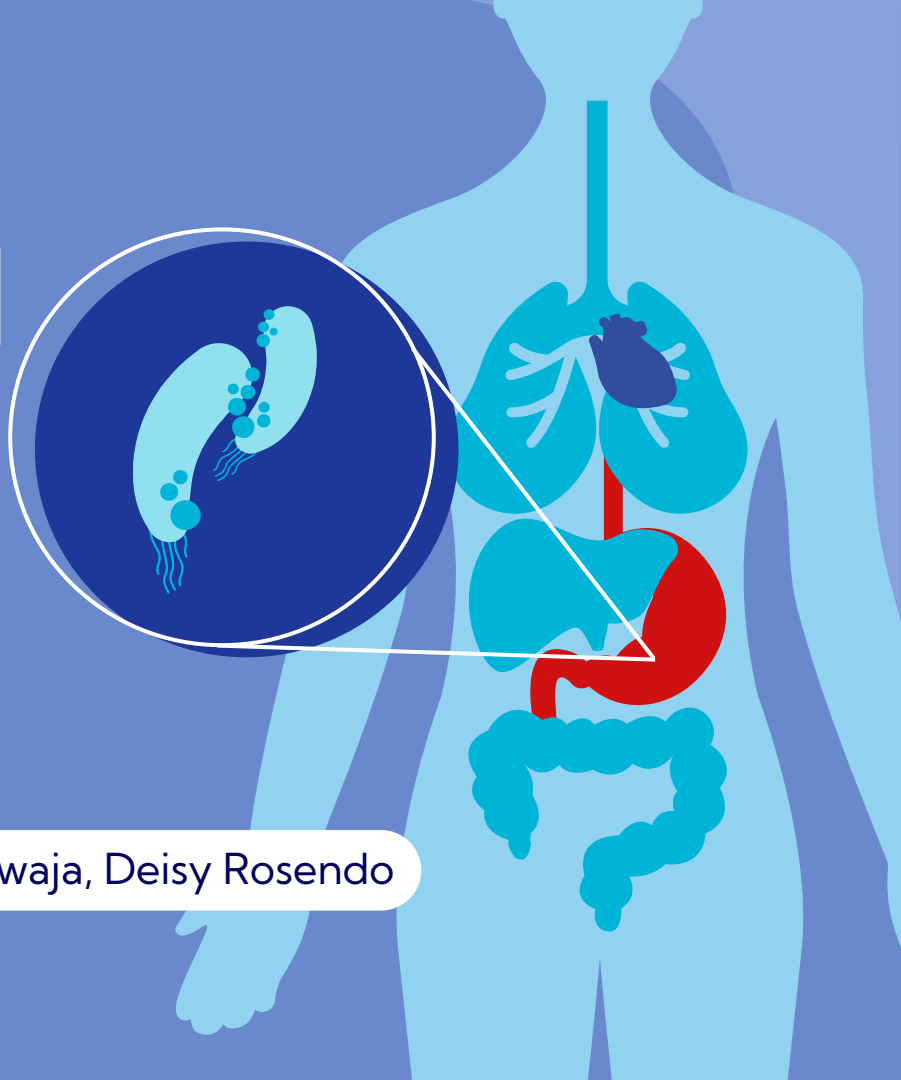
Gut dysbiosis and its Association with ASD



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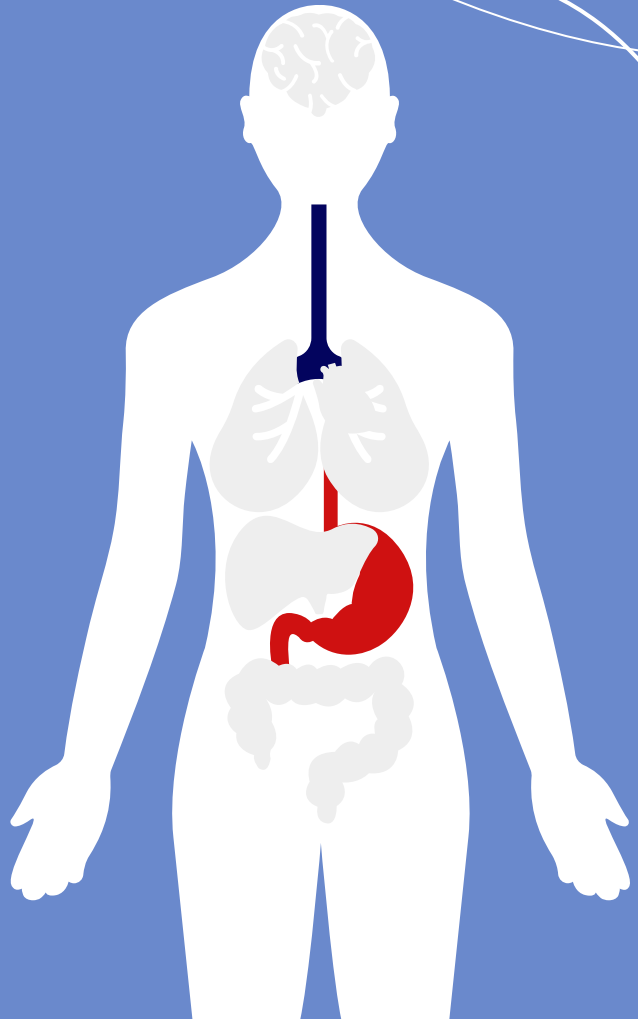
REACH Program
August 2022

