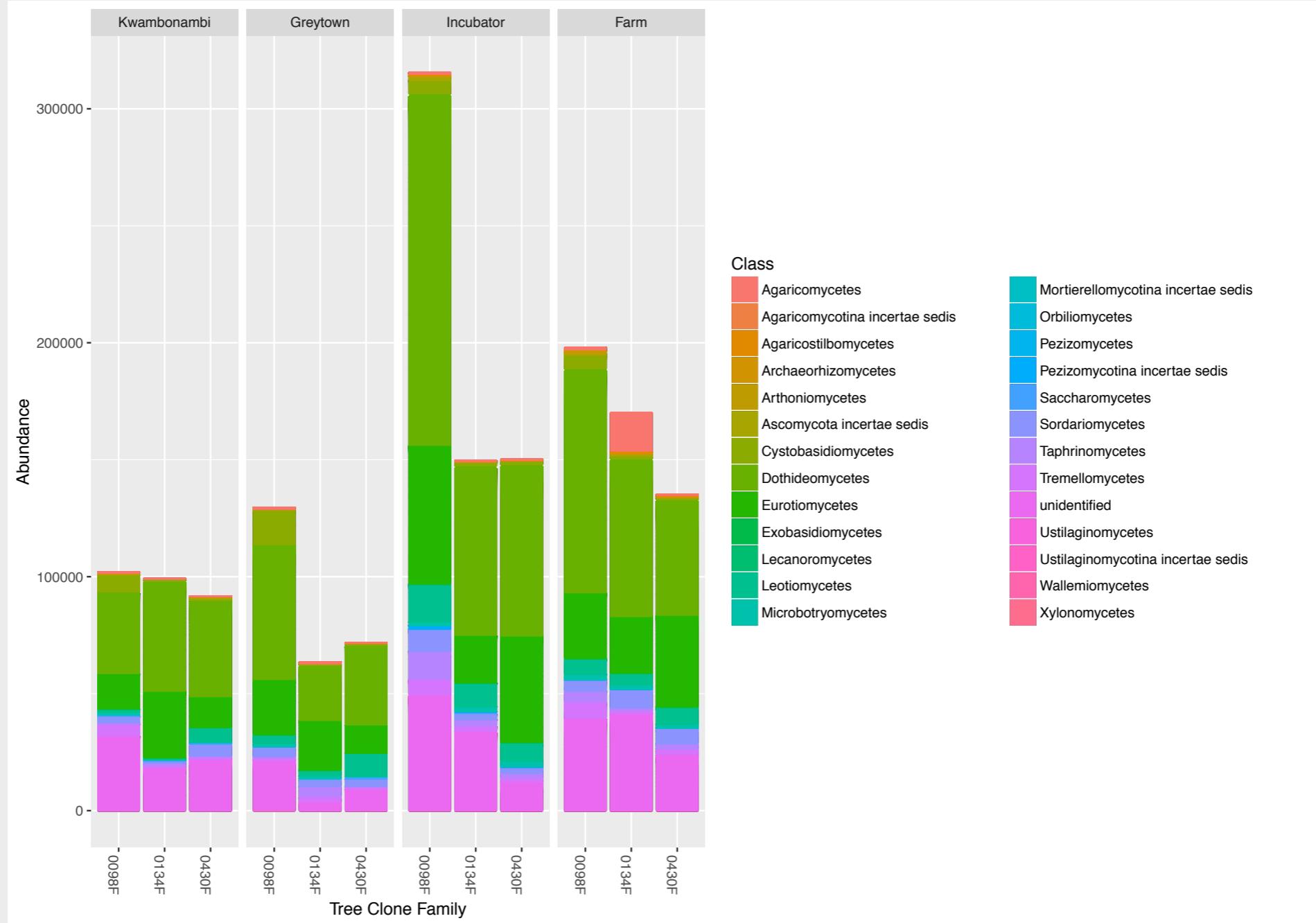


RUHR-UNIVERSITÄT BOCHUM

Analysis of NGS amplicon sequencing data for fungal community assessment

Martin Kemler

New Dimension of Fungal Diversity



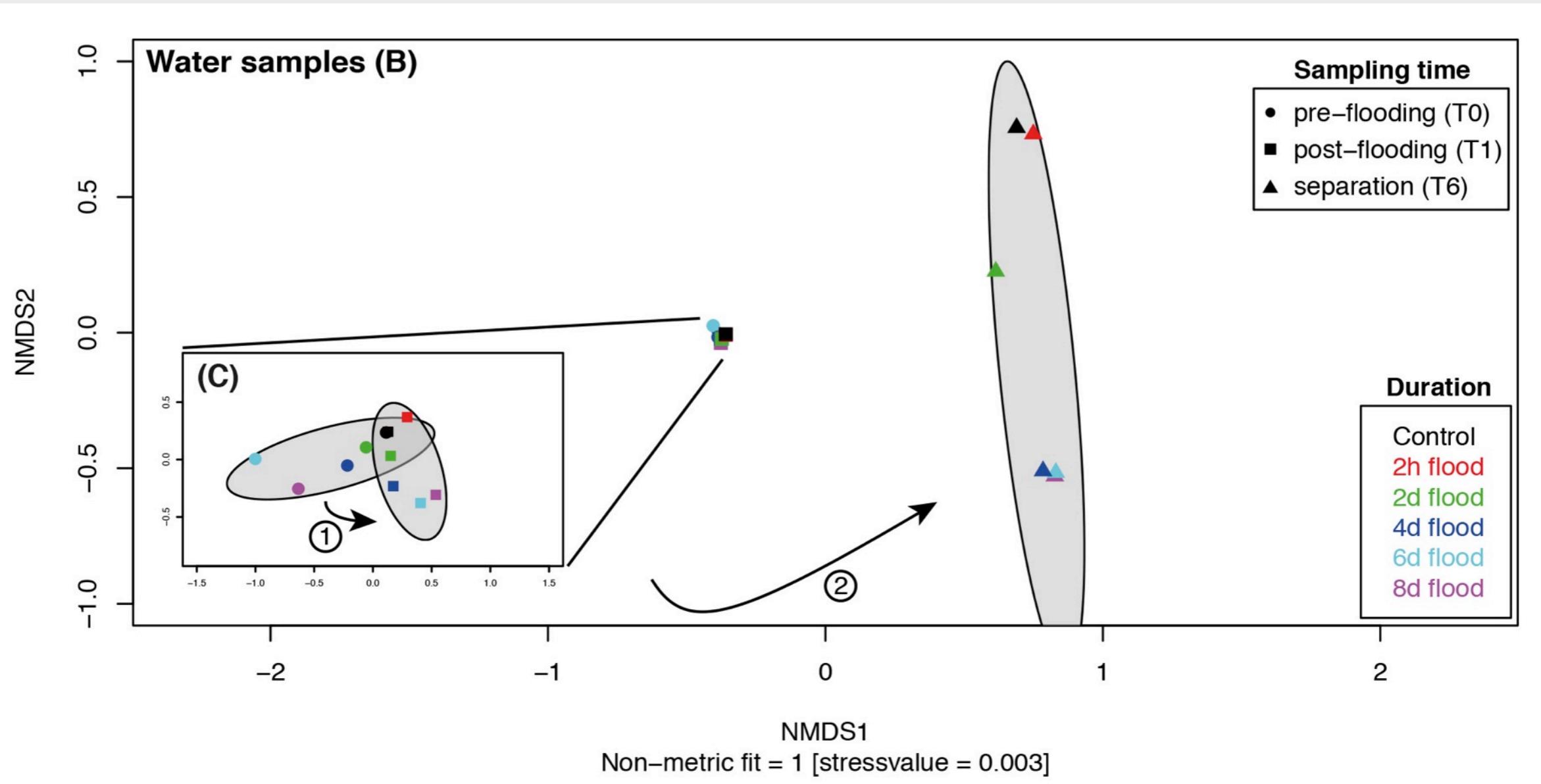
New Dimension of Fungal Diversity

Fungal group	Kemler et al. 2013 'perfect match'/ 95%		Fisher et al. 1993 ^a		Smith et al. 1996 ^a	
	Amount of sequences	% of total sequences	Amount of isolates	% of total isolates	Amount of isolates	% of total isolates
Mycosphaerellaceae	28318	32,84	-	-	11	1,75
Botryosphaeriaceae	27370	31,74	288	18,57	158	25,12
Pleosporaceae	1630	1,89	66	4,26	155	24,64
Amphisphaeriaceae	718	0,83	48	3,09	23	3,66
Epicoccum	1200	1,39	28	1,81	43	6,84
Phoma	130	0,15	-	-	8	1,27
Trichosphaerales	84	0,10	28	1,81	72	11,45
Xylariaceae	131	0,15	-	-	12	1,91
Dothioraceae	26	0,03	65	4,19	50	7,95
Chaetomiaceae	38	0,04	-	-	16	2,54
Leptosphaeriaceae	-	-	54	3,48	-	-
Glomerellaceae	-	-	-	-	2	0,32
Valsaceae	-	-	673	43,39	34	5,41
Hypocreaceae	-	-	40	2,58	6	0,95
Davidiellaceae	-	-	37	2,39	27	4,29
Schizophyllaceae	-	-	36	2,32	-	-
Aspergillaceae	-	-	129	8,32	-	-
Helotiaceae	-	-	30	1,93	-	-
Sordariaceae	-	-	29	1,87	6	0,95
Cytonema	-	-	-	-	4	0,64
Spegazzinia	-	-	-	-	1	0,16
Bulgariaceae	-	-	-	-	1	0,16
Total	86242	69,16 ^b	1551	100	629	100

^a isolates that consisted only of sterile mycelium were omitted from this table

^b the total does not add up to 100% as many more taxa were recovered that are not included in the table

