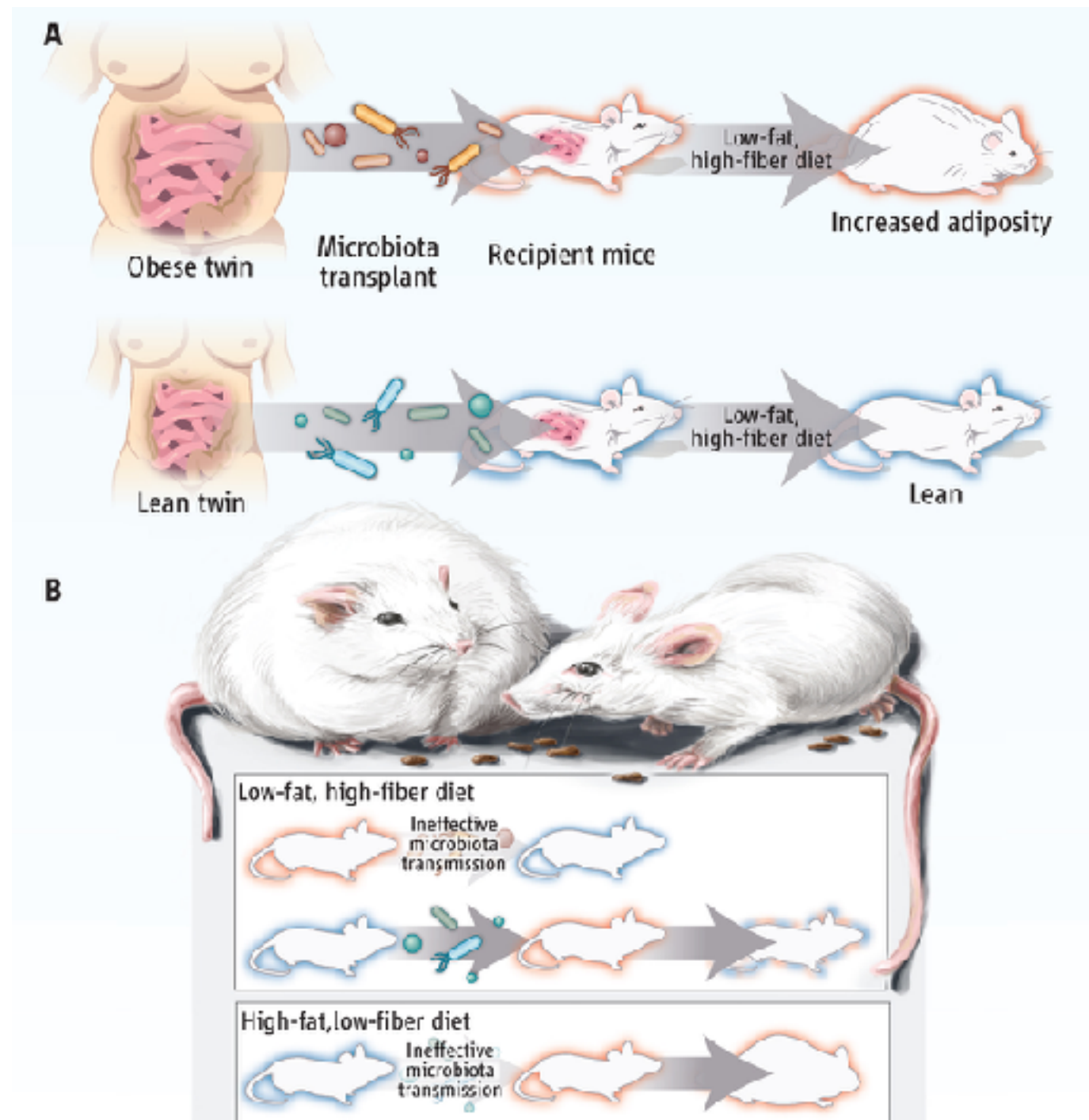# Microbiome Analysis Overview

Josh Granek

# Microbiomes in Human Health

- Acne
- Asthma/allergies
- Autism
- Autoimmune diseases
- Cancer
- Dental cavities
- Depression and anxiety
- Diabetes
- Eczema
- Gastric ulcers
- Hardening of the arteries
- Inflammatory bowel diseases
- Malnutrition
- Obesity
- Parkinson's Disease
- Drug Metabolism
- Vaccine Effectiveness
- …

