# Feb 5, 2024 - Agenda

1:15-1:25 - finalize research question
1:25-1:45 - discussion of next steps: project proposal planning, literature review and types of downstream analysis
1:45-1:55 - Github and project documentation review
1:55-end - remaining questions for Chris about assignments, R, etc

Parkinsons - what are the effects of dopamine agonists pramipexole and amantadine, and monoamine oxidase-B inhibitor rasagiline on PD patients?

Idea: Look to see if treatments brings microbiome back to what normal person would have. Some microbes associated with health vs disease.

Idea: look at different BMI groups or different alcohol consumption categories. Do these factors affect response to treatment?

Aim 1: getting people with multi-treatments into their own group
First do α β diversity: control, PD untreated, PD treated (1, 2, 3, 4), PD combo

- Min sample size = 5 individuals

Move forward with only interesting group(s)
Aims 3-5: do all taxonomic analyses: core microbiome, indicator taxa, differential abundance

Aim 6 (optional): confounding factors. What other variables could affect efficacy of treatments

Data needs to be processed before proposal → aka complete aim 1

- Add 1 column: treatment = healthy, PD_untreated, drug1, drug2, drug3, drug4, drug5(combo)

Look at efficiency on how these treatments are recovering the microbiota of PD patients

Make another folder for R script.

- Follow the same process as qiime.

Documenting qiime process: make a .sh script

- Another folder in the qiime folder for qza files etc…

6+ combinational →

- We can keep a low sample size and keep it as a limitation of the study
- May end up being a supplementary figure

Only have ¾ drugs → no need for MVA

1. Processing/filtering
    a. Stratifying by treatment/multi-drug treatment
2. Basic alpha and beta alpha diversity analysis in these
    a. 5 groups: healthy, PD untreated, 4 drug groups
    b. From the alpha/beta → see what groups are interesting and move forward with those
3. Do ALL the taxonomic analyses
    a. Core microbiome, indicator taxa, differential abundance
4. Explore confounding factors

End presentation " we looked at how diff treatment plans → found only 1 was interesting → went deeper to see what bacteria are actually changing in these patients

- Must have QIIME processing done before proposal submission because part of the proposal is an overview of the dataset
    - Need to complete aim 1 before it
    - Generate diversity metrics in QIIME and R
    - Create a single column with our 7 categories already labeled

<u>Github</u>

- For QIIME processing
    - Have a folder for QIIME and folder for R
        - Have a .sh script for QIIME where it has all of our commands
        - Have another folder within QIIME for .qza, .qzv files
        - **You can edit the .sh file on github**
- No specifics for how to interface it
- Within R folder
    - Have different folders for the different aims/R analyses
- Stay on main branch?
-

# Feb 12, 2024 - Agenda

1:15-1:35 - Review aim 1 and troubleshooting
1:35-1:55 - Project proposal planning and outline
1:55-end - Remaining questions and misc.

- 1 person do intro, 1 experimental aims, 1 research objectives
- **Intro:**
    - Lots of lit search
    - Include background on PD, PD microbiome, other probiotic papers, bg on different treatments being investigated
    - Level of detail: reference to other literature that might support hypotheses or trends
    - 1.5 page ish
    - Convey importance of research
    - Lead into research gap
    - Mention significance of work at the end
- **Research question:**
    - Might be a bit repetitive compared to intro
    - Why are we asking these questions?
    - “Because of the gap, we want to look into …”
- **Experimental aims:**
    - Important: reference literature that have same methods we are using

- **Overview chart:** generate a flowchart
    - Highlight how we are addressing research questions through aims

- **Timeline:** include a week for troubleshooting in gaant chart
- **Next meeting:**
    - **Diversity metrics:** Richness, shannon, all of them?