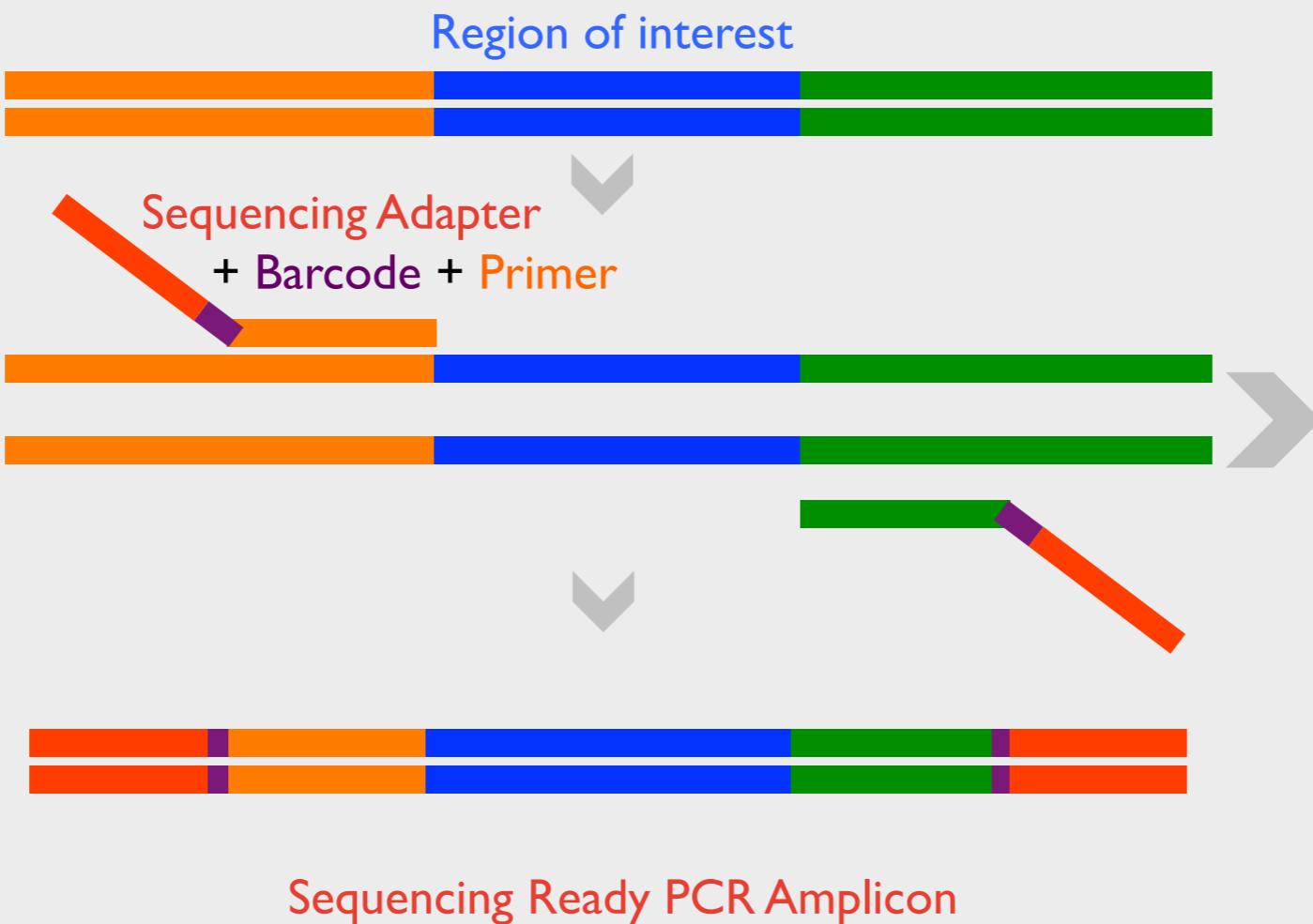
New Dimension of Fungal Diversity



Marker assisted community assessments

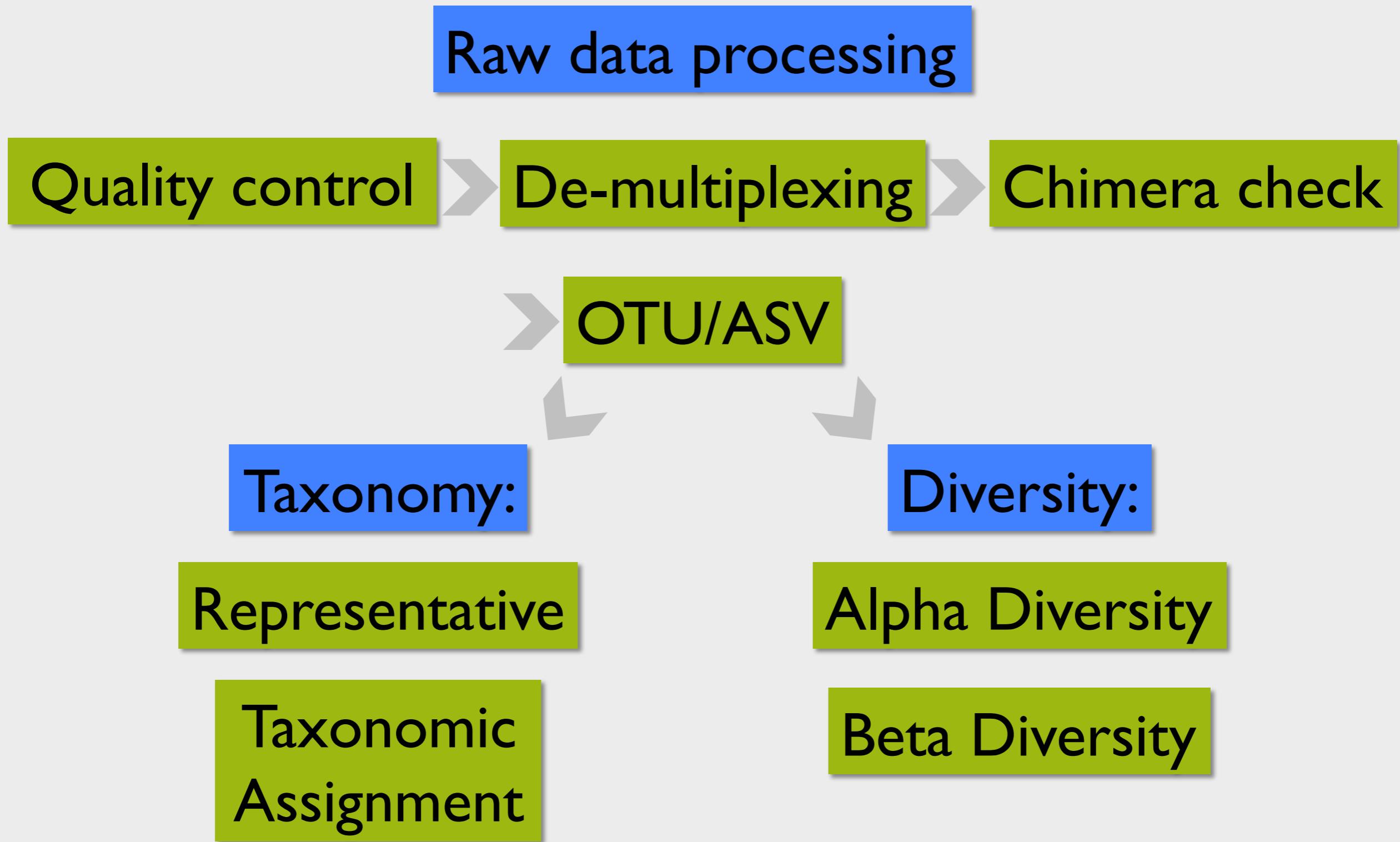
DNA
Isolation
from env.
Sample

Amplification



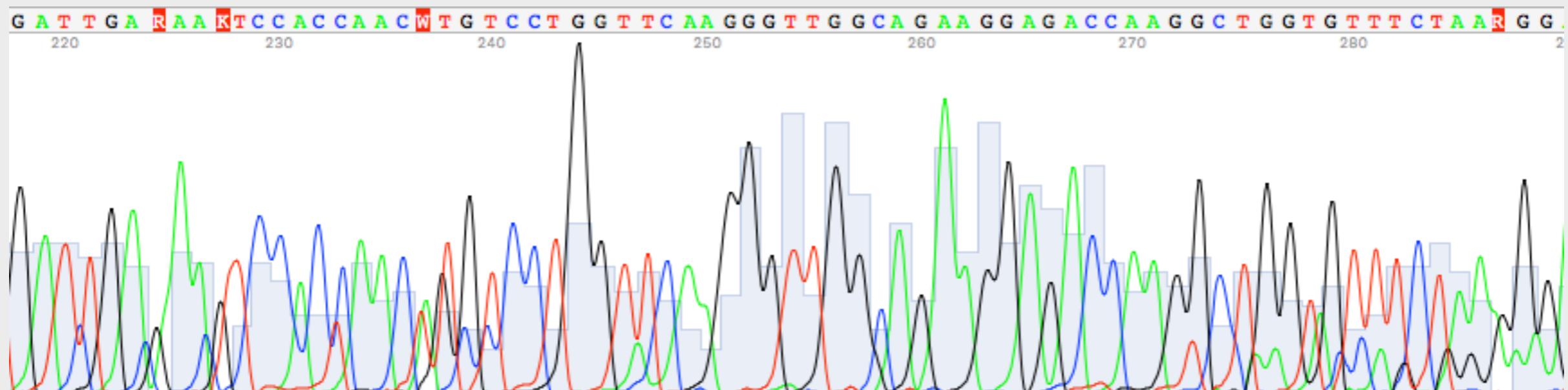
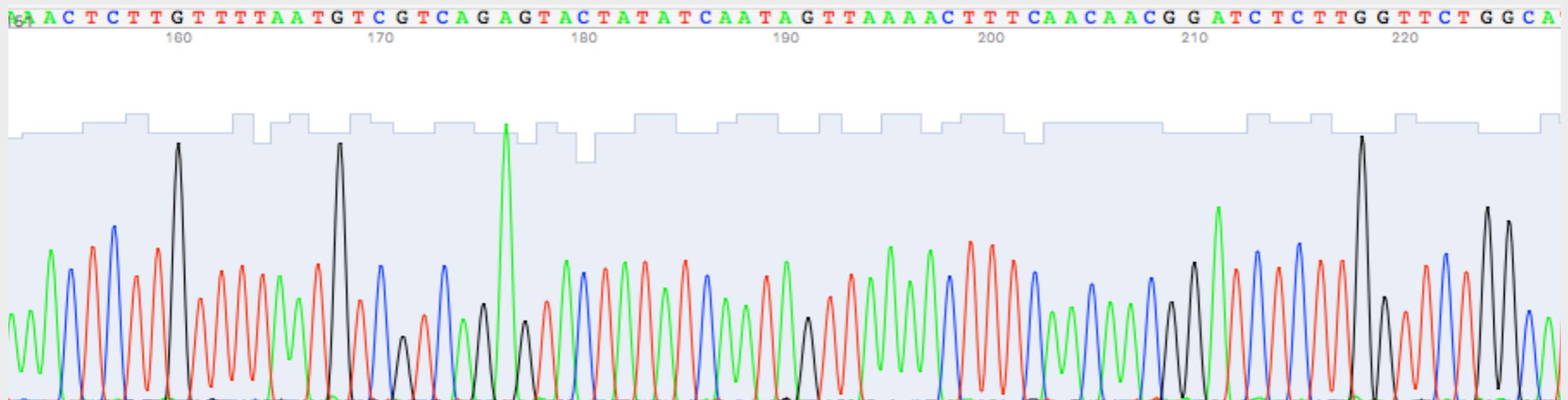
Sequencing





Quality control – sequence formats

Trace files



Quality control – sequence formats

Fasta

>M02542:21:000000000-AAMB0:I:1101:9896:1055

ATGGAACGCTTGGTCATTAGAGGAAGTAAAGTCGTAACAAGGTTCCGTAGGTGAAC

< Header

< DNA sequence

Qual

>M02542:21:000000000-AAMB0:I:1101:9896:1055

34 34 34 34 24 31 36 37 38 38 38 38 38 38 38 38 38 38 37 38 38 34 37 38 37 38 38

< Header

< Quality Value

Fastq

@M02542:21:000000000-AAMB0:I:1101:9896:1055 I:N:0:3

ATGGAACGCTTGGTCATTAGAGGAAGTAAAAGTCGTAACAAGGTTCCGTAGGTGAAC

+

CCCC9@EFGGGGGGGGGGGFGGGCFGFGGGGGGGGGGGGGFG<FEGGGGGG

< Header

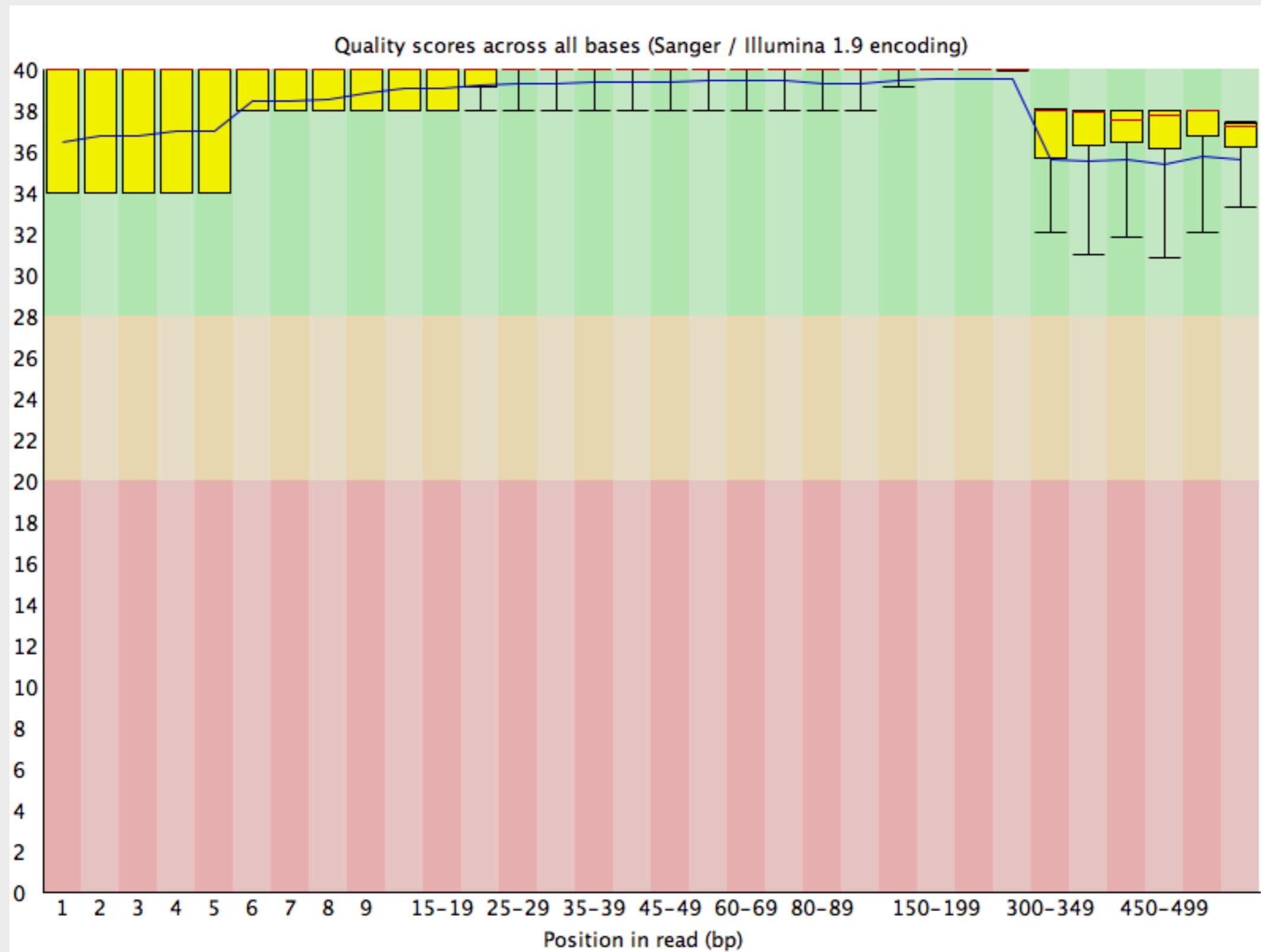
< DNA sequence

< Separator

< Quality Value

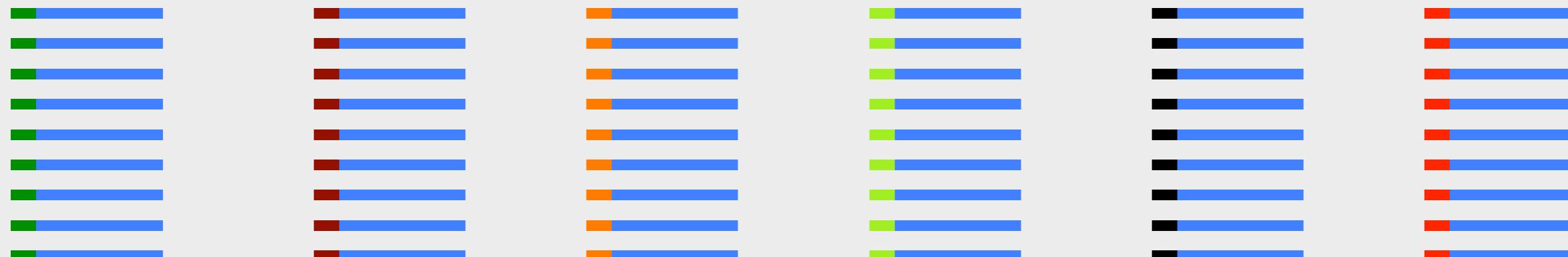
Quality control – fastq encoding

Quality control – FastqC



De-multiplexing

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description
K1.M	ATGGAACG	CTTGGTCATTAGAGGAAGTAA	GCTGC GTTCTTCATCGATGCA	Kwambonambi
K2.M	ATGGAAGC	CTTGGTCATTAGAGGAAGTAA	GCTGC GTTCTTCATCGATGCA	Kwambonambi
K3.M	ATGGATCC	CTTGGTCATTAGAGGAAGTAA	GCTGC GTTCTTCATCGATGCA	Kwambonambi
G9.M	ATGGATGG	CTTGGTCATTAGAGGAAGTAA	GCTGC GTTCTTCATCGATGCA	Greytown
G1.M	ATGCCAT	CTTGGTCATTAGAGGAAGTAA	GCTGC GTTCTTCATCGATGCA	Greytown
G2.M	ATGGCCTA	CTTGGTCATTAGAGGAAGTAA	GCTGC GTTCTTCATCGATGCA	Greytown



K1 K2 K3 G9 G1 G2

De-multiplexing



K1

K2

K3

G9

G1

G2



>KI_1

ATGGAACGCTTGGTCATTAGAGGAAGTAAAAGTCGTAAACAAGGTTCCGTAGGTGAAC

>KI_2

TAAAAGTCGTAAACAAGGTTCCGTAGGTGAACATGGAACGCTTGGTCATTAGAGGAAG

...