Outline

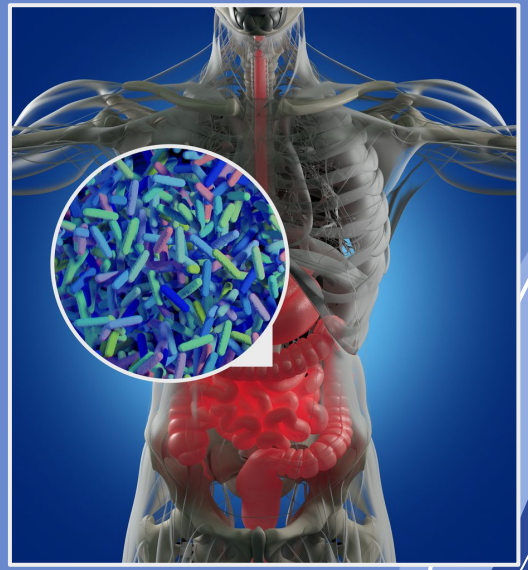
- Introduction
- Specific Aims
- Research Plan
- Results and Conclusion



Introduction

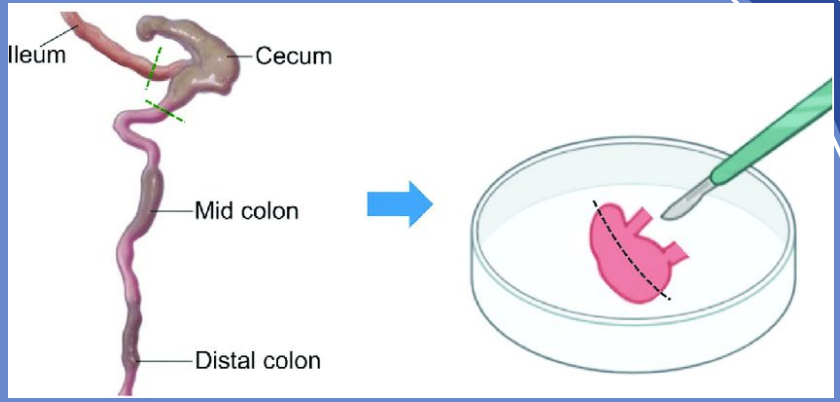
Gut Microbiota

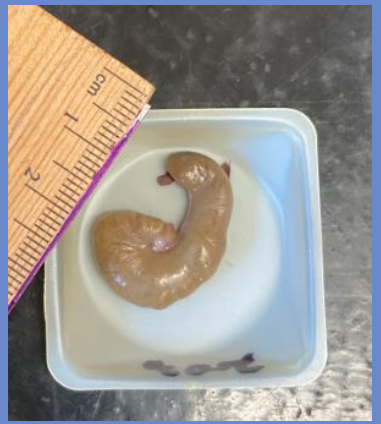
- A system of trillions of microorganisms
- Function in the body
- Diet and microbiota
- Cecum



Cecum

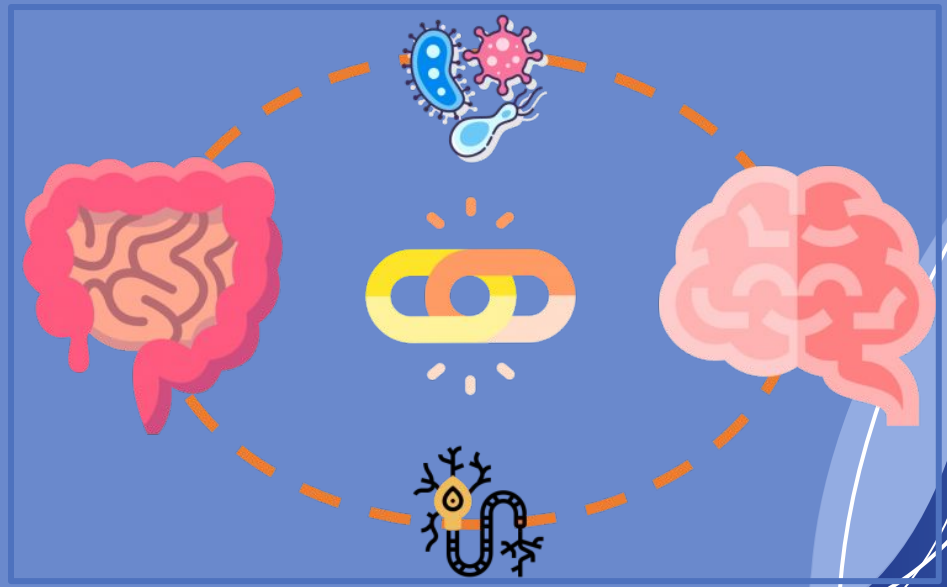
- Located in the lower left side of the abdominal cavity
- Cecum in mice is enlarged
- Contains bacteria that aid in digestion of plant matter
- Facilitates nutrient absorption