# Feb 21, 2024 - Agenda

1:00-1:20 - review QIIME analysis, discuss lack of significance in overall diversity and refine research objective if necessary (confounders, functional analysis)
1:20-1:45 - review experimental aims 1-5 and their approaches, and discuss modifying/adding aims based on previous discussion

- Purpose of PD drugs should be to specifically affect species that are involved in PD
    - Changes may not be reflected in α β diversity
- Boxplots of Alpha Diversity Measure may need to be analyzed through stats tests to determine significance → one-way ANOVA?
    - Run the stats on boxplots → negatives are still results
- Beta diversity test weighted unifrac plot
- Do everything on R
- See if any of 7 diversity metrics are significant; if none, then show observed and shannons
- Aim 1 is done
- Aim 2
    - Done when informs us about next steps to do
    - If some comparisons are interesting, focus more on those
- Move on to Aim 3, 4, 5
    - Understand which core microbiomes that we want to compare, how it overlaps
- Chris will provide code for comparisons two way ANOVA and generating plots
    - 2 categorical variables independent on 1 dependent variable
- Aims 3, 4, 5 will take more thought
    - E.g. indicator taxa: generate table is easy, but have to think about comparing them; what does it actually mean if indicator table different between groups, or if certain groups are missing
- DESeq: need to already know which comparisons we want to do
    - Group 2 vs group 3, 4, 5, 6, 7
    - Control: 1 vs 3, 4, 5, 6, 7
        - If 1vs3 and 2vs3 are same, then not rlly interesting; not because of the treatment or PD
        - If positive value in a 2vs3 comp, it means more upreg in group 2
        - Assumption: bacteria have to be present in both treatments/groups
    - 7 more of a for fun comparison, may not have biological significance
    - Shows upregulation of specific bacteria to see how it compares between groups
- Could just focus on ~5 bacteria of interest

Which section:

- No more than 1 paragraph. Cite enough literature to justify hypothesis
- Explain novelty

- Research question -> Cite literature for hypothesis,-> state hypothesis in terms of this proposal -> mention impact (3-4 citations)

Research objective: half a page minimum

Intro:

- Parkinson's -> microbiome -> drugs -> knowledge gap (no one has looked at effect of drugs w.r.t microbiome) small paragraph -> significance of research
- Audience = people who don't know topic. Explain PD, what is it?
- Transition into microbiome
- PD microbiome has been studied, has been found to be different. Some markers include … certain bacteria/microbes
- Go into background of drugs a bit → there are dopaminergic drugs (focus of study) out there (don't need to go into detail for each individually)
- Write about significance of research; why important; what could we potentially find
- Explain what PD is → explain that PD microbiome has been studied and is distinctly different from that of healthy individuals → some markers include _____ → explain the background of the drugs → identify knowledge gap → write about significance of research, importance, potential findings/implications

Talking about any tools like QIIME or R, no abbreviations, and include references,
Link: https://docs.qiime2.org/2023.9/citation/

For each aim: use point format for each aim; write out what the aim is as a sentence

- (e.g. Aim 2 = to assess and compare the … between… with α and β diversity → under that, have a paragraph explaining why it's important to look at α and β diversity, and how does it inform us)
- E.g. DEseq: why are we doing deseq, what info will it give us, why is that significant to studying PD
- E.g. "To determine if Hi-SEAS reliably mimics the microbial environment of the ISS, with the ultimate goal of assessing the utility of Hi-SEAS as an Earth-based analog for space-based studies. This will involve validating the Hi-SEAS model using indicator taxa analysis and differential abundance analysis to determine commonalities."

Need figure legends for any figures

Explaining aims: have citations from other papers that have used the same methods.

# Feb 26, 2024 - Agenda

1:15 - 1:40 - Review core microbiome (aim 3) and indicator taxa (aim 4) analysis/code in the context of our project and plan out workflow for the next 1-2 weeks

- Question: rarefaction reduces sample numbers. Do we have to work with reduced numbers for core, indicator, deseq

1:40 - end - Any questions for Chris and Evelyn re: project, modules, proposal etc. if necessary

- Set deliverables for next week?
- Each meeting someone different presents what they did
- Put timeline chart on github readme
- Next immediate thing: look at stats for alpha diversity for aim 2
    - Some may be significant/interesting
    - Will give idea of which drugs are more or less important → follow up with core microbiome and indicator taxa analyses
    - Plot richness function to make the graphs (part of phyloseq package)

