



VANDERBILT
UNIVERSITY
MEDICAL
CENTER



Credits: Servick/Science 2016

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Topics to discuss...

- Factors that affect experimental reproducibility

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- Establishing a gnotobiotic facility

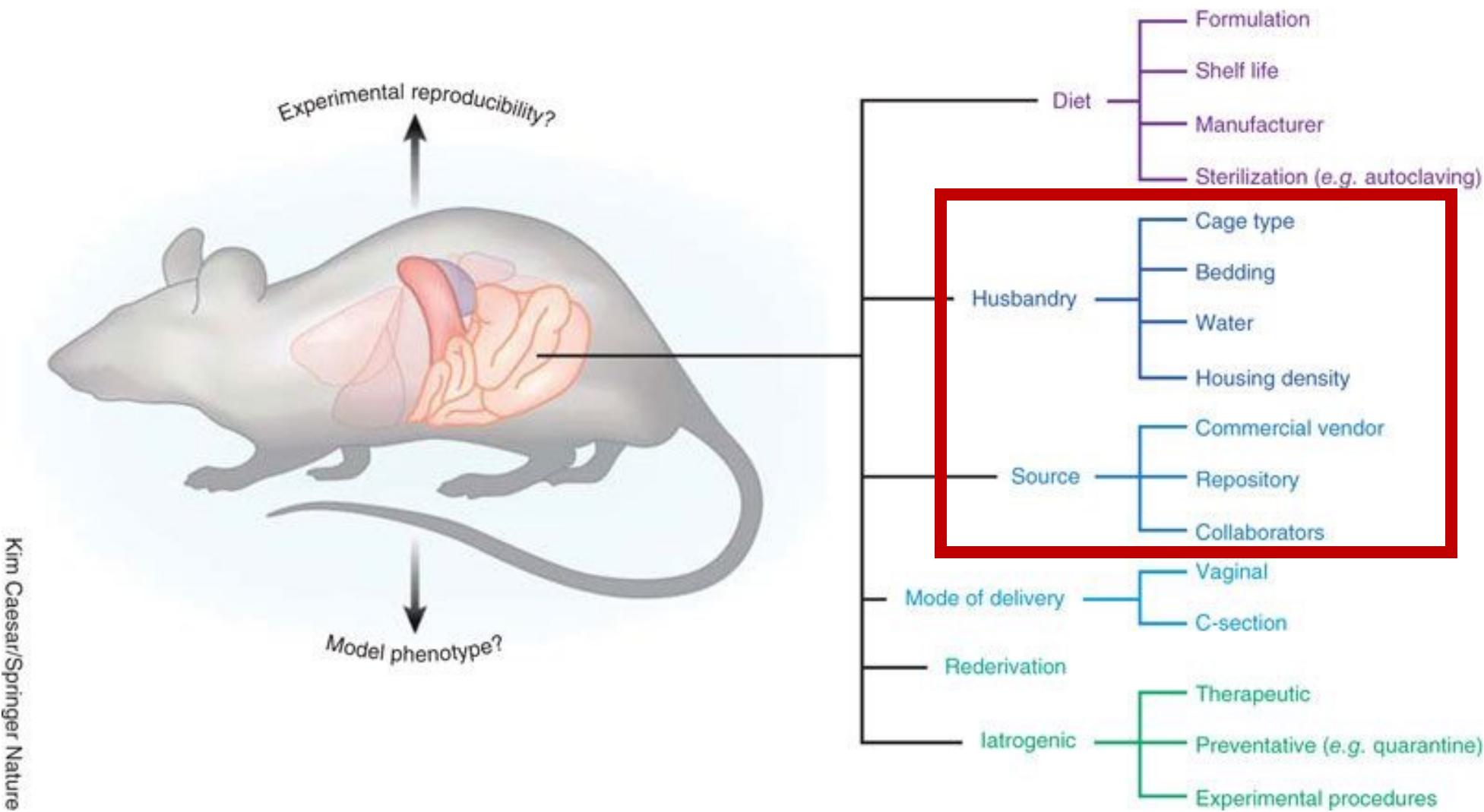
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- Use of gnotobiotic mice and defined microbiota communities in microbiota research

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- Factors that affect experimental reproducibility

Ready to start a microbiome study involving animal models?



Kim Caesar/Springer Nature

Where are you obtaining your animals from?