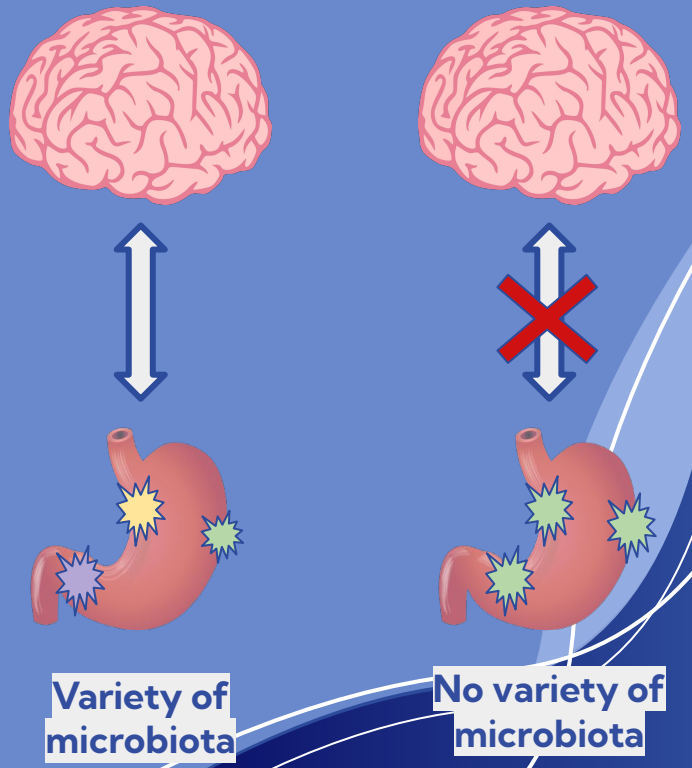
Gut-Brain Axis

- Two-way biochemical signaling that takes place between the GI tract and the CNS