# Causation

Walker, et al. Science 2013. https://doi.org/10.1126/science.1243787

Ridaura1, et al. Science 2013. https://doi.org/10.1126/science.1241214

# Metagenomics

| | What | Information | Analogy | Target Size | Cost |
|---|---|---|---|---|---|
| **Amplicon** | Marker Gene | Who is Present | Name | 100bp - 1kb | Low |
| **Shotgun Metagenome** | Genomes | What Genes are Present | CV | 100kb - 100Mb | High |
| **Shotgun Metatranscriptome** | All RNA | What Genes are Expressed | Twitter Feed | 100kb - 100Mb | High |

[Fungal genome and mating system transitions facilitated by chromosomal translocations involving intercentromeric recombination.](#)

Sun S, Yadav V, Billmyre RB, Cuomo CA, Nowrousian M, Wang L, Souciet JL, Boekhout T, Porcel B, Wincker P, **Granek JA**, Sanyal K, Heitman J.

PLoS Biol. 2017 Aug 11;15(8):e2002527. doi: 10.1371/journal.pbio.2002527. eCollection 2017 Aug.

PMID: 28800596 **Free PMC Article**

[Similar articles](#)

Select item 28376087

2.

[Elucidation of the calcineurin-Crz1 stress response transcriptional network in the human fungal pathogen Cryptococcus neoformans.](#)

Chow EW, Clancey SA, Billmyre RB, Averette AF, **Granek JA**, Mieczkowski P, Cardenas ME, Heitman J.

PLoS Genet. 2017 Apr 4;13(4):e1006667. doi: 10.1371/journal.pgen.1006667. eCollection 2017 Apr.

PMID: 28376087 **Free PMC Article**

[Similar articles](#)

Select item 26133796

3.

[Evidence for distinct brain networks in the control of rule-based motor behavior.](#)

**Granek JA**, Sergio LE.

J Neurophysiol. 2015 Aug;114(2):1298-309. doi: 10.1152/jn.00233.2014. Epub 2015 Jul 1.

PMID: 26133796 **Free PMC Article**

[Similar articles](#)

Select item 26112692

4.

[Rapid mapping of insertional mutations to probe cell wall regulation in Cryptococcus neoformans.](#)

Esher SK, **Granek JA**, Alspaugh JA.

Fungal Genet Biol. 2015 Sep;82:9-21. doi: 10.1016/j.fgb.2015.06.003. Epub 2015 Jun 23.

PMID: 26112692 **Free PMC Article**

[Similar articles](#)

Select item 25920978

5.

[Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and reverse genetic analysis of Chlamydia.](#)

Kokes M, Dunn JD, **Granek JA**, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ.

Cell Host Microbe. 2015 May 13;17(5):716-25. doi: 10.1016/j.chom.2015.03.014. Epub 2015 Apr 23.

PMID: 25920978 **Free PMC Article**

[Similar articles](#)

Select item 25079329

6.

[Antifungal drug resistance evoked via RNAi-dependent epimutations.](#)

Calo S, Shertz-Wall C, Lee SC, Bastidas RJ, Nicolás FE, **Granek JA**, Mieczkowski P, Torres-Martínez S, Ruiz-Vázquez RM, Cardenas ME, Heitman J.

Nature. 2014 Sep 25;513(7519):555-8. doi: 10.1038/nature13575. Epub 2014 Jul 27.

PMID: 25079329 **Free PMC Article**

[Similar articles](#) [1 comment](#)

Select item 24392035

7.

