # iterate over async results and print result of succesful jobs
    while 1:
        for job in jobs:
            # skip over the job for now if not ready yet
            if job.ready():
                if job.successful():
                    print('{} was successful'.format(job))
                else:
                    print('{} had an error'.format(job))
                # remove job from queue
                jobs.remove(job)

        # end loop if all jobs are finished
        time.sleep(0.5)
        if len(jobs) == 0:
            break
```

```
<multiprocessing.pool.ApplyResult object at 0x7f7a58dcedd8> had an error
<multiprocessing.pool.ApplyResult object at 0x7f7a58dce9e8> was successful
```

Genomic assembly and analysis with ipyrad:

And a dive into API structure

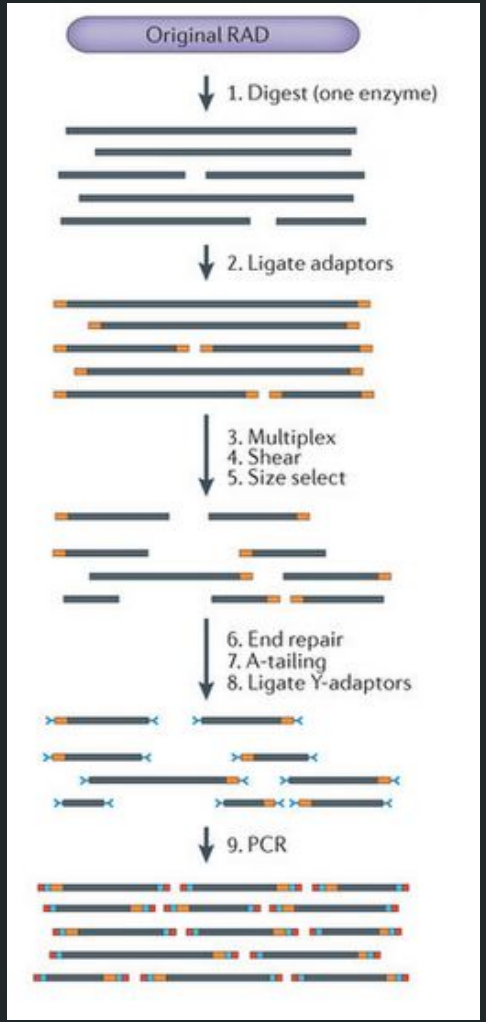
RAD-seq genomics

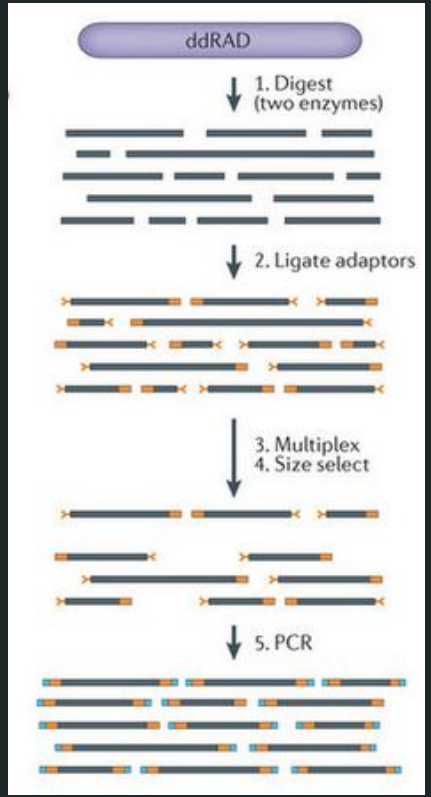
Restriction-site associated DNA is a type of genomic data that samples the genome at places where certain recognition sites occur.

Review

Harnessing the power of RADseq for ecological and evolutionary genomics

Kimberly R. Andrews ✉, Jeffrey M. Good, Michael R. Miller, Gordon Luikart & Paul A. Hohenlohe

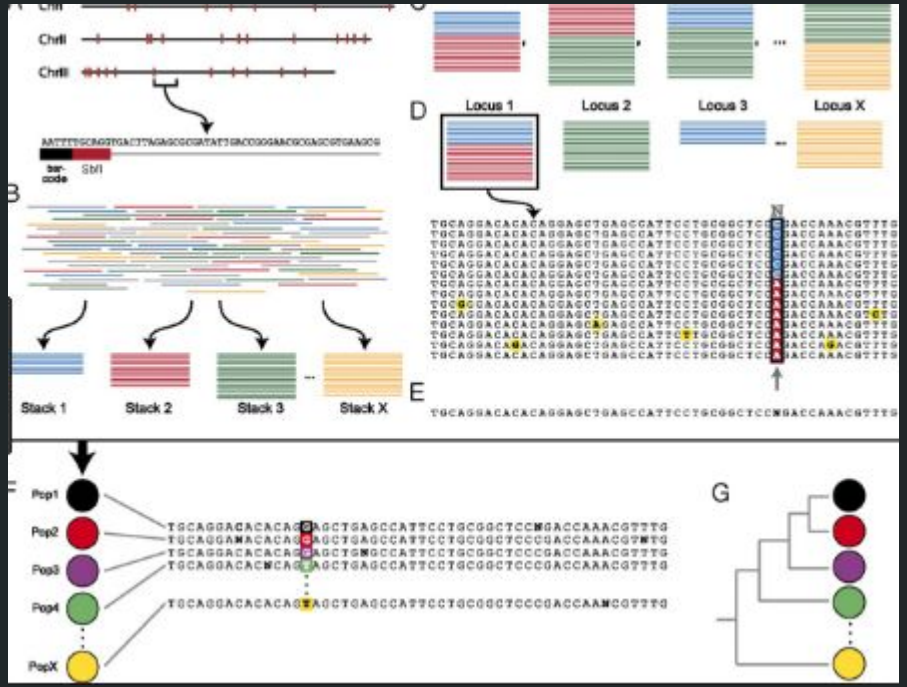




RAD-seq genomics

It is a convenient method for sampling thousands of loci from hundreds or thousands of individuals.

