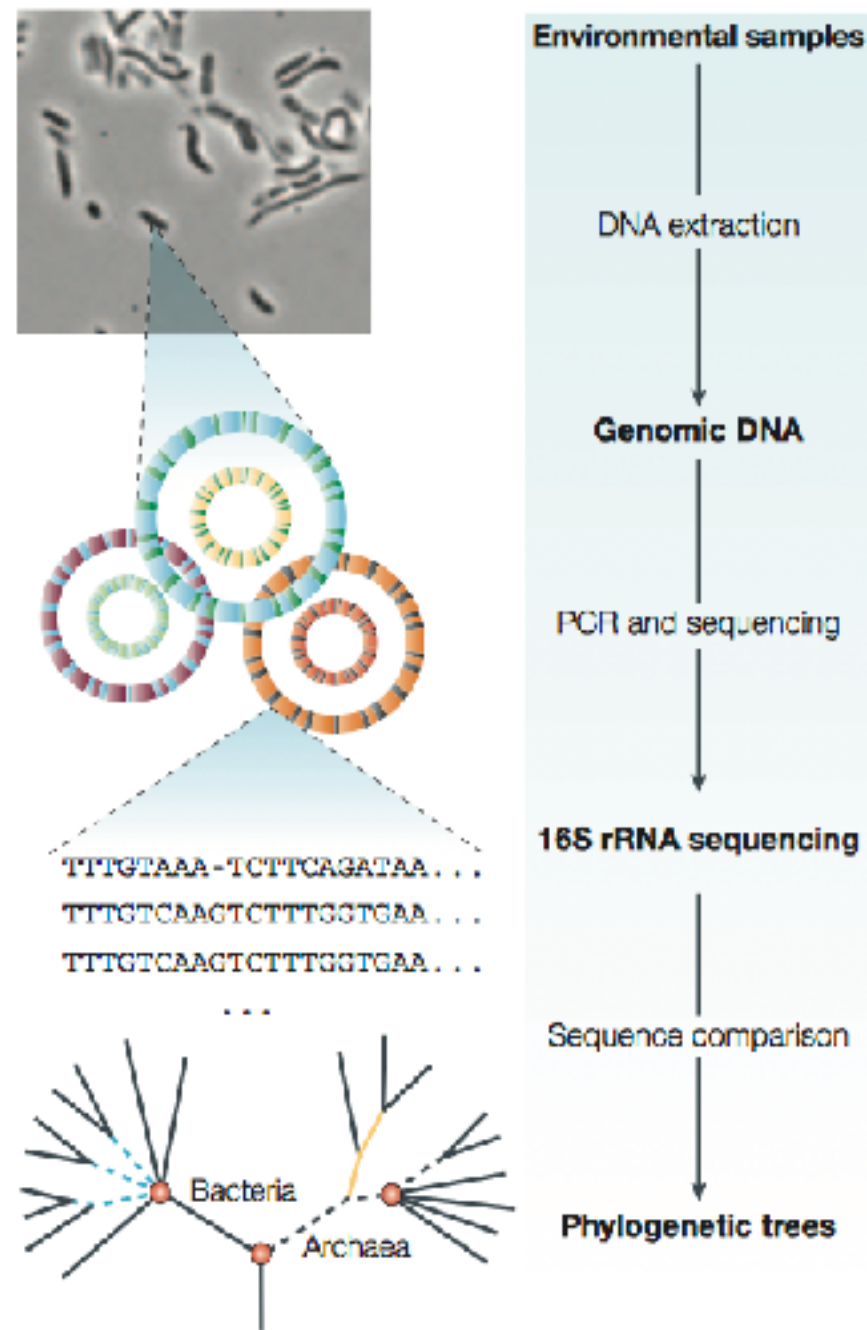


Microbiome Analysis Using R

Scott A. Handley
Assistant Professor
Pathology & Immunology
August 23, 2017

16S rRNA Gene Sequencing



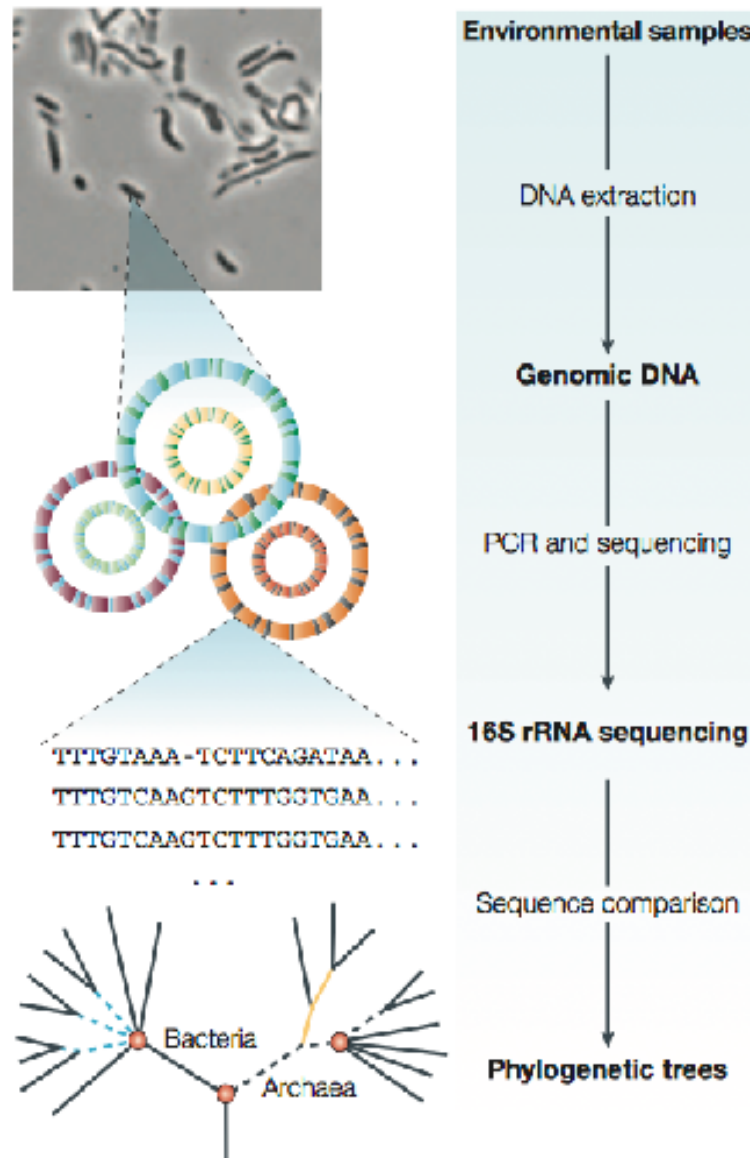
Tringe, S.G., Rubin, E.M. Nat
Rev Genet. 2005 Nov;6(11):
805-14