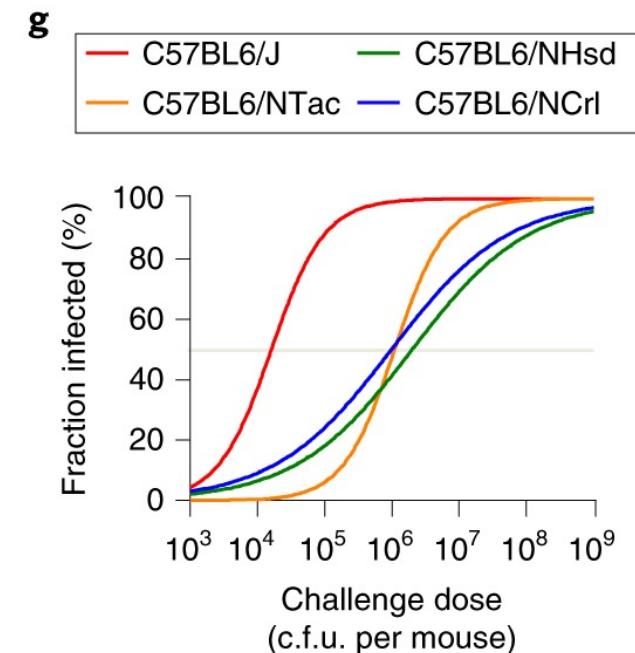
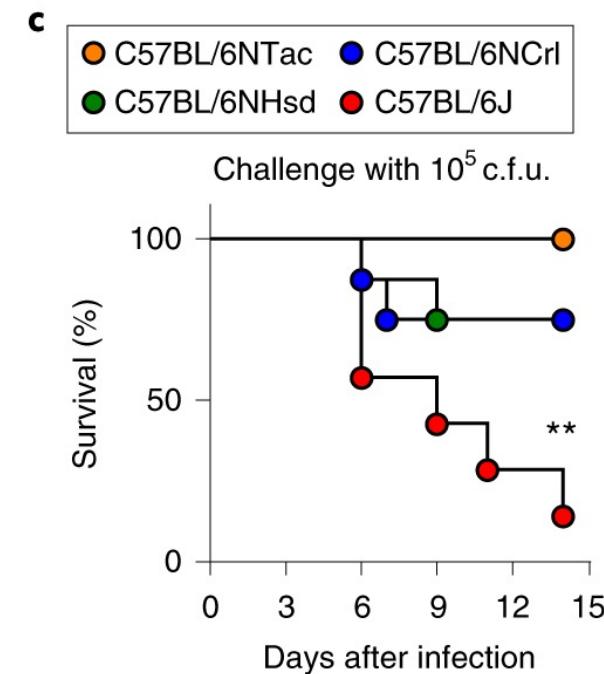
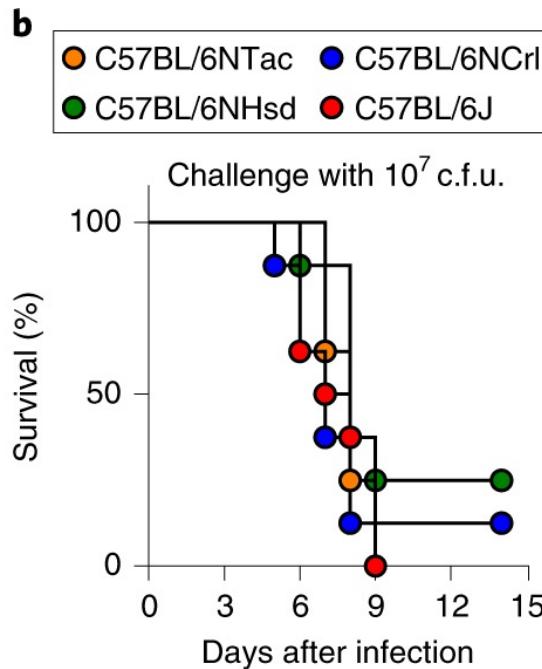
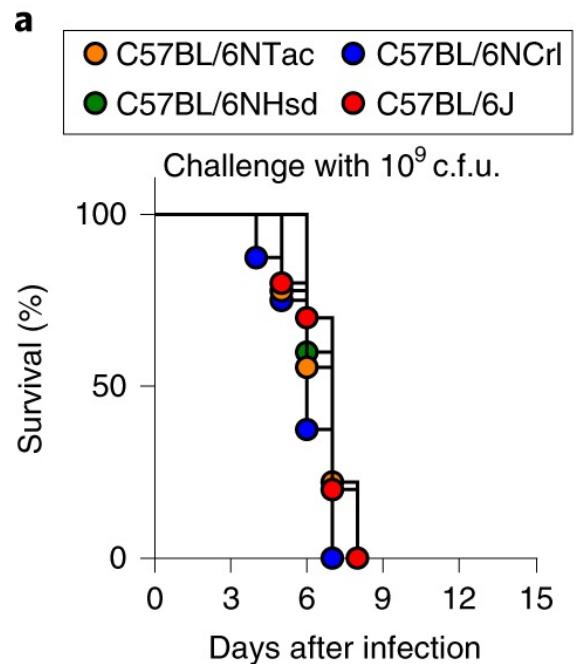