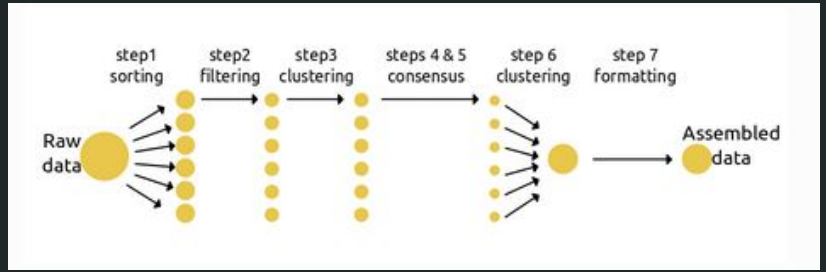
Short-read sequencing technologies yield reads that are ~50-150 bp long, which we need to *assemble* bioinformatically to identify which reads are from the same genomic region, to correct for base-calling errors, and to identify homologous sequences across samples.

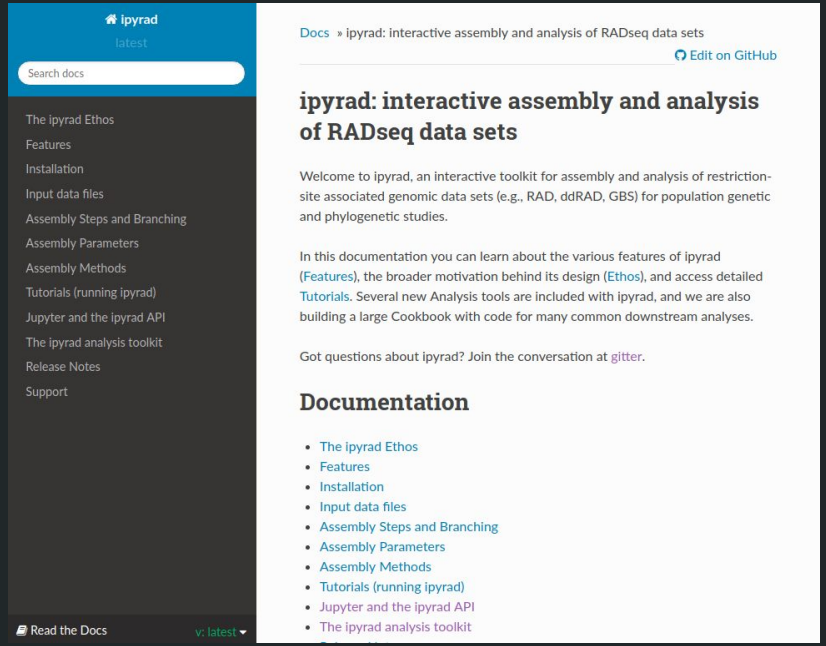


RAD-seq genomics

One program for this is *ipyrad,* which I've developed over the last 5 years, and with the help of a collaborator have now expanded to include complex assembly options and analysis tools.

Ipyrad uses *ipyparallel* to distribute jobs over arbitrarily large computing clusters while also allowing users to work interactively and perform their analysis in reproducible jupyter notebooks.





RAD-seq genomics

Installation: ipyrad is written for Python 2.7

http://ipyrad.readthedocs.io/installation.html

Install a python2 conda environment and install with a simple conda command. This pulls in all dependencies, of which there are many, and allows users to easily install the software locally.

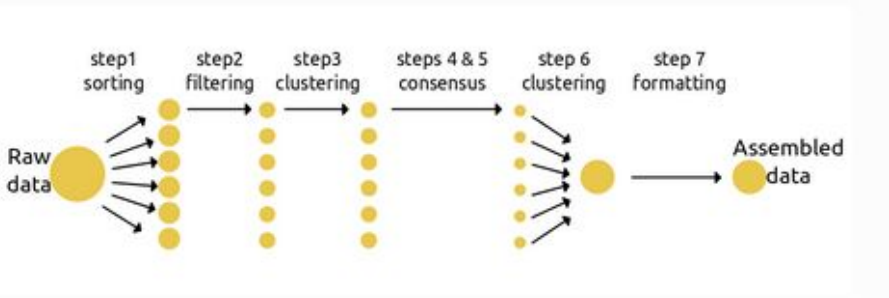
```
# install ipyrad from conda
conda install ipyrad -c ipyrad
```

RAD-seq genomics

Step 1:

Sorting/Loading reads.

Load data from .fastq or .fastq.gz files. If the reads are not sorted into samples yet then you can provide a barcodes file with index information to demultiplex reads.




