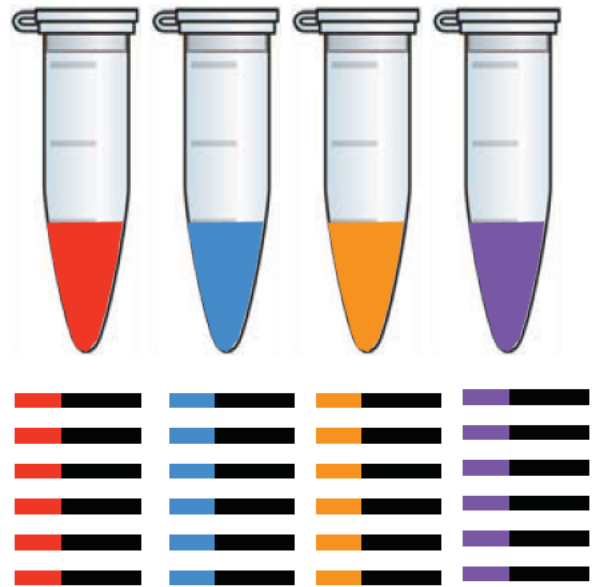


Extract DNA and
PCR amplify with
barcoded primer



Pool amplicons



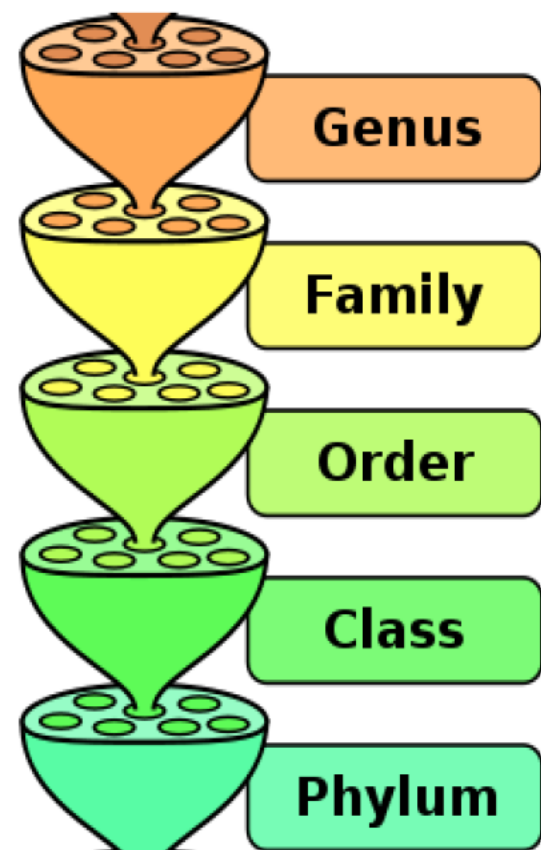
Pyrosequence
amplicons using 454's
GS FLX instrument



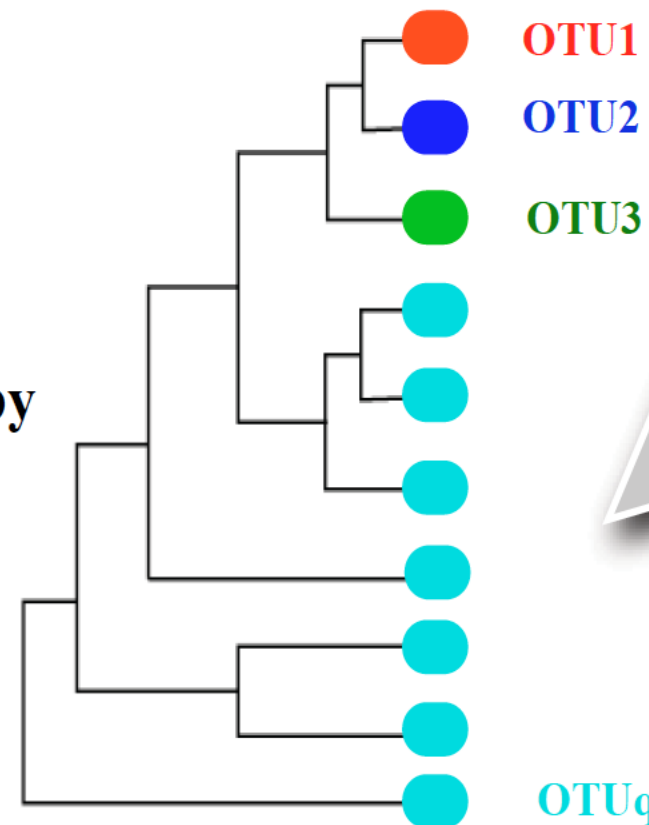
Quality control, assign
sequences to samples
using barcode and denoise



Species level OTUs can be
summarized into higher levels



Assign lineages by
RDP classifier



Thousands of OTUs,
100's of samples

> AGTGAGAGAAGCAGGGTCGTAATGTT ... OTU1
> AGTGCGATGCGTAGGGTCGTAATGCG ...
> AGTGCGATGCGTAGGGTCGTAATGTA|... OTU2
> AGTGGATGCTCTAGGGTCGTAATGCA ...
> AGTGTCACGGTGAGGGTCGTAATGGG ... OTU3
> AGTGGATGCTCTAGGGTCGTAATGTT ...
> AGTGTCACGGTGAGGGTCGTAATGCC ...
> AGTGAGAGAAGCAGGGTCGTAATCAC ...
...

Cluster sequences into OTUs (97% level),
align to reference alignment (e.g., using
PYNAST), infer phylogeny (FastTree)

OTU: Operational Taxonomic Unit