Introduction to Metagenomics Analysis for Next Generation Sequencing Data

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DIVISION OF RESEARCH
TEXAS A&M UNIVERSITY

Primary NGS Applications

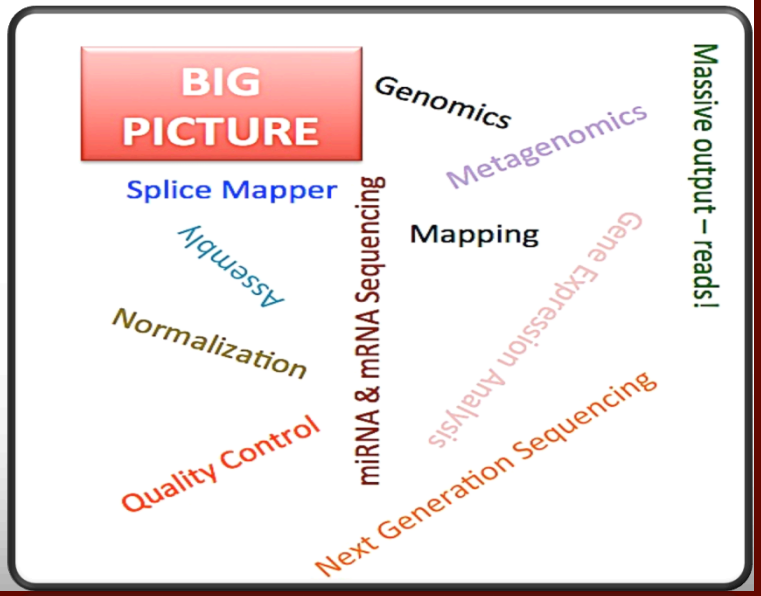
Last weeks

1. Alignment
2. Assembly(no reference/with a reference)
   - Genome
   - Transcriptome
3. RNA-Seq

This class → **4. Metagenomics**

5. ChIP-Seq

Next week → 6. RADSeq



Outline

- Background
  - Sequencing
- Application of Next Generation Sequencing in Research

Why sequencing?

Determining the sequence of nucleotides within a DNA (or RNA) fragment

How?

Using sequencing methods, such as Sanger sequencing, next generation sequencing and single-molecule techniques