```
less /rigel/edu/w4050/files/example_empirical_rad/29154_superba.fastq.gz
```

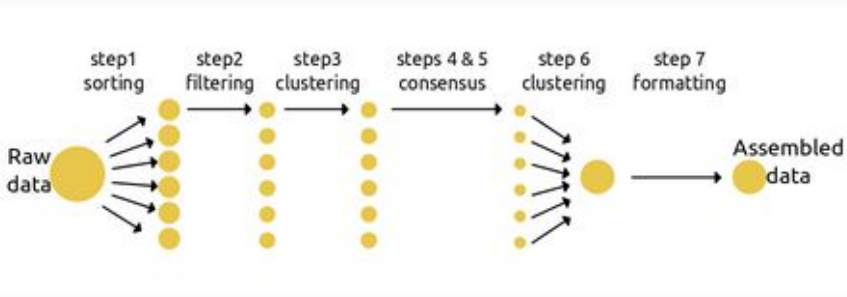
```
@29154_superba.3 GRC13_0027_FC:4:1:19092:1179 length=74
TGCAGGCTCTGACAAAGAANTCGACTGANNNNNNNNNNNNNNNCACNGGTTCNNGNNNATGTCAATGTGGTANAN
+29154_superba.3 GRC13_0027_FC:4:1:19092:1179 length=74
GGGGHHHHBHHBHEB?B@############################################################
@29154_superba.4 GRC13_0027_FC:4:1:1248:1210 length=74
TGCAGAACTGCTCCCAGAATCTCCAGAAATTGCGAGATTACCCCCAAAATCTCCAGAAATTTCTAGATTACCTC
+29154_superba.4 GRC13_0027_FC:4:1:1248:1210 length=74
HHHHHDHHHFHHHDHBGHGHHHHHHHGDHE<GGE<D>DGHGGHHHDHHHHHHGHHHHFHHHHHHEHGHHEDEF>
@29154_superba.5 GRC13_0027_FC:4:1:5242:1226 length=74
TGCAGCAGTCACCAGTCTGGCCCCTACCTCACAAAGTAGCTTGATGGCCGAACCACTCCCAAGGTGAATAGTGC
+29154_superba.5 GRC13_0027_FC:4:1:5242:1226 length=74
HHHHHHHHHGGGGGHHHGHHHGHGGGGGBHHHBHEE>BGADDDDFHEHFDBGBDCFHBHBB8DD4????::?::?
@29154_superba.6 GRC13_0027_FC:4:1:12660:1232 length=74
TGCAGGCCCAAAATCAACAATTATGCATAATACAACAAAGTTAATTAATTAATTATATTAAAAAAAGAAAAAGA
+29154_superba.6 GRC13_0027_FC:4:1:12660:1232 length=74
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHGGHHHHFHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
@29154_superba.7 GRC13_0027_FC:4:1:13459:1226 length=74
TGCAGTCAATCACATTACTCTATACTCTTCTGCGTCAGGGATTTGATGGGAAACCAATGGTTAAAAAAGCGATC
+29154_superba.7 GRC13_0027_FC:4:1:13459:1226 length=74
HHHGGGEGGDGGG@HHHHHHEHHHGHHHG>DDGGEGGGGGBBGG>8CE>FEDGEDBFDEFGHHHHFHH<EBFEC
@29154_superba.8 GRC13_0027_FC:4:1:14162:1226 length=74
TGCAGTAACGTGAATTGCACTACGAAAACCTTCCAAACATAGGGCTATCACAACTTCATCATCACTTTGGTCAA
+29154_superba.8 GRC13_0027_FC:4:1:14162:1226 length=74
IIIIIIIIIIIIIIIIIIIIDIIIIIIHIHIIIIIIDIIIIIIIIIIIIIIHHFIIFIIGIIIEHIHHHEIIG
@29154_superba.9 GRC13_0027_FC:4:1:17619:1233 length=74
TGCAGTGGCCAATGATGACATGGTCACCCTCCTTAACACAAAAGCATGGGATATGAGCGTAGCAATATTTTGAA
+29154_superba.9 GRC13_0027_FC:4:1:17619:1233 length=74
IIIIIIIIIIIIIIIIEIIIIHIIIIIIIIIIIHIGIIIIIDIIHIIHHGFHFHHIHIEIIIGBHCIHHIHBE
@29154_superba.10 GRC13_0027_FC:4:1:14537:1250 length=74
TGCAGCGGTTTTTTGAAGAGGTAGAATGGTCCGGACTGTTGGAGTAGGCAGCAGTTGGATCAGAAACTCGAAAA
+29154_superba.10 GRC13_0027_FC:4:1:14537:1250 length=74
IIIIIIIIIIIIIIIIIHIIEIIIIIIHEIIIIFHIIIIIIEIEIIIIHIIEIGGIICGIHHEHHGIHHEEGE
```

RAD-seq genomics

Step 2:

Filter reads

Trim illumina adapter sequences and low quality bases from the ends of reads.