Study Design

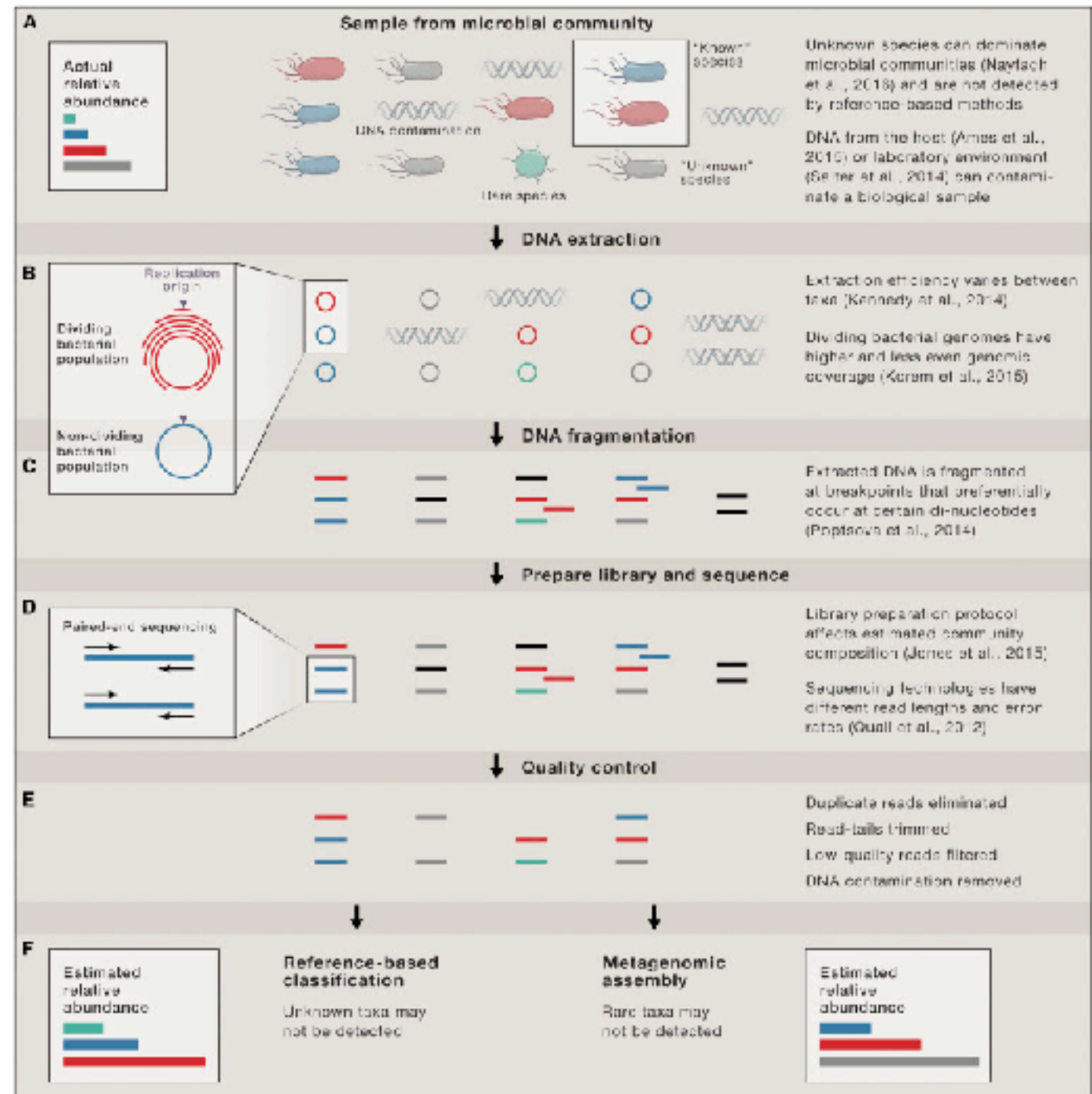
Laboratory

Bioinformatics, Ecological
Analysis and Statistics

16S Amplicon Surveys vs Metagenomics?



