

Metagenome Analysis of Preterm Birth

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Overview

1 Introduction

2 Materials

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1. Introduction

Microbiome

- Microbiota: the microorganisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome: 10^{13} to 10^{14} microorganisms whose collective genome (Gill et al., 2006)



Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

- Ribosomal RNA
- Well-known as a key to phylogeny (Olsen & Woese, 1993)

Preterm Birth (PTB)

PTB:

- ① PTB < 37 GW (Gestational week)
- ② Normal ≥ 37 GW

Detailed PTB:

- ① Early PTB < 34 GW
- ② 34 GW \leq Late PTB < 37 GW
- ③ Normal ≥ 37 GW

(J. Tucker & McGuire, 2004; Voronkov, Solonovych, Liashenko, & Revenko, 2018)

2. Materials

16S rRNA Sequencing

16S rRNA sequencing is the *reference method* for bacterial taxonomy & identification (Mignard & Flandrois, 2006)

Three main reasons (Janda & Abbott, 2007):

- 16S rRNA exists in almost all bacteria
- Functions of the 16S rRNA has not changed over evolution.
- 16S rRNA is large enough for bioinformatics

Data Composition I

Data composition

50 pregnant women & 59 newborns

PTB

- Mother ⇒ Early PTB: 17 & Late PTB: 22 & Normal: 29
- Newborn ⇒ Early PTB: 13 & Late PTB: 24 & Normal: 22

Data Composition II

Table: Clinical characteristics of mothers

Clinical	<34 GW (n=11)	≥34 GW (n=39)	p-value	Remarks
Cholesterol	273.4±37.5	285.3±63.4	0.667	
DBP	84.3±18.1	80.1±11.1	0.863	
Glucose	105.4±38.4	87.4±18.3	0.228	
HDL	82.2±20.6	84.1±26.5	0.957	
Hb	11.8±1.0	11.8±1.5	0.916	
Hct	35.1±2.5	35.0±3.8	0.833	
LDL	162.8±35.0	143.7±33.7	0.345	
Mother Age	31.5±4.8	33.4±4.5	0.264	
SBP	144.2±33.0	132.8±16.9	0.541	
Weight gain	6.7±3.7	12.3±5.8	0.002	*
Advanced maternal age	3 (27.3%)	12 (30.8%)	1.000	
C-section	6 (54.5%)	33 (84.6%)	0.048	*
Gestational Diabetes	0 (0.0%)	3 (7.7%)	1.000	
Hypertension	3 (27.3%)	10 (25.6%)	1.000	
Mother Antibiotics	5 (45.5%)	8 (20.5%)	0.126	
Mother Steroid	11 (100.0%)	1 (2.6%)	0.000	*
Obesity	3 (27.3%)	9 (23.1%)	1.000	
PROM	5 (45.5%)	7 (17.9%)	0.105	
Preterm Labor	4 (36.4%)	8 (20.5%)	0.424	
Too much weight gain	0 (0.0%)	7 (17.9%)	0.324	

Data Composition III

Table: Clinical characteristics of newborns

Clinical	<34 GW (n=13)	≥34 GW (n=46)	p-value	Remarks
Apgar Score	7.7±0.7	9.5±0.8	0.000	*
Gestational Week	30.1±2.7	36.5±1.7	0.000	*
Hospitalized Day	25.5±15.2	8.4±6.3	0.003	*
Weight	1505.1±407.3	2795.4±476.9	0.000	*
CPAP	7 (53.8%)	5 (10.9%)	0.002	*
Dyspnea	11 (84.6%)	6 (13.0%)	0.000	*
Gender	6 (46.2%)	22 (47.8%)	1.000	
Neonate Antibiotics	8 (61.5%)	8 (17.4%)	0.003	*
PROM	7 (53.8%)	7 (15.2%)	0.008	*
Respirator	8 (61.5%)	1 (2.2%)	0.000	*
Sepsis	4 (30.8%)	6 (13.0%)	0.204	

Statistical tests

- Continuous: M.W.W.
- Categorical: Fisher exact

3. Methods

Qiime 2 Workflow

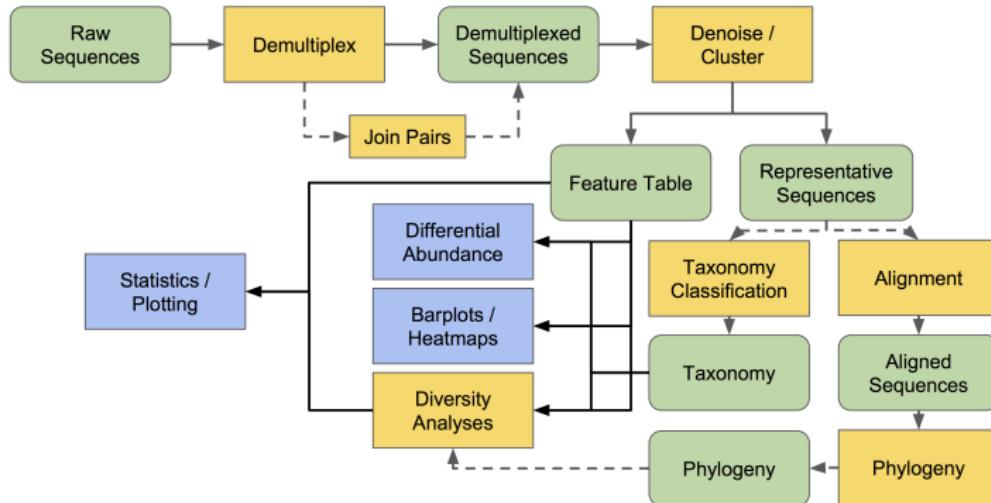


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal et al., 2015; McDonald et al., 2012)

4. Results

4. Results

4.1. Taxonomy Overview

Proportion Distribution

Proportion

- Minimum: 0.0
- Mean: 0.00008
- Median: 0.0
- Maximum: 0.793

Proportion without Zero

- Minimum: 0.00002
- Mean: 0.00008
- Median: 0.00153
- Maximum: 0.793