Sanger

Classic Sequencing

Third Generation Sequencing Platforms

PacBio

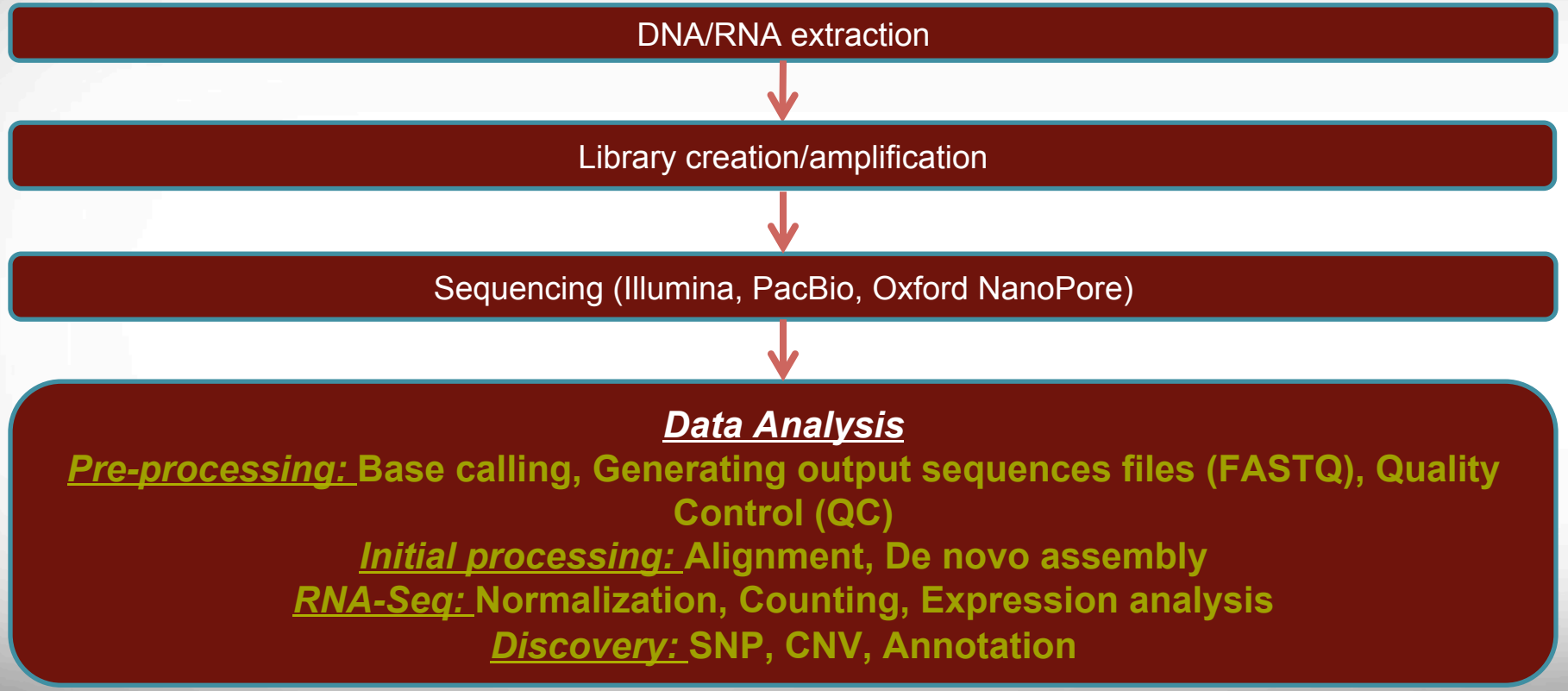
Next Generation Sequencing Platforms

Illumina

MinION

http://nextgenseek.com/2014/01/illumina-announces-new-sequencers-hiseqx-nextseq-500-at-jpm-2014/

NGS Sequencing Workflow



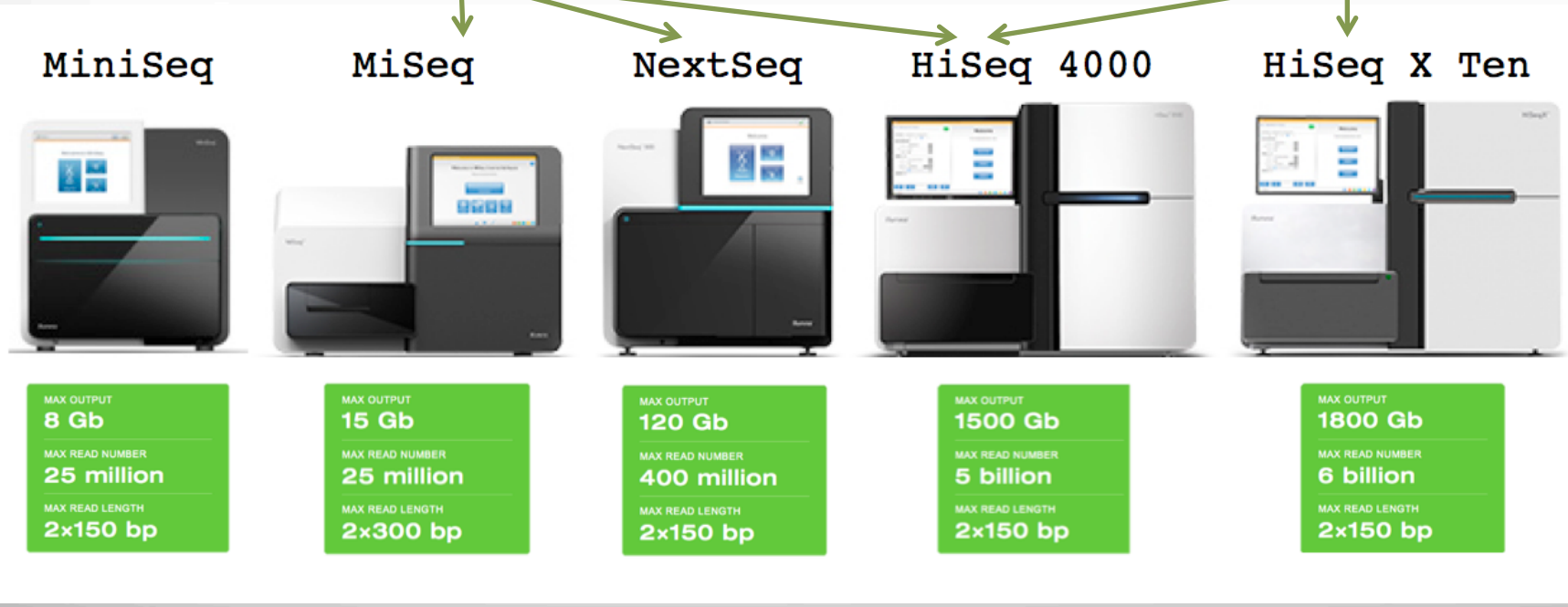
Comparing Sequencing Platforms

Platform	Read length	Error rates	Technology	Portable?
Illumina	< 400 bp	Low	Sequencing by synthesis	No
PacBio	~ 10-15 Kb	High	SMRT – ZMW	No
Oxford Nanopore Technologies	~ 5-8 Kb	High	Nanopore protein – strand sequencing	Yes

Choosing among Illumina Sequencers

Metagenomics 16S rRNA

Metagenomics WGS



http://core-genomics.blogspot.com/2016/01/meet-newest-members-of-family-miniseq.html

Metagenomics

What is Metagenomics?