[Decoupled visually-guided reaching in optic ataxia: differences in motor control between canonical and non-canonical orientations in space.](#)

**Granek JA**, Pisella L, Stemberger J, Vighetto A, Rossetti Y, Sergio LE.

PLoS One. 2013 Dec 31;8(12):e86138. doi: 10.1371/journal.pone.0086138. eCollection 2013.

PMID: 24392035 **Free PMC Article**

[Similar articles](#)

Select item 23172850

8

# Amplicon Sequencing

## PCR amplify and sequence a marker gene

| | Marker Gene |
|---|---|
| **Bacteria** | 16s rRNA |
| **Fungi** | 18s or ITS rRNA |
| **Archaea** | 16s rRNA |
| **Protozoa** | 18s rRNA |
| **Viruses** | ????? |

# Metagenomics

| | What | Information | Analogy | Target Size | Cost |
|---|---|---|---|---|---|
| **Amplicon** | Marker Gene | Who is Present | Name | 100bp - 1kb | Low |
| **Shotgun Metagenome** | Genomes | What Genes are Present | CV | 100kb - 100Mb | High |
| **Shotgun Metatranscriptome** | All RNA | What Genes are Expressed | Twitter Feed | 100kb - 100Mb | High |

# Metagenomics

| | What | Information | Analogy | Target Size | Cost | Discovery? |
|---|---|---|---|---|---|---|
| **Amplicon** | Marker Gene | Who is Present | Name | 100bp - 1kb | Low | +/- |
| **Shotgun Metagenome** | Genomes | What Genes are Present | CV | 100kb - 100Mb | High | ++ |
| **Shotgun Metatranscriptome** | All RNA | What Genes are Expressed | Twitter Feed | 100kb - 100Mb | High | ++ |

# Sequencing Details

# Amplicon Sequencing

Primers

Microbes

Amplicon Library

P1

P2

+

=
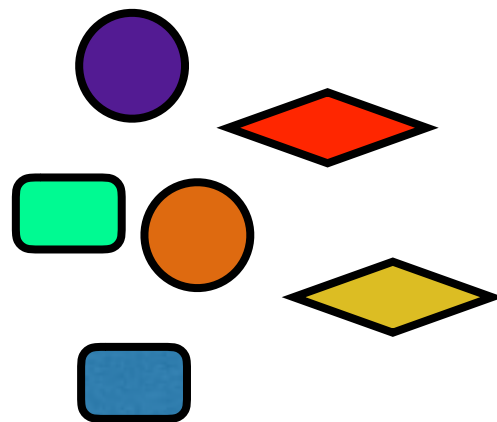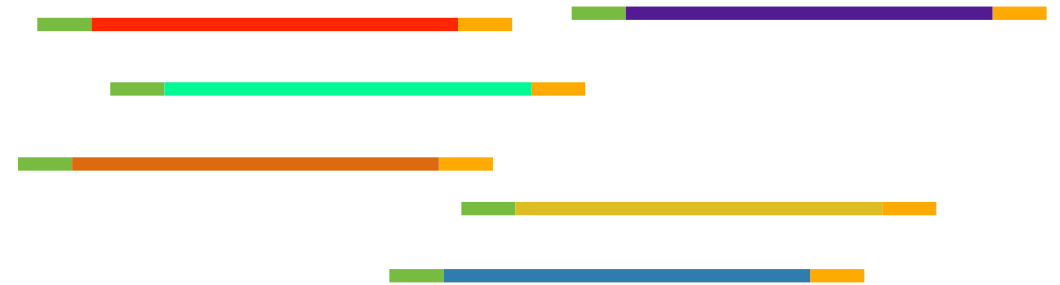
# Sequencing Library

