Metagenomic analysis using NanoDJ Notebooks in CyVerse

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September 27th, 2019

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Outline of Today’s Focus Forum

- Introduction to metagenomics using long reads.
- Classification with BLAST and custom databases.
- Rapid taxonomic classification using Kraken2.

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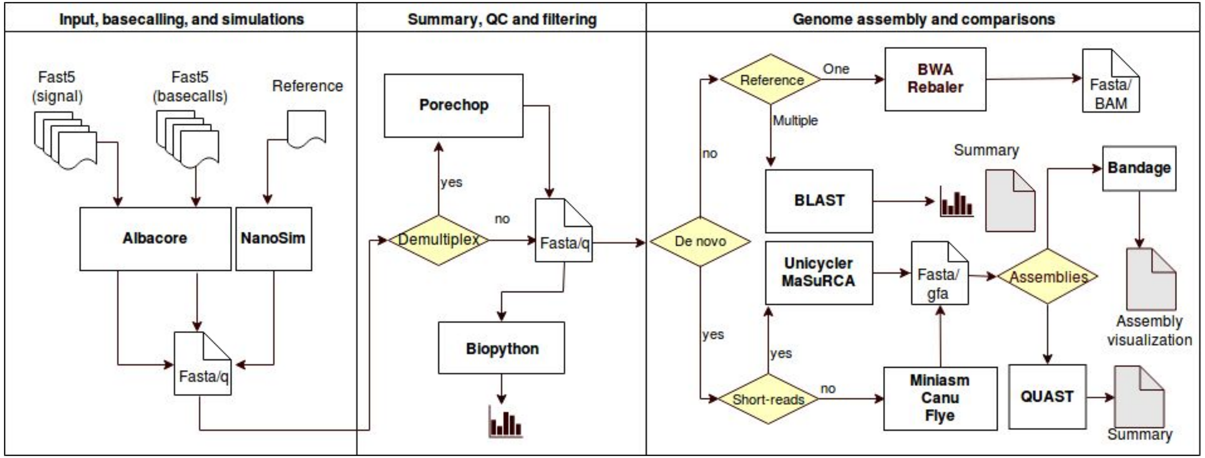
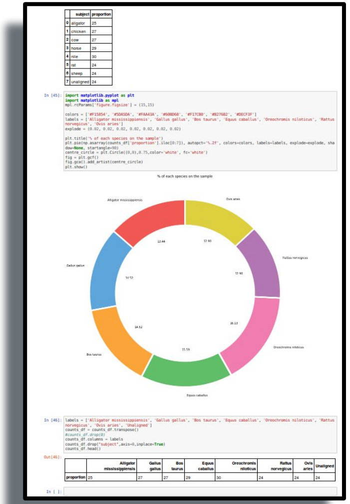
- **Integration of bioinformatics tools for Nanopore data analysis.**
- **Jupyter notebooks with genome assembly and metagenomics pipelines step by step.**





	subject	proportion
0	aligator	25
1	chicken	27
2	cow	27
3	horse	29
4	nile	30
5	rat	24
6	sheep	24
7	unaligned	24

```
In [45]: import matplotlib.pyplot as plt
         import matplotlib as mpl
         mpl.rcParams['figure.figsize'] = (15,15)

         colors = ['#F15854', '#5DA5DA', '#FAA43A', '#60BD68', '#F17CB0', '#B276B2', '#DECF3F']
         labels = ['Alligator mississippiensis', 'Gallus gallus', 'Bos taurus', 'Equus caballus', 'Oreochromis niloticus', 'Rattus
         norvegicus', 'Ovis aries']
         explode = (0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02)

         plt.title('% of each species on the sample')
         plt.pie(np.asarray(counts_df['proportion'].iloc[0:7]), autopct='%.2f', colors=colors, labels=labels, explode=explode, sha
         dow=None, startangle=90)
         centre_circle = plt.Circle((0,0),0.75,color='white', fc='white')
         fig = plt.gcf()
         fig.gca().add_artist(centre_circle)
         plt.show()
```

```
In [46]: labels = ['Alligator mississippiensis', 'Gallus gallus', 'Bos taurus', 'Equus caballus', 'Oreochromis niloticus', 'Rattus
         norvegicus', 'Ovis aries', 'Unaligned']
         counts_df = counts_df.transpose()
         #counts_df.drop(0)
         counts_df.columns = labels
         counts_df.drop("subject",axis=0,inplace=True)
         counts_df.head()
```