Each vendor has completely different husbandry and monitoring systems

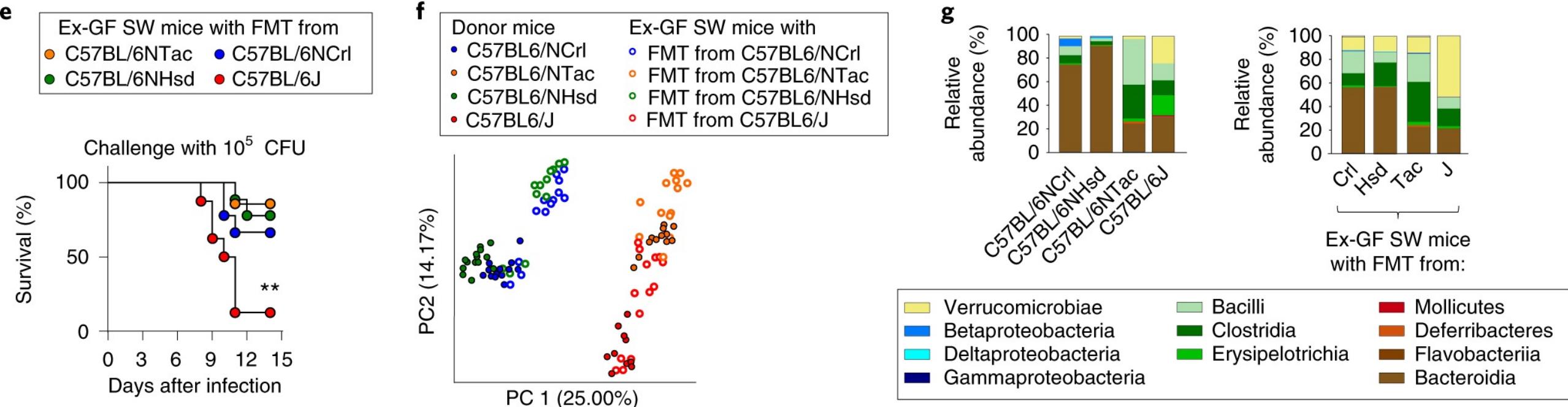


Differences in gut microbiota

Mice from different vendors have different susceptibility to enteric pathogens



Susceptibility to *Salmonella* infection is determined by the microbiota



What to do?

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What to do?

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- Request that the mice come from the same room
- IDEAL: breed your own mice
- Use littermate controls
- Maintain mice in sterile housing conditions during experiments
- Perform husbandry yourself during experiments (especially in shared rooms)
- Consider cage effects when deciding animal density

MOST IMPORTANT:

**BE CONSISTENT IN EVERY ASPECT OF YOUR
RESEARCH!**

- Consider cage effects when deciding animal density

Topics to discuss...

- Factors that affect experimental reproducibility
- Use of germ-free mice and defined microbiota communities in microbiota research

Culture-independent methods

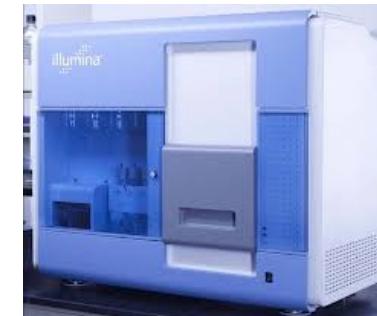
Microbiology

Culture-dependent
Methods



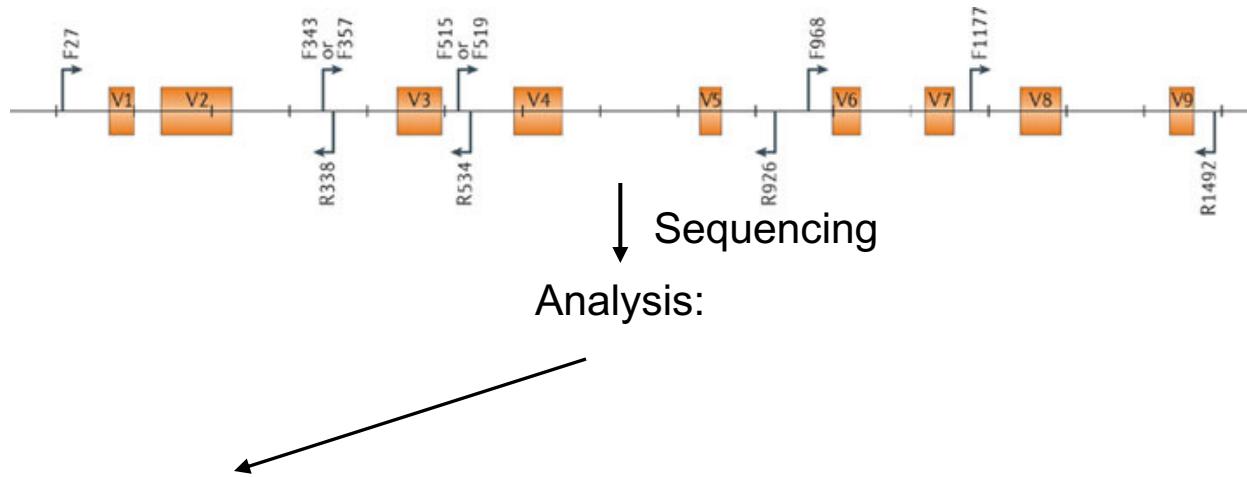
Germ theory
(pathogens cause disease)

