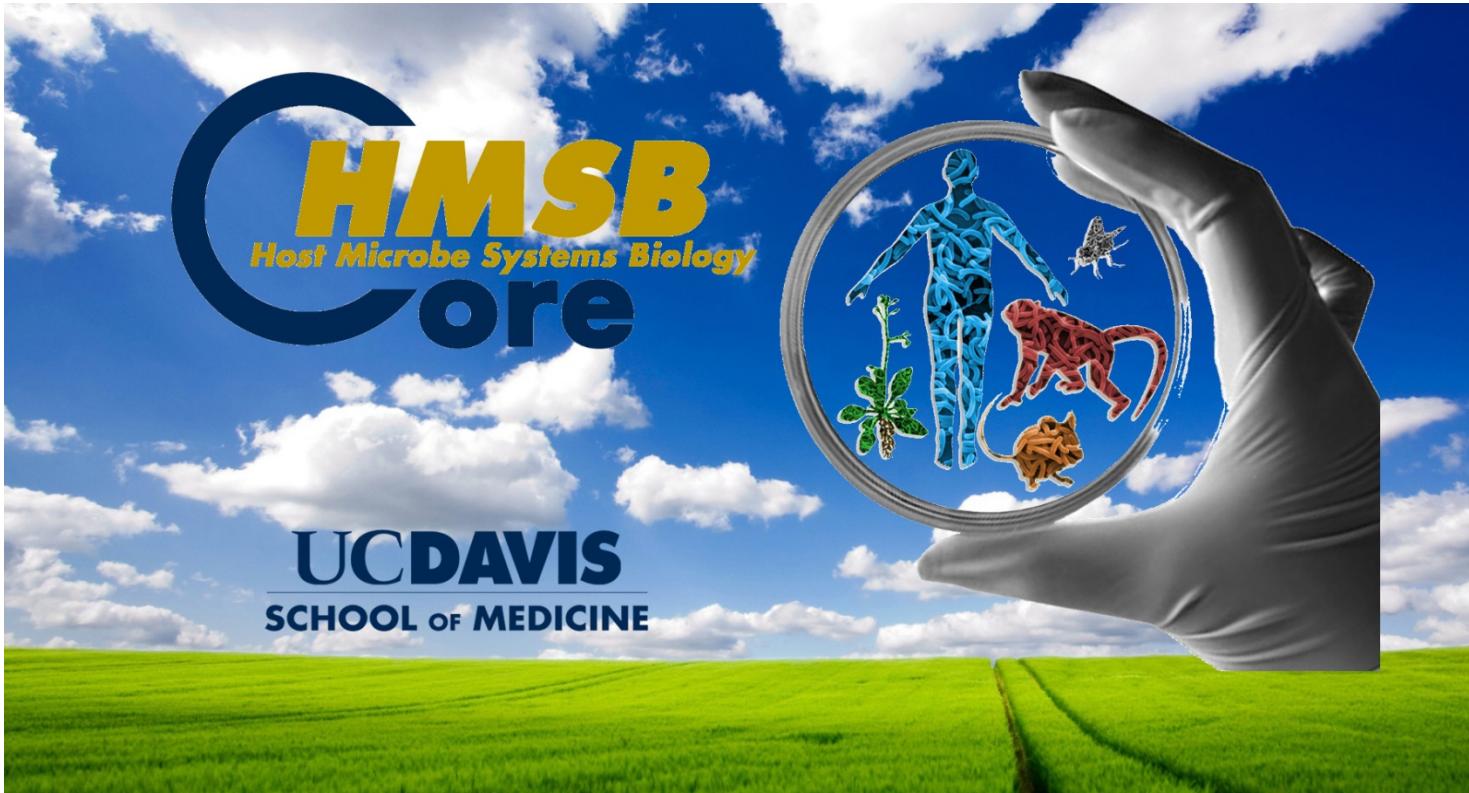


Host Microbe Systems Biology Core



Tupper Hall Room 3439 (530) 754-7850

Director: Satya Dandekar, Ph.D.

Associate Director: Loubna Tazi, Ph.D.