Out[46]:

	Alligator mississippiensis	Gallus gallus	Bos taurus	Equus caballus	Oreochromis niloticus	Rattus norvegicus	Ovis aries	Unaligned
proportion	25	27	27	29	30	24	24	24

In [ ]:

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Materials

- **NanoDJ (included in VICE)**: Integration of bioinformatics tools for Nanopore data analysis on top of a Jupyter Lab environment.
    - Python programming language (Biopython, Numpy, Pandas, Matplotlib).
    - Command line tools (BLAST tools, Kraken2, Bracken).
- **2 notebooks:**
    - Classification with BLAST and custom database.
    - Rapid taxonomic classification with Kraken2.
- **Example sequence datasets:**
    - MtDNA of different species.
    - 16S rRNA sequences of a saliva sample.

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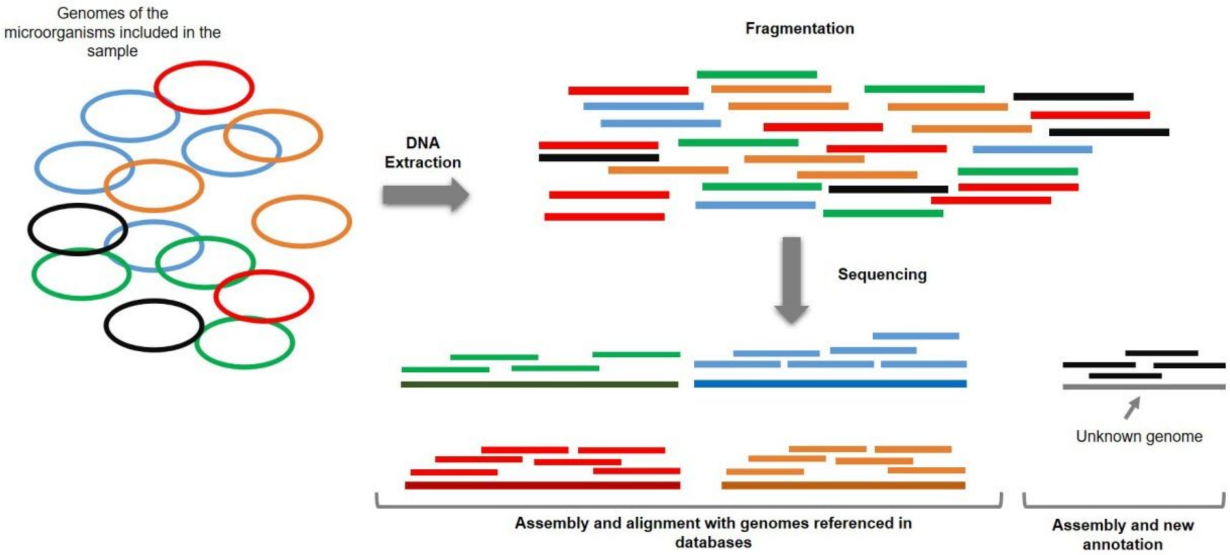
Metagenomics