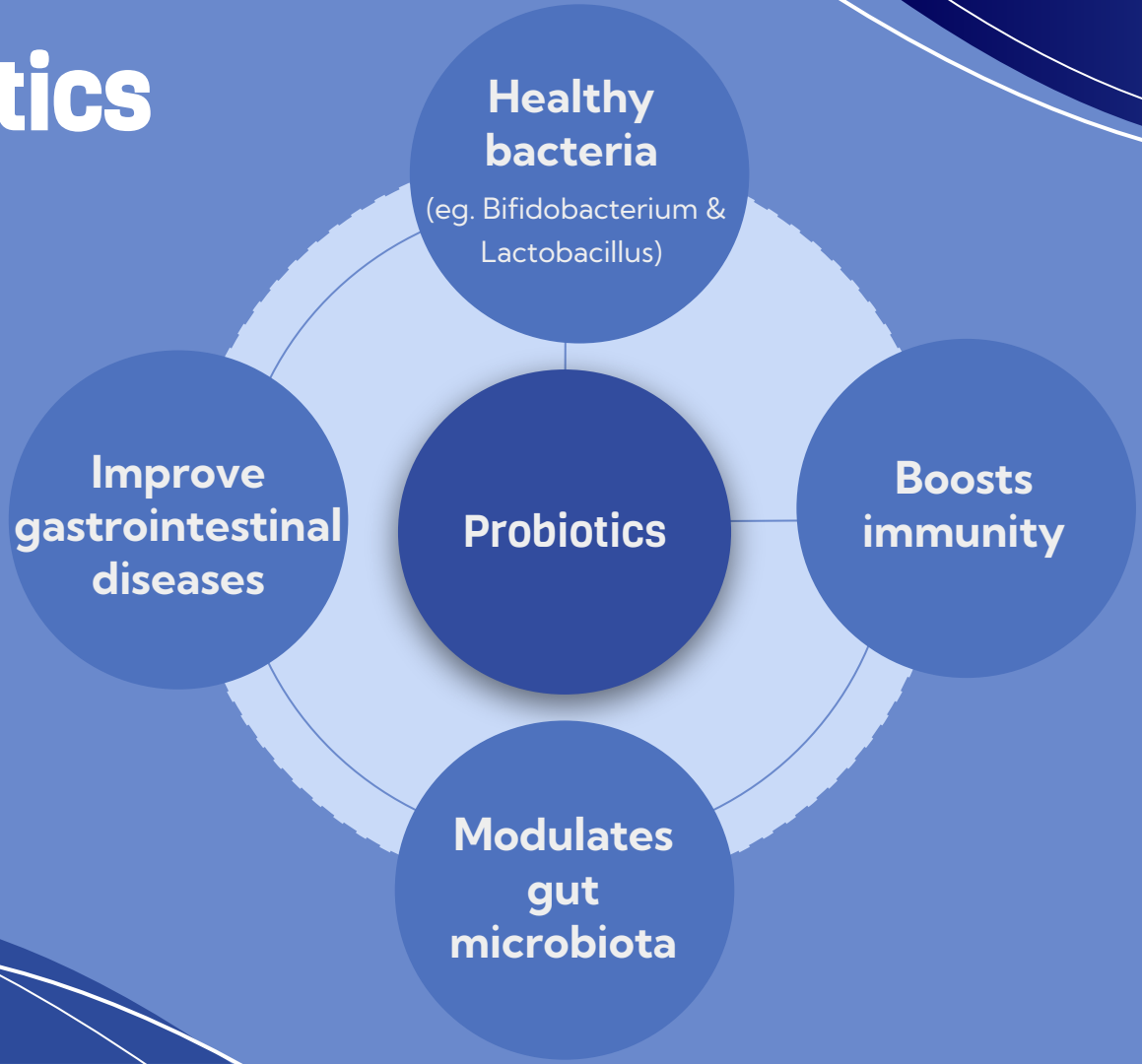


Gut Dysbiosis and ASD

- Imbalance of gut microbiota
- Lifestyle choices can have an impact
- Correlation between severity of gut issues and ASD
- Affects certain signaling pathways
- Disrupts Blood Brain Barrier
- Probiotics role

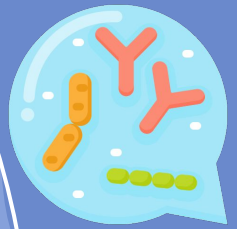


Probiotics


Healthy bacteria
(eg. Bifidobacterium & Lactobacillus)
Improve gastrointestinal diseases
Probiotics
Boosts immunity
Modulates gut microbiota

Bifidobacterium Bifidum

- Live in the GI tract
- Digests food, absorb nutrients and prevents infection
- Some *Bifidobacterium* species generate GABA

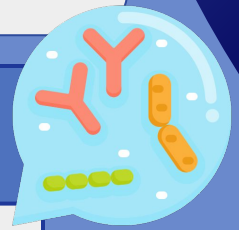


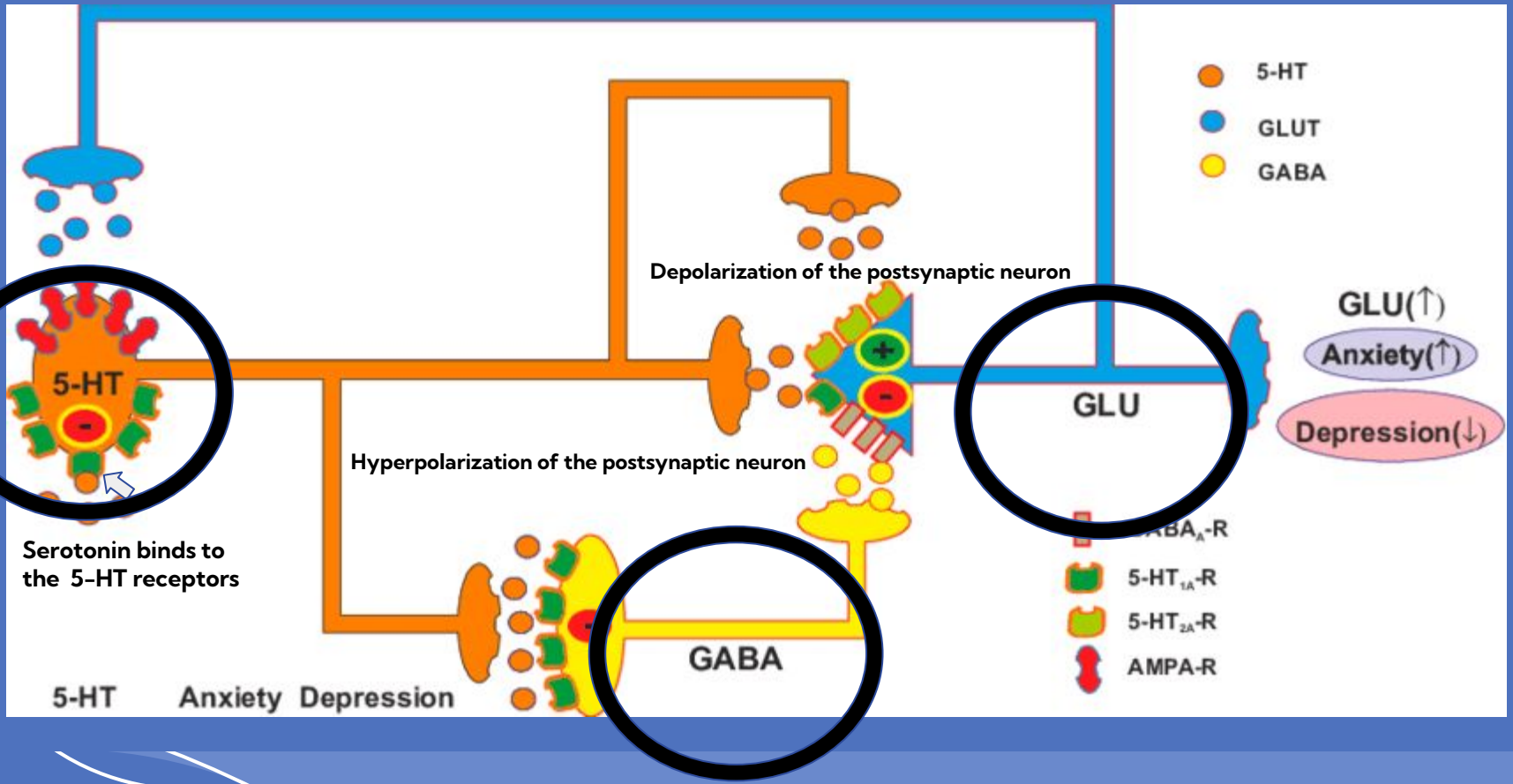
- GABA (Gamma-Aminobutyric Acid) :
  - Inhibitory neurotransmitter
  - Decreasing nerve cell hyperactivity (fear, stress and anxiety)
- Children with ASD
  - Lower levels of GABA → high levels of anxiety