Culture-independent
Methods



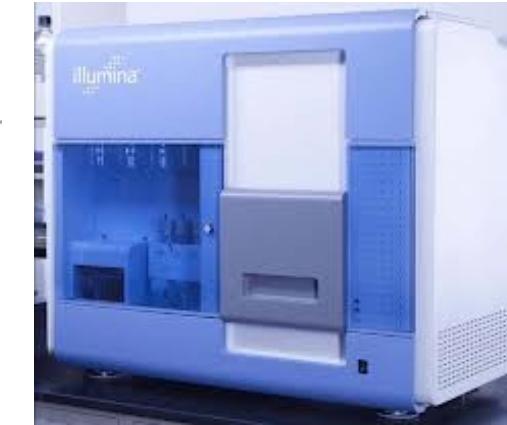
Role of the microbiota in diseases

16S rRNA gene (16S rDNA)



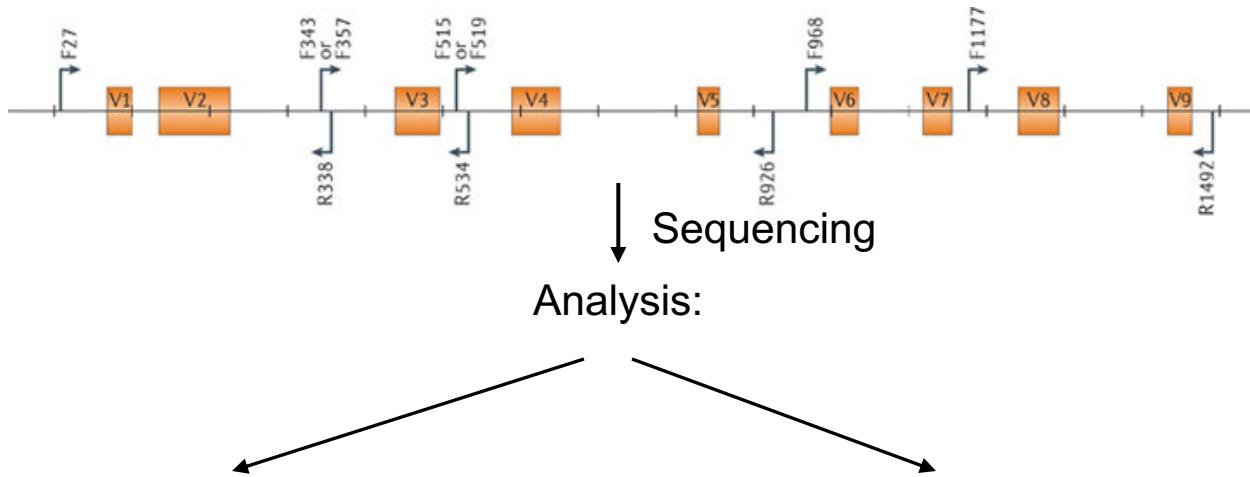
Within one sample:

- Alpha-diversity: Richness / Diversity of the community
e.g. 798 OTU in the sample