```
less /rigel/edu/w4050/files/analysis-ipyrad/Pedicularis_edits/\
29154_superba.trimmed_R1_.fastq.gz
```

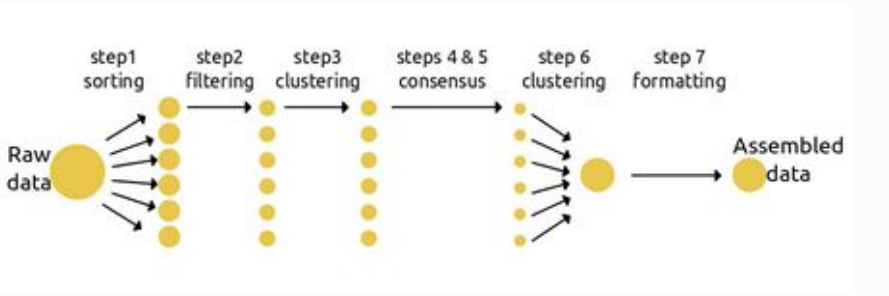
```
@29154_superba.4 GRC13_0027_FC:4:1:1248:1210 length=74
TGCAGAACTGCTCCCAGAATCTCCAGAAATTGCGAGATTACCCCCAAAATCTCCAGAAATTTCTAGATTACCTC
+29154_superba.4 GRC13_0027_FC:4:1:1248:1210 length=74
HHHHHDHHHFHHHDHBGHGHHHHHHHGDHE<GGE<D>DGHGGHHHDHHHHHHGHHHHFHHHHHHEHGHHEDEF>
@29154_superba.5 GRC13_0027_FC:4:1:5242:1226 length=74
TGCAGCAGTCACCAGTCTGGCCCCTACCTCACAAAGTAGCTTGATGGCCGAACCACTCCCAAGGTGAATAGTGC
+29154_superba.5 GRC13_0027_FC:4:1:5242:1226 length=74
HHHHHHHHHGGGGGHHHGHHHGHGGGGGBHHHBHEE>BGADDDDFHEHFDBGBDCFHBHBB8DD4???::?::?
@29154_superba.6 GRC13_0027_FC:4:1:12660:1232 length=74
TGCAGGCCCAAAATCAACAATTATGCATAATACAACAAAGTTAATTAATTAATTATATTAAAAAAAGAAAA
+29154_superba.6 GRC13_0027_FC:4:1:12660:1232 length=74
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHGGHHHHFHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
@29154_superba.7 GRC13_0027_FC:4:1:13459:1226 length=74
TGCAGTCAATCACATTACTCTATACTCTTCTGCGTCAGGGATTTGATGGGAAACCAATGGTTAAAAAAGCGATC
+29154_superba.7 GRC13_0027_FC:4:1:13459:1226 length=74
HHHGGGEGGDGGG@HHHHHHEHHHGHHHG>DDGGEGGGGGBBGG>8CE>FEDGEDBFDEFGHHHHFHH<EBFEC
@29154_superba.8 GRC13_0027_FC:4:1:14162:1226 length=74
TGCAGTAACGTGAATTGCACTACGAAAACCTTCCAAACATAGGGCTATCACAACTTCATCATCACTTTGGTCAA
+29154_superba.8 GRC13_0027_FC:4:1:14162:1226 length=74
IIIIIIIIIIIIIIIIIIIIDIIIIIIHIHIIIIIIDIIIIIIIIIIIIIIHHFIIFIIGIIIEHIHHHEIIG
@29154_superba.9 GRC13_0027_FC:4:1:17619:1233 length=74
TGCAGTGGCCAATGATGACATGGTCACCCTCCTTAACACAAAAGCATGGGATATGAGCGTAGCAATATTTTGAA
+29154_superba.9 GRC13_0027_FC:4:1:17619:1233 length=74
IIIIIIIIIIIIIIIIIEIIIIHIIIIIIIIIIIHIGIIIIIDIIHIIHHGFHFHHIHIEIIIGBHCIHHIHBE
@29154_superba.10 GRC13_0027_FC:4:1:14537:1250 length=74
TGCAGCGGTTTTTTGAAGAGGTAGAATGGTCCGGACTGTTGGAGTAGGCAGCAGTTGGATCAGAAACTCGAAAA
+29154_superba.10 GRC13_0027_FC:4:1:14537:1250 length=74
IIIIIIIIIIIIIIIIIIHIIEIIIIIIHEIIIIEHIIIIIIEIEIIIIHIIEIGGIICGIHHEHHGIHHEEGE
```

RAD-seq genomics

Step 3:

Cluster/map reads

Identify reads that are from the same RAD locus within each sample and cluster them together by sequence similarity (denovo) or by mapping to a reference genome (reference).



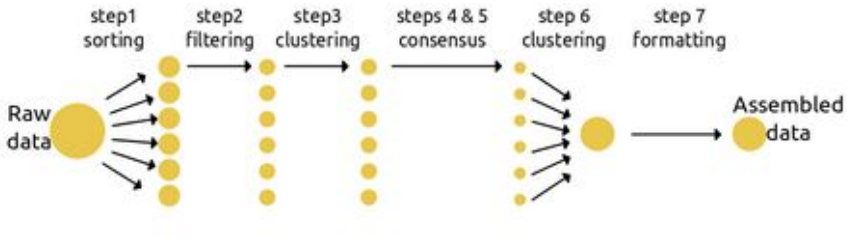

```
less w4050/files/analysis-ipyrad/Pedicularis_clust_0.9/\
32082_przewalskii.clustS.gz
```