Lactobacillus

- Rod-shaped bacteria
- Produces lactic acid
- Production of fermented dairy products

Lactobacillus Helveticus	*Lactobacillus Paracasei*
• Regulates the 5HT that balances the excitatory and inhibitory neurotransmission in the PNS and CNS	• Increases glutamate and GABA in ASD patients.




5-HT
GLUT
GABA
Depolarization of the postsynaptic neuron
GLU(↑)
Anxiety(↑)
Depression(↓)
5-HT
GLU
Hyperpolarization of the postsynaptic neuron
Serotonin binds to
the 5-HT receptors
GABA
5-HT Anxiety Depression

Gut Dysbiosis and ASD

Knowns

- The human gut is home to a variety of microbes
- ASD is associated with an unbalanced gut microbiota
- Children with ASD are at a greater risk of GI concerns
- Probiotics exert beneficial effects in both the gut and the brain

Questions

- Is there a cause- effect relationship between ASD and gut microbiota?
- Is gut microbiota in ASD patients different from someone who does not have ASD ?
- Does adding probiotics alter the gut microbiota in ASD patients or impact their behaviors ?

Hypothesis

16s rRNA gene can help us identify gut microbiota and manipulating microbiota by administering probiotics can be used as a treatment for ASD by decreasing autism-related behaviors.

Specific Aims

Specific Aims

1 Specific Aim 1

Identification of gut microbiota through 16S rRNA gene in BTBR and C57 mice.

2 Specific Aim 2

Evaluation of autism-related behavioral phenotypes and cecum measurement in BTBR and C57 mice after administering probiotics.

Research Plan

Experimental approach

1. Identifying Microbiota

Collection of stool samples

↓

DNA extraction

↓

qPCR

2. Modification of diet

Probiotics administration

↓

Behavioral changes and cecum measurement

Animal models

3 BTBR mice

- ❏ Absence of corpus callosum
- ❏ Smaller Hippocampal Commissure
- ❏ Exhibits autism-like behaviors

3 C57BL/6 mice

- ❏ Most widely used strain in biomedical research
- ❏ Exhibits wild type behaviors

Identifying Microbiota

1. Collection of stool samples

Place mouse in cage

Disinfect cage with 70% ethanol

Wait for mice to defecate

Up to 40 minutes of waiting.

