

mothur tutorial

STAMPS 2014

Tracy K. Teal

Assistant Professor

Microbiology & Molecular Genetics

Michigan State University

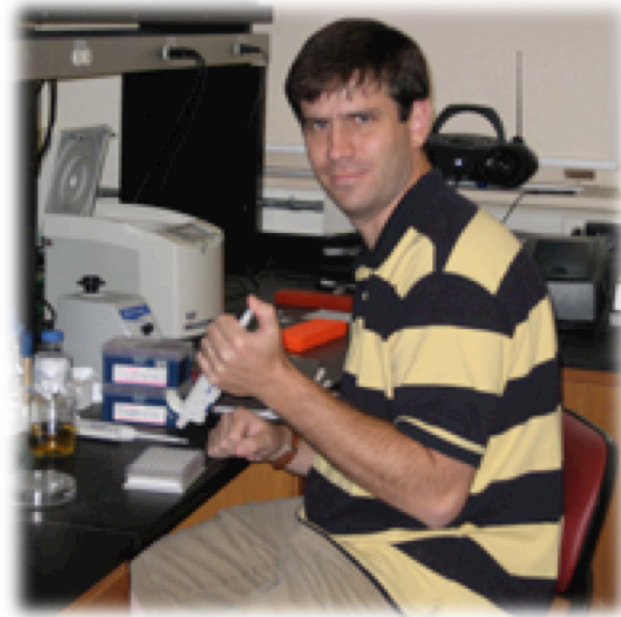
mothur

Pat Schloss

Microbiology & Immunology
University of Michigan

Sarah Westcott

<http://www.mothur.org>



Outline

- Case study – microbial communities in soil
- mothur workflow
- mothur MiSeq SOP
http://www.mothur.org/wiki/MiSeq_SOP

How to get your data in, get your data out
(an OTU table), and get help

- Some preliminary statistics



mothur

- Updated versions are released every few months
 - First release, v1.1.0, March 2009
 - Last release, v1.32.1, October 2013
- Approaches
 - Taxonomy
 - OTUs
 - Phylogeny
- Sequencing systems
 - Sanger
 - 454
 - Illumina
- Tutorials for OTU-based approach
 - 454 http://www.mothur.org/wiki/454_SOP
 - Illumina http://www.mothur.org/wiki/MiSeq_SOP

mothur and QIIME

- Both are open source and on github
- Both aim to enable advances in microbial ecology and are actively maintained and developed
- Both require alignment
- mothur is not an acronym
- While QIIME connects multiple tools, mothur reimplements algorithms, so that it is all one program

Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities

Schloss et al, AEM, 2009

- mothur in C++, QIIME connections in Python
- QIIME has mothur and mothur has Unifrac, but the default behavior for mothur is to do clustering based on sequence distance
- mothur's clustering can be very memory intensive, Uparse as used in QIIME requires less memory