KI

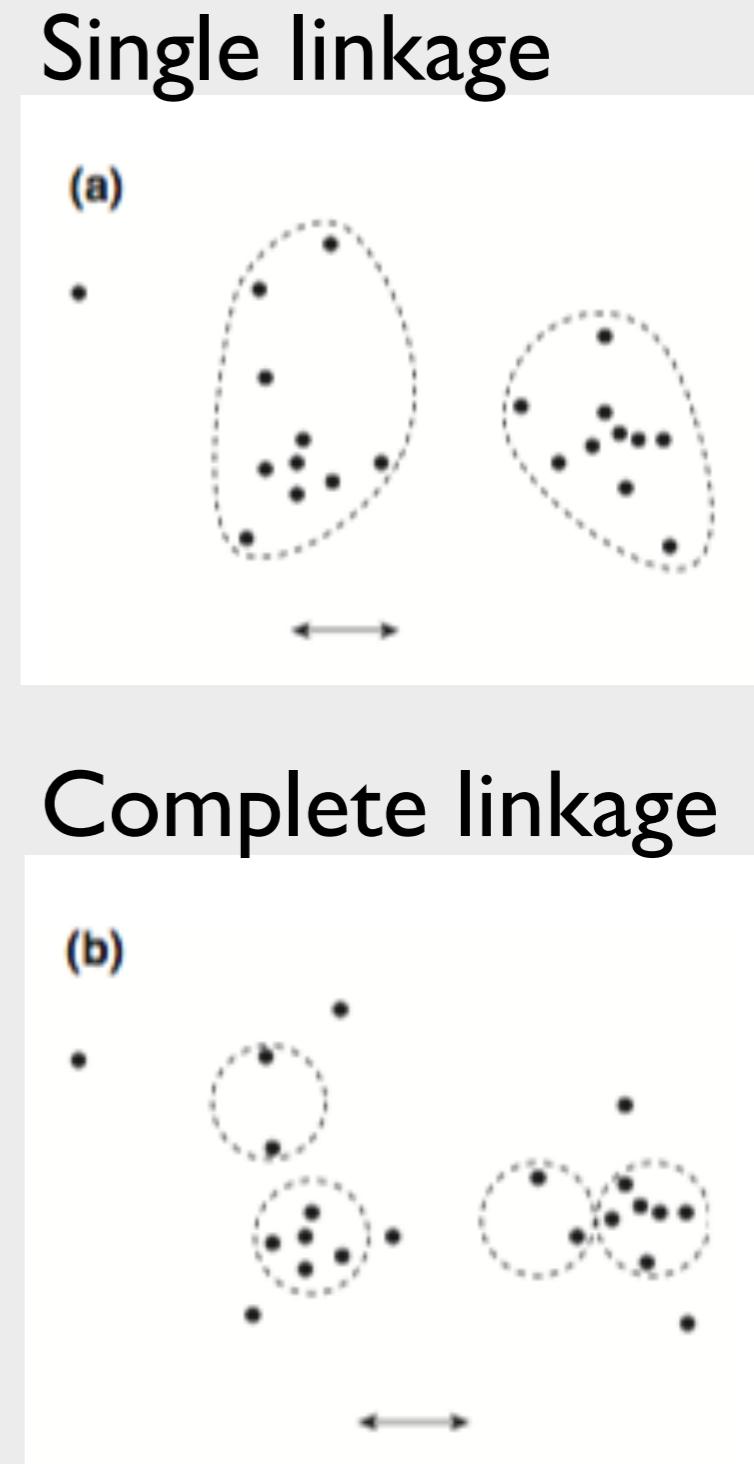
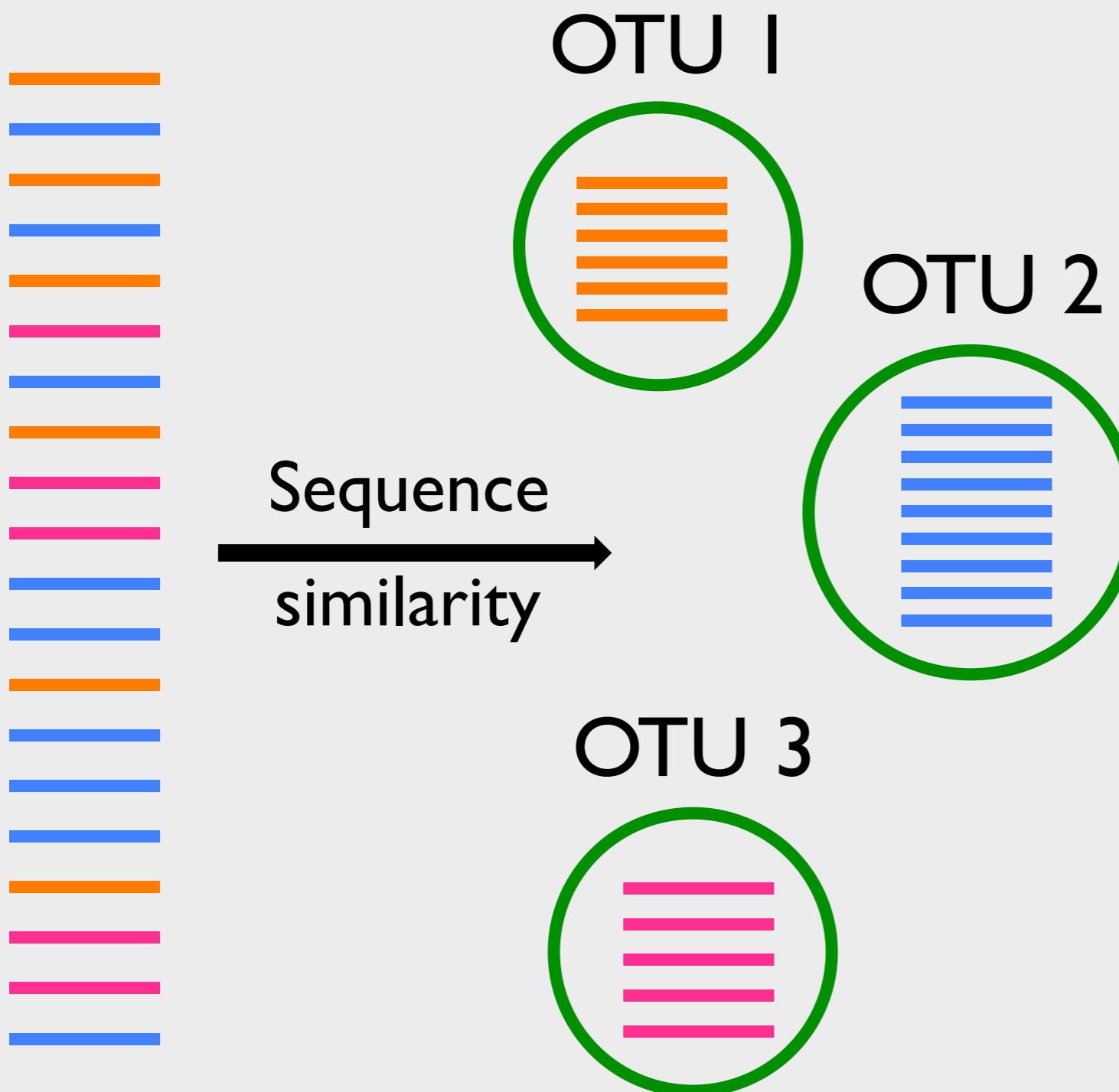
Chimera checking