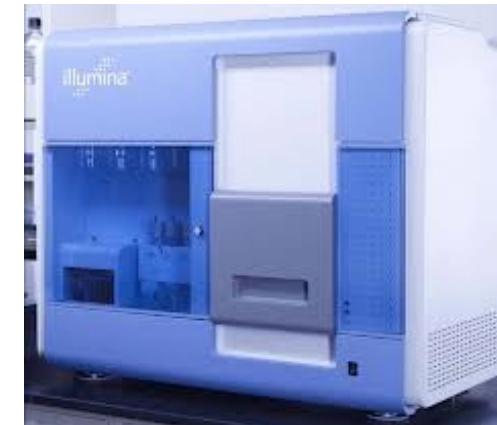
Next-generation sequencing

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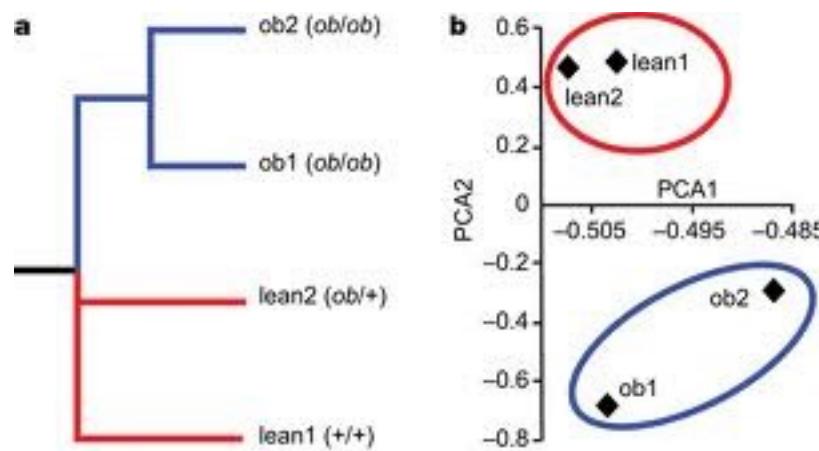


Within one sample:
- Alpha-diversity

Compare different samples:
- Beta-diversity: Similarity between two communities
- Principal Component Analysis



Next-generation sequencing



How can we determine cause and effect?

Germ-free animals (mice)
reared in the absence of any microbiota
(except endogenous viruses)