Study of communities of microbial organisms directly in their natural environments
Without the need for isolation and lab cultivation of individual species

Moved from traditional BAC cloning to NGS long reads or high coverage short reads

• Chen K and Pachter L, Bioinformatics for Whole-Genome Shotgun Sequencing of Microbial Communities, *PLoS Comput Biol*,

Metagenomics Studies

- PathoMap
  - Research project by Weill Cornell Medical College to study the microbiome and metagenome of the built environment of NYC
- Cow rumen microbiome study
  - 220 bacterial and archaeal genomes assembled directly from 768 GB rumen sequenced data
  - Majority unsequenced strains and species of bacteria and archaea
  - Over 13,000 proteins predicted to be involved in carbohydrate metabolism, over 90% of which do not have a good match in the public databases
  - Assembly of hundreds of microbial genomes from the cow rumen reveals novel microbial species encoding enzymes with roles in carbohydrate metabolism

Metagenomics Techniques

1. Whole Genome Shotgun (WGS)
2. Marker Gene
   - 16S Ribosomal RNA (rRNA)
     - Bacteria, Archaea
   - 18S rRNA
     - Fungus, Eukaryotes

- Chen K and Pachter L, Bioinformatics for Whole-Genome Shotgun Sequencing of Microbial Communities, *PLoS Comput Biol,* 1(2), 2005.

Whole Genome Shotgun (WGS) Metagenomics

- Sequencing the whole genome of the organisms present in the sample
- Facilitates discovering gene/gene function, genome structure
- Studying the evolutionary relationships for microbiomes
- Steps
  - Genome Assembly
  - Binning
  - Predicting and Annotating Genes

• Chen K and Pachter L, Bioinformatics for Whole-Genome Shotgun Sequencing of Microbial Communities, *PLoS Comput Biol*,

WGS Metagenomics Tools

Assembly

- Velvet, MetaVelvet, MetaVelvet-SL
- IDBA-UD
- MetAMOS pipeline: selecting assembly, scaffolding, annotating
- Genome Assemblers such as ALLPATHS, SOAP and ABySS

Binning

- LikelyBin
- PHYSCIMM
- MetaCluster
- MetaWatt
- MetaPhyler
- PhymmBL

Annotation

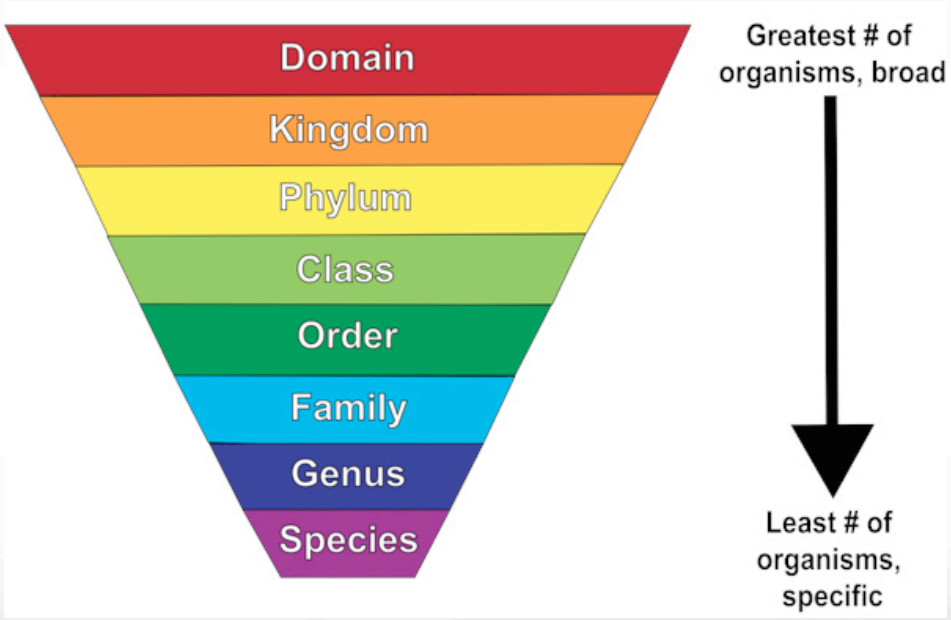
- MetaGeneAnnotator
- Glimmer-MG
- FragGeneScan
- MetaGeneMark
- Kraken