Collect sample and place in tube

Later on used for DNA extraction

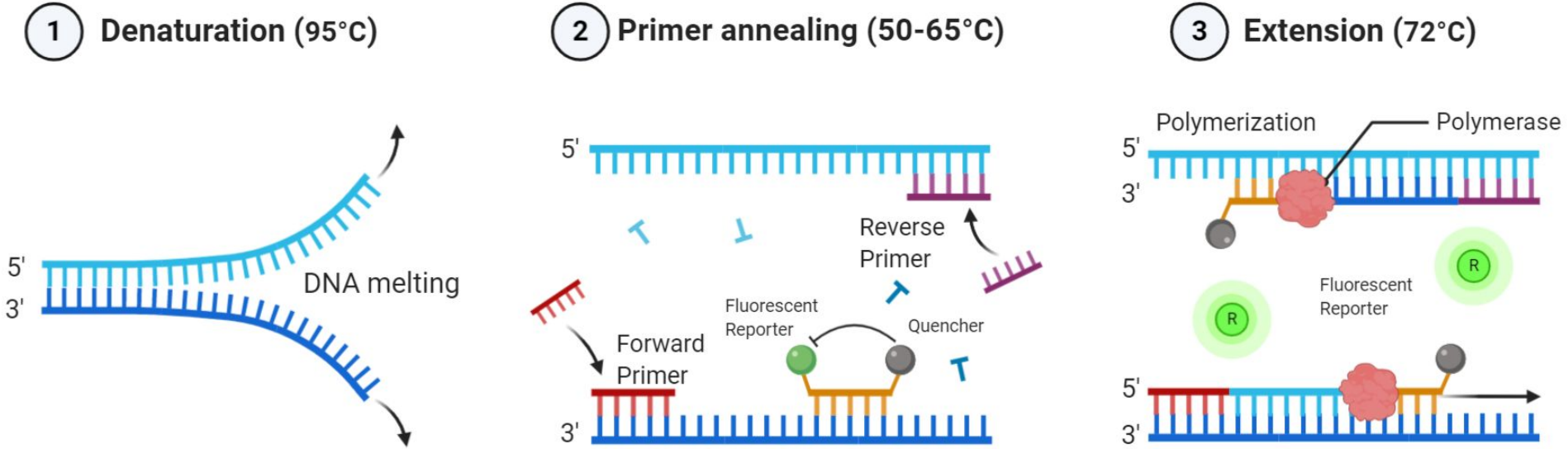
Identifying Microbiota

2. DNA extraction

1. Disruption of cells to release DNA for collection
2. Removal of Contaminating Biomolecules with buffers and washers

Identifying Microbiota

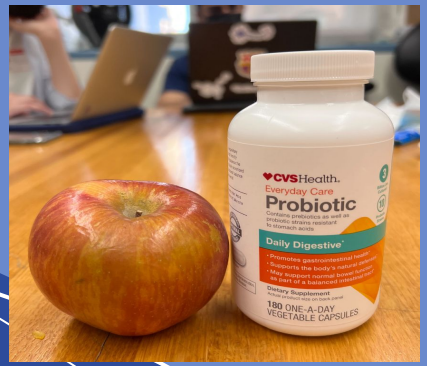
3. qPCR



Modification of diet

1. Probiotics administration

- 10^6 CFU of probiotic for a total of 3 days
- Including Lactobacillus and Bifidobacterium



Modification of diet

2. Behavioral data collection process

- 10 minute home cage recordings
- Behaviors
  - Excessive Grooming
  - Rearing
  - Nibbling
  - "Spidermouse"
  - Cage location

Results

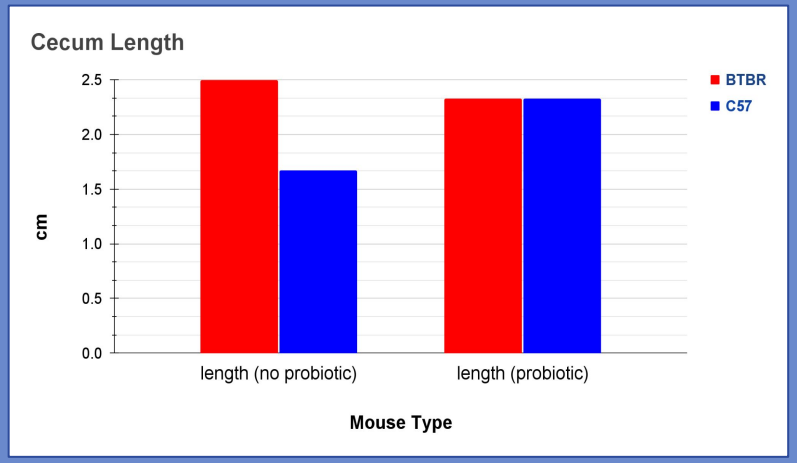
qPCR Microbiota Identification

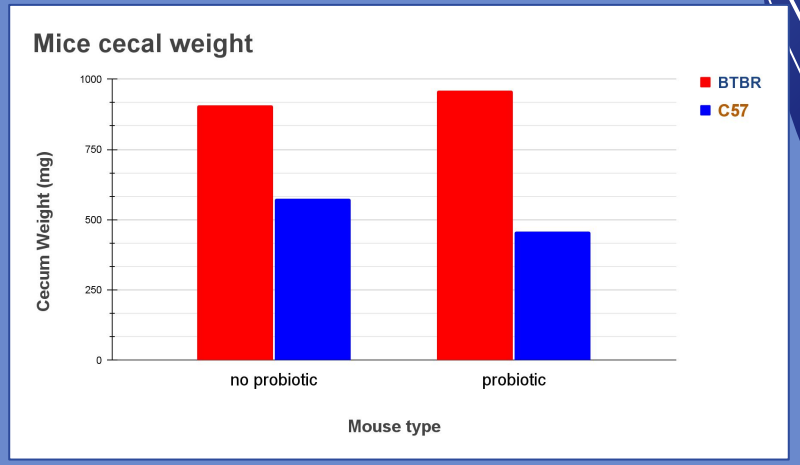
Qualitative – qPCR *Identification* shows us whether or not a microbiota is present

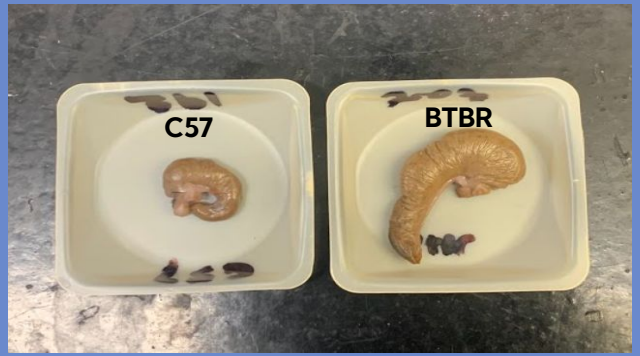
Next steps – qPCR Profiling which would give us *quantitative results* of how much of a specific microbiota is expressed