- Shotgun metagenomics
- Targeted metagenomics



Genomes of the microorganisms included in the sample

DNA Extraction

Fragmentation

Sequencing

Unknown genome

Assembly and alignment with genomes referenced in databases

Assembly and new annotation

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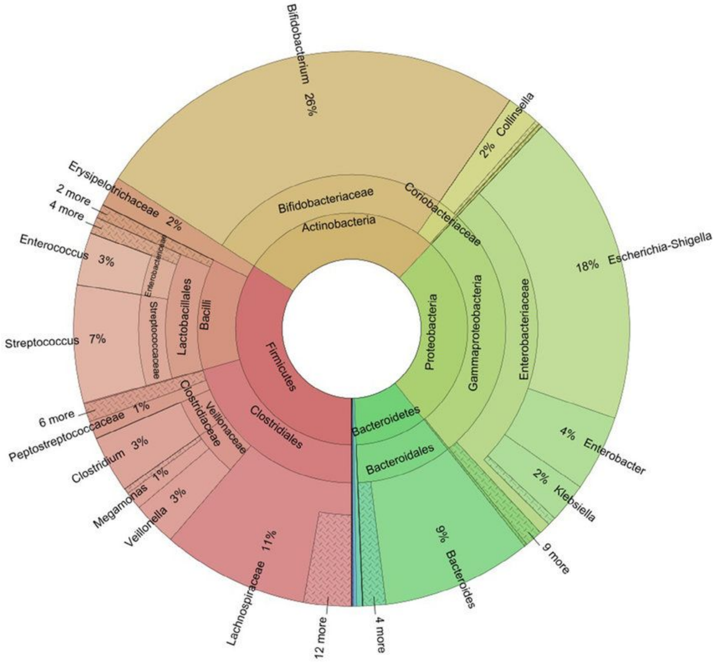
Metagenomics applications

- Ecology.
- Agriculture.
- Medicine:
    - ○ Gut microbe characterization.
    - ○ Infectious disease diagnosis.



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Metagenomic analysis using long reads

- Cost-efficient approach to metagenomics.
- Rapid library preparation and real time data output.
- Long reads enable sequencing of informative genes (e.g. 16S rRNA) in their entirety (Full 16s on one read).

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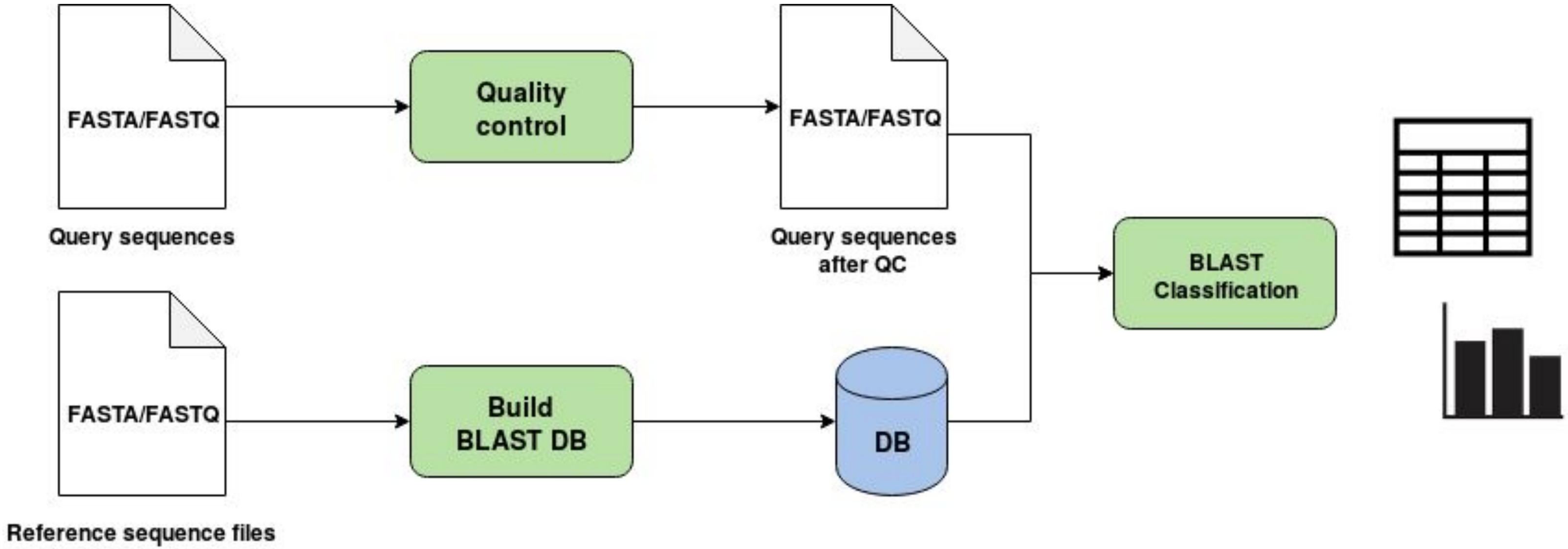
Classification with custom database using BLAST



FASTA/FASTQ

Query sequences

Quality control

FASTA/FASTQ

Query sequences after QC

FASTA/FASTQ

Reference sequence files

Build BLAST DB

DB

BLAST Classification

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Rapid taxonomic classification of long read data

Why is rapid taxonomic classification of long reads challenging?