Marker Gene Metagenomics

- Usually based on 16S rRNA
  - Conserved within species
  - Greatly different between species
  - Widely used for microbial ecology
- Needs a reference database to match the Operational Taxonomic Units (OTU)
  - Silva
  - Ribosomal Database Project
  - Unite
- Steps
  - Preprocessing to remove noise
  - OUT clustering and taxonomic assignment
  - Alpha diversity analysis – within sample diversity
  - Beta diversity analysis – between sample diversity

Metagenomics - Outcomes

- OUT clustering
- Taxonomic rank assignment
- Alpha diversity analysis – within sample diversity
- Beta diversity analysis – between sample diversity



https://d2gne97vdumgn3.cloudfront.net/api/file/QooG1lg6RLGdDVIi9oOg

Marker Gene Metagenomics Tools

Microbial community analysis

- QIIME
- Mothur
- SILVAngs
- MG-RAST
- MEGAN

Diversity analysis

- Chao
- UniFrac
- PCoA

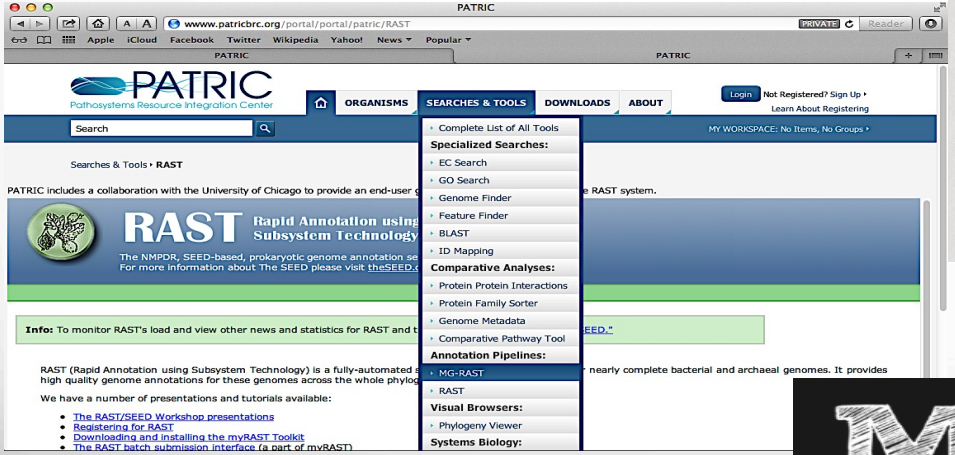
Visualization

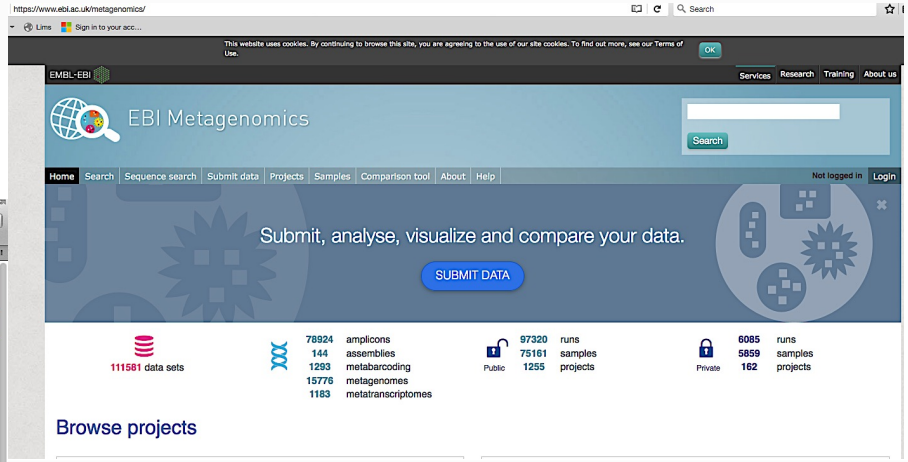
- QIIME
- MEGAN
- FigTree

Metagenomics Web-Based Tool

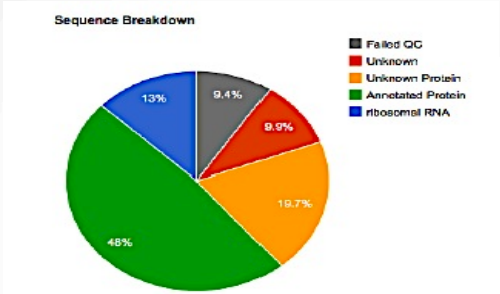
MG-RAST

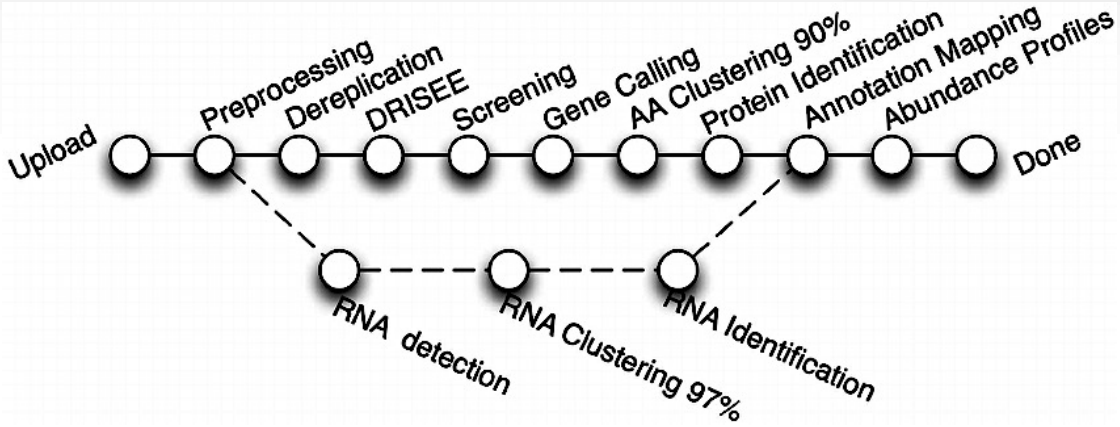
- Available tools, via PATRIC
- RAST: Rapid Annotation using Subsystem Technology
- Annotating the assembled contigs of a bacterial and archaeal genomes
- Quantitative insights for microbial populations, based on NGS data

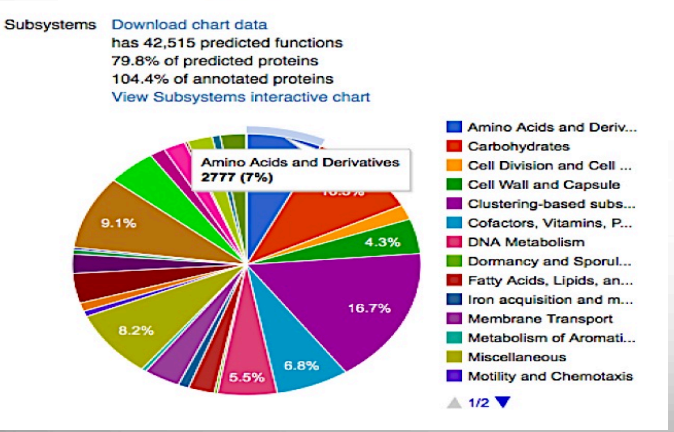


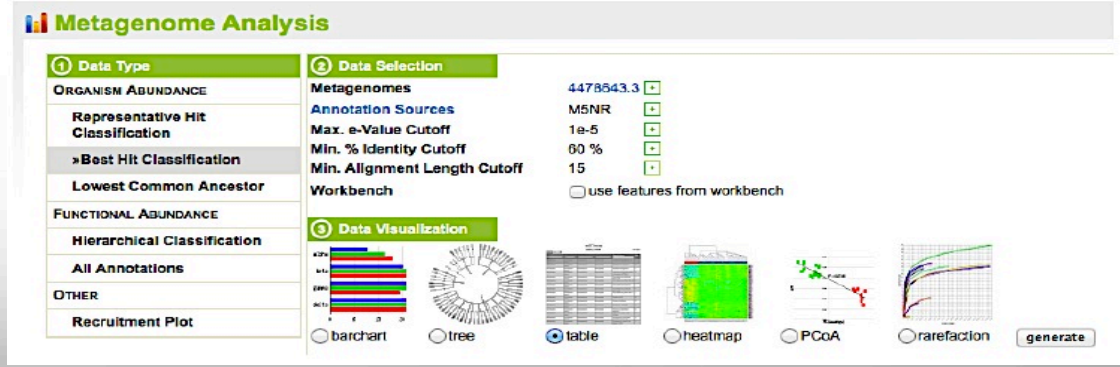


MG-RAST Pipeline









MG-RAST Example

Amplicon Based 16S Ribosomal RNA Sequencing and Genus Identification

*J. Risinger, *L. Renken, +J. Hill,
+N. Ghaffari, +R P. Metz, PhD,
+C. D. Johnson,*M. M. Toloue,
*Bioo Scientific
+AgriLife Genomics and Bioinformatics Service, Texas A&M University