	C57	C57	C57	BTBR	BTBR	BTBR
Bacteroides fragilis	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+
Candida albicans	+	+	+	+	+	+
Streptococcus agalactiae	+	+	+	+	+	+
Bifidobacterium longum	+	+	+	+	+	+
Enterococcus faecalis	+	+	+	+	+	+
Helicobacter pylori	+	+	+	+	+	+
Streptococcus mitis	+	+	+	+	+	+
Citrobacter freundii	+	+	+	+	+	+
Clostridium perfringens	+	+	+	+	+	+

Cecum measurements

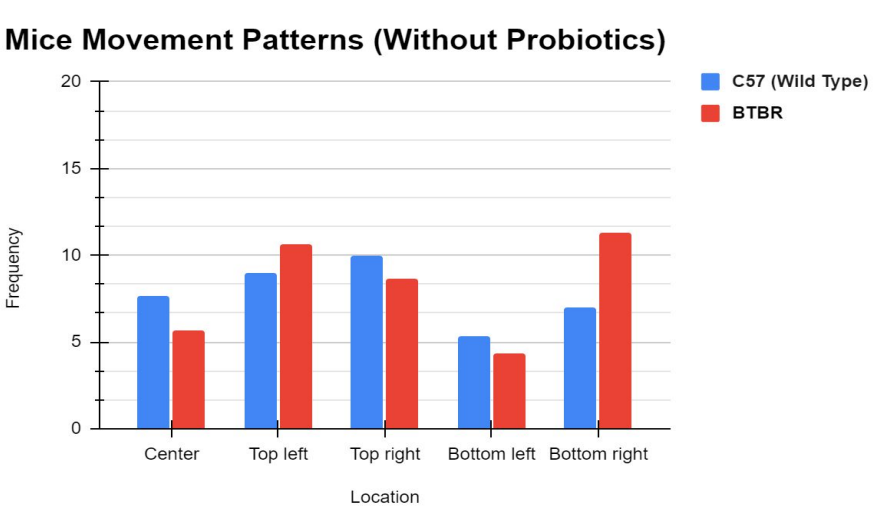


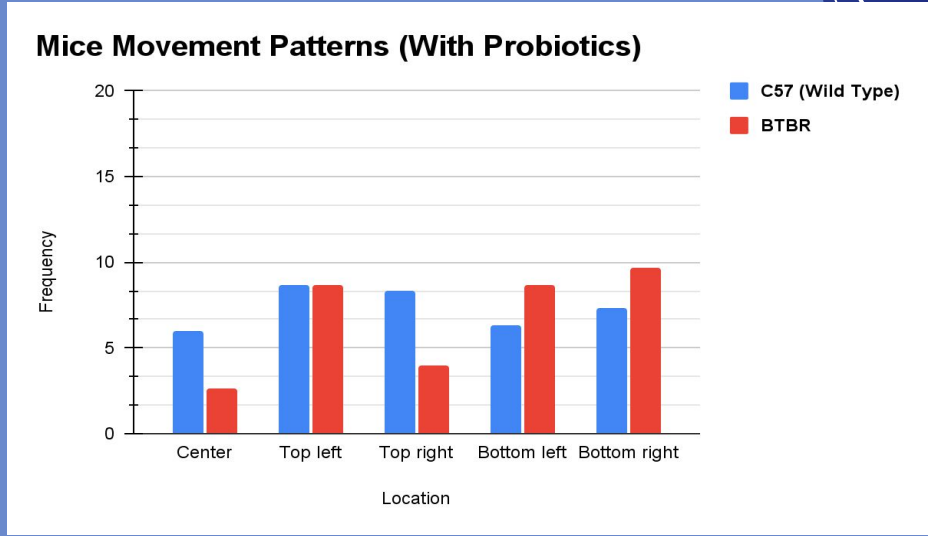




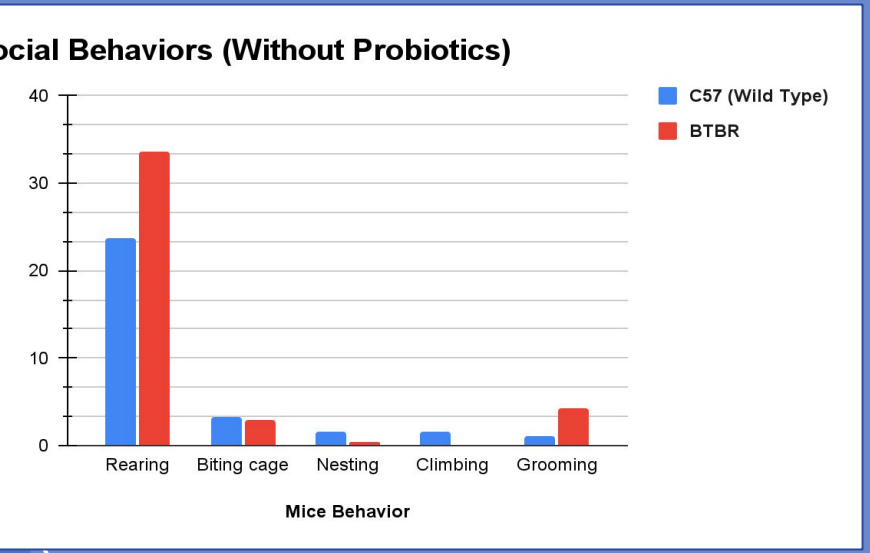
Behavioral observations

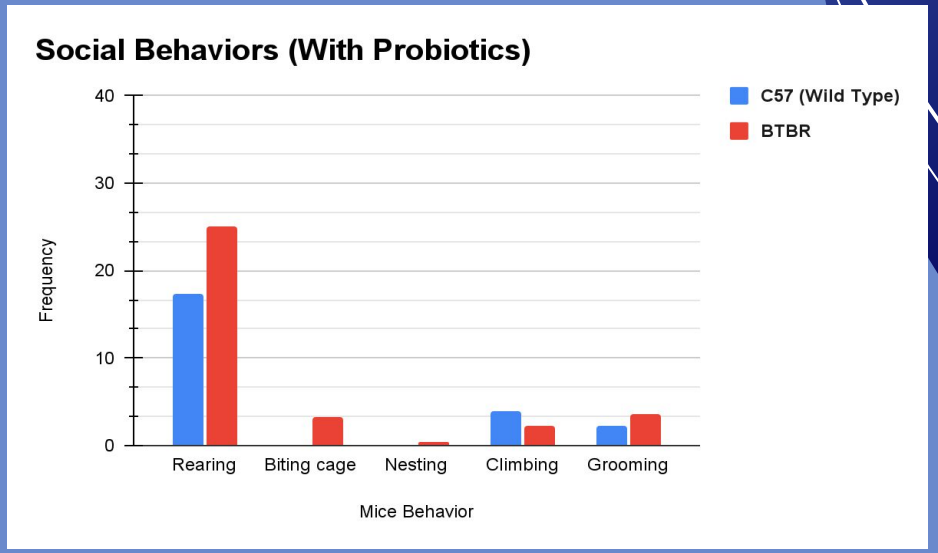
Mice Movement Patterns





Social Behaviors





Conclusion

qPCR Identification

- The data showed us that gut microbiota associated with ASD is present in both mice

Behavioral observation