- Much longer reads (300bp vs <1kbp) and higher error-ratio.
- BLAST scales badly in time and space. Not suitable for high-throughput data.
- Databases are not perfect (not even close) on accuracy and species diversity representation.

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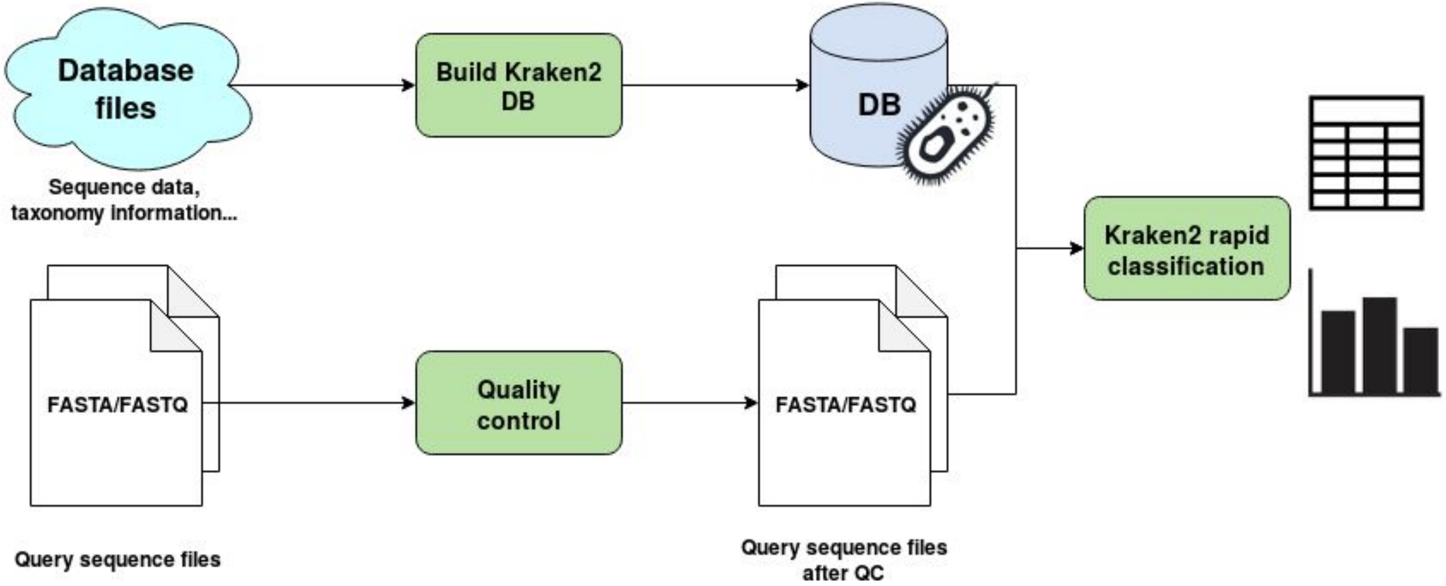
Rapid taxonomic classification of complete 16S reads



Database files

Sequence data, taxonomy information...

Build Kraken2 DB

DB

FASTA/FASTQ

Query sequence files

Quality control

FASTA/FASTQ

Query sequence files after QC

Kraken2 rapid classification

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Things to keep in mind

- Classification of long reads and high-throughput sequencing data can be done using rapid approaches. Modern tools focus on increasing classification speed and reducing memory consumption.

- Database choice is important. Kraken2 includes commands for building NCBI RefSeq databases, 16S databases and custom databases.

- This tools are included in NanoDJ and work fine with Nanopore long read data but they also work also with short read data.

★ <u>Check NanoDJ repository for updates. More notebooks coming!</u>

`https://github.com/genomicsITER/NanoDJ`

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Thank you for attending

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CyVerse is supported by the National Science Foundation under Grants No. DBI-0735191, DBI-1265383 and DBI-1743442.

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