## Amplicon Library



## Shotgun Library

# Read Length

Read
Length

100bp

150bp
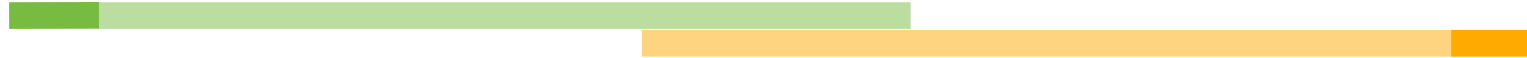
250bp
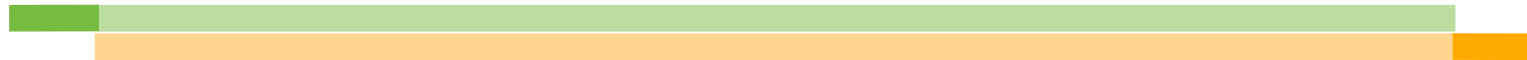
250bp Amplicon

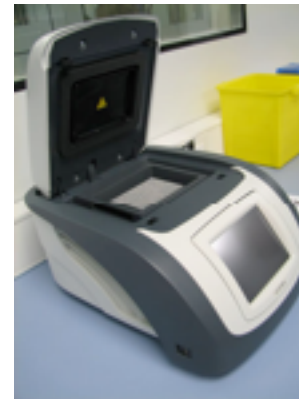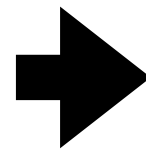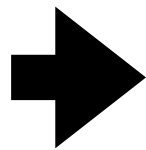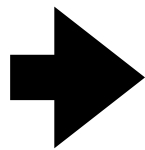# 16s Amplicon Analysis Overview

# Big Picture
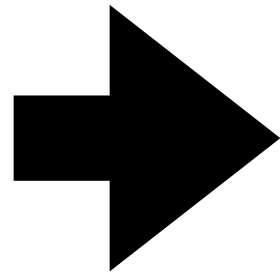


1. What is present?

2. How much?

3. Are there differences between treatments, host species, …?
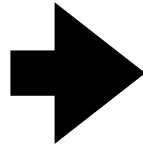
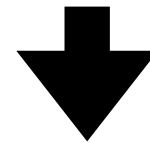4. What are the differences?
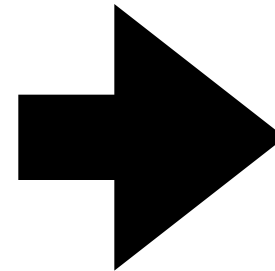
# Molecular Biology

# Bioinformatic Analysis



```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGHHHHHHHHHHHGGG
@M00698:36:000000000-AFBEL:1:1101:15928:1413 1:N:0:0
GTAAAGTCCTGAGTGATACCGGCAACTTTTACCCCCAGTCCCACTTTCGAACCGGCAAACATATCGGCAAAAGAGGCCGTGCCTGATTTAAAGCCGTAGGT
+
```

| | Sample 1 | Sample 2 | ... | Sample N |
|---|---|---|---|---|
| Bacteria 1 | | | | |
| Bacteria 2 | | | | |
| ... | | | | |
| Bacteria N | | | | |

# Statistical Analysis

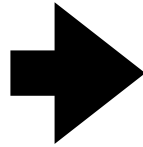| | Sample 1 | Sample 2 | ... | Sample N |
|---|---|---|---|---|
| Bacteria 1 | | | | |
| Bacteria 2 | | | | |
| ... | | | | |
| Bacteria N | | | | |

1. What is present?

2. How much?

3. Are there differences between treatments, host species, …?

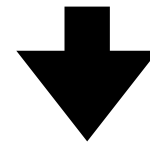4. What are the differences?

# Caveat

# Bioinformatic Analysis



```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGHHHHHHHHHHHGGG
@M00698:36:000000000-AFBEL:1:1101:15928:1413 1:N:0:0
GTAAAGTCCTGAGTGATACCGGCAACTTTTACCCCCAGTCCCACTTTCGAACCGGCAAACATATCGGCAAAAGAGGCCGTGCCTGATTTAAAGCCGTAGGT
+
```

| | Sample 1 | Sample 2 | ... | Sample N |
|---|---|---|---|---|
| Bacteria 1 | | | | |
| Bacteria 2 | | | | |
| ... | | | | |
| Bacteria N | | | | |

# The End