Manager: Matt Rolston

mrrolston@ucdavis.edu

<http://www.ucdmucdavis.edu/medmicro/hmsbcore/index.html>

Why do we utilize 16S sequencing?

All bacteria have a 16S gene

Highly conserved because it is necessary for ribosomes to translate mRNA

Because this gene has changed very little over time we can construct phylogenies to examine the evolution of bacteria and their relationships to one another

This conserved nature also allows us to construct universal primers that will amplify gene sequences across many different bacterial groups

Sequence information can be compared to known reference databases and identification can be accurately described down to the genus level (or species, or sub-species, depending on who you ask)

What 16S Sequencing IS and what it IS NOT

IS

Tool for Generating New
Hypotheses

Powerful when combined with
other techniques like qPCR and
Gene Expression data

Qualitative with some
quantitative elements
depending on experimental
design

The Beginning

IS NOT

Tool for Generating the Final
Answer

A standalone technique that
requires no other validation

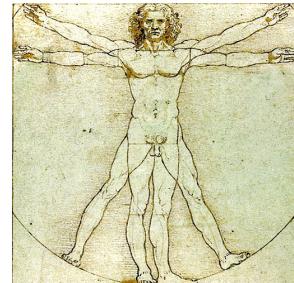
A purely quantitative tool that
will solve all of your problems
and wrap them up with a nice
little bow