Tringe, S.G., Rubin, E.M.
Nat Rev Genet. 2005 Nov;
6(11):805-14



Nayfach S., Pollard KS. Cell. Aug 25;166(5):1103-16

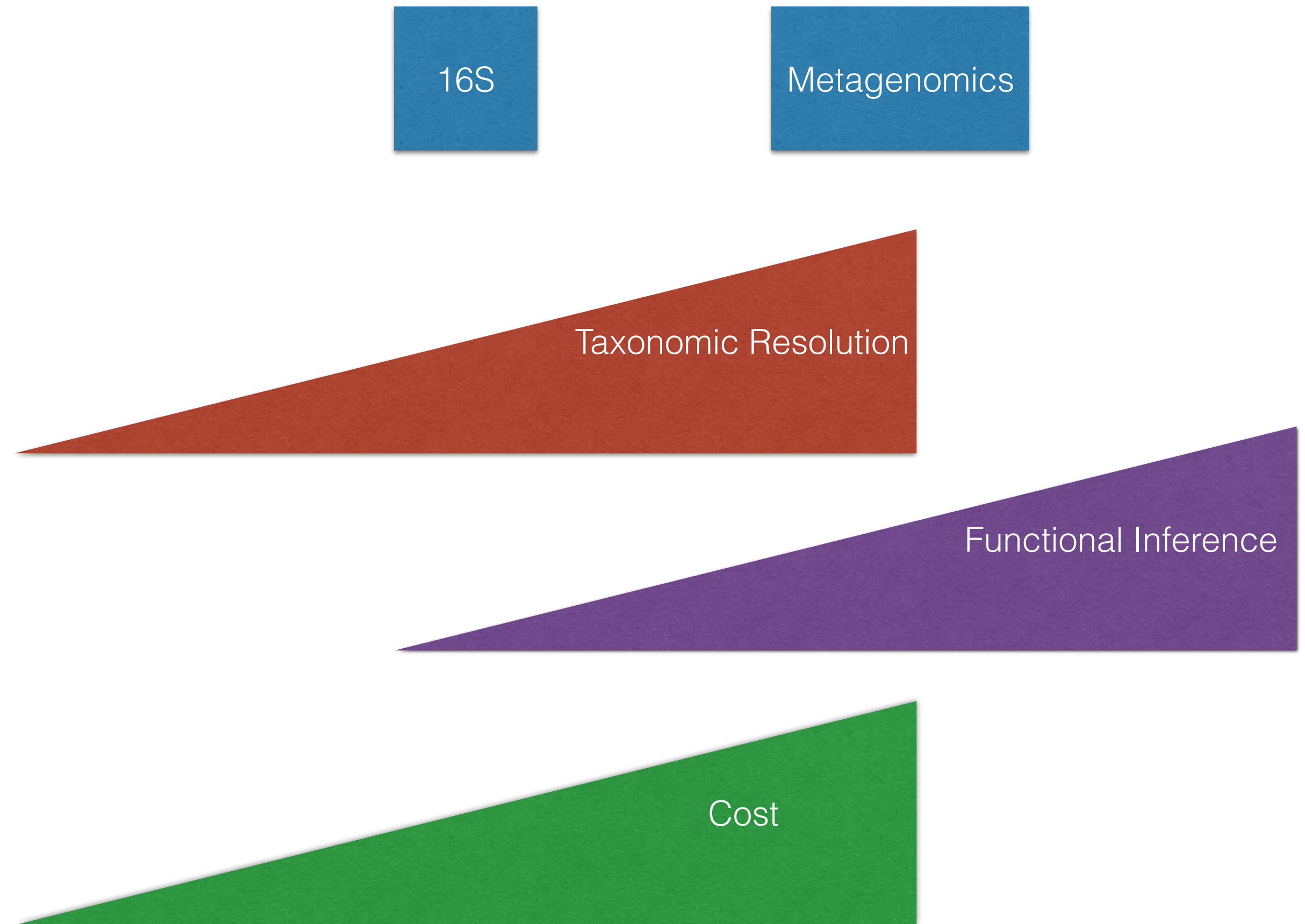
16S

Metagenomics

Taxonomic Resolution

Functional Inference

Cost



Most of Your Decision Will Boil Down to \$\$\$

- Our labs per sample costs:
 - 16S = \$17.50 per sample