```
0001b983dbc693d31d9d971be660771a;size=8;*
TGCAGGAGAAATCATAGCAGCATCAGATGCCATGGCATTTATCATTACAACAGCATGCAAATTAATATAGAATT
0f09d01ef88543a687f35db247a18c0f;size=8;+
TGCAGGAGAAATCATAGCAGCATGAGATGCCATGGCATTTATCATTACAACACCATGCAAATTAATATAGAATT
bb012c69ef74409a44b5581ffa8dc260;size=1;+
TGCAGGAGAAATCATAGCCGCATGAGATGCCATGGCATTTATCATTACAACACCATGCAAATTAATATAGAATT
9f97cb575f6b85b9ed83dc2dacf10aec;size=1;+
TGCAGGAGAAATCATAGCAGCATGAGATGCCATGGCATTTATCATT----------------------------
//
//
00024f7687a7bb684a2c2397ec2a0d0d;size=14;*
TGCAGCTCCTTCGACTCCTCCGAAGCTTCGAAGGCCGTTCAAGTCCCTGTTTCGAACAATCTCGGCGATGATGA
1bbedead1d4ba95aa97184863d69423c;size=4;+
TGCAGCTCCTTCGAATCCTCCGAAGCTTCGAAGGCCGTTCAAGTCCCTGTTTCGAACAATCTCGGCGATGATGA
7c95ba9bb40f3234d910fbc51ded6cc7;size=1;+
TGCAGCTCCTTCGACTCCTCCGAAGCTTCGAAGGCCGTTCAAGTCCCTGTTTCGAACAATCTCGGCG-------
a9196acfb3f2758dde0d826c8e040b2f;size=1;+
TGCAGCTCCTTCGACTCCTCCGAAGCTTCGAAGGCCGTTCAAGTCCCTGTTTCGAACAA---------------
5021dd9126bb4b8b608071337a174c32;size=1;+
TGCAGCTCCTTCGAATCCTCCGAAGCTTCGAAGGCCGTTCAAGTCCCTGTTTCGAACAATCTCGGCGATGATG-
//
//
0003b73fa2fd642a64a64bac96eff0fa;size=599;*
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCCGTCTTAGGAAATGACTTAGTTTAACTTAAAAGACAGATGCC
5ce0ab38773024648fffd091c903fd37;size=3;+
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCCGTCTTAGGAAATGACTTAGTTTAACTTAAAAGACAGATGC-
a2aaa64f49fb7fbb579cb9a54cc068d3;size=2;+
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCNGTCTTAGGAAATGACTTAGTTTAACTTAAAAGACAGATGCC
e99f8f219794191e541dcb752f7f519c;size=2;+
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCCGGCTTAGGAAATGACTTAGTTTAACTTAAAAGACAGATGCC
8d439f4880315957101 70fe4c518ff0b;size=2;+
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCCGTCTTAGGAAATGACTTAGTT--------------------
cbd277bbf2e12ae570e46ec56c845003;size=2;+
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCCGTCTTAGGAAATGACTTAGTTTAACT---------------
77bc94ead6597d65a17d5603e468eeba;size=2;+
```

RAD-seq genomics

Steps 4-5:

Estimate parameters and make consensus base calls and haplotype calls.



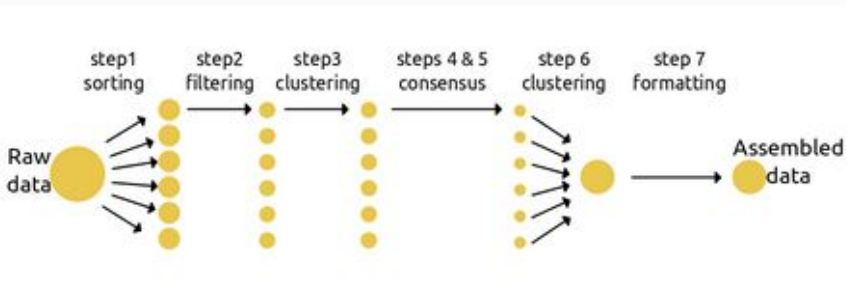

```
less w4050/files/analysis-ipyrad/Pedicularis_consens/29154_superba.consens.gz
```

```
>29154_superba_0
TGCAGGATGATAACAAAGCATTGGACCGGCTCACCAAATCCAAAGAGGCTGCTCTACTAGAAACAGAGAGAACT
>29154_superba_1
TGCAGCTTGGATTATCGTAGAATAAGAGGGGCCGTCTTAGGAAATGACTTAGTTTAACTTAAAAGACAGATGCC
>29154_superba_2
TGCAGCGGCGTCTAGAAGGTGTCGAYGAATTTAAGGTYTGTCTATTGATTTATTCATTTCAAAATAGGAACTAA
>29154_superba_3
TGCAGATTGGAATTCCCAGAAAGAATTGTTCTATCAAYAAACGGGAATTGRTCGTMTMTCTGTTMCAACAGTAM
>29154_superba_4
TGCAGTAAATTTAGTGAAGTTTATTGTAATGTTTATTCAGGAAGCAGAGAGGAAAATCCCAATTAATTATGCCT
>29154_superba_5
TGCAGTCGCTGMCYAGAAAAATGATGTTGGTTTCGATGTTGGCAACATCAGACSCAANATYRATGTCKGYTRCTC
>29154_superba_6
TGCAGAACTATCAAATTTTTCAATCAACCCATTAACTTTATGTTCATTTATTAAAAAAAGAAAATCACAAGATA
>29154_superba_7
TGCAGTGCCAAAAAAAAAAAKGTCGAAAAANAAAyGCTTTACCAAAAATCCACACyGTAGTGCGTGTTTTGTGTGT
>29154_superba_8
TGCAGCTTGTGGGGAGACAATCATTTTGACAAAGTGCAAGATGGAGTACTAGTGTGGAGGTTAAGGTGAAGAGG
>29154_superba_9
TGCAGTATTGAAGAGAGCCTTGSKCGCTATCGTGCTGTAsTTAATGCCGCTAAGAAGCTTTCGATTCCCGTGCG
>29154_superba_10
TGCAGNATTGYAGGTACTTGCGTArAANAAAAATAAGATAAACGCAAACYGATGTTGTCAGTGGTGTTGGCAACACCANTCAC
>29154_superba_11
TGCAGCTGTTGATTGATTGCCAGCAACTCCTCCGGCAACTCTGGCTCGTCGACACTAACTCCATTCCAATTTAA
>29154_superba_12
TGCAGCAATTTAAAAGAACACTCGGGTCCCAAGGAACAAAAGGATATAAAAGTCCATGTGTTGTCATCCTCAGT
>29154_superba_13
TGCAGAGTCCAGACATTACAGAAAACCTGTCATTCTAACAAAACCACACTTTTCYGCTCTAACGACTTCTTACT
>29154_superba_14
TGCAGCATTTTCGAACTCAAGTAGATATGGCTGCAACCGCAACTCCGGGNCCACAAATTGTATCACCAAGGCCT
>29154_superba_15
TGCAGATTATCAGAACTTTGCCATATCGCATGATGCTGACTCGATCTTGAGTAAGGCTCATGATGATCAAGTGA
```

RAD-seq genomics

Steps 6:

Cluster/Map across samples -- identify *homologous* sequences derived from the same genomic locus.