Outline

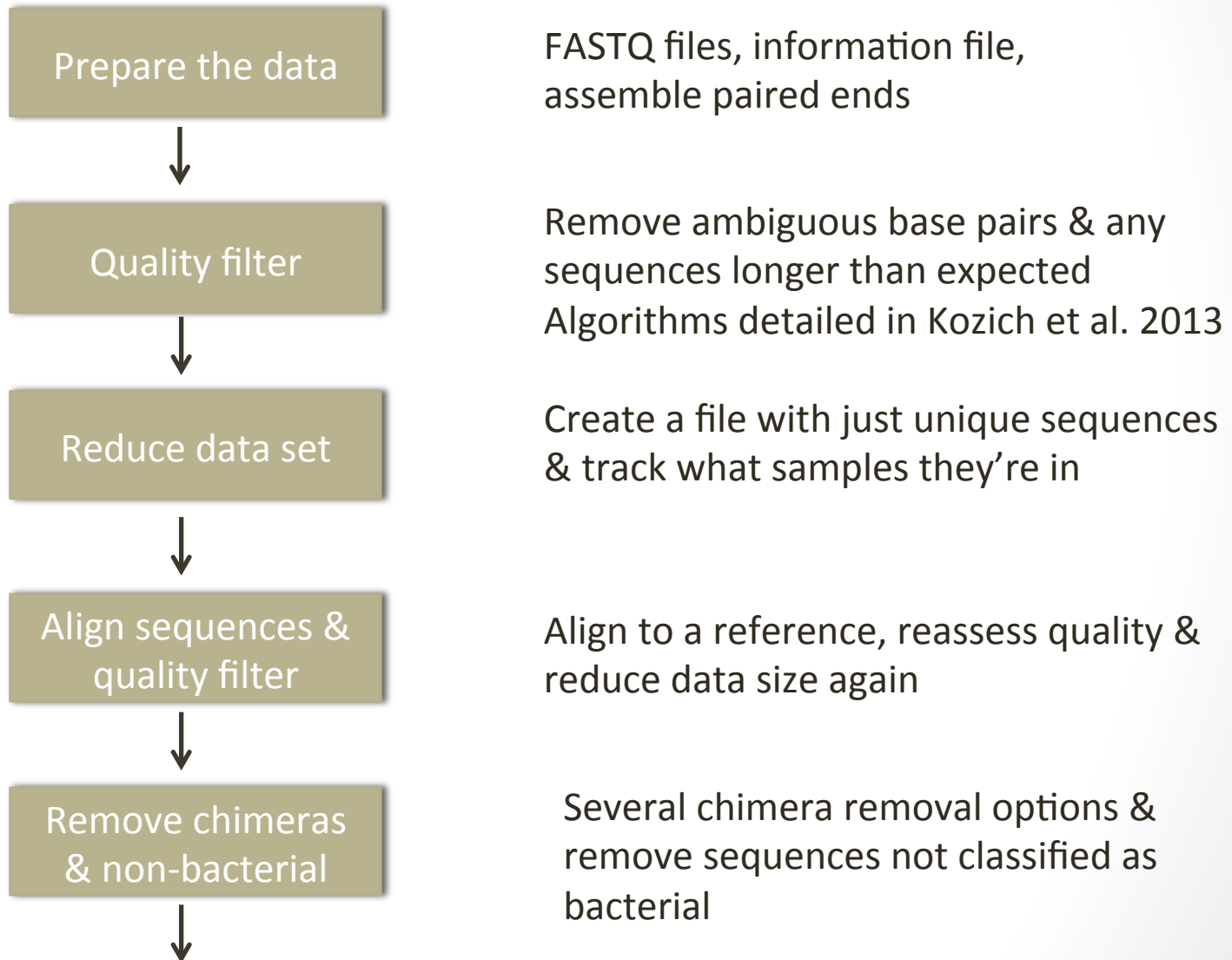
- Case study – microbial communities in soil
- mothur workflow
- mothur MiSeq SOP
http://www.mothur.org/wiki/MiSeq_SOP