 - Metagenome = \$225.00 per sample
 - Has been estimated to be as low as \$100 per sample
- Study we will discuss today: 270 samples
 - \$4,725 vs. \$27 - \$60,750
- Other considerations:
 - Understanding analytical space
 - Data storage

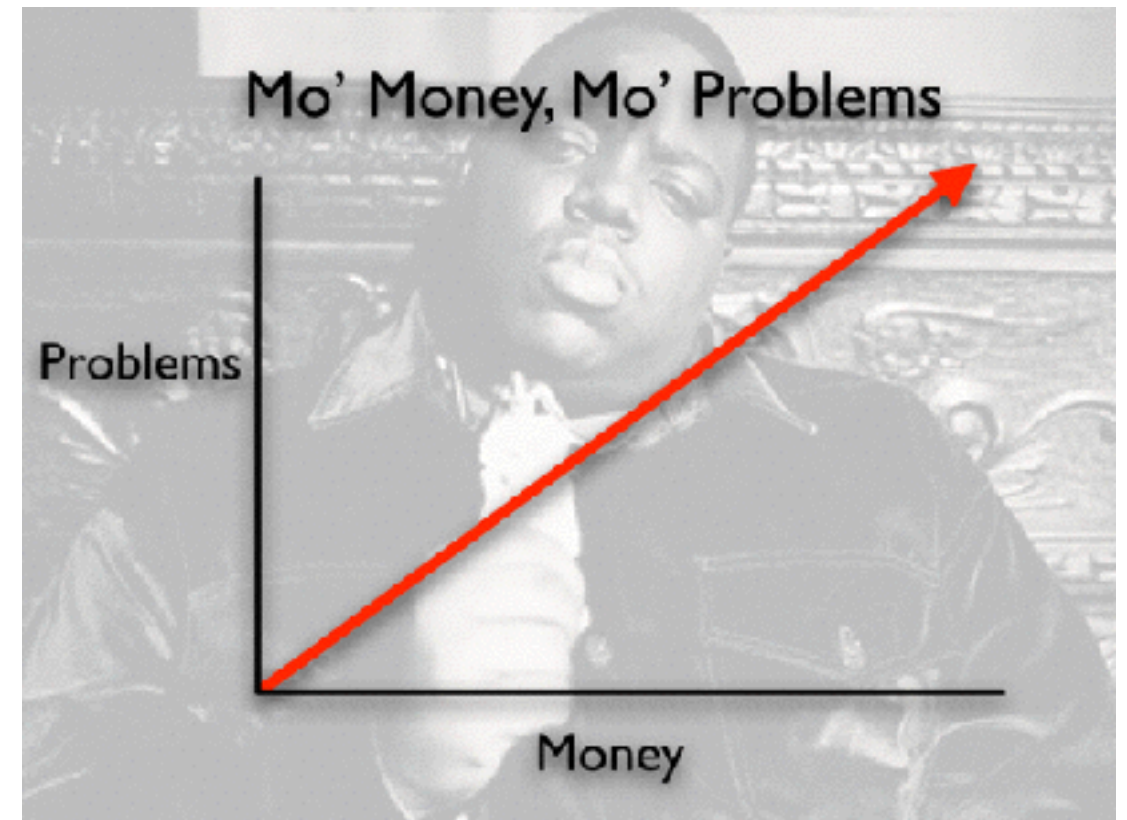
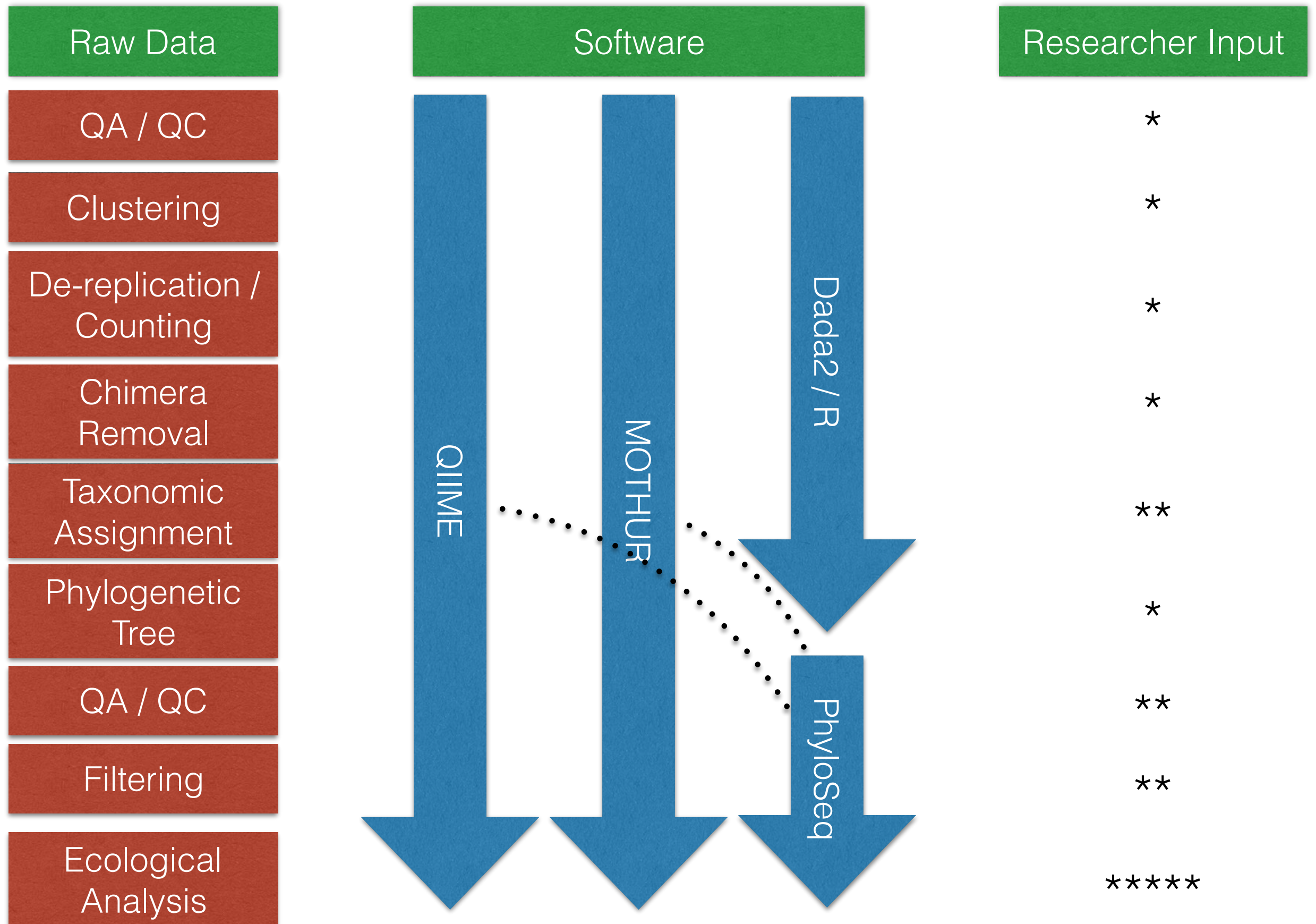