```
less w4050/files/analysis-ipyrad/Pedicularis_across/\
Pedicularis_catclust.gz
```

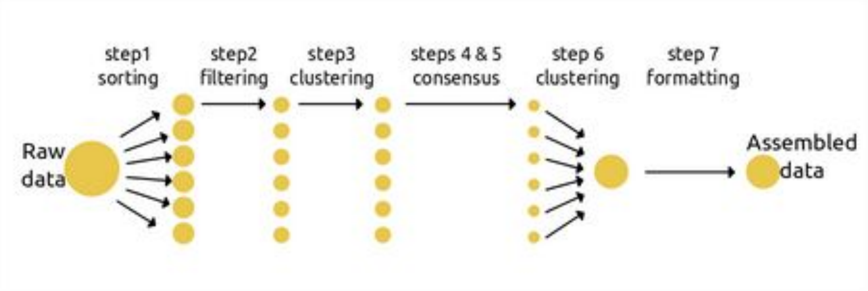
```
29154_superba_1045
TGCAGGTGAGACGATCTTGCACTTTT--TTTTTCATCCATACAAAAGAAAAAATGGATTTT-AAGTTCAATATAAATA
30556_thamno_65359
TGCAGGTGAGAGGATCTTGCATTTTTT-TTTTTTATCCATACAAAAGAAAAAATGGATTTA-AAGTTCAATATAAA--
30686_cyathophylla_71328
TGCAGGTGAGACGATCTTGCATTTTTTCTTTTTTATCCATACAAAAGAAAAAATGGATTTT-AAGTTCAATATAA---
41478_cyathophylloides_61181
TGCAGGTGAGACGATCCTGCACTTT---TTTTTCATCCATACAAAAGAAAAAATGGATTTTAAAGTTCAATATAAAT-
41954_cyathophylloides_67315
TGCAGGTGAGACGATCCTGCACTTT---TTTTTCATCCATACAAAAGAAAAAATGGATTTTAAAGTTCAATATAAAT-
//
//
29154_superba_105
TGCAGGCCAGCCCGATCCATCAGGCCTCGTCGGCTTTATGCGCGGGCCTAAATAACCGGCATTCYAGCCGGGTT
30686_cyathophylla_40275
TGCAGGCCAGCCCGATCCATCAGGCCTCGTGGGCTTTATGCACGGGCCAAAATAACCCGCATTCCAGCCGGAAT
//
//
29154_superba_107
TGCAGGATCATCATTATGTACCGGATAATAAAGAACCTCACCAATCTGTTCATCTTTTAAAATGTCAGAATACT
35236_rex_1103
TGCAGGATCATCATTATGTACCGGATAATAAAGAACCTCACCAATCTCTTCATCTTTTAAAATGTCCGAATACA
35855_rex_24476
TGCAGGATCATCATTATGTAMCGGATAATAAAGAACCTCACCAATCTSTTCATCTTTTAAAATGTCAGAATACT
38362_rex_18814
TGCAGGATCATCATTATGTACCGGATAATAAAGAACCTCACCAATCTCTTCATCTTTTAAAATGTCAGAATACT
39618_rex_14308
TGCAGGATCATCATTATGTACCGGATAATAAAGAACCTCACCAATCTCTTCATCTTTTAAAATGTCAGAATACT
41478_cyathophylloides_359
TGCAGGATCATCATTATGTACCGGATAATAAAGAACCTCACCAATCTGTTCATCTTTTAAAATGTCAGAATACT
41954_cyathophylloides_77279
TGCAGGATCATCATTATGTACCGGATAATAAAGAACCTCACCAATCTGTTCATCTTTTAAAATGTCAGAATACT
//
```

RAD-seq genomics

Steps 7:

Filter and format output files.

You can apply many different filters at this stage to reduce error-prone regions, or to exclude data with too much missing information.