The End

16S sequencing is a valuable resource IF you understand its limitations

Human Health and Disease

Primate Models



Development Studies



Mouse Models



Insect Models



The Built Environment



16S
Microbial
Analysis



Nutrition Studies



Agriculture Studies



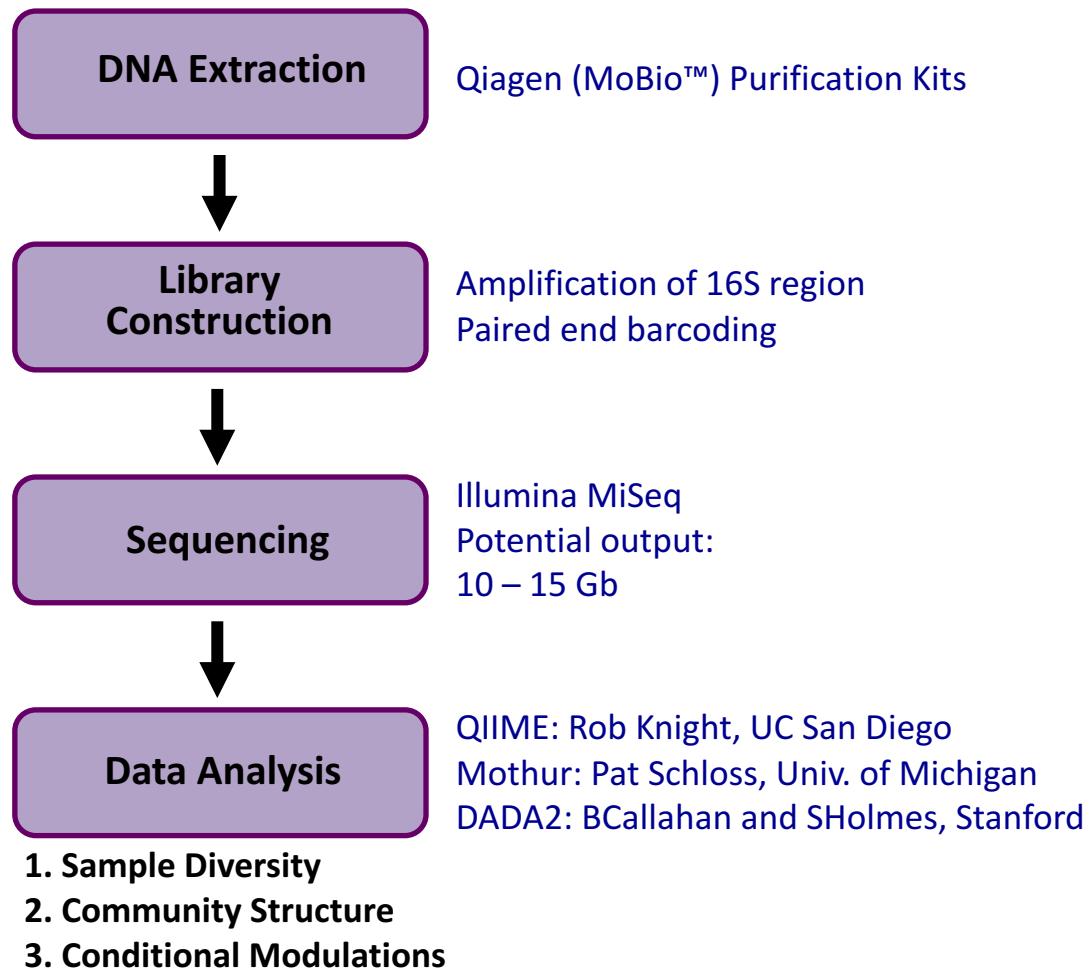
Environmental Communities



UC Davis Host Microbe Systems Biology Core

Typical 16S Microbial Analysis

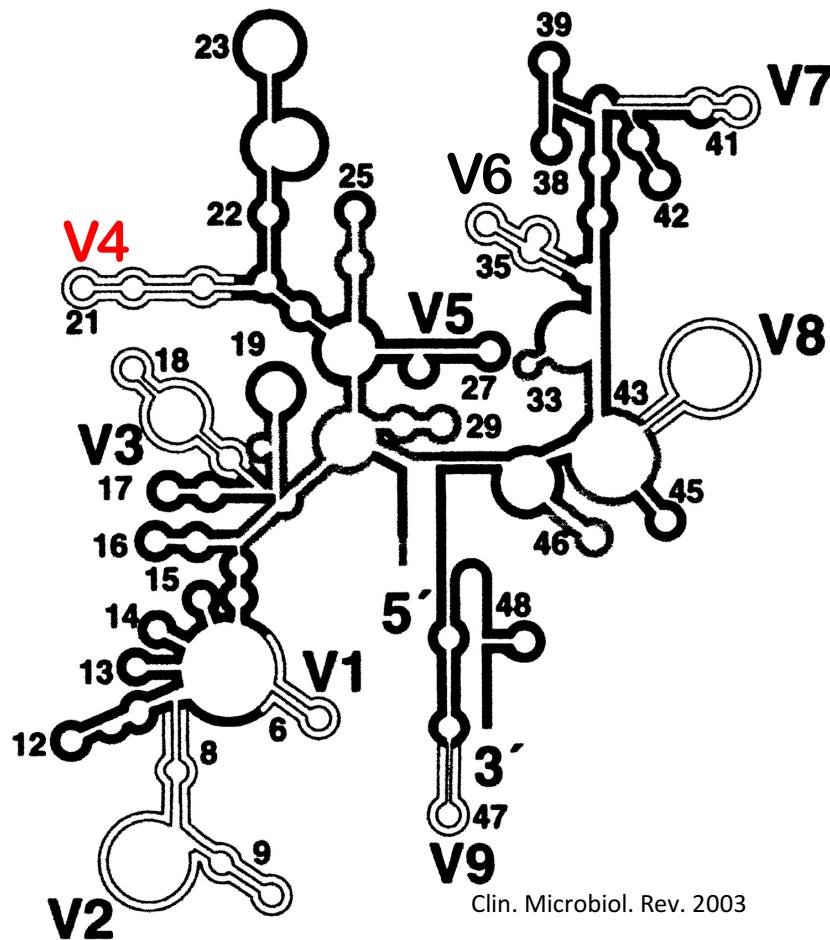
Workflow



Why the MiSeq for 16S?



16S Ribosomal RNA



Clin. Microbiol. Rev. 2003

0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 bp

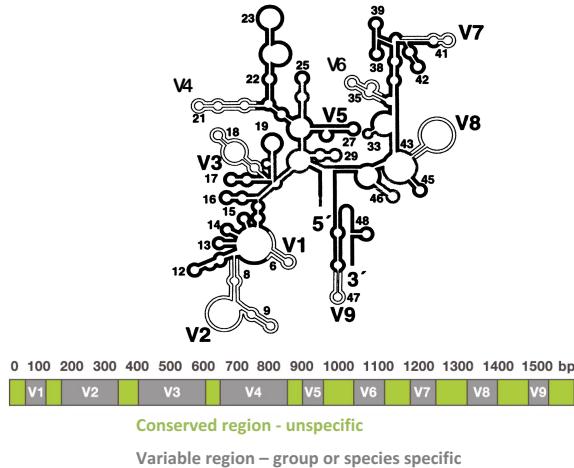


Conserved region - unspecific

alimetrics.net

Variable region – group or species specific

MiSeq is Flexible



- PE250 and PE300 allows for flexibility when selecting target primers
- Can sequence barcodes of variable length
 - 6, 8, 12nt possible
- Can sequence inline, single or dual indices