Image credit: The Internet
Quote credit: Notorious B.I.G.

Stages of Bioinformatics



Count Tables: The Analytical Substrate

Raw Data

QA / QC

Clustering

De-replication /
Counting

Chimera
Removal

Taxonomic
Assignment

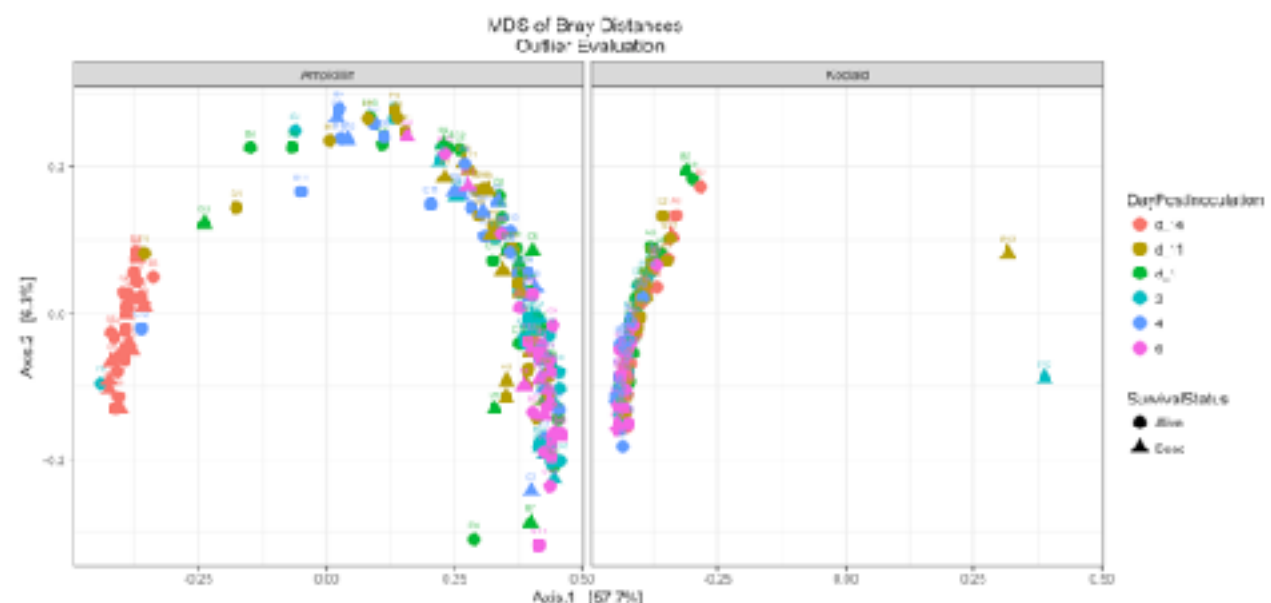