- Try to do aim 3 as well
- Use rarefied final phyloseq object for core, indicator, deseq??? → CHRIS WILL GET BACK ON THIS. **ASSUME WE DONT RAREFY FOR NOW**
- For α stats, use kruskal-wallis
    - Do kruskal wallis + tukey/hsd post-hoc test
- For β diversity, run with all the distance matrices (brae curtis, jacquard, weighted and unweighted)
    - PERMANOVA → 4 different stats total
    - If including one, weighted unifrac would be best → but probably not including any

<u>Microbiome</u>

- Run core microbiome for all 7 groups
    - Decide on detection and prevalence limit
    - Good way to decide: use a package (CHRIS WILL EMAIL TO US, WITH SCRIPT) to generate heatmap. Has different prevalence and detections, can see how core microbiome changes as you adjust parameters
    - Create venn diagram and UpSet plot for next week
        - Ali can create UpSet plot once code is completed

<u>Indicator species</u>

- David will lead → leave for next week
- Looking at the indicator taxa table from group 2 (PD) vs all the other groups
    - Looking at if any species that are missing/present in drug treatment groups vs PD group
    - Indicator taxa table tells us which species are missing
- Table 2 vs 3, 4, 5,
    - Which bacterias present in 3, missing in 2, or other way

# Mar 4, 2024 - Agenda

1:15 - 1:35 - review comments on project proposal (if marked)
1:35 - end - troubleshoot heatmap code, double check if we need to rarefy for aims 3-5, review how to implement Upset plot code

- Aim to decide on detection and prevalence limit by tuesday night (50%?)
- Generate venn diagrams around wednesday (Alicia) troubleshoot if necessary

Meeting Notes:

<u>Core microbiome</u>
Pick a cutoff where core microbiome of all the groups can be encompassed, and compare where the bacteria are, which ones are unique, what they're associated with → health?

Another possibility: The effect of the drugs (combined) create completely different core microbiomes

Research what the different bacteria are, blast unidentified species

- Interested mostly in people without parkinsons?
- Conclusion: how does the drug manipulate the microbiome towards or away from a disease state, or does it create something completely different?

<u>Aim 3</u>

- Make venn diagrams
    - Prevalence: 50%, detection threshold at least 2%
    - Mostly between groups 1 vs 3-7, 2 vs 3-7
    - Do some lit review on identified bacteria

- Ali do alpha or beta diversity stats
    - Bar plot with all treatments
- David do aim 4
- **Keep un-rarefied for aims 3-5**

# Mar 11, 2024 - Agenda

1:15 - 1:20 - Diversity metrics presentation (Ali)
1:20 - 1:35 - Core microbiome presentation (Alicia)
1:35 - 1:50 - Indicator taxa presentation (David)
1:50 - end - Discuss aim 5, potential aim 6 and which genera/species to focus on moving forward for the paper

2024-Mar-11

- Increase permutations for PERMANOVA to 10,000 → Number of permutations from 999
- Filter before run β diversity to look at 2 specific comparisons
    - Or run it 4 separate times, each time with a different comparison. 1-2, 1-4, 1-5, 1-6
- Also make sure its the drug and not PD status

Indicator taxa

- Provide more context for what indicator taxa means
- Name drop the 30 sps in comp of healthy vs PD if they are already associated with PD
- Barplot: have groups 1, 3, 4 .. with low abundance, and then group 2 with high abundance
    - Make abundance plot for the 2 species in Group 1,3,4,5,6
- Measure the abundance of the two species in the combined group
    - **Oscillospiraceae Colidextribacter and Firmicutes Clostridia**

**Coremicrobiome**

- Change detection to 1%, keep prevalence at 50%
-

**Followup on Amantadine**
***F. Prausnitizii***

# Mar 18, 2024 - Agenda

1:15 - 1:35 - DESeq2 presentation (Cayden) and discussion to interpret results
1:35 - 1:45 - Core microbiome presentation (Alicia)
1:45 - end - Discuss next steps, figures for paper and presentation

DEseq used more to comment on up/down regulation of bacteria that may be health/disease associated.