Manageable amount of Data for Lower Costs

- 10-15 million reads per run
- \$1300-1800 per run

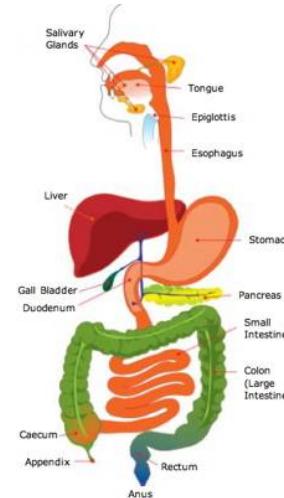


DNA Extraction

DNA Extraction Methods and Considerations

What type of sample do I have?

- Gut Contents/Fecal
- Soil/Environmental
- Skin swabs
- BAL

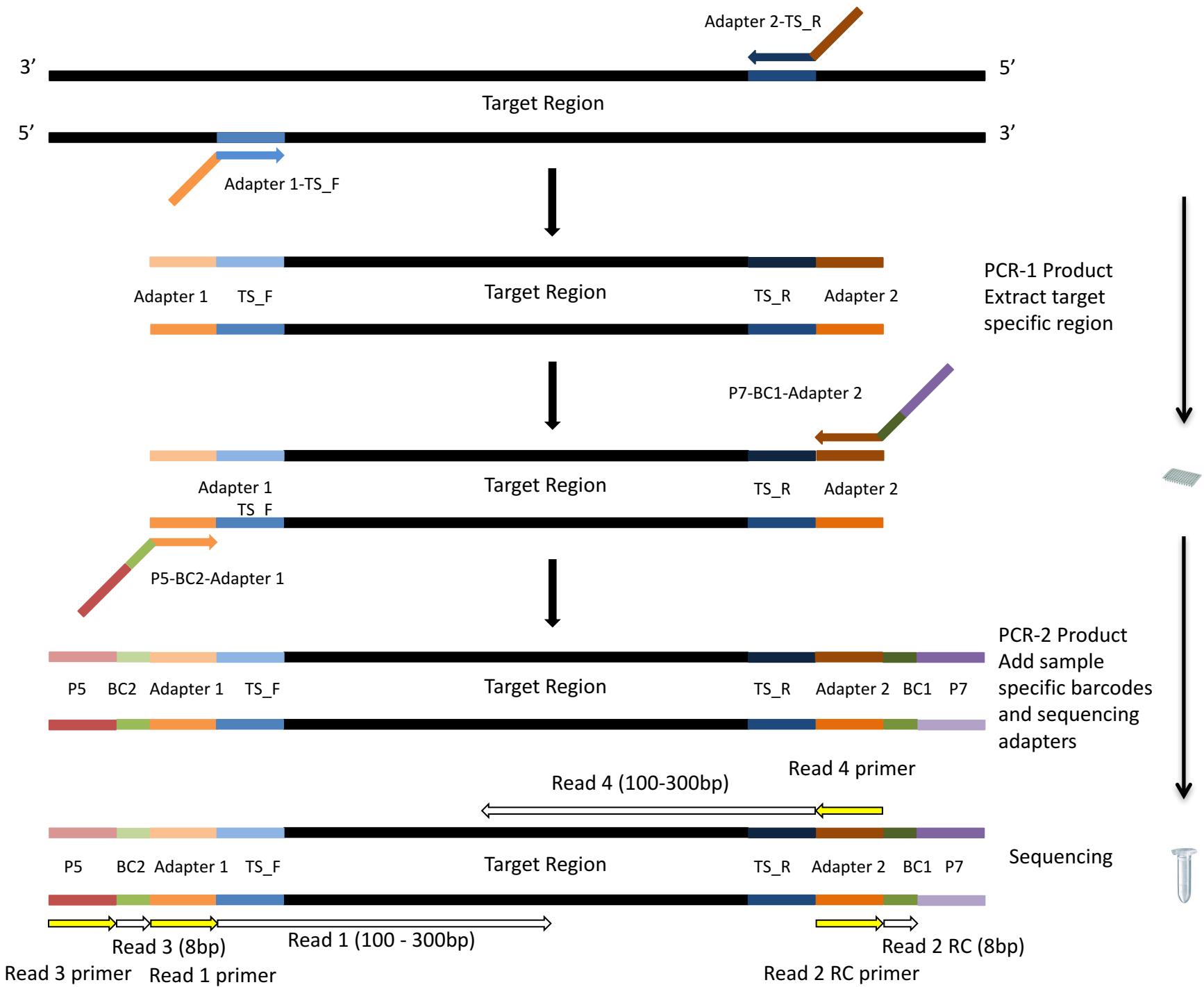


How much of each sample do I have?

- Hummingbird fecal material