Phylogenetic
Tree

QA / QC

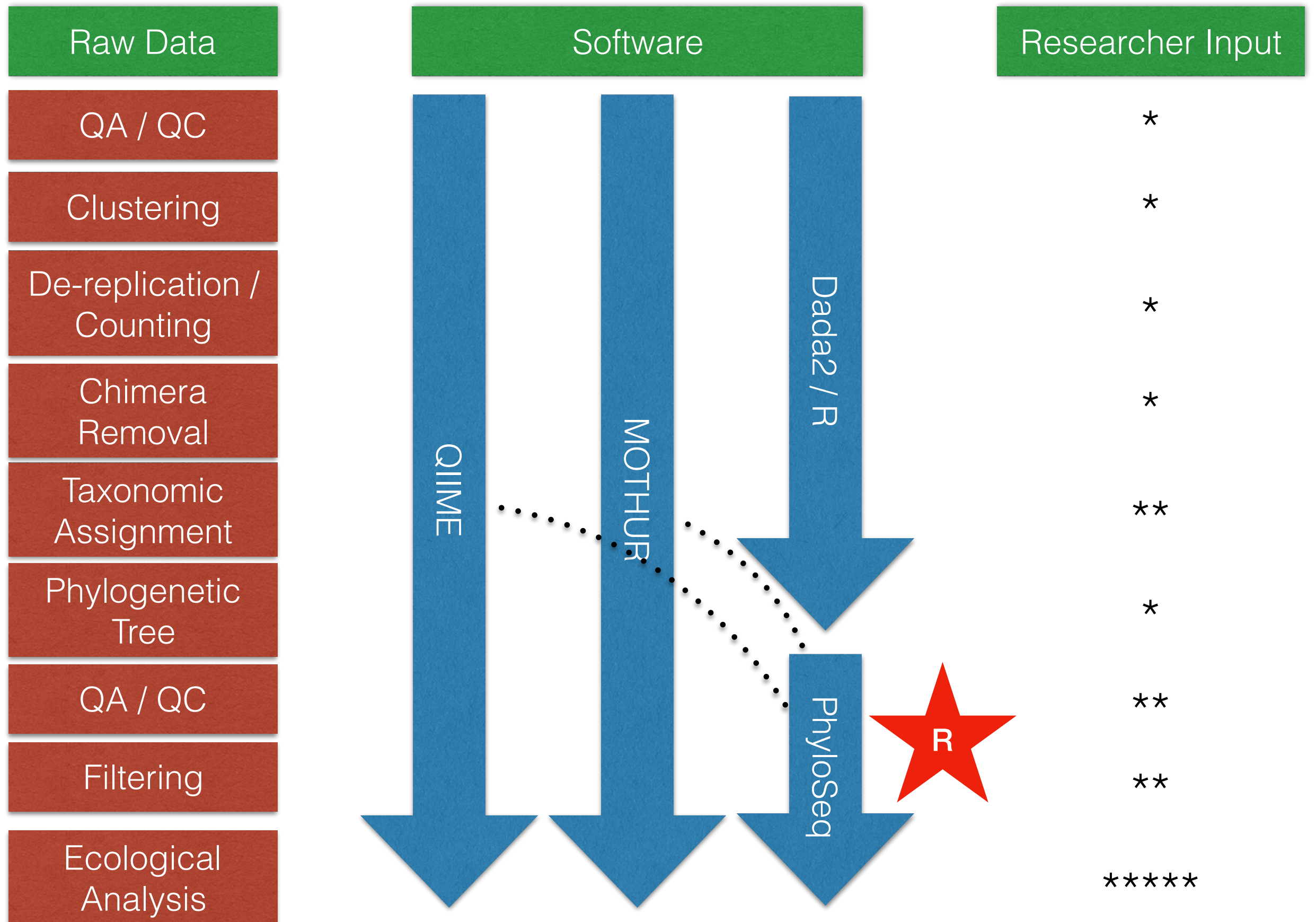
Filtering

Ecological
Analysis

ID	Sample 1	Sample 2	Sample 3	Sample 4
RSV 1	0	0	2	0
RSV 2	12	8	8	456
RSV 3	112	101	98	10
RSV 4	435	435	382	3
RSV 5	76	83	68	145