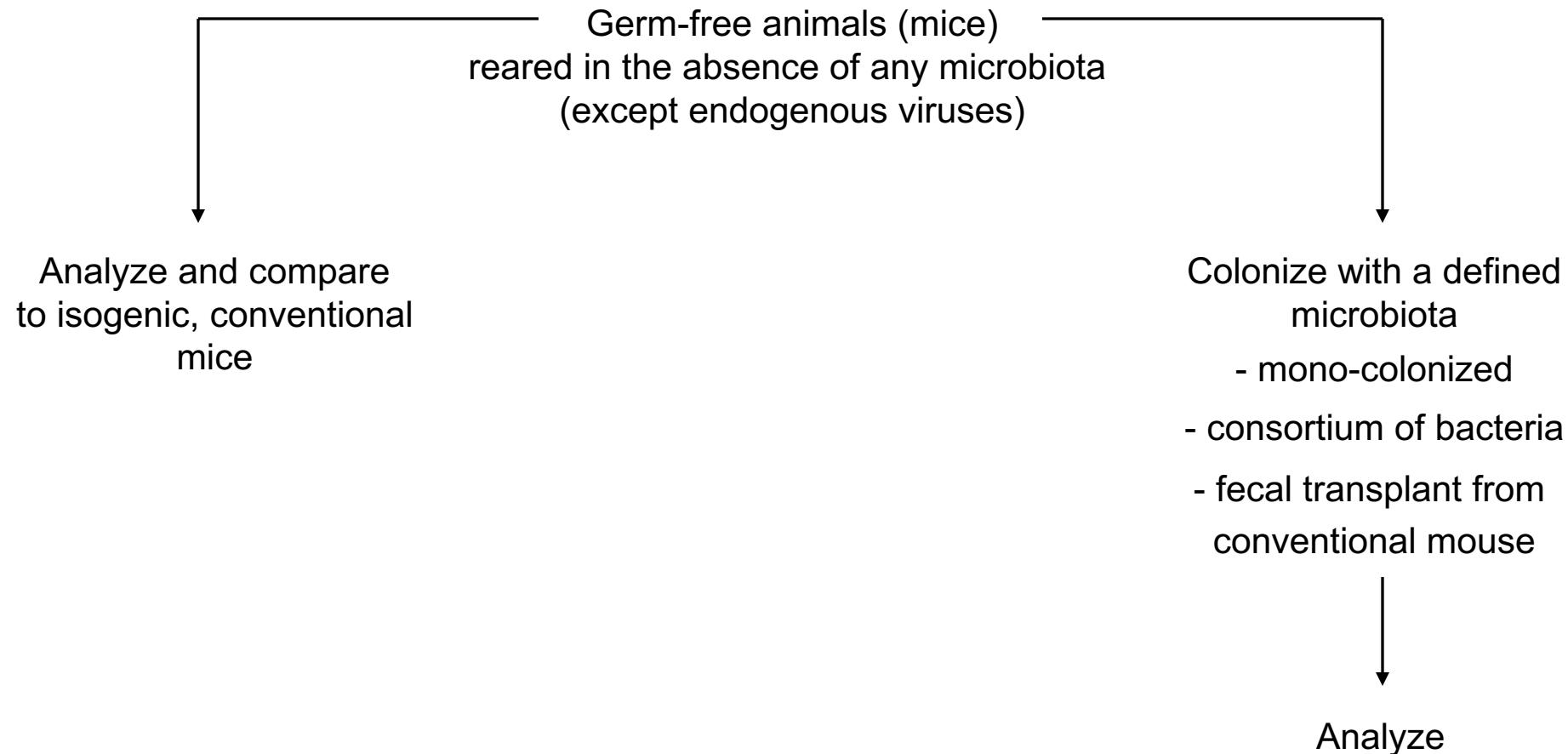


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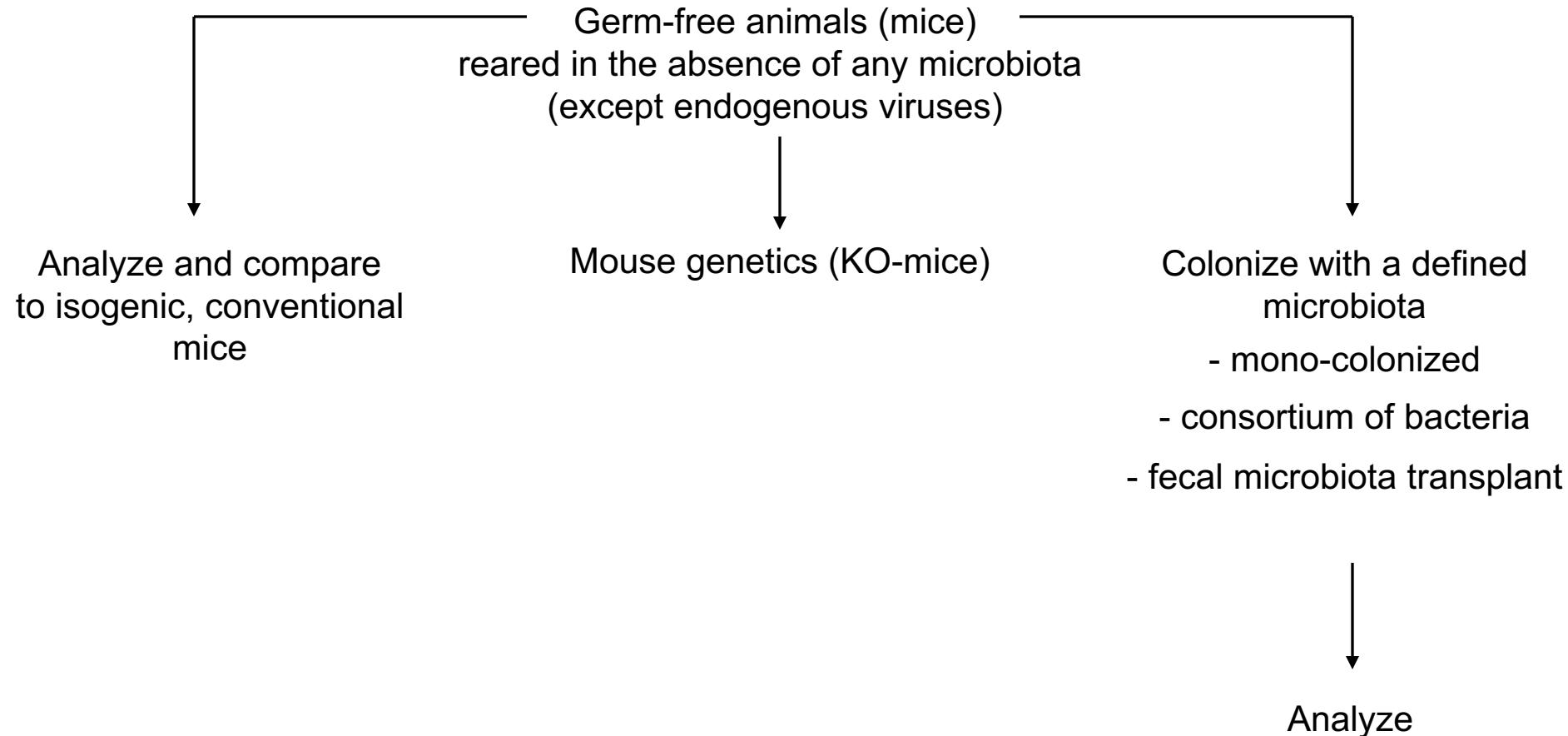
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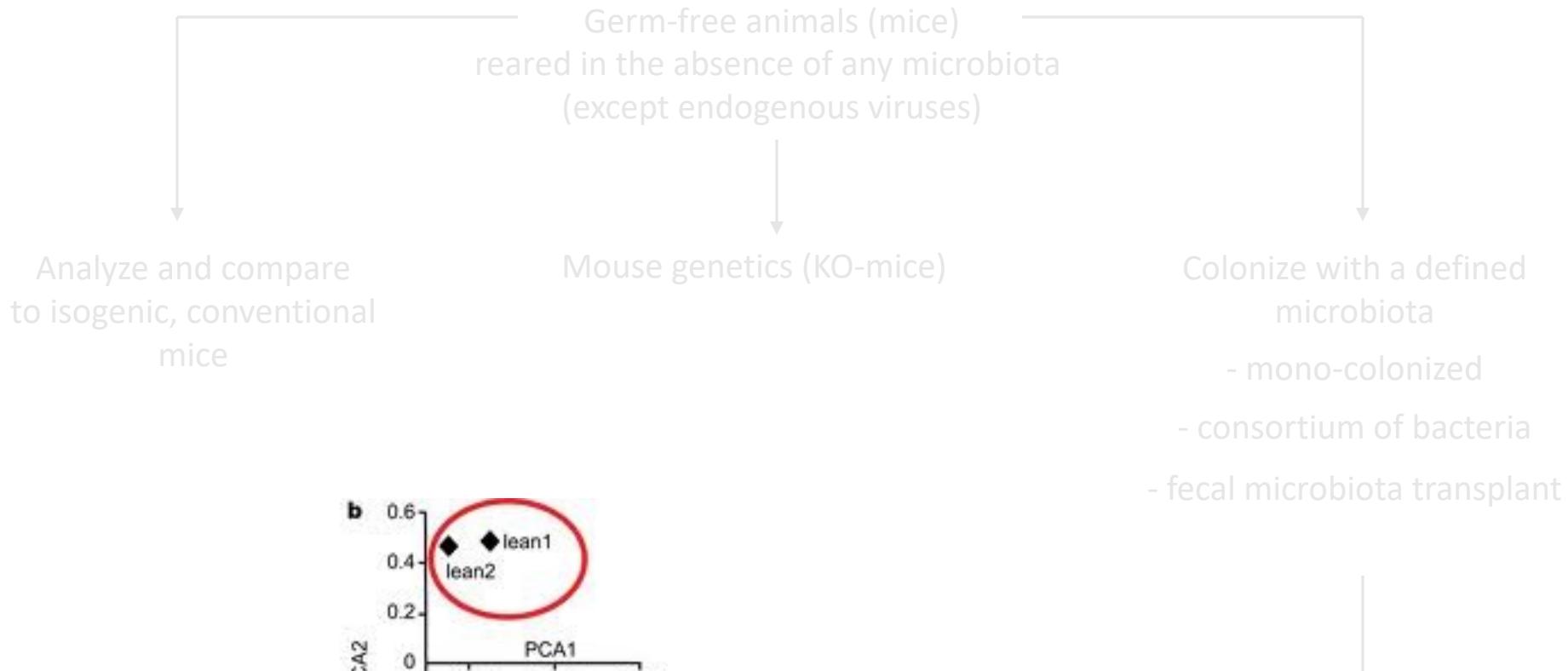