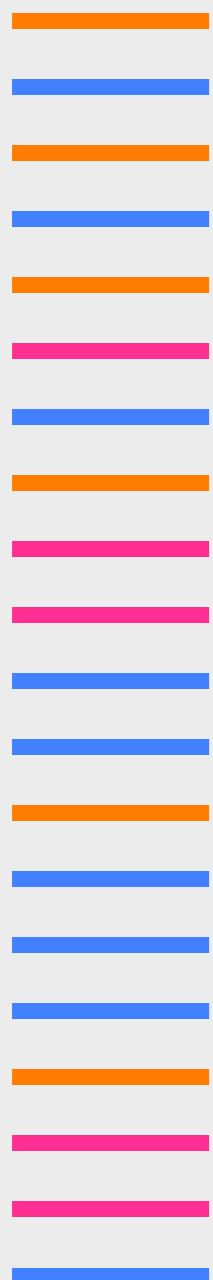
I) Reference based chimera checking

2) *De novo* chimera checking

De novo Operational Taxonomic Unit (OTU) picking

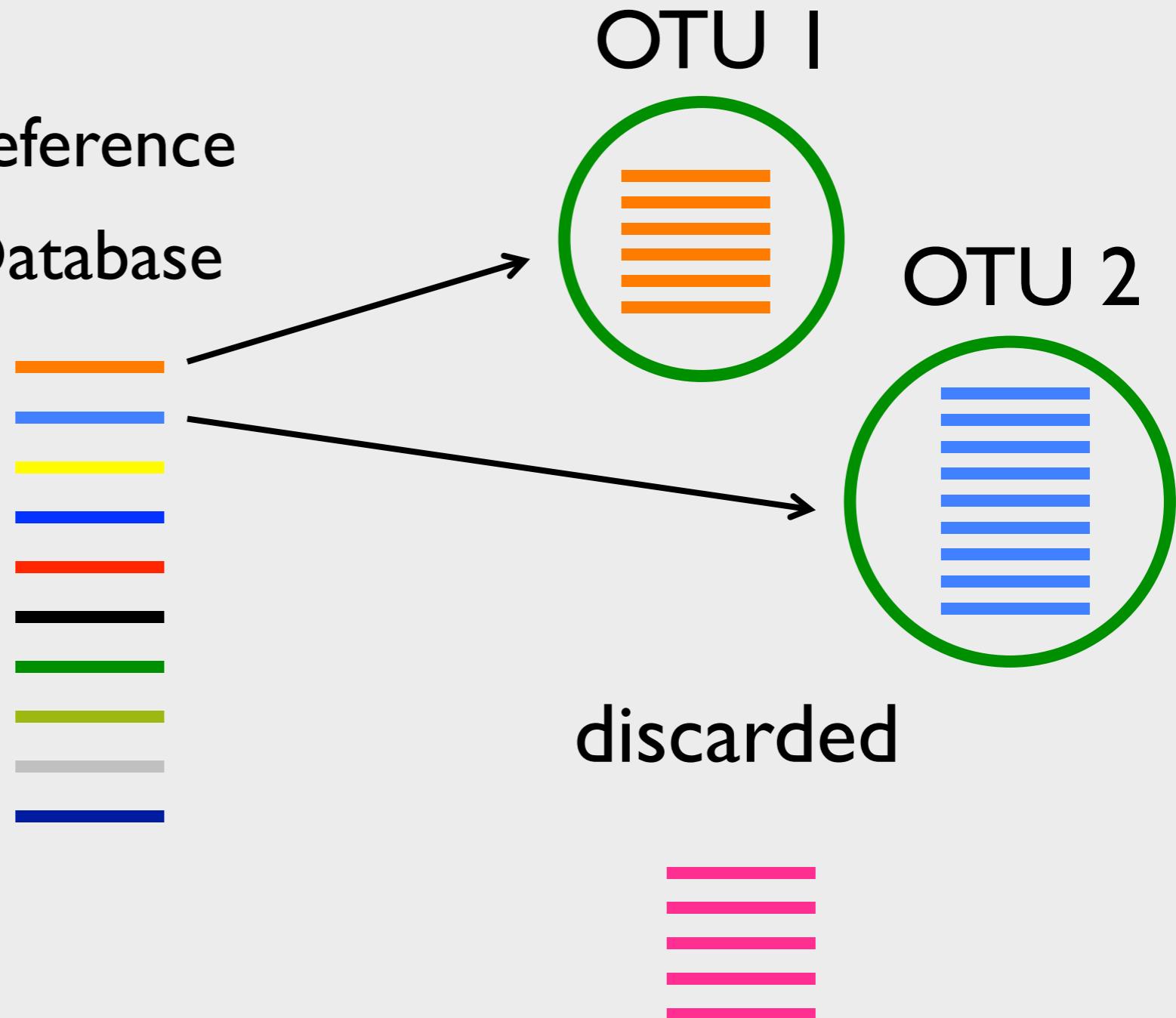


Reference-based OTU picking

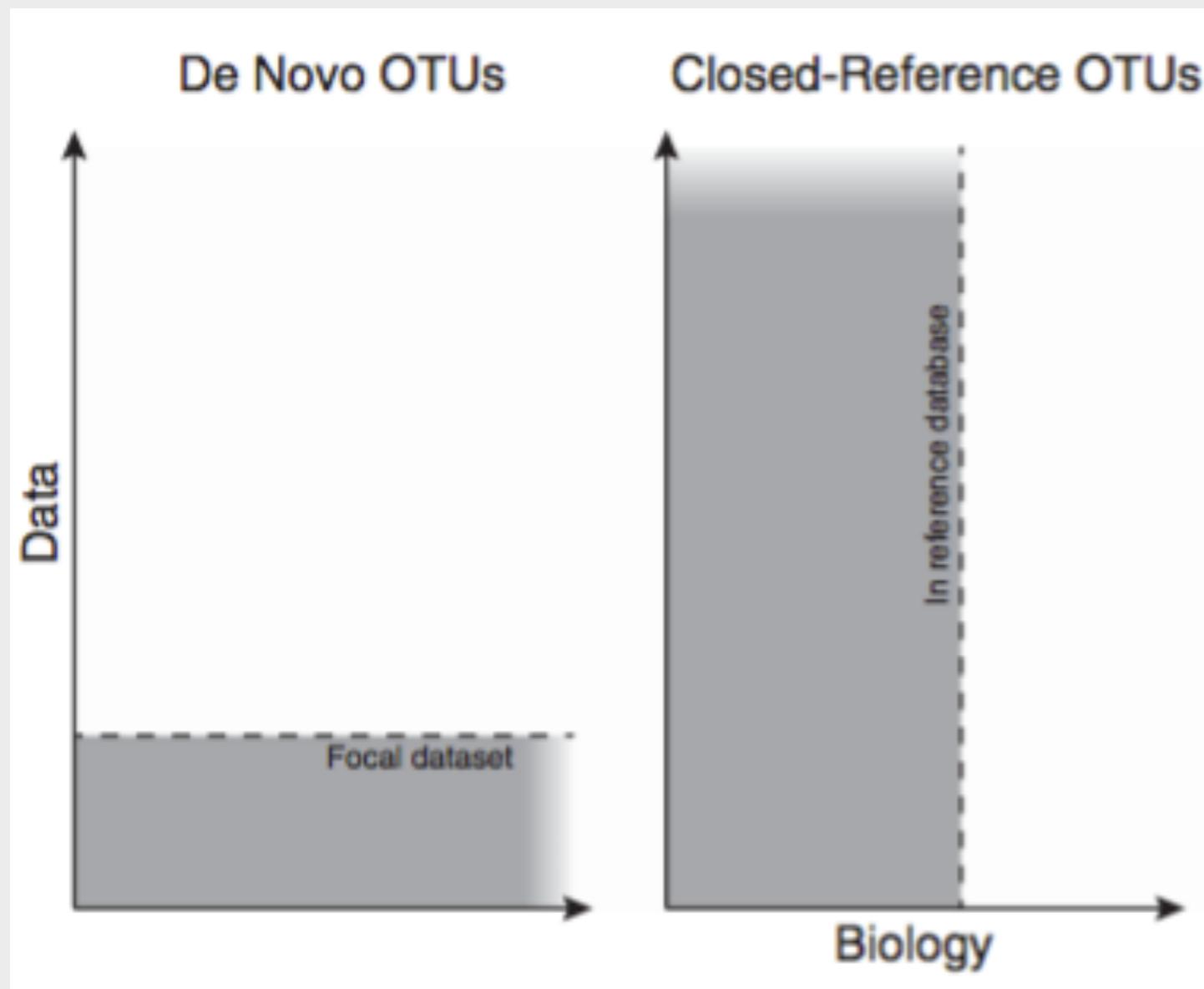


Reference
Database

Sequence
similarity →

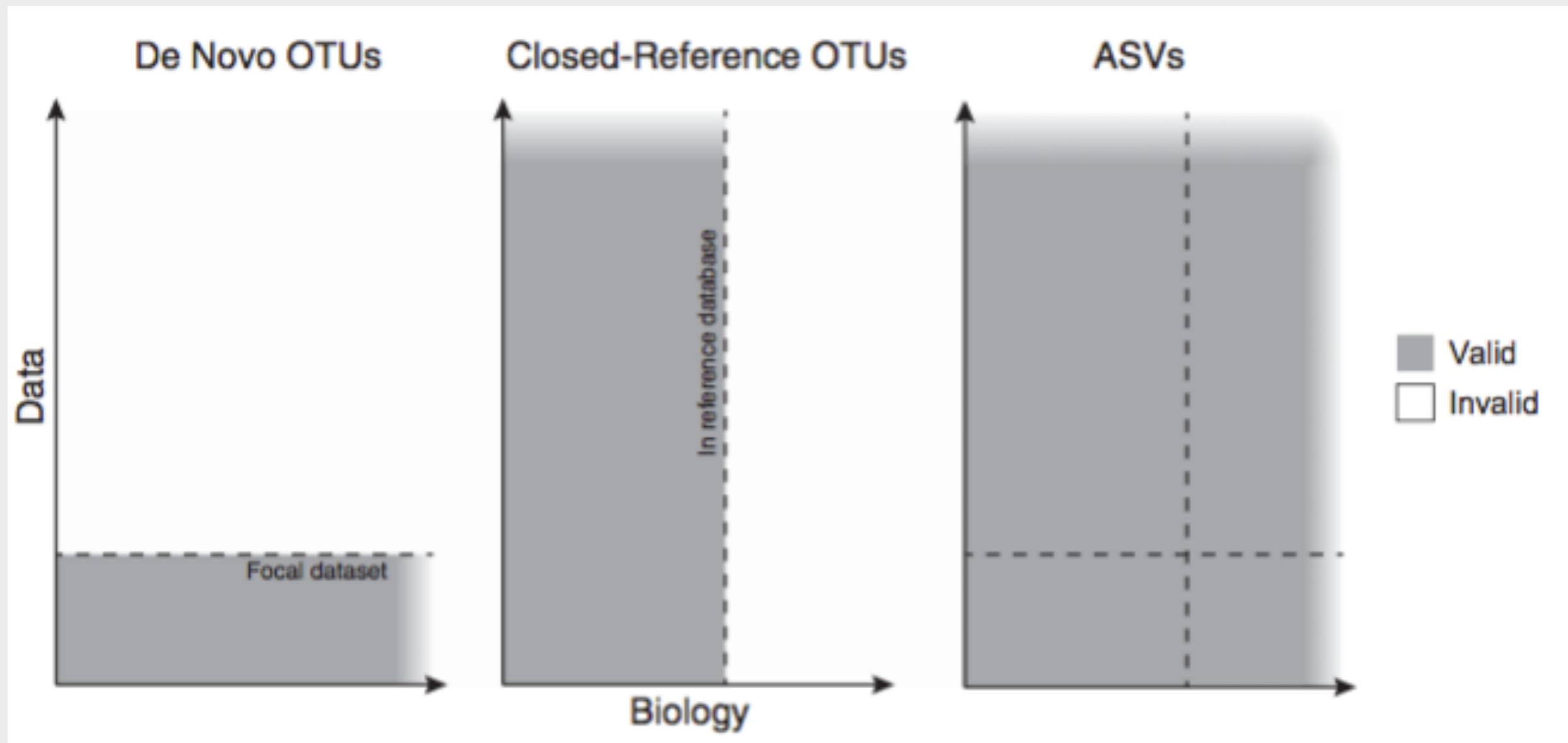


OTU picking is not without issues

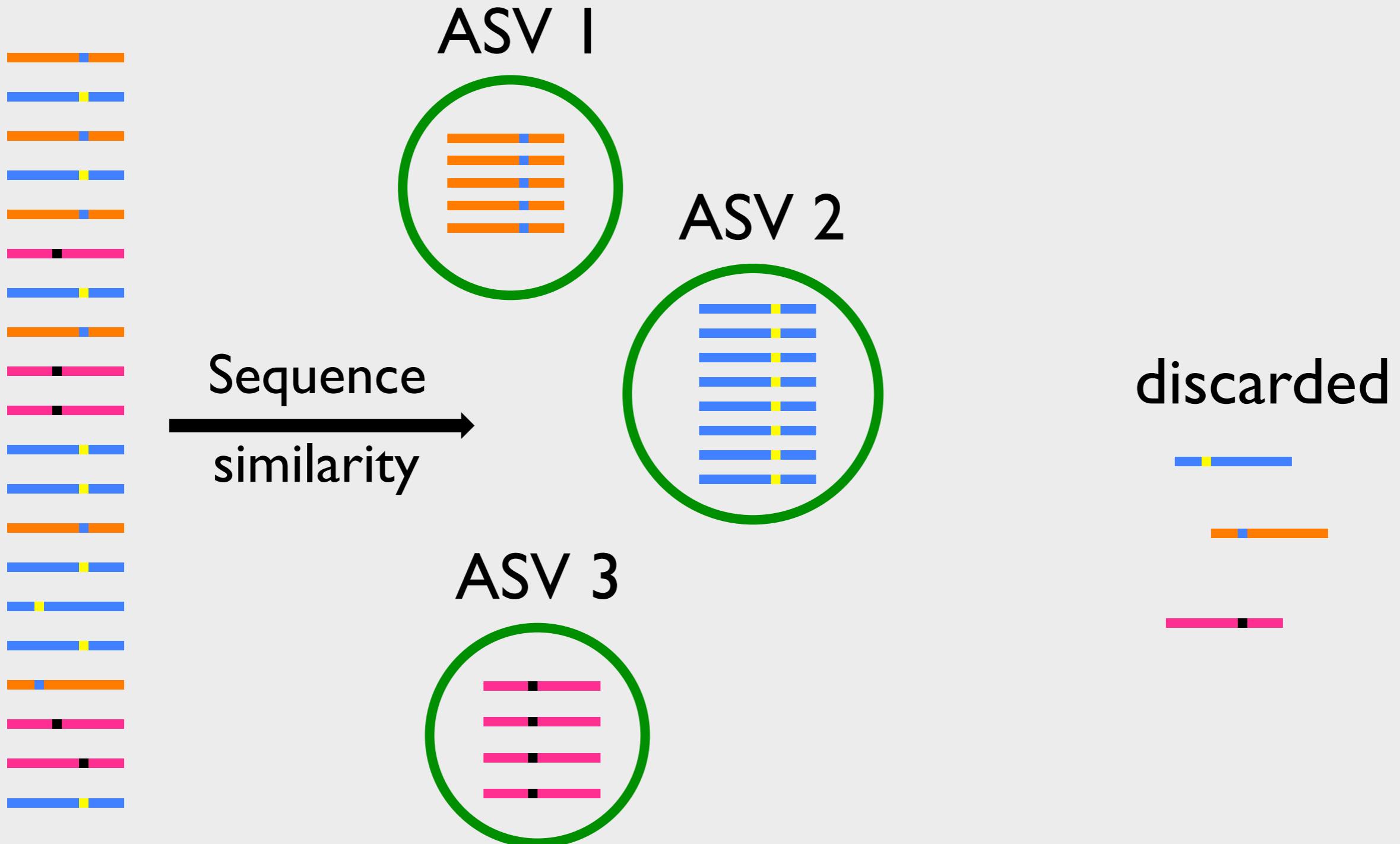


Callahan et al. 2017

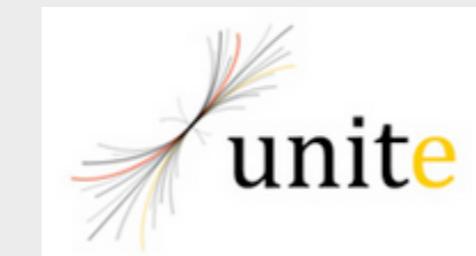
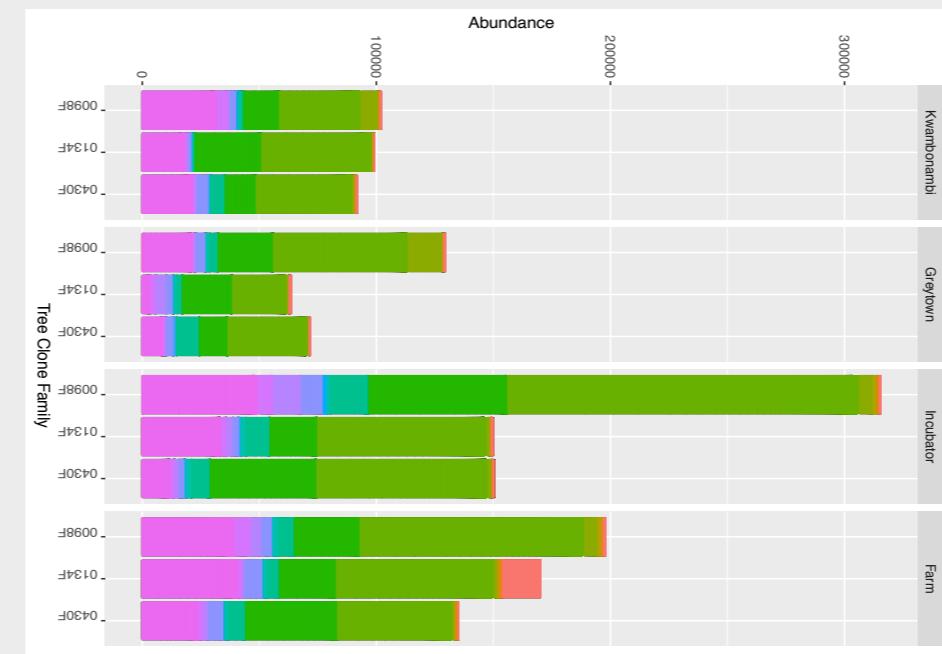
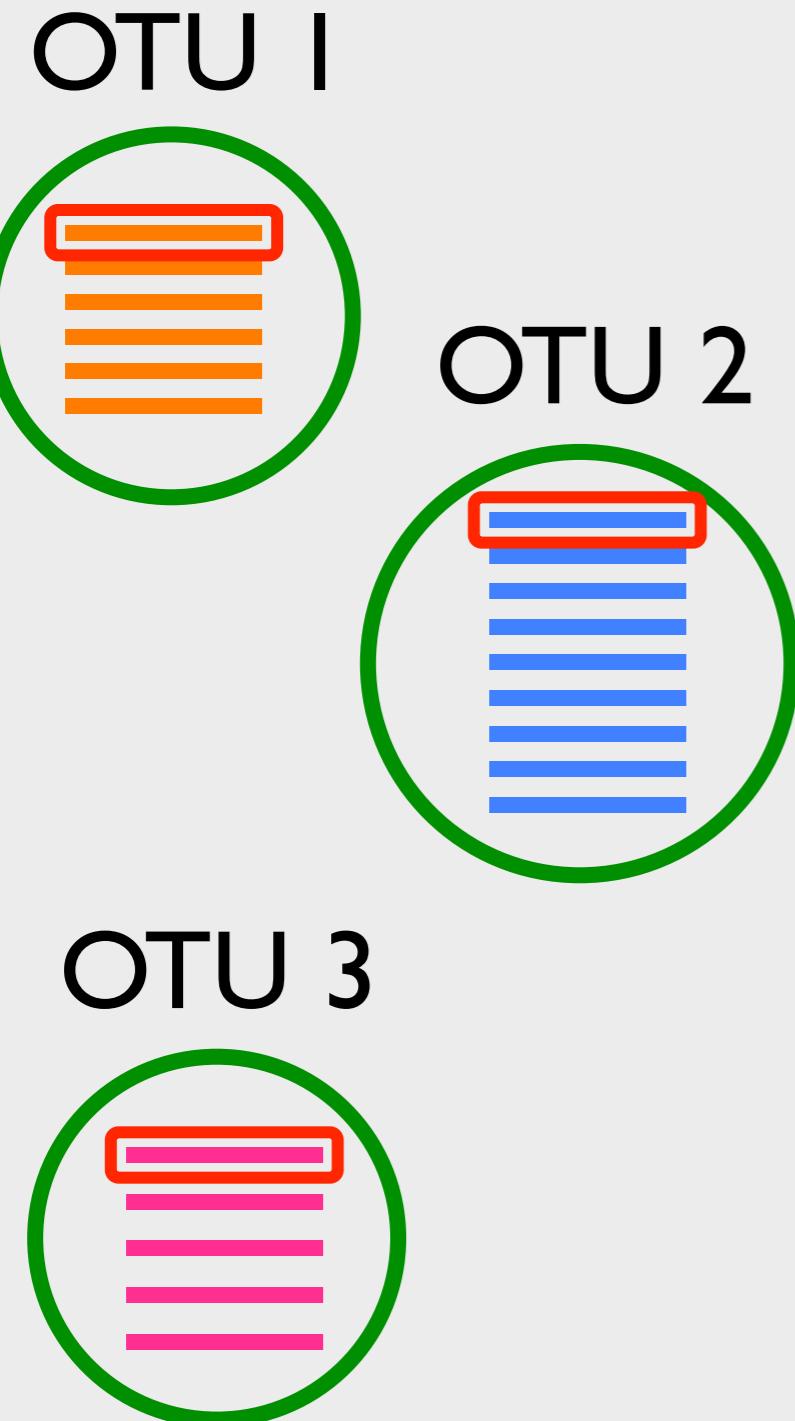
Amplicon Sequence Variants (ASVs)



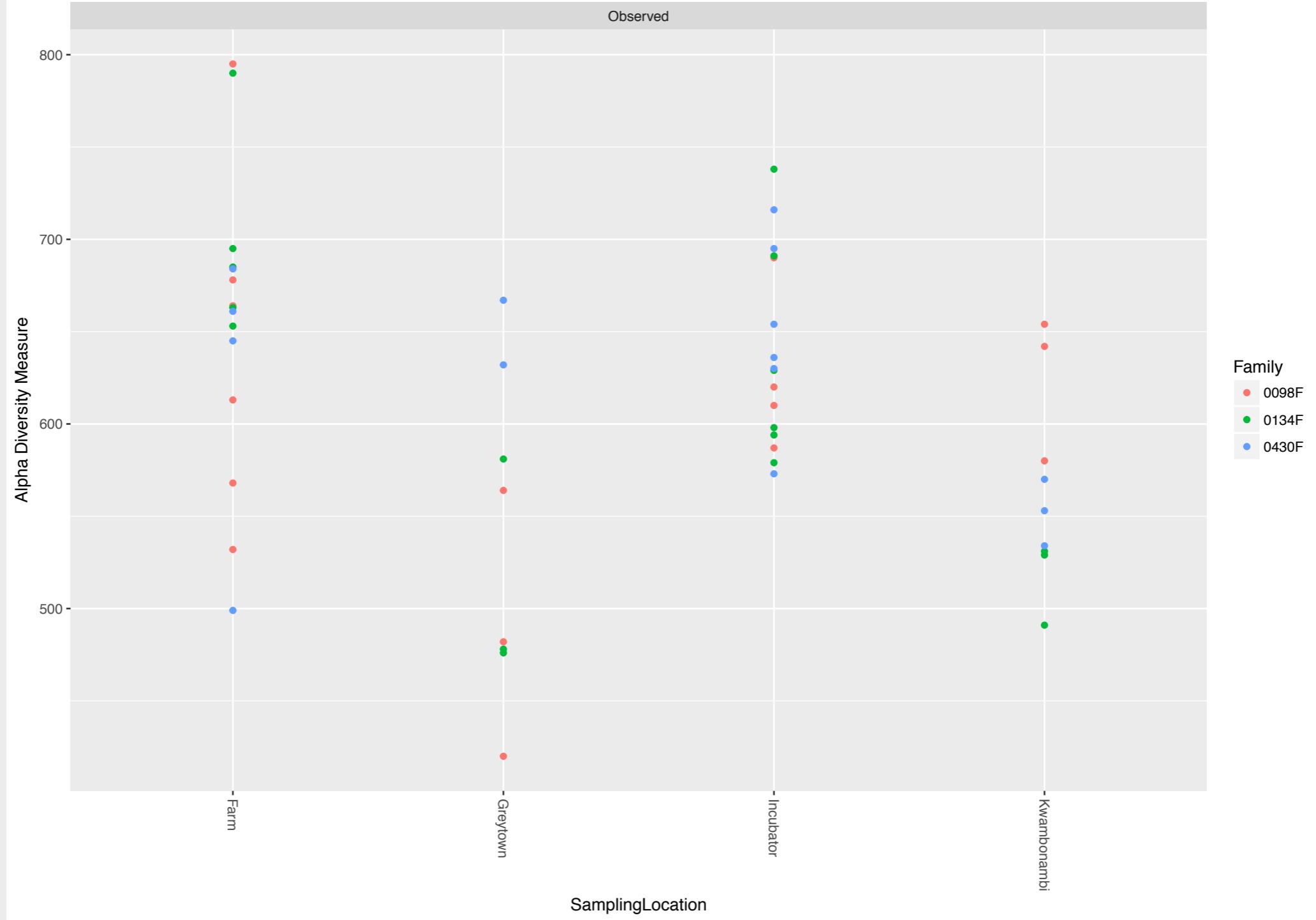
Amplicon Sequence Variants (ASVs)