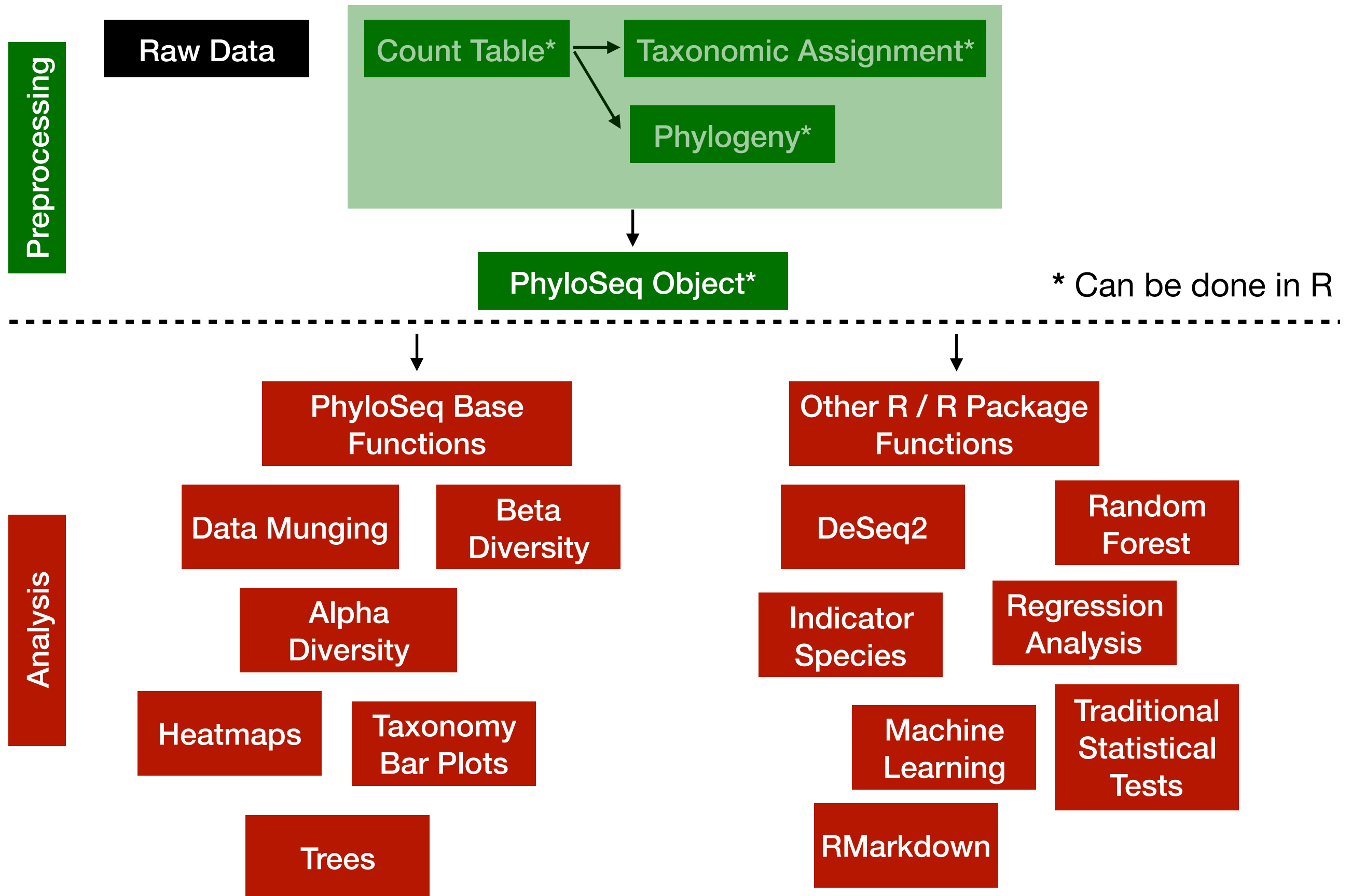
Stages of Bioinformatics



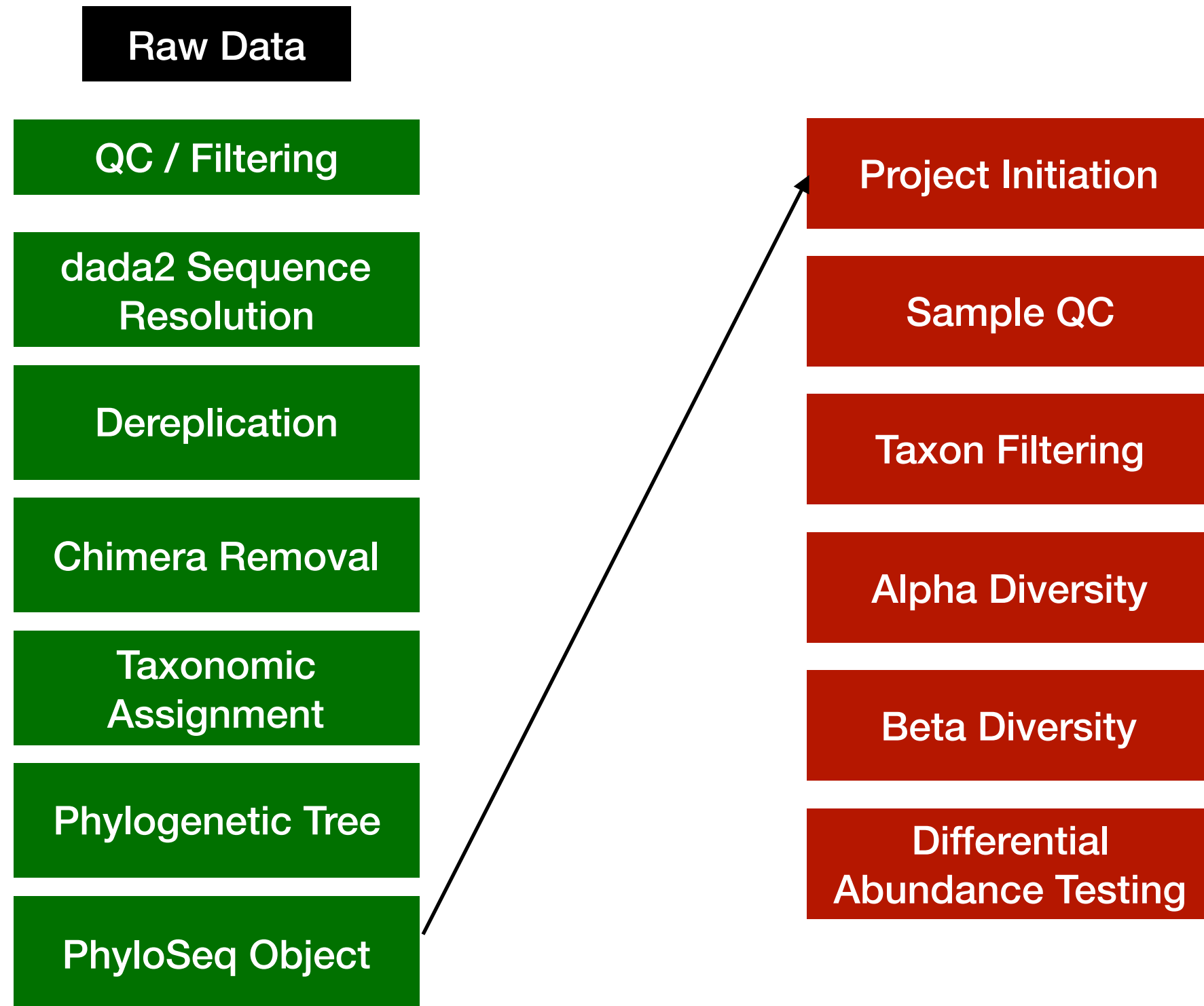
Why You Should Consider Using R for Microbiome Analysis

- R is a multitasker
- Huge development community in unrelated disciplines
- Integrated command, lab notebook, graphing environment and reporting environment
 - Easily integrated with GitHub for collaboration and post-publication reporting

Why You Should Consider Using R for Microbiome Analysis



Basic R Analysis Workflow Detail



- Very basic R / RMarkdown
- Analysis Workflow
 - Goal is to move quickly to provide an overview and allow time for specific questions
 - Remember: We are now a community! All examples will be shared and happy to meet and discuss outside
 - Remember part 2: Identify future topics for discussion

Case Study

- Effect of antibiotic treatment on West Nile Virus pathogenesis