- Repetitive behaviors associated with anxiety decreased while interactive and explorative behaviors increased

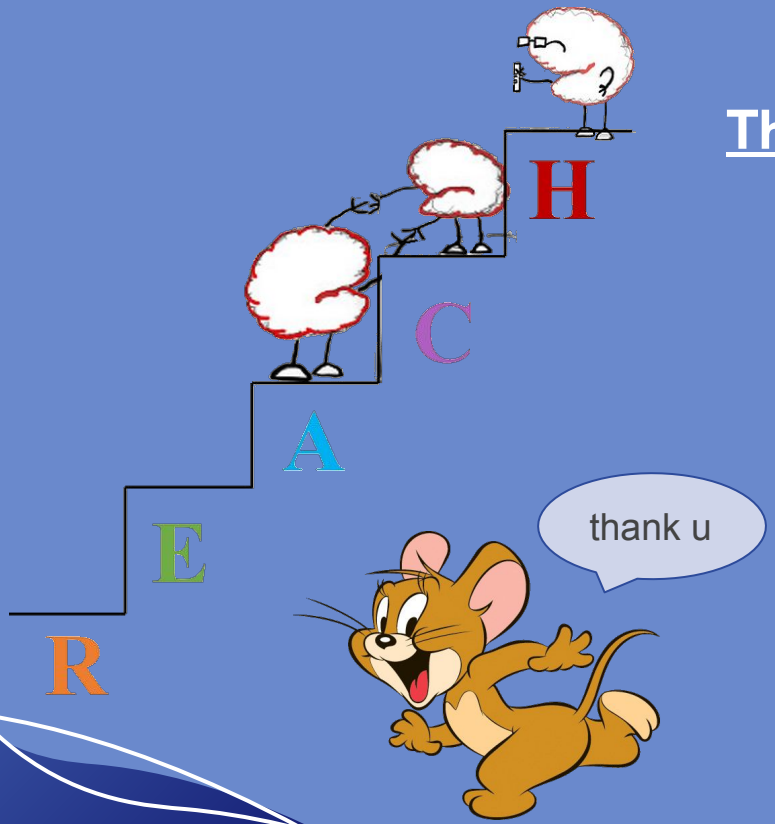
Cecum measurements

- Probiotics caused a slight increase BTBR cecal size probably due to an influx of microbiota
- C57 cecal size minor fluctuation due to already normal function

Limitations/Future Steps

- Mice might not eat the probiotics
- Time (conducting the procedure within a specific time frame)
- Explore Different types of behavioral analysis experiments
- We will experiment on different types of probiotic strains

Acknowledgements



Thank you to the REACH Program:

Pargol Mashati
Joanne Andre
Dan Rosenthal
Juan Marcos
Craig Kelly
Daniel Mishan
Isaac Vingan

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Thank you! :)

Questions?