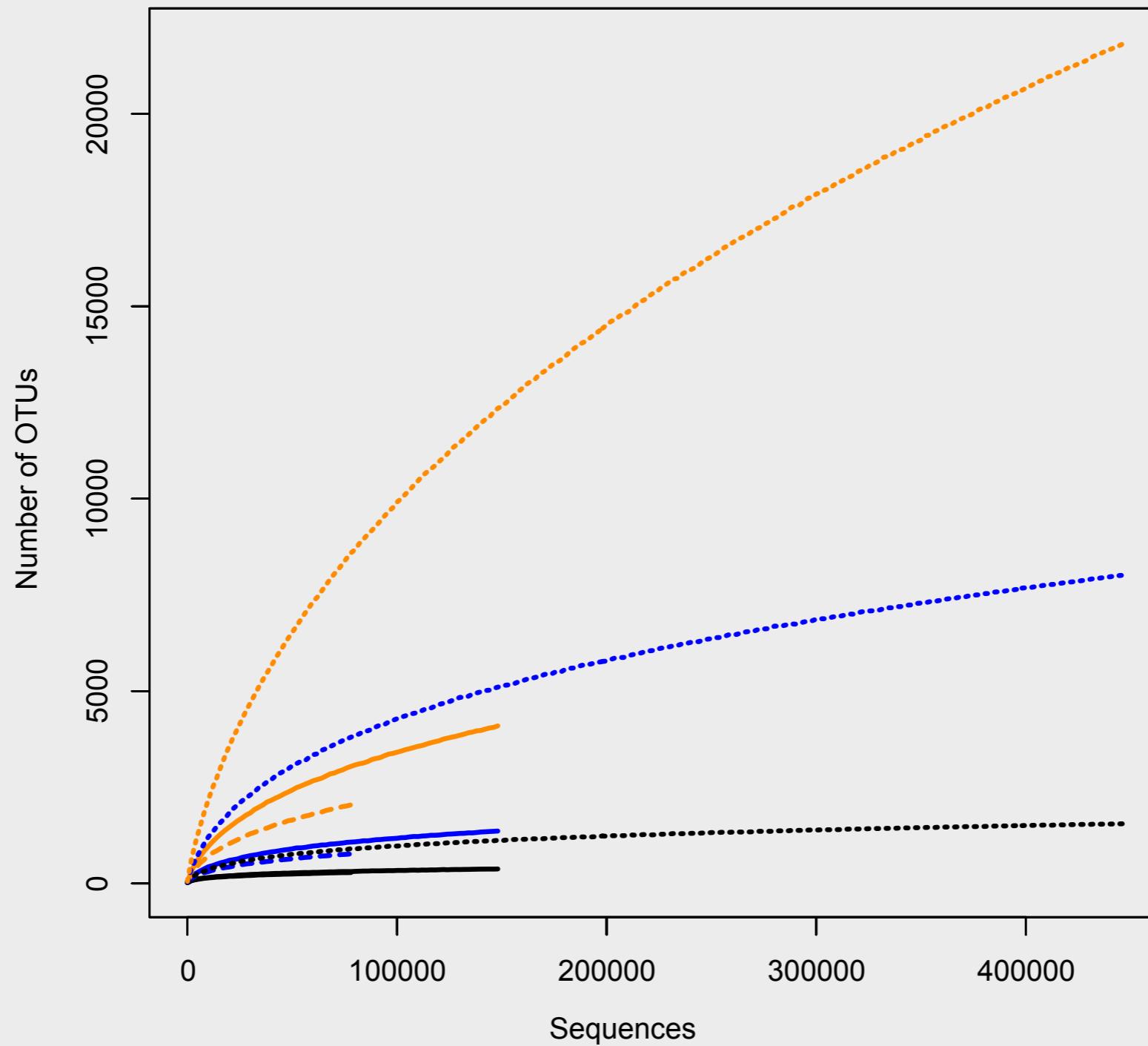
Taxonomy



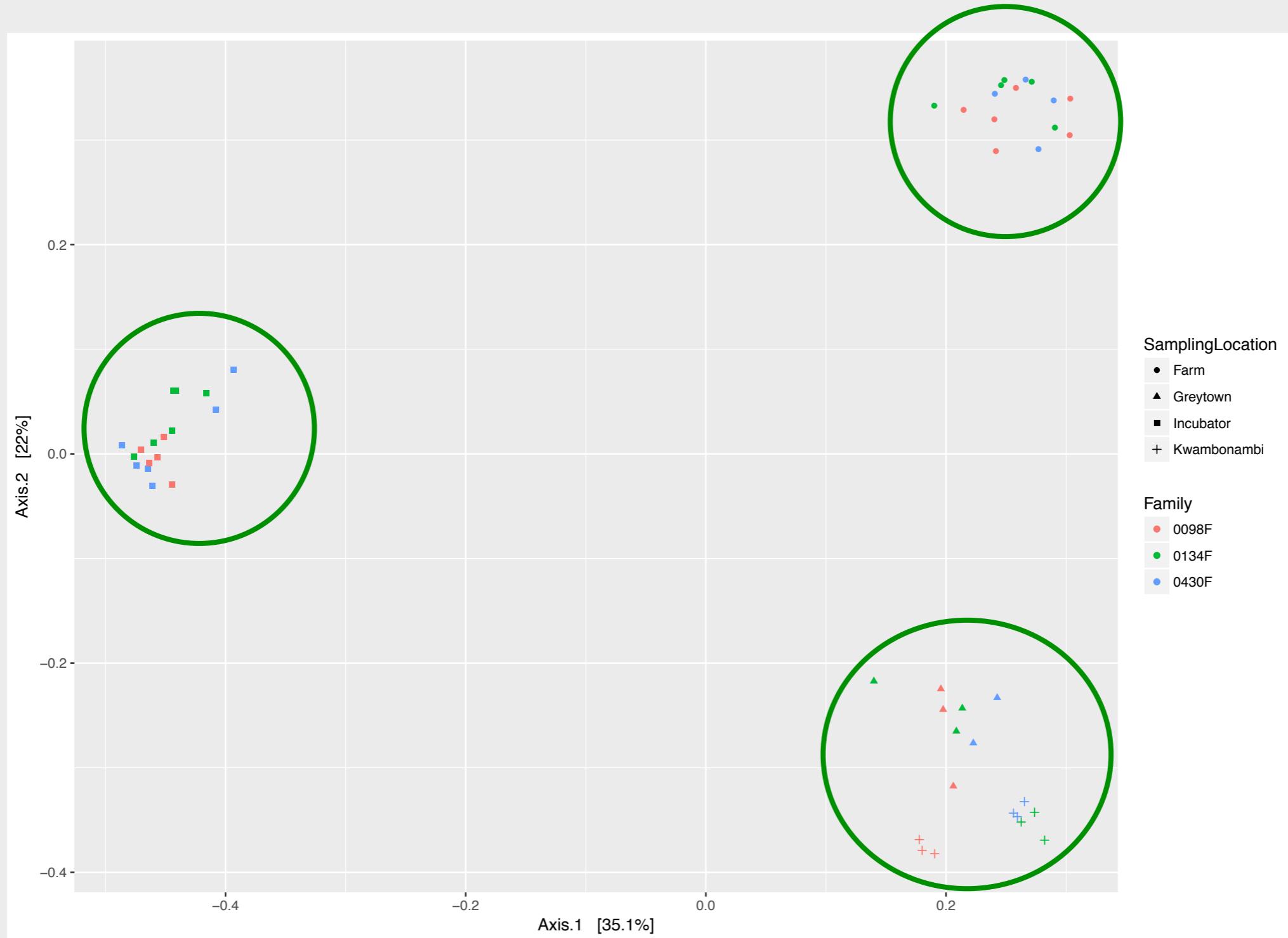
Diversity – Alpha diversity



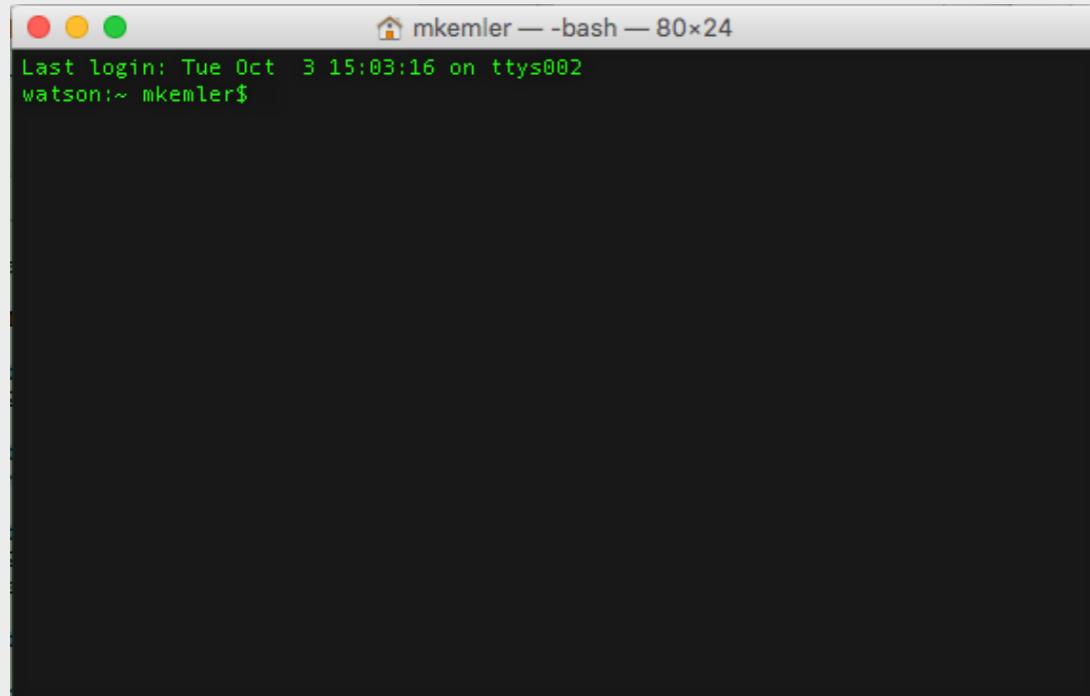
Diversity – Alpha diversity



Diversity – Beta diversity



Shell & QIIME



```
mkemler@mkemler: ~ - bash - 80x24
Last login: Tue Oct  3 15:03:16 on ttys002
Watson:~ mkemler$
```

Text Editor



```
sh_refs_qiime_ver7_97_s_28.06.2017.fasta
~/Documents/.../sh_qiime_release_s_28/sh_refs_qiime_ver7_97_s_28.06.2017.fa...
1 >SH465869.07FU_UDB004660_reps_singleton
2 GGAAGGACATTATCGAAACAAATGGGGGGAAAGACTGTCGCTGGCCCTCGGCATGTGCACGTC
3 >SH488012.07FU_KT328615_reps_singleton
4 ACGTGTGTTGGTCCCCTCGGGGCCGACATCCCACCCCTTGTTGTCACTTCAATGCGTTGCT
5 >SH012276.07FU_U68322_reps_singleton
6 ACGTGTGTCGTACGGTCTGGTCAATCGCCGGCGTGCACCTCCCACCCCTTGCTATCTTACCT
7 >SH492372.07FU_KR673632_reps_singleton
8 TATCTTCTTTGTGACATTTTAGTCTCAAAGTCGAAAAGTGAACCCCTCTCGCAGCAATG
9 >SH027529.07FU_GQ985425_reps
10 GATATTAAATTGCTCACCTTCAGTGCTGGCTTCATGCATGTGCACGTTGGAGGCATATATA
11 >SH009881.07FU_EF634088_reps
12 CCGAACTGTCGACACGAGTTGCTGGCCTCTCAAACGGGGGGCATGTGCACACTCTGTTA
13 >SH492954.07FU_DQ656654_reps_singleton
14 CATGAGCCTTGATCTGCCGGTTAACAGAGGCCCTCACGGTCCGCGGGTAATCTGCCGGCCG
15 >SH628622.07FU_LC131409_reps
16 TCGAAGAAGCACACTTCTCCAACCCCTGTGAACCGTGTCCGAGCATGATGCTCGGACGC
17 >SH003789.07FU_KC966369_reps_singleton
18 CCGAGTGTACTGCTCACAAACCCCTGTGAACCTACTTTGTTGCTCGCGGGCCGTCCAG
19 >SH487811.07FU_KU535777_reps_singleton
```



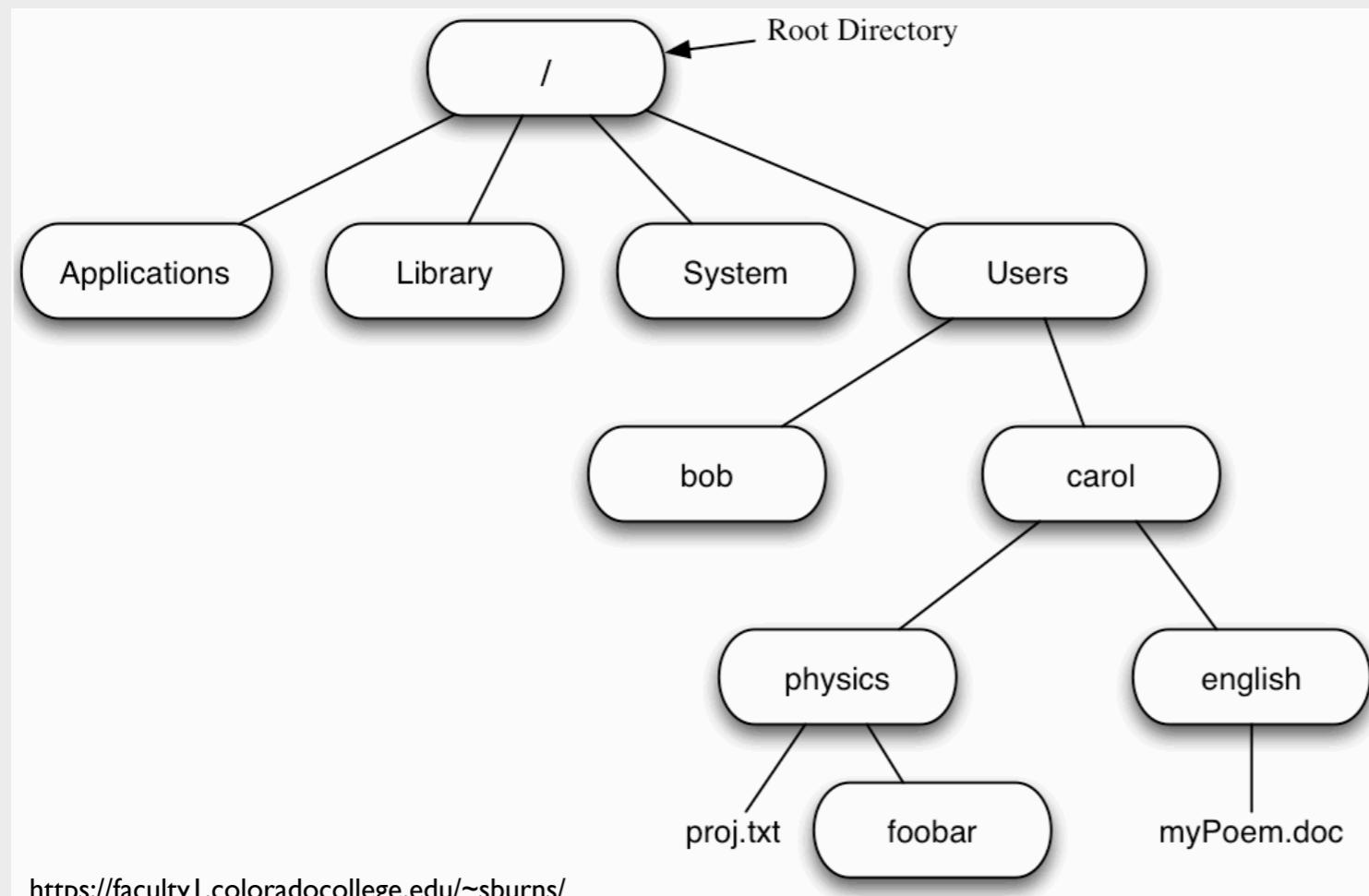
Quantitative Insights into Microbial Ecology (QIIME) – Caporaso et al. 2010