Presented at PAG 2015

MG-RAST Example - 2

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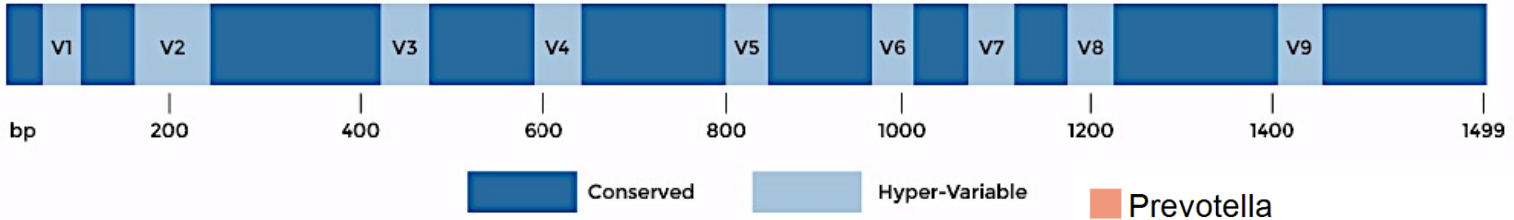
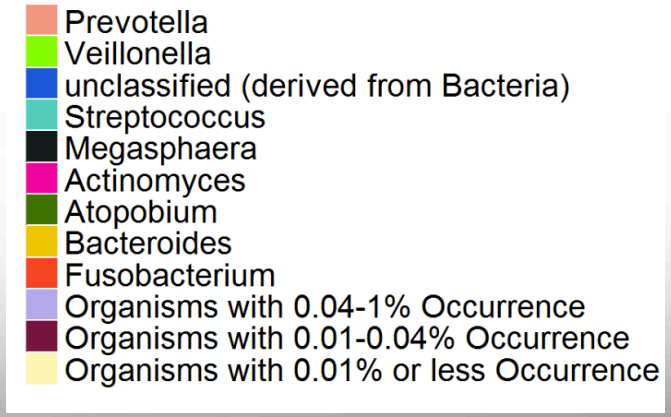
We demonstrate the utility of the NEXTflex™ 16S ***V1-V3 Amplicon***-Seq Kit combined with the longer read chemistry of Illumina MiSeq (2x300) for enabling accurate identification of genera present in highly complex microbial communities across a vast number of samples



- Common practice: V4 region
- 7 human saliva samples
- The top ten represented genera in this study reflect
- proportions expected to be found in oral microbiome of a healthy individual



Metagenomics Tools - Mothur

- Open-source
- Serves the microbial ecology community
- DOTUR and SONS programs
- Data: Sanger, PacBio, IonTorrent, 454, and Illumina MiSeq and HiSeq
- Most cited bioinformatics tool for analyzing 16S rRNA gene sequences

Metagenomics Tools – Qiime 2

- Qiime: Quantitative Insights Into Microbial Ecology
- Open-source, community developed
- NGS microbial bioinformatics platform
  - Interactive visualizations and data exploration
  - Automatically tracks analysis
  - Facilitate easy sharing
  - Plug-in based
- Multiple interfaces
  - Command line interface: q2cli
  - Data scientist's interface: Artifact API
  - the graphical user interface: q2studio (PROTOTYPE)
- Artifact: contain data and metadata

Qiime2 - Continued

Input Data

- SE or PE FastQ files, multiplexed or demultiplexed
  - Name of the input files should have a specific format: Sample1_Barcode1_L001_R1_001.fastq.gz
    - sample identifier_barcode sequence/barcode identifier_the lane number_read number_set number.fastq(.gz)
- FastQ Manifest
  - CSV manifest file, columns are
    - Sample ID, file-path, direction of sequencing (forward/reverse)
- Feature Table Data
  - BIOM format, based on HDF5
    - Id, type, format-url, format-version, generated-by, creation-date, shape, nnz (non-zero elements)
- Per-feature unaligned sequence data
- Phylogenetic trees (unrooted)

Qiime2 - Continued

Meta Data

- Tab-separated text (TSV) file
  - Example: https://docs.google.com/spreadsheets/d/1bHXutGx07HnYUGE1O4lFn9yItt6BEXQEZn276xqPid0/edit#gid=0