- Single skin swab
- 1 Liter of wastewater
- All the dirt you could carry



Library Preparation



Common Universal Primers

16S - Bacteria

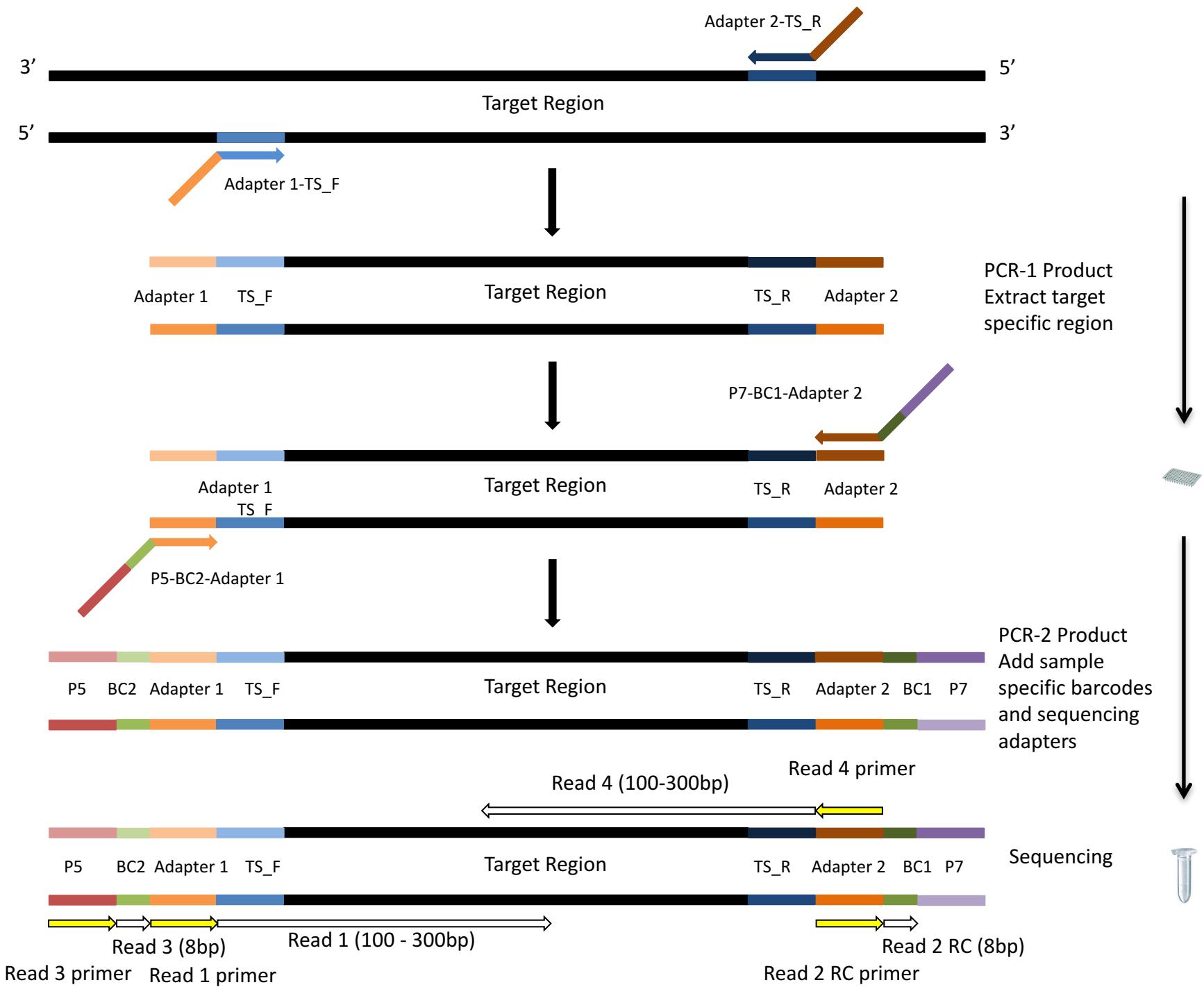
- 27F and 1492R – Full Length (Sanger Sequencing)
- 27F and 534R – V1 to V3
- 319F and 806R – V3 to V4
- 515F and 806R – V4 (Earth Microbiome Project)
- 515F and 926R – V4 to V5 (Earth Microbiome Project)