- Need to compare with core microbiome analysis

*****Presenting order**

- **Alpha beta diversity showed no significant differences → changes are more minute than compositional**
- **Core microbiome trends with one getting rescued**
- **Indicator species different lists with 3 and 6**
    - **Differences between treatment 3 vs treatment 6**

***Barchart with actual abundance values for *Prevotella* see abundance across different groups.**

Different naming schemes show different genus, but may be part of the same family. Definitely different species. E.g. Bacteroides.2 is distinct compared to Bacteroides.1

Genus with .1 are distinct enough to be categorized differently. Treat them as separate.
Uncultured -> don't know what the genus is

Each result should have a conclusion. Could be simple but want to go into detail

- E.g. **Drug 3 influences/downregs PD associated bacteria**

Major result from DEseq: *prevotella* downregulation.

- Despite all drugs downregulating prevotella, actually these drugs have different effects. For example: core microbiome, indic species results

**April 8 meeting:** finalize presentation and present it to Chris for feedback

Change graph titles to be more informative

**Core Microbiome stick with 2% but look at g_Faecalibacterium. Don't mention other %s**
Core - which of these bacteria are the same in all the indiv
DEseq - shows effect of change
Conclusion:

- Treatment 4&6 may be effective in terms of bringing microbiome back to normal

- Show how the drugs bring g_Faecalibacterium back to normal levels compared to PD patients and healthy individuals
-

Furhter analysis:

- Abundance of specific bacteria

# Mar 25, 2024 - Agenda

1:15 - 1:30 - Finalize figure types for manuscript and formatting (color palette, font etc.)
1:30 - 1:45 - DESeq abundance bar plots and literature review progress
1:45 - 1:55 - Discuss potential aim 6 addition (correlation or regression with abundance and UPDRS)
1:55 - 2:00 - Remaining questions

Meeting Notes 3/25/2024:

Figure 1: alpha beta diversity 4 panels 2 each
- Shannons and Faiths

Figure 2: core microbiome 4 panels, **heatmaps supplemental fig1.**

Table 1: indicator taxa, just show number of identified taxa
- Initial comparison of healthy and PD
- Comment on how indicator species found initially disappear

Figure 3: DESeq2
- A lot of panels → 8 have all barplots
    - Good colors
- Volcano plots supplemental

Figure 4: specific abundance plots (panels?)
- Bifidobacterium
- Prevotella
- Abundance barplots only to complement DEseq2

- **Complete draft of slides by next meeting for review**

Additional aim:
- Regression analysis of identified genera abundances and PD UPDRS disease scale

April 3, 2024 - Agenda

3:00 - 3:30 discuss proposal
3:30 - 4:00 presentation

Meeting notes:

Title: kinda vague?

- Proposal will be remarked by Avril

**Changes to presentation to be completed by Monday:**

- **Change non-PD to healthy**
- Put red box around drugs of interest in intro slide, delete AADC, cut out everything we are not talking about
- GET RID OF ALL DATA PROCESSING STUFF. NO QIIME2
- Get rid of overall workflow slide
- Venn diagram slide too busy
- Core microbiome: get rid of parameters
    - Remove treatment group numbers
    - Make title more concise → less busy
    - For presentation: tabulate + make a table, put percentages only. Could do heatmap?. for manuscript, keep as is
- Indicator taxa:
    - Briefly mention __???idk___, elaborate in discussion
    - Hammer home the indicator taxa slide with presenting group
- DESeq
    - Table format possibly ONLY FOR PRESENTATION??
        - Would give more representation, shows better trend
    - 8 rows, 2 columns for increase decrease
    - ~~For PD-untreated, draw dashed line across the graph~~
    - ~~Cut out akkermansia~~
- Cut out *Lachnospiraceae* slide (supp fig 2.)

Apr 8, 2024 - Agenda

1:15 - 1:30 - Present oral presentation changes to Chris and finalize for submission
1:30 - 1:45 - Discuss and finalize manuscript roles
1:45 - end - remaining questions

**MEETING NOTES**

- Wouldn't include alpha diversity for slides → delete
- Important to note transition from slide 11→12. Highlight to presenters
- Shifts core microbiome to healthy individuals take home msg → highlight in red box
- Keep DEseq slides