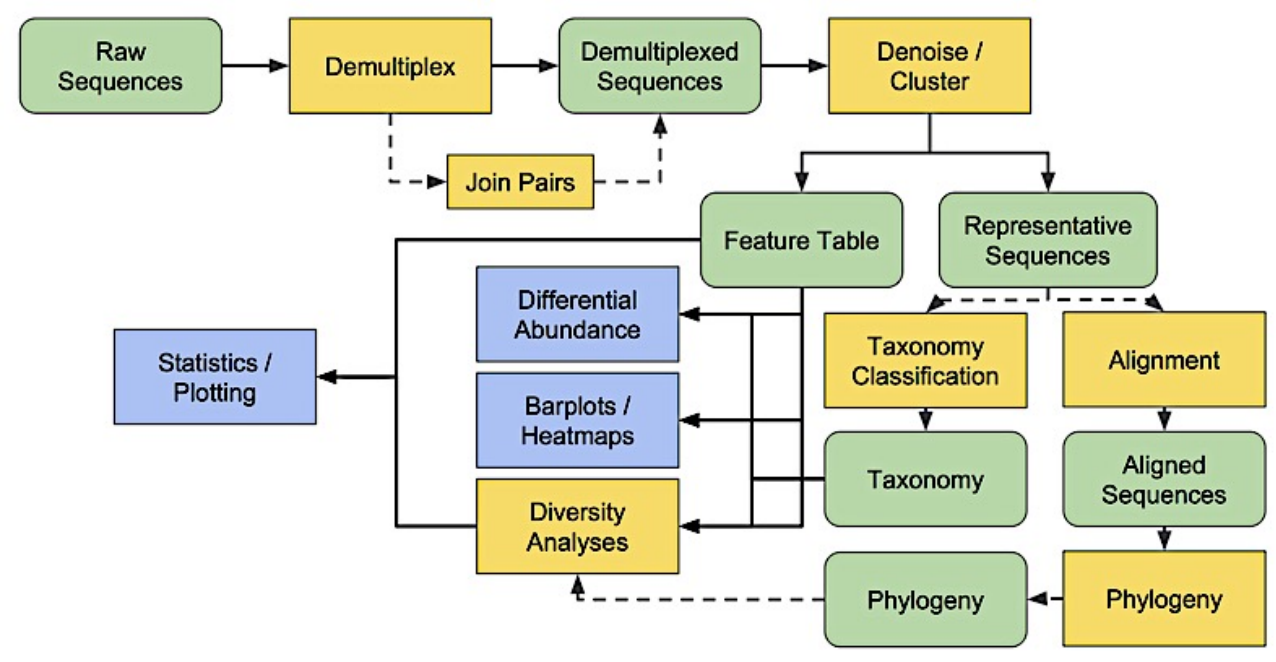
Artifacts

- Name.qza, zipped archives files containing data and its related files
- Name.qzv, visualization files
  - Visualize online at: https://view.qiime2.org/
  - Command: "qiime tools view file.qzv" on HPRC portal > VNC session by logging into: portal.hprc.tamu.edu

Classification

- Naive Bayes classifier can be trained based on sequenced data or can be downloaded based pre-trained from Qiime2 “Data resources”: https://docs.qiime2.org/2018.8/data-resources/

Qiime2 - Continued



- Sample QC: DADA2 R Package. Only SE, thus, ran for R1 and R2 files separately and then results are merged.

https://docs.qiime2.org/2018.8/tutorials/overview/

Qiime 2 - Practice

- https://docs.qiime2.org/2018.2/tutorials/

- ***Practical portion (***based on different tutorials)

  - Data: Fecal microbiota transplant (FMT)
  - Children under the age of 18 with autism and gastrointestinal disorders
  - Treated with ***fecal microbiota transplant*** in attempt to reduce the severity of their behavioral and gastrointestinal symptoms
    - collection of weekly fecal swab samples
    - stool samples
  - 18 treated individuals, 20 control
  - Subset data for exercise***: 5 treated, 5 control:*** Between six and sixteen samples are included per individual, including stool and fecal swab samples for each individual, and samples before and after FMT treatment. Five samples of the transplanted fecal material are also included.
  - 2 Illumina MiSeq sequencing runs