How to get your data in, get your data out
(an OTU table), and get help

- Some preliminary statistics

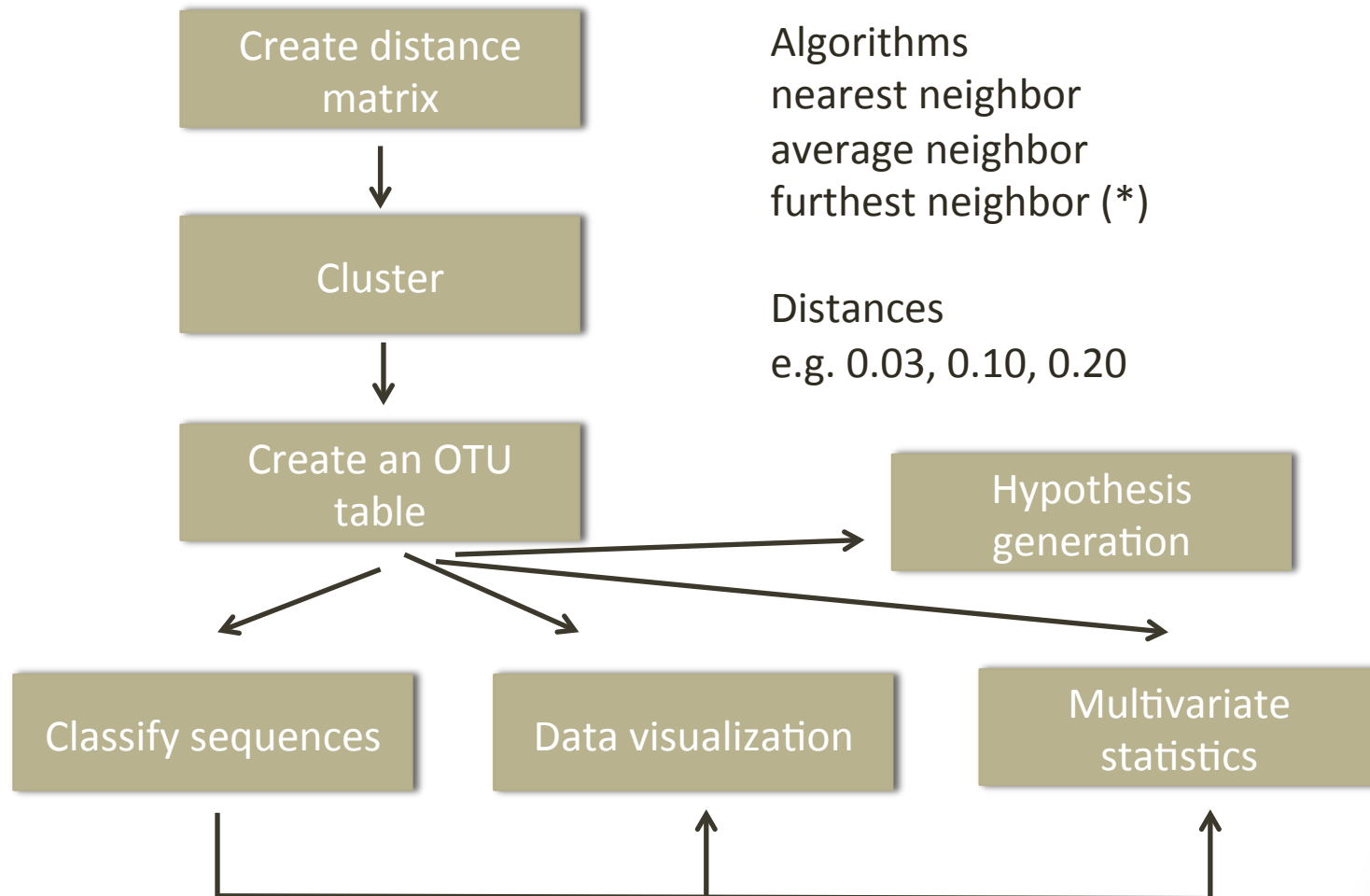
mothur workflow

(Schloss 2009; Schloss 2010; Schloss 2013; Pruesse et al. 2007, doi:10.1093/nar/gkm864; Pruesse et al. 2012, doi:10.1093/bioinformatics/bts252)



mothur workflow

(Schloss 2009; Schloss 2010; Schloss 2013; Pruesse et al. 2007, doi:10.1093/nar/gkm864; Pruesse et al. 2012, doi:10.1093/bioinformatics/bts252)



Working in mothur

Learn how to:

- Get in (get data in)
- Get out (Come home with an OTU table or on it)
- Get help

<https://github.com/tracykteal/tutorials/tree/master/mothur>

http://www.mothur.org/wiki/MiSeq_SOP

Specific file types worth noting

Illumina

- .files
- .count_table

Options for running mothur

- http://www.mothur.org/wiki/Download_mothur
- Windows, Mac, Linux
 - Interactive
 - Batch
 - GUI
- In my test runs, I used 2 processors
 - Both 454 & Illumina tutorials took ~ 1 – 1.5 hrs
 - 454, start with trim.seqs (the output files have been provided)
 - You will not do the “using quality scores” approach
- You can copy & paste all commands but, as Sue correctly noted with R, you won't learn it this way



mothur tutorial

- Is your sample coverage sufficient for meaningful analyses?
- Do mouse fecal microbiota differ between weanling and adult mice?
 - alpha, beta diversity
 - OTUs responsible?
- Is variation in fecal microbiota greater among weanlings than adults?



Use both graphical and statistical tools to answer each question.
Ignore the obvious pseudoreplication and simultaneous lack of replication.