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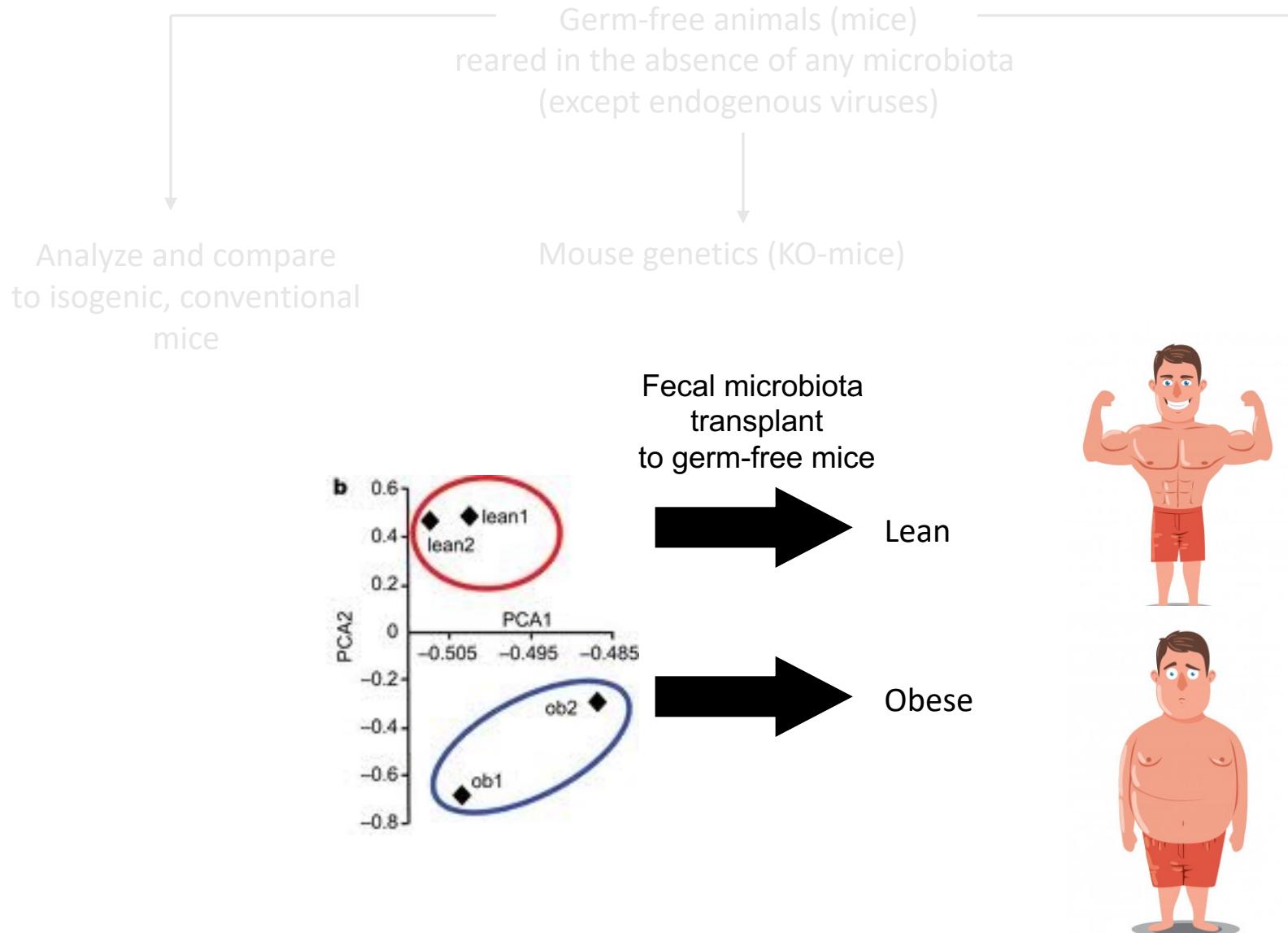
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How can we determine cause and effect?