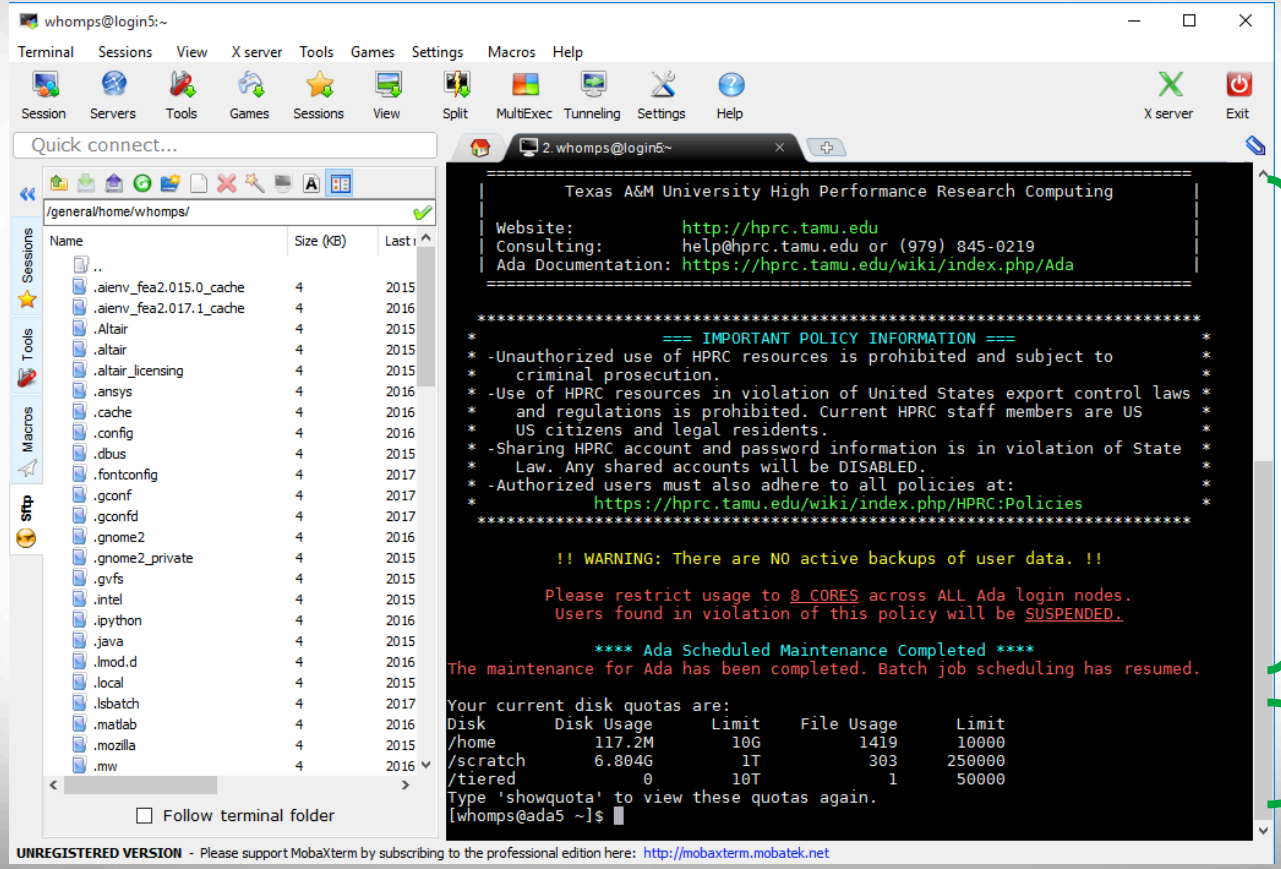
Practical Portion

Logging in to the system

- SSH (secure shell)
  - The only program allowed for remote access; encrypted communication; freely available for Linux/Unix and Mac OS X hosts;
- For Microsoft Windows PCs, use *MobaXterm*
    - https://hprc.tamu.edu/wiki/HPRC:MobaXterm
      - You are able to view images and use GUI applications with MobaXterm
  - or *Putty*
    - https://hprc.tamu.edu/wiki/HPRC:Access#Using_PuTTY
      - You can not view images or use GUI applications with PuTTY

- Both state of Texas law and TAMU regulations prohibit the sharing and/or illegal use of computer passwords and accounts
- Don't write down passwords
- Don't choose easy to guess/crack passwords
- Change passwords frequently

Using SSH - MobaXterm (on Windows)



message of the day

your quotas

Using SSH to Access Ada

```
ssh –X user_NetID@ada.tamu.edu
```

https://hprc.tamu.edu/wiki/Ada:Access

You may see something like the following the first time you connect to the remote machine from your local machine:

```
Host key not found from the list of known hosts.
Are you sure you want to continue connecting (yes/no)?
```

Type yes, hit enter and you will then see the following:

```
Host 'ada.tamu.edu' added to the list of known hosts.
user_NetID@ada.tamu.edu's password:
```