ITS – Internal Transcribed Spacer - Fungi

- ITS1f and ITS2
- ITS3 and ITS4
- ITS86F and ITS4

18S – Microbial Eukaryotes

- Euk1391f and EukBr



Benefits of Utilizing a Core Facility

We navigate the pitfalls so you don't have to

- Technology and methods rapidly evolve
- Spend your time on your project instead of chasing best techniques

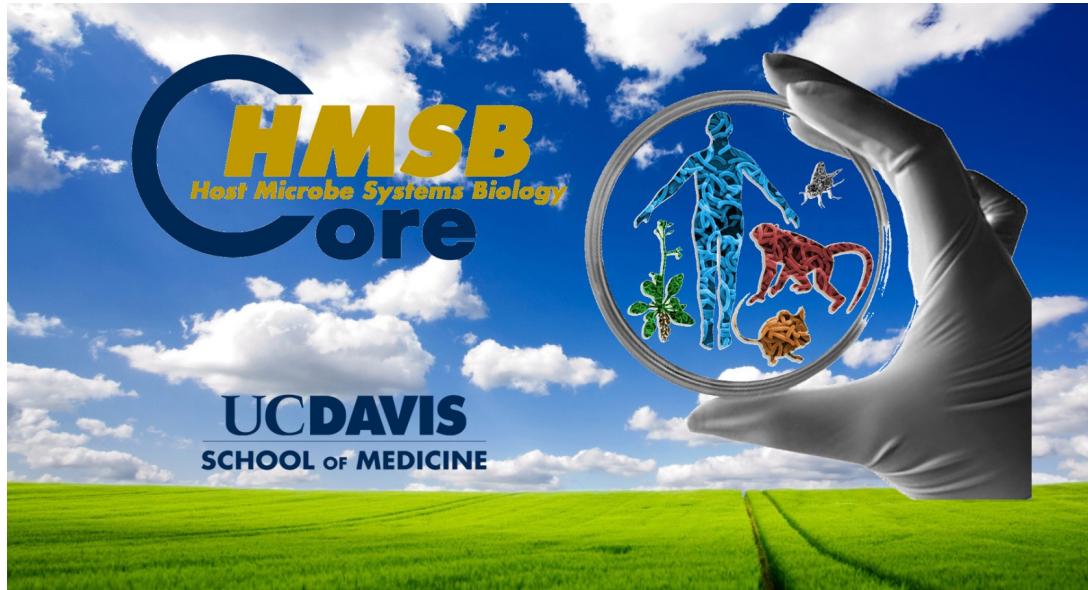
Leverage the Power of Scale

- We can combine your project with others to decrease costs
- We validate all consumables and order larger volumes than a typical lab

Proven Expertise

- This is what we do day in, day out

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Thanks for Listening!!!

For More Information contact: Matt Rolston

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