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- Factors that affect experimental reproducibility
- Establishing a gnotobiotic facility

Budget

Equipment:

- Isolators
- Cylinders
- Replace parts
- Maintenance (autoclave and isolators)

Personnel:

- Salary
- Training

Animals:

- *Per diem*
- Purchase
- Generation of knockout mice

Quality Control:

- Sterilant
- Microbiological Media
- Supplies for qPCR

Core Facility vs. Investigator Maintained Colony

What to consider:

- Types of isolator vs. how much space available

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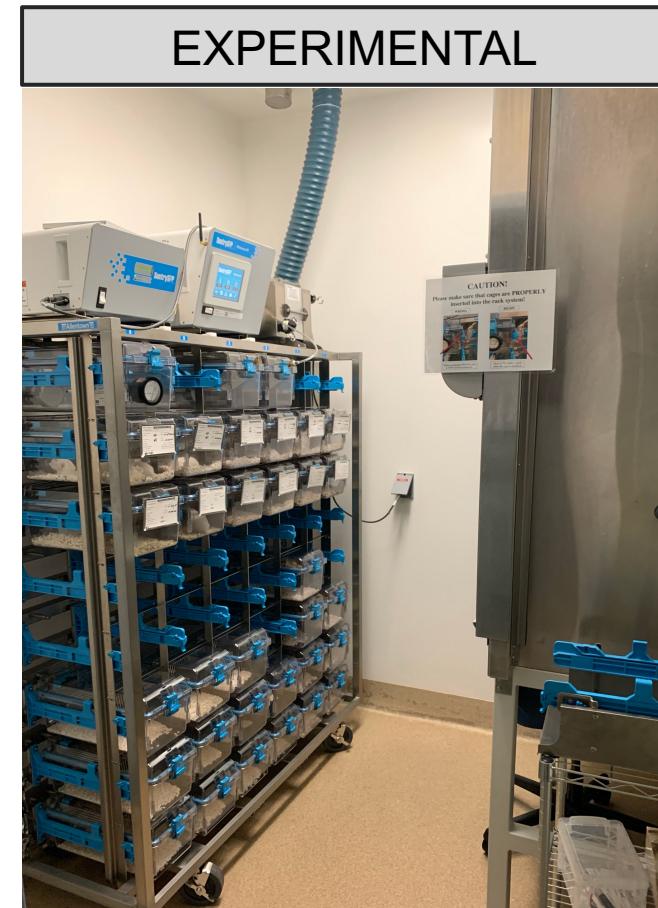
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- Types of isolator vs. how much space available
- Small number of big isolator vs. multiple small isolators
- Number of isolators to decrease impact of contamination
- Types of isolator for breeding vs. experimental animals



Standard Operating Procedures

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Defined microbiota communities in microbiota research

- Communities ranging from 8 (Altered Schaedler Flora) to up to 100 species

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- Genome sequenced: easier to figure out metabolic capacity
- Microbiota composition can be determined by qPCR
- Some members can be genetically modified
- *Limitations:*
 - Access to anaerobic chamber for cultivation
 - Simplified communities
 - Does not account for members of the microbiota that cannot be cultured



QUESTIONS?