RUB

- Open source bioinformatics pipeline for microbiome analysis
- Individual Python scripts are used to process raw sequencing data up to diverse analyses

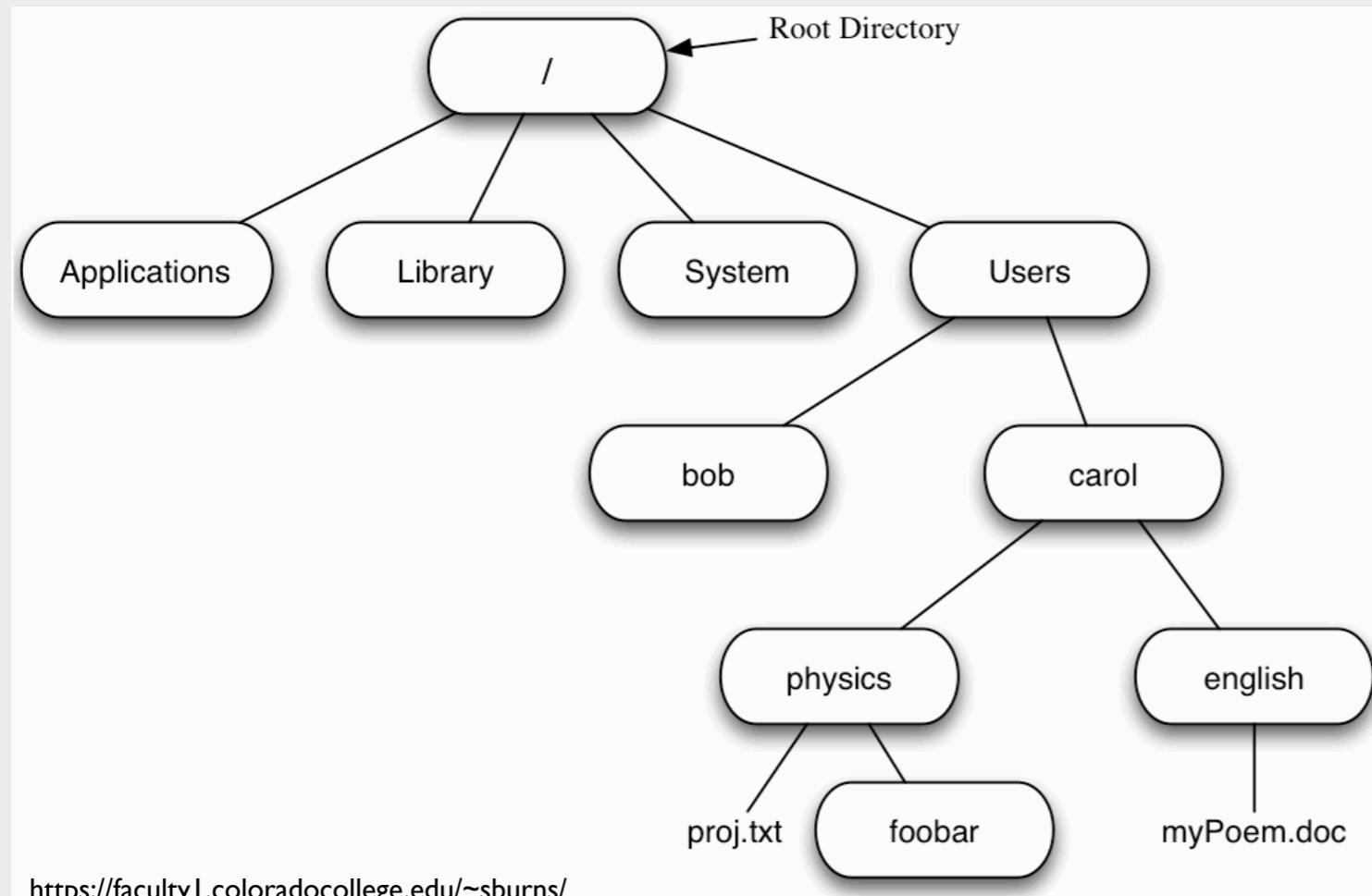


Present working directory (pwd)



Start directory:
/Users/carol/

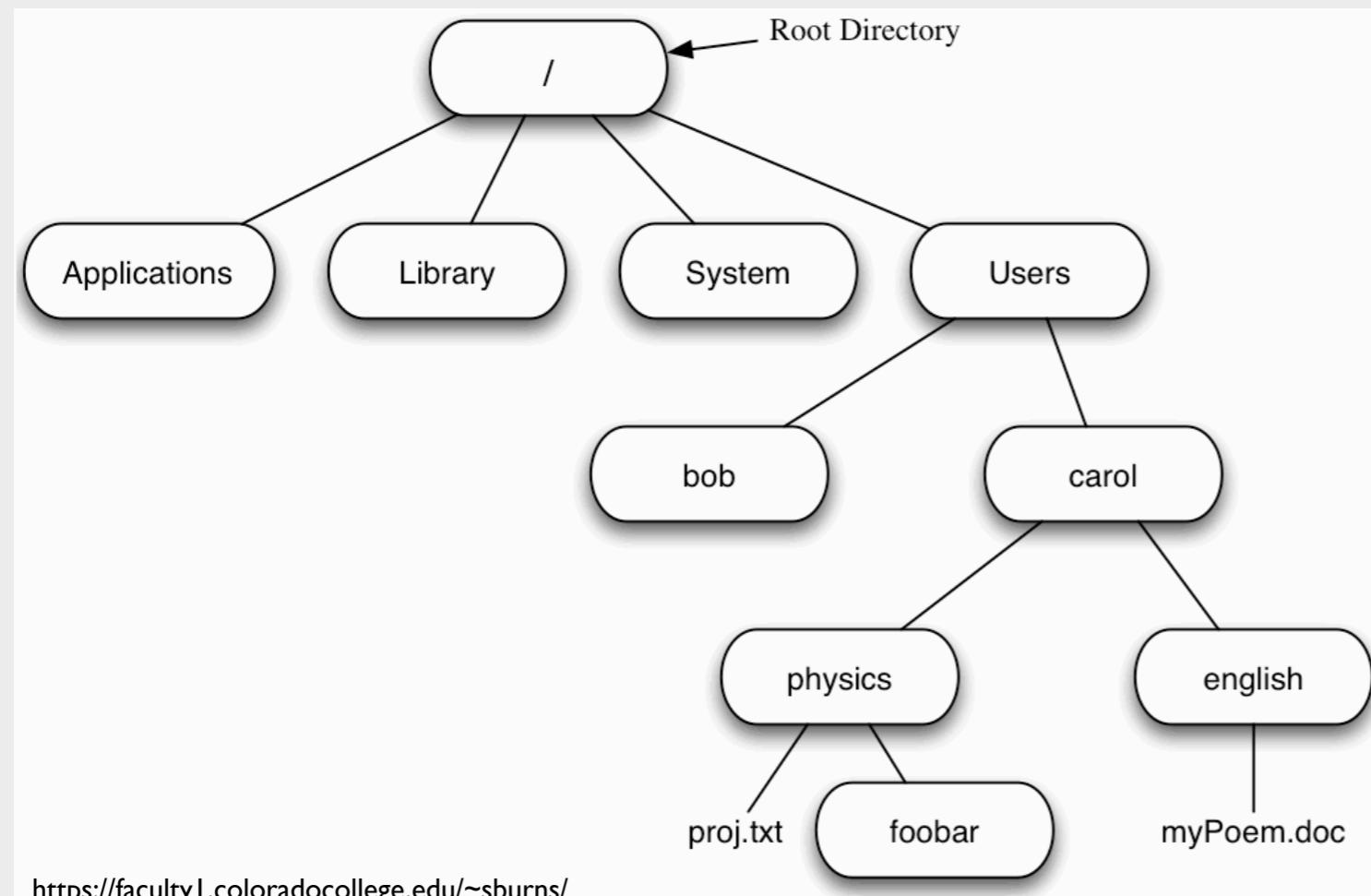
list (ls)



<https://faculty1.coloradocollege.edu/~sburns/>

Start directory:
physics
english

Change directory (cd)



<https://faculty1.coloradocollege.edu/~sburns/>

Start directory:

cd .. > Users

cd english > english

cd > carol

```
split_libraries.py -m Fasting_Map.txt -f
```

Split library output

Log file

```
Number raw input seqs 200000
Length outside bounds of 200 and 1000 3592
Num ambiguous bases exceeds limit of 6 0
Missing Qual Score 0
Mean qual score below minimum of 30 637
Max homopolymer run exceeds limit of 6 29658
Num mismatches in primer exceeds limit of 0: 29583

Number of sequences with identifiable barcode but without identifiable reverse primer:
1374

-z truncate_only option enabled; sequences without a discernible reverse primer as well as
sequences with a valid barcode not found in the mapping file may still be written.

Sequence length details for all sequences passing quality filters:
Raw len min/max/avg 230.0/547.0/329.4
Wrote len min/max/avg 180.0/512.0/280.8

Barcodes corrected/not 0/115740
Uncorrected barcodes will not be written to the output fasta file.
Corrected barcodes will be written with the appropriate barcode category.
Corrected but unassigned sequences will not be written unless --retain_unassigned_reads is
enabled.

Total valid barcodes that are not in mapping file 0
Sequences associated with valid barcodes that are not in the mapping file will not be
written.

Barcodes in mapping file
Num Samples 6
Sample ct min/max/mean: 1922 / 6147 / 3465.00
Sample Sequence Count Barcode
K3.M 6147 ATGGATCC
K1.M 3973 ATGGAACG
G9.M 3392 ATGGATGG
K2.M 3341 ATGGAAGC
G2.M 2015 ATGCCCTA
G1.M 1922 ATGGCCAT

Total number seqs written 20790
```

Histogram

# bins	raw sequence lengths, length of se	processing, and lengths of sequences that	
Length	Raw	Before	After
50	6	0	0
60	11	0	0
70	20	0	0
80	17	0	0
90	53	0	0
100	44	0	0
110	66	0	0
120	55	0	0
130	86	0	0
140	72	0	0
150	98	0	0
160	116	0	0
170	139	0	0
180	245	0	118
190	393	0	58
200	667	0	86
210	388	0	795
220	1116	0	2862
230	4586	135	1395
240	2229	73	2546
250	4910	92	5474
260	19921	846	623
270	21333	2962	669
280	24079	1463	244
290	20433	2565	360
300	31701	5511	66
310	19703	557	40
320	15222	511	24
330	3158	222	67
340	2930	358	7
350	7192	46	16
360	688	7	6
370	2332	21	100
380	843	78	4414
390	337	7	89
400	507	5	642
410	1152	22	8
420	2178	300	22
430	5540	4671	2
440	366	43	5
450	1737	229	34
460	212	7	2
470	128	2	3
480	221	2	0

Chimera checking

Split_library

```
Number raw input seqs 200000
Length outside bounds of 200 and 1000 3592
Num ambiguous bases exceeds limit of 6 0
Missing Qual Score 0
Mean qual score below minimum of 30 637
Max homopolymer run exceeds limit of 6 29658
Num mismatches in primer exceeds limit of 0: 29583

Number of sequences with identifiable barcode but
1374

-z truncate_only option enabled; sequences without
sequences with a valid barcode not found in the ma

Sequence length details for all sequences passing
Raw len min/max/avg 230.0/547.0/329.4
Wrote len min/max/avg 180.0/512.0/280.8

Barcodes corrected/not 0/115740
Uncorrected barcodes will not be written to the ou
Corrected barcodes will be written with the approp
Corrected but unassigned sequences will not be wri
enabled.

Total valid barcodes that are not in mapping file
Sequences associated with valid barcodes that are
written.

Barcodes in mapping file
Num Samples 6
Sample ct min/max/mean: 1922 / 6147 / 3465.00
Sample Sequence Count Barcode
K3.M 6147 ATGGATCC
K1.M 3973 ATGGAACG
G9.M 3392 ATGGATGG
K2.M 3341 ATGGAAGC
G2.M 2015 ATGGCCTA
G1.M 1922 ATGGCCAT

Total number seqs written 20790
```

Chimera output

```
input_seqs_fp
/Users/mkemler/Documents/workshops/NGS_amplicon_Pretoria2017/split_library/seqs.fna
output_dir
/Users/mkemler/Documents/workshops/NGS_amplicon_Pretoria2017/chimaera_detection
reference_seqs_fp None
suppress_usearch61_intermediates False
suppress_usearch61_ref True
suppress_usearch61_denovo False
split_by_sampleid False
non_chimeras_retention union
usearch61_minh 0.28
usearch61_xn 8.0
usearch61_dn 1.4
usearch61_mindiffs 3
usearch61_mindiv 0.8
usearch61_abundance_skew 2.0
percent_id_usearch61 0.97
minlen 64
word_length 8
max_accepts 1
max_rejects 8
HALT_EXEC False

ref_non_chimeras 0
ref_chimeras 0
denovo_chimeras 1144
denovo_non_chimeras 19646
```

Log File

```
UclustOtuPicker parameters:  
Application:uclust  
Similarity:0.95  
enable_rev_strand_matching:True  
exact:False  
max_accepts:1  
max_rejects:8  
new_cluster_identifier:denovo  
optimal:False  
output_dir:uclust_picked_otus  
prefilter_identical_sequences:True  
presort_by_abundance:True  
save_uc_files:True  
stable_sort:True  
stepwords:8  
suppress_sort:True  
word_length:9  
Num OTU:603  
Result path: uclust_picked_otus/seqs_nc_otus.txt
```

denovo0	K3.M_12599					
denovo1	K3.M_17868					
denovo2	K3.M_5349					
denovo3	G2.M_20007	K1.M_8024				
denovo4	K1.M_4659					
denovo5	K2.M_14419					
denovo6	K1.M_1302	K1.M_2109	K1.M_882	K1.M_17853	K1.M_10027	K3.M_23
denovo7	K1.M_5003					
denovo8	G9.M_759	G9.M_1109	G2.M_1445	G9.M_1534	G9.M_2057	G2.M_24
denovo9	G9.M_758	G9.M_1411	G9.M_5370	G9.M_7070	G9.M_8163	G9.M_90
denovo10	G9.M_754	G2.M_10007	K3.M_10083	K2.M_11747	G2.M_13623	K2.M_11747
denovo11	G2.M_3699					
denovo12	K3.M_13834					
denovo13	K1.M_9985					
denovo14	G2.M_11885					
denovo15	K2.M_17886					
denovo16	K3.M_16192					
denovo17	K3.M_16190					
denovo18	G9.M_6451					
denovo19	K1.M_14509					
denovo20	G1.M_12392					
denovo21	K3.M_1610	K1.M_11176	K2.M_12021	K3.M_12333	K3.M_15678	K2.M_11176
denovo22	K2.M_13304					
denovo23	K3.M_6483	K3.M_10078	K2.M_12274	K3.M_15201	G1.M_16024	K1.M_13304
denovo24	G9.M_6691					
denovo25	G2.M_9075					

Taxonomic assignment

Log File

```
BlastTaxonAssigner parameters:  
Application:blastn/megablast  
Max E value:1e-30  
Min percent identity:90.0  
id_to_taxonomy_filepath:/Users/mkemler/Documents/data  
reference_seqs_filepath:/Users/mkemler/Documents/data  
Number of sequences inspected: 603  
Number with no blast hits: 229  
Result path: /tmp/assign-taxWDqAX9
```

Assignment output

```
denovo448 k_Fungi;p_Ascomycota;c_unidentified;o_unidentified;f_uniden  
denovo449 k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Dothideomycetes_ord  
denovo446 k_Fungi;p_Basidiomycota;c_Tremellomycetes;o_Tremellales;f_T  
denovo447 No blast hit None None  
denovo444 k_Fungi;p_Basidiomycota;c_Ustilaginomycetes;o_Ustilaginales;  
denovo445 No blast hit None None
```

denovo444

< OTU name

k_Fungi;p_Basidiomycota;c_Ustilaginomycetes;o_Ustilaginales
;f_Ustilaginaceae;g_Macalpinomyces;s_Macalpinomyces_tristac
hyae

< Taxonomic assignment

< e-value

Ie-112
SH005407.07FU_AY740164_reps_singleton < Accession no.

OTU Table

# Constructed from biom file							
#OTU ID	K3.M	G2.M	K1.M	K2.M	G9.M	G1.M	taxonomy
denovo3	0.0	1.0	1.0	0.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo6	2.0	0.0	6.0	0.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Sordariomycetes; o_
denovo8	0.0	6.0	0.0	0.0	24.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo9	0.0	3.0	0.0	0.0	10.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo10	2.0	2.0	2.0	2.0	2.0	1.0	k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Le
denovo23	4.0	1.0	4.0	1.0	7.0	1.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo29	38.0	0.0	23.0	2.0	0.0	1.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo34	9.0	0.0	0.0	0.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo36	4.0	1.0	2.0	3.0	2.0	2.0	k_Fungi; p_Ascomycota; c_Taphrinomycetes; o_
denovo37	1.0	2.0	1.0	3.0	0.0	0.0	k_Fungi; p_Basidiomycota; c_unidentified; o_un
denovo40	0.0	0.0	0.0	0.0	3.0	0.0	k_Fungi; p_Basidiomycota; c_Tremellomycetes; o
denovo44	1.0	0.0	0.0	0.0	1.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo46	0.0	0.0	8.0	0.0	0.0	1.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo48	0.0	2.0	3.0	0.0	0.0	2.0	k_Fungi; p_Basidiomycota; c_Tremellomycetes; o
denovo50	7.0	0.0	0.0	0.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo52	1.0	21.0	0.0	1.0	249.0	0.0	k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Le
denovo57	0.0	0.0	4.0	0.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo60	1.0	1.0	0.0	2.0	3.0	1.0	k_Fungi; p_Ascomycota; c_Leotiomycetes; o_He
denovo63	0.0	0.0	0.0	2.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo64	0.0	0.0	0.0	0.0	3.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_
denovo65	1.0	0.0	0.0	1.0	0.0	0.0	k_Fungi; p_Ascomycota; c_Dothideomycetes; o_

Summary

```
Num samples: 6
Num observations: 205
Total count: 8972
Table density (fraction of non-zero values): 0.460

Counts/sample summary:
  Min: 622.0
  Max: 2317.0
  Median: 1528.000
  Mean: 1495.333
  Std. dev.: 563.560
Sample Metadata Categories: ReversePrimer; LinkerPrimerSequence; BarcodeSequence; Description
Observation Metadata Categories: taxonomy

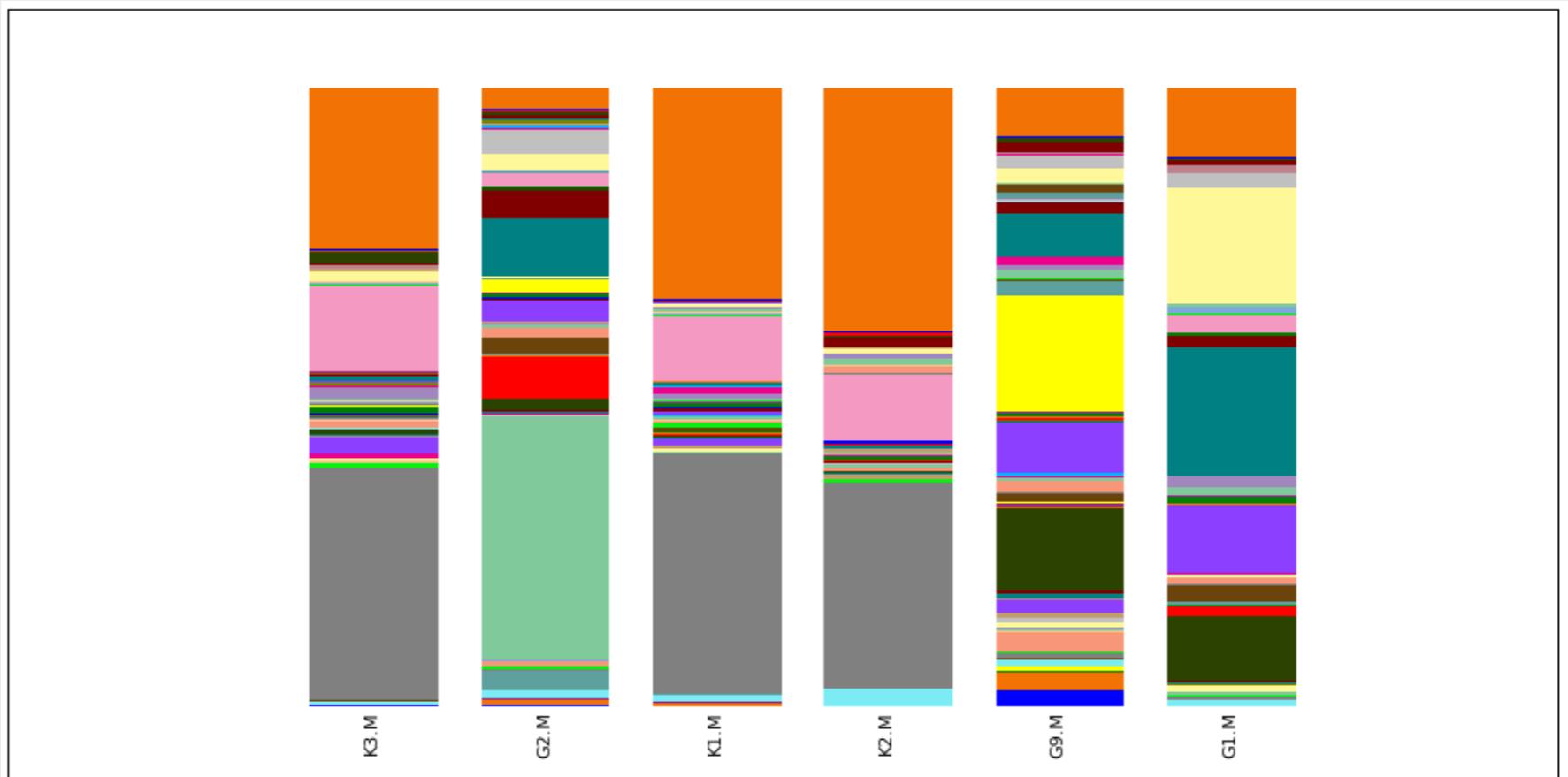
Counts/sample detail:
  G1.M: 622.0
  G2.M: 1049.0
  G9.M: 1329.0
  K2.M: 1727.0
  K3.M: 1928.0
  K1.M: 2317.0
```

Taxonomy plot

```
# Constructed from biom file
#OTU ID K3.M      G2.M      K1.M      K2.M      G9.M      G1.M
k__;p__;c__;o__;f__;g__Archaeorhizomyces    0.00103734439834    0.0 0.0 0.0 0.0 0.0 0.0
```

.

.



[View Table \(.txt\)](#)

Legend	Taxonomy						Total	K3.M	G2.M	K1.M	K2.M	G9.M	G1.M
	%	%	%	%	%	%							
k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizomycetaceae;g_Archaeorhizomyces	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%							
k_Fungi;p_Ascomycota;c_Arthoniomycetes;o_unidentified;f_unidentified;g_unidentified	0.5%	0.1%	0.4%	0.0%	0.0%	2.5%							

Alpha diversity

OTU table

```
Num samples: 6
Num observations: 205
Total count: 8972
Table density (fraction of non-zero entries): 0.022540000000000002

Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Reversely oriented
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0
```

Rarify

```
Num samples: 6
Num observations: 205
Total count: 8972
Table density (fraction of non-zero entries): 0.022540000000000002

Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Reversely oriented
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0

Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Reversely oriented
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0

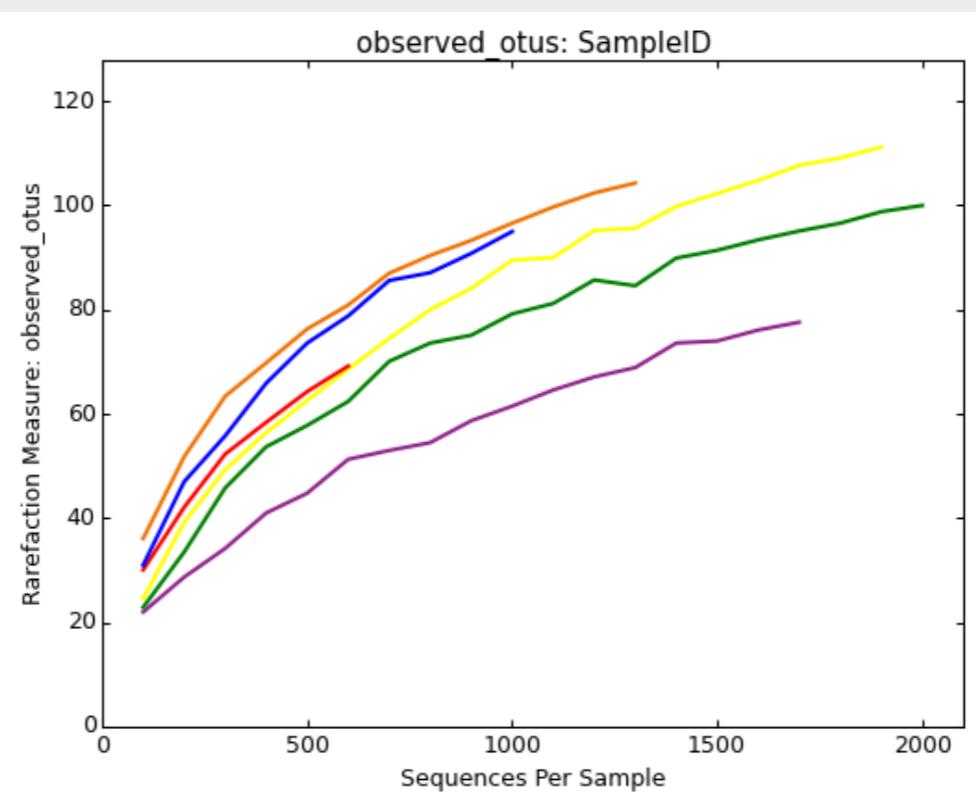
Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Reversely oriented
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0
```

Diversity index

Observed OTUs
Chao I, etc ..

Plot



Beta diversity

OTU table

```

Num samples: 6
Num observations: 205
Total count: 8972
Table density (fraction of non-zero entries): 0.0223

Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Revert
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0
  
```

Rarify

```

Num samples: 6
Num observations: 285
Total count: 8972
Table density (fraction of non-zero entries): 0.0223

Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Revert
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0

Num samples: 6
Num observations: 285
Total count: 8972
Table density (fraction of non-zero entries): 0.0223

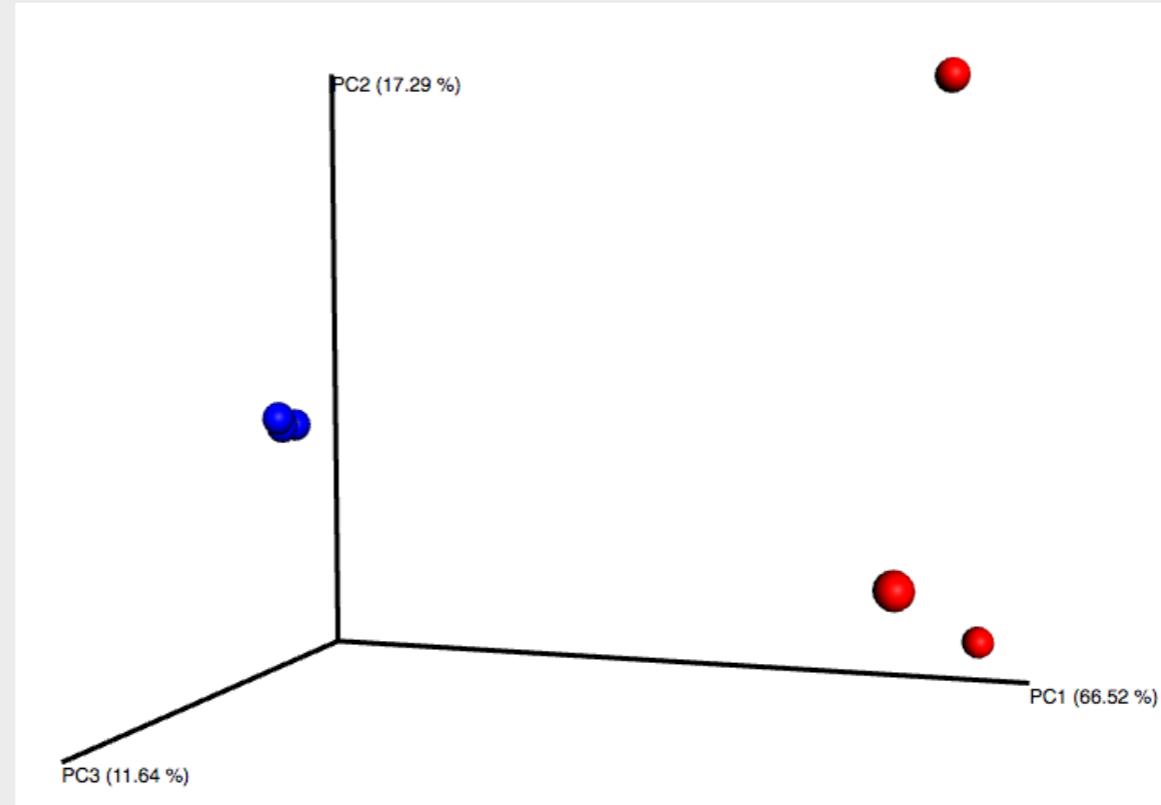
Counts/sample summary:
Min: 622.0
Max: 2317.0
Median: 1528.000
Mean: 1495.333
Std. dev.: 563.568
Sample Metadata Categories: Revert
Observation Metadata Categories: t

Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0

Num samples: 6
Counts/sample detail:
G1.M: 622.0
G2.M: 1049.0
G9.M: 1329.0
K2.M: 1727.0
K3.M: 1928.0
K1.M: 2317.0
  
```

Observed OTUs
Diversity Chao I, etc ..
index

PCoA
NMDS

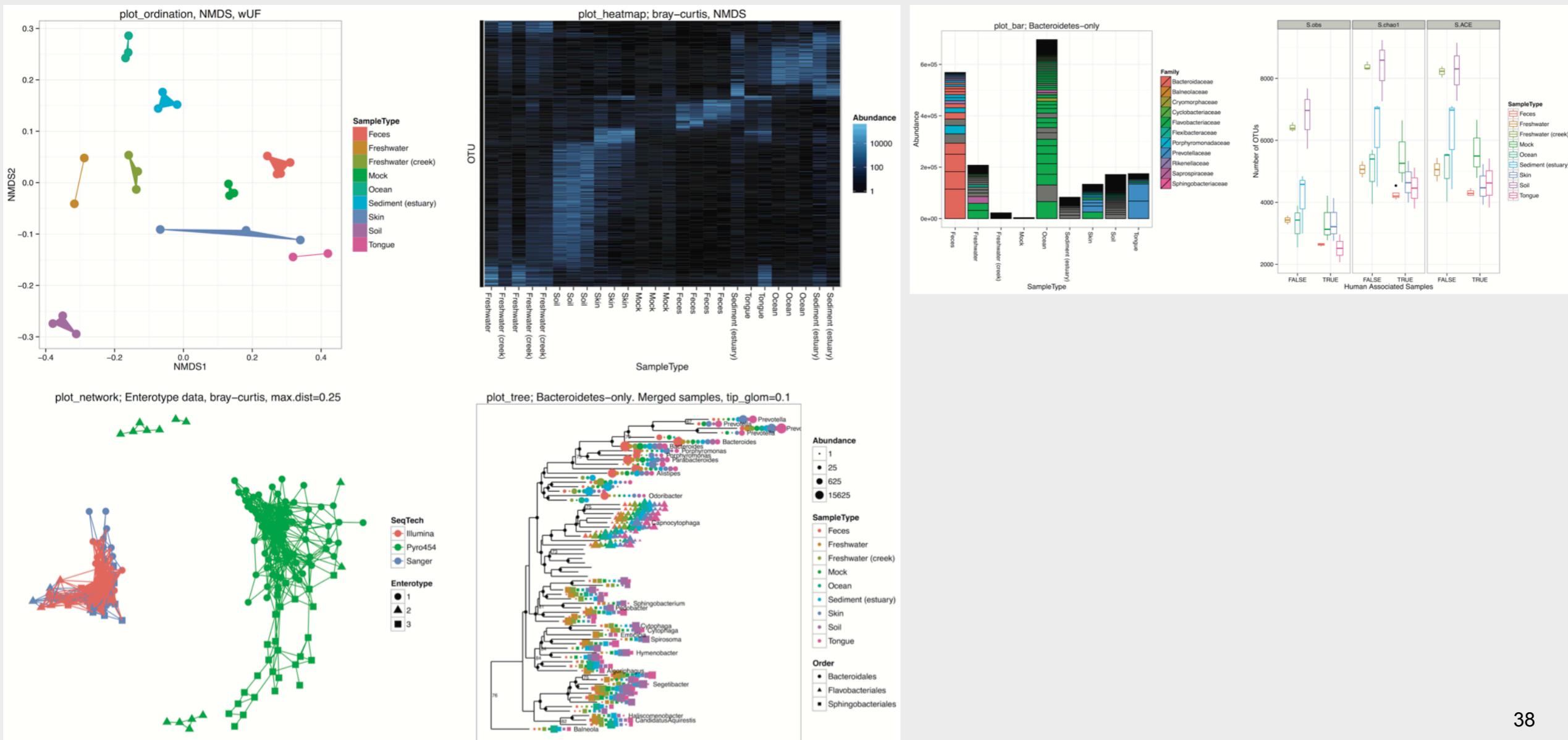


Downstream analyses

phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data

Paul J. McMurdie, Susan Holmes*

Department of Statistics, Stanford University, Stanford, California, United States of America

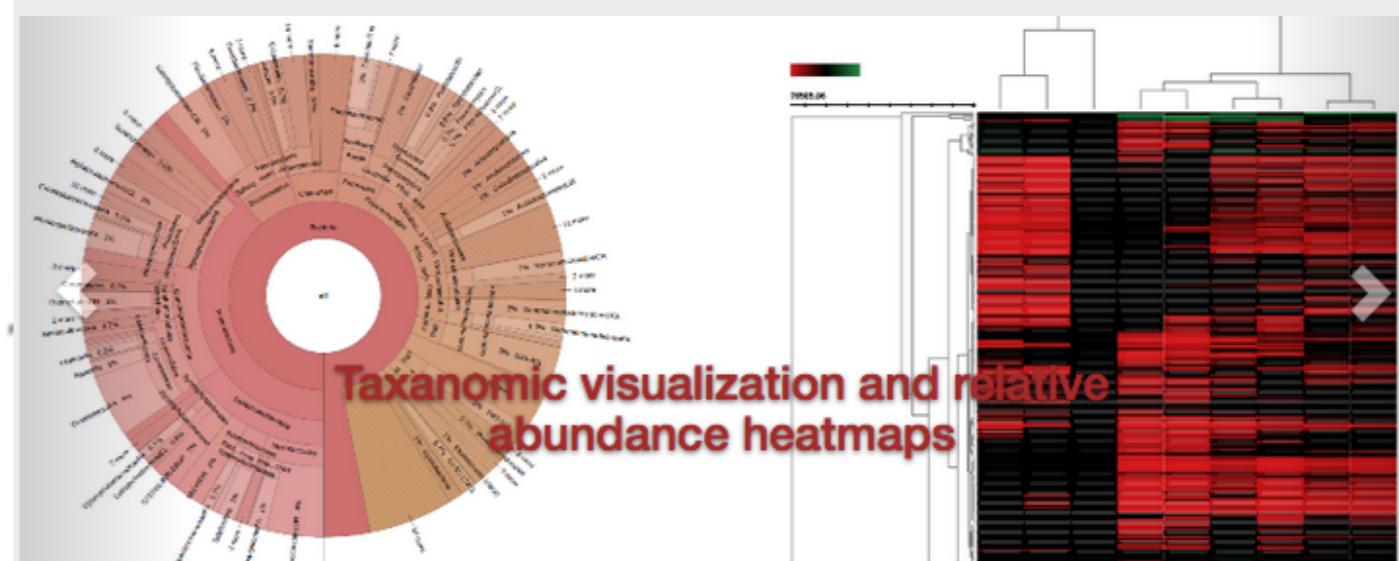
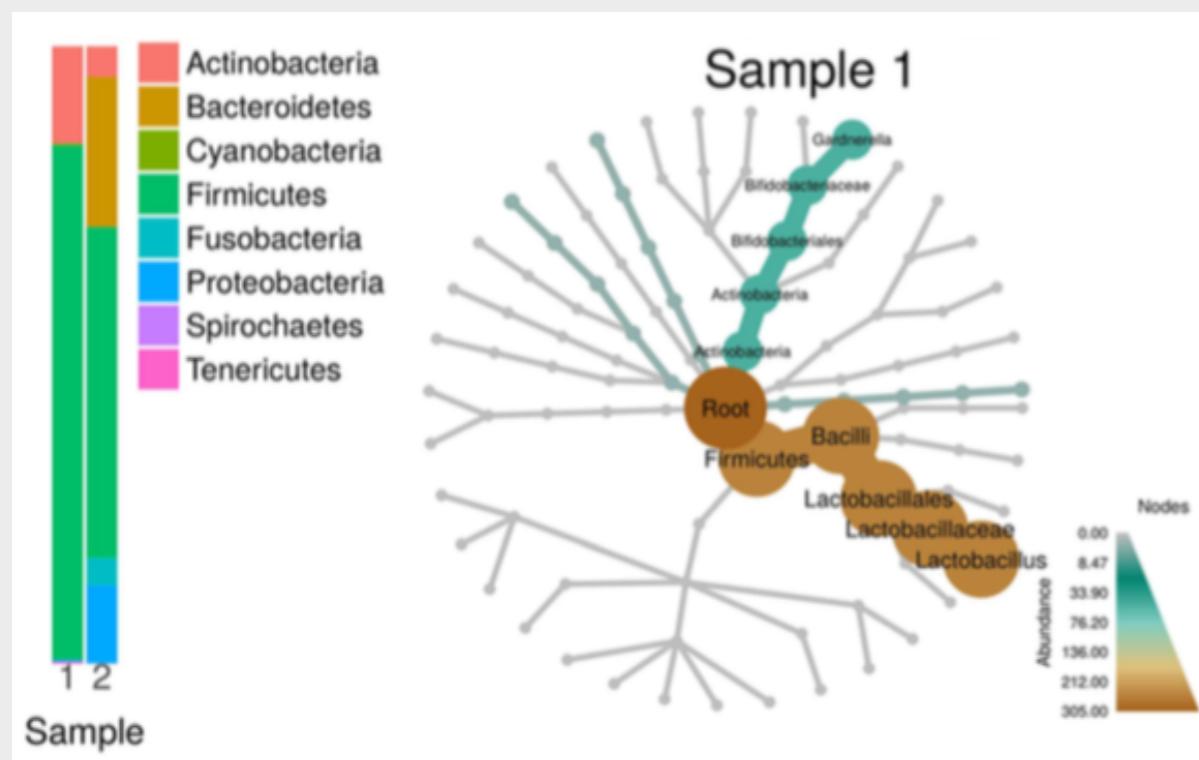


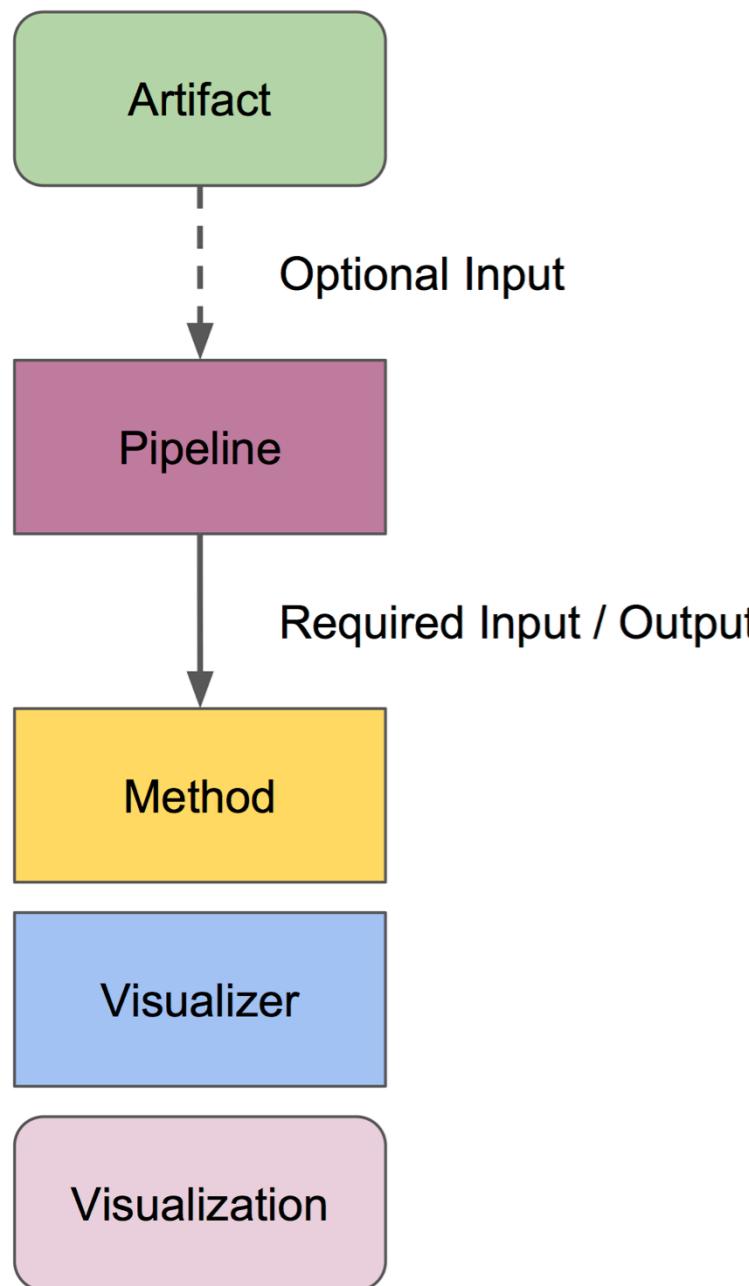
Downstream analyses software

RESEARCH ARTICLE

Metacoder: An R package for visualization and manipulation of community taxonomic diversity data

Zachary S. L. Foster¹, Thomas J. Sharpton^{2,3,4}, Niklaus J. Grünwald^{5*}





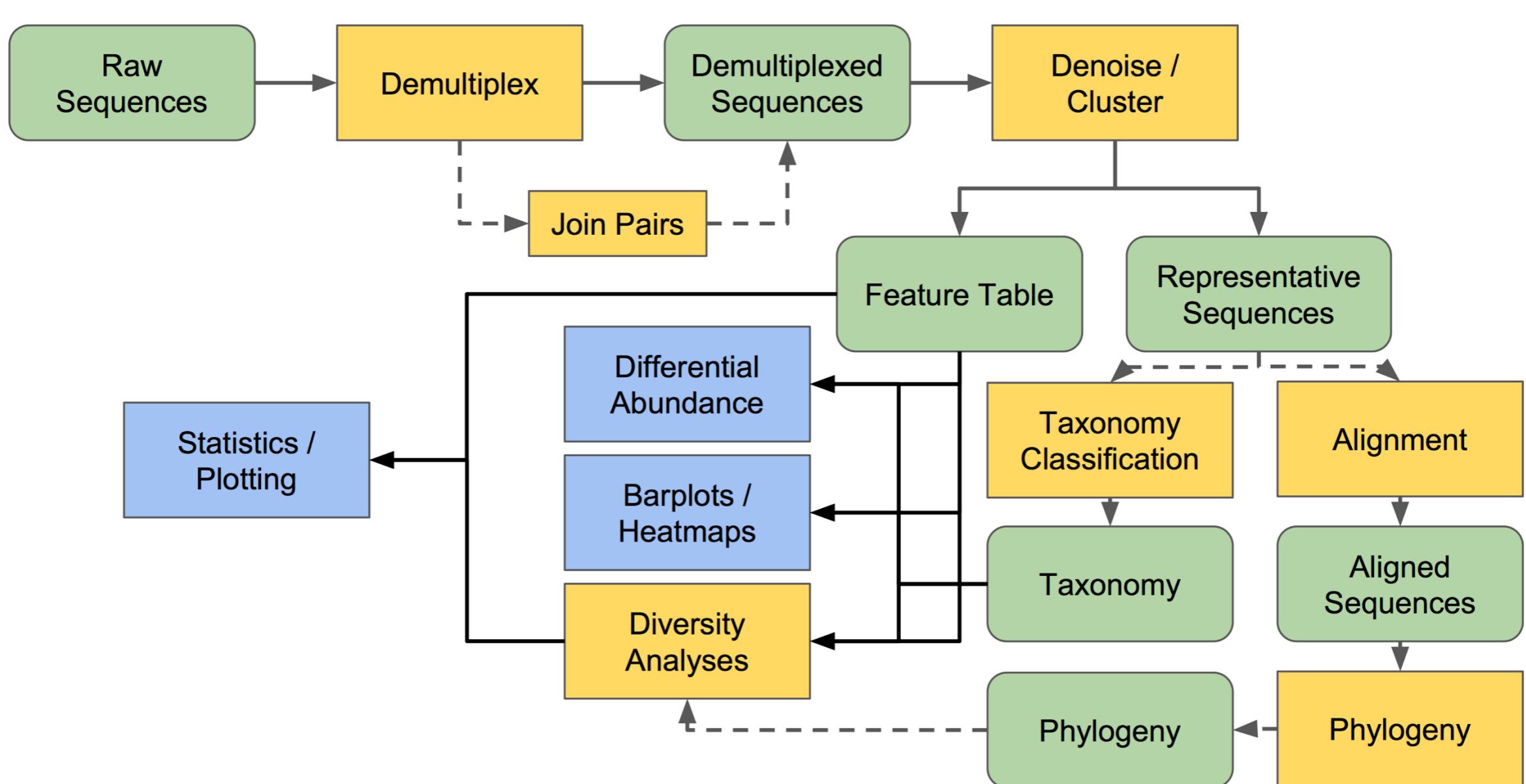
Artifact: Input or output data (.qza)

Pipeline: Combination of actions

Method: Action of combined Artifact
+ Parameters to produce Artifact

Visualizer: Method that produces
Visualization

Visualization: Output of Visualizer
.qzv)



```
qiime tools import \  
  --type EMPSingleEndSequences \  
  --input-path emp-single-end-sequences \  
  --output-path emp-single-end-sequences.qza
```

For any questions email me:

martin.kemler@rub.de