```
less /rigel/edu/w4050/files/analysis-ipyrad/\
min10_outfiles/min10.loci
```

```
29154_superba              ATATAACGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
30556_thamno               ATATAATGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
30686_cyathophylla         ATATAACGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
35236_rex                  ATATAATGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
35855_rex                  ATATAACGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
38362_rex                  ATATAATGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
39618_rex                  ATATAATGAGGCCACAGCCGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
40578_rex                  ATATAACGAGGCCACAGCYGGTTTTAAGCCTTACAGACAAAAAGATGGCGCCCTTCTTGCCATTGGWGC
41478_cyathophylloides     ATATAATGAGGCCACAGCCGGTTTTAAGCCTTATAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
41954_cyathophylloides     ATATAATGAGGCCACAGCCGGTTTTAAGCCTTATAGACAAAAAGATGGCGCCCTTCTTGCCATTGGAGC
//                               *           -               *                                -  |7|
29154_superba              GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
30556_thamno               GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
30686_cyathophylla         GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
32082_przewalskii          GCGGAACGATGGACC-TTTACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
33413_thamno               GCGGAAGGATGAACGATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
35236_rex                  GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
35855_rex                  GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
38362_rex                  GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
40578_rex                  GCGGAACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCTGCTCCAGCAAGGTCCACAAT
41478_cyathophylloides     GCGGGACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
41954_cyathophylloides     GCGGGACGATGAACCATATACCTGATTGCCGGTCTTCTTTTCTCTCTCCGCTCCAGCAAGGTCCACAAT
//                             * -    -  -  -                               -                    |17|
29154_superba              AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
30556_thamno               AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
30686_cyathophylla         AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAATTGTATGTTGCAT
33413_thamno               AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
35236_rex                  AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
35855_rex                  AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
38362_rex                  AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
39618_rex                  AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
40578_rex                  AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
41478_cyathophylloides     AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
41954_cyathophylloides     AGGTTGAGCTATAGGCTGAATTAGAGTCCTGTGTGTAGTTTTGATACTGATGAGAACTGTATGTTGCAT
//                                                                                  -            |20|
29154_superba              AGCAAATTCGTTTGGCGCCTGAGAAAATGTAACCAGCTCTATTAAATGCCAGTTTCATTTATTTTGGCTC
30556_thamno               AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
30686_cyathophylla         AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
32082_przewalskii          AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
35236_rex                  AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
35855_rex                  AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAAACRCCAGTTTMATTTATTTTGGCTC
38362_rex                  AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAATCGCCAGTTTCATTTATTTTGGCTC
40578_rex                  AGCAAATTCGTTTGGCGCCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
41478_cyathophylloides     AGCAAATTCGTTTGGCACCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
41954_cyathophylloides     AGCAAATTCGTTTGGCACCTGAG-AAATGTAACCAGCTCTATTAAACGCCAGTTTCATTTATTTTGGCTC
//                                         *                             ---       -              |46|
```

RAD-seq genomics

The command-line interface (CLI)

ipyrad analyses can be run using a simple command line tool to run steps of the assembly.

The params file

A single params file describes all the parameters needed to assemble a dataset from steps 1 - 7 .

```
ipyrad -p params-data.txt -s 1234567
```

RAD-seq genomics

The parameter settings:

http://ipyrad.readthedocs.io/parameters.html

We will walk through the most important of these. They include options for entering the location of your raw data, and where you want the assembled data to be stored, as well as filtering options, and parameters that affect the efficiency and accuracy of assemblies.

When working with the command line you can create a default params file with the -n argument and then edit it with a text editor.

```
# create a new params file for 'new'
ipyrad -n new

# edit it with a text editor
subl params-new.txt
```

```
------ ipyrad params file (v.0.7.23)-----------------------------------------
new                            ## [0] [assembly_name]: Assembly name. Used to na
./                             ## [1] [project_dir]: Project dir (made in curdir
                               ## [2] [raw_fastq_path]: Location of raw non-demu
                               ## [3] [barcodes_path]: Location of barcodes file
                               ## [4] [sorted_fastq_path]: Location of demultipl
denovo                         ## [5] [assembly_method]: Assembly method (denovo
                               ## [6] [reference_sequence]: Location of referenc
rad                            ## [7] [datatype]: Datatype (see docs): rad, gbs,
TGCAG,                         ## [8] [restriction_overhang]: Restriction overha
5                              ## [9] [max_low_qual_bases]: Max low quality base
33                             ## [10] [phred_Qscore_offset]: phred Q score offs
6                              ## [11] [mindepth_statistical]: Min depth for sta
6                              ## [12] [mindepth_majrule]: Min depth for majorit
10000                          ## [13] [maxdepth]: Max cluster depth within samp
0.85                           ## [14] [clust_threshold]: Clustering threshold f
0                              ## [15] [max_barcode_mismatch]: Max number of all
0                              ## [16] [filter_adapters]: Filter for adapters/pr
35                             ## [17] [filter_min_trim_len]: Min length of read
2                              ## [18] [max_alleles_consens]: Max alleles per si
5, 5                           ## [19] [max_Ns_consens]: Max N's (uncalled bases
8, 8                           ## [20] [max_Hs_consens]: Max Hs (heterozygotes)
4                              ## [21] [min_samples_locus]: Min # samples per lo
20, 20                         ## [22] [max_SNPs_locus]: Max # SNPs per locus (R
8, 8                           ## [23] [max_Indels_locus]: Max # of indels per l
0.5                            ## [24] [max_shared_Hs_locus]: Max # heterozygous
0, 0, 0, 0                     ## [25] [trim_reads]: Trim raw read edges (R1>, <
0, 0, 0, 0                     ## [26] [trim_loci]: Trim locus edges (see docs)
p, s, v                        ## [27] [output_formats]: Output formats (see doc
                               ## [28] [pop_assign_file]: Path to population ass
```

Ipyrad API

The ipyrad API let's you do all of these same things but in a way that is much cleaner, and more programmatic and reproducible.

http://ipyrad.readthedocs.io/API_user-guide.html

Copy notebook from the Habanero cluster:

```
cp /rigel/edu/w4050/files/nb-12-example.ipynb ~
```

Setting parameters of the assembly

```
data = ip.Assembly("Pedicularis")
```

```
New Assembly: Pedicularis
```

```
data.set_params("project_dir", "/rigel/edu/w4050/files/analysis-ipyrad")
data.set_params("sorted_fastq_path", "/rigel/edu/w4050/files/example_emp
data.set_params("clust_threshold", 0.90)
data.set_params("mindepth_majrule", 10)
data.set_params("mindepth_statistical", 10)
data.set_params("filter_adapters", 2)
data.set_params("output_formats", "*")
```

Run ipyrad assembly

```
data.run("12", ipyclient=ipyclient, show_cluster=True)
```

```
host compute node: [24 cores] on node009
Assembly: Pedicularis
[####################] 100%  loading reads          | 0:00:04 | s1 |
[####################] 100%  processing reads       | 0:01:09 | s2 |
```

Ipyrad API

Using ipyrad with jupyter and ipyparallel

http://ipyrad.readthedocs.io/HPC_Tunnel.html

```
cp /rigel/edu/w4050/files/nb-12-example.ipynb ~
```

Setting parameters of the assembly

```
data = ip.Assembly("Pedicularis")
```

```
New Assembly: Pedicularis
```

```
data.set_params("project_dir", "/rigel/edu/w4050/files/analysis-ipyrad")
data.set_params("sorted_fastq_path", "/rigel/edu/w4050/files/example_emp
data.set_params("clust_threshold", 0.90)
data.set_params("mindepth_majrule", 10)
data.set_params("mindepth_statistical", 10)
data.set_params("filter_adapters", 2)
data.set_params("output_formats", "*")
```

Run ipyrad assembly

```
data.run("12", ipyclient=ipyclient, show_cluster=True)
```

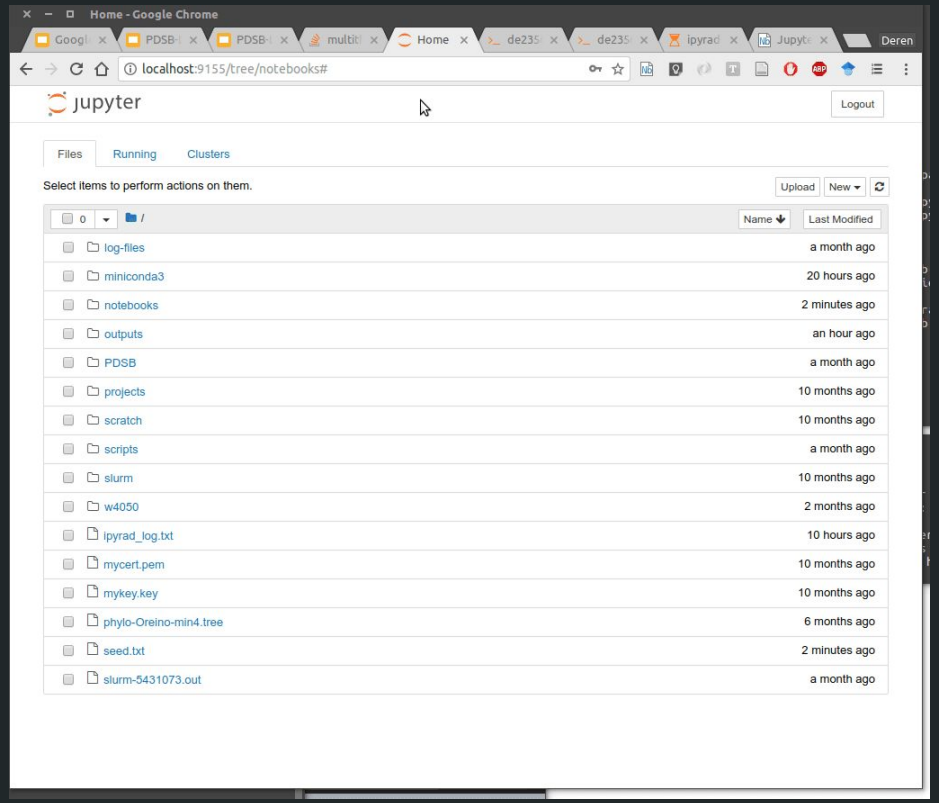
```
host compute node: [24 cores] on node009
Assembly: Pedicularis
[####################] 100%  loading reads          | 0:00:04 | s1 |
[####################] 100%  processing reads       | 0:01:09 | s2 |
```

RAD-seq genomics

After submitting jobs to run on ipcluster you can check that your jobs are running correctly by using the `top` command from a terminal in you jupyter dashboard.

Press the ‘q’ key to quit out of top and return to the normal terminal.

The ability to check your running jobs with top makes working interactively on the cluster much easier to use than submitting batch jobs with sbatch.



Assignments notebook is in assignments/
Assigned reading in syllabus:

Assignment: Link to Session 12 repo
Readings: See syllabus
Collaborate: Work together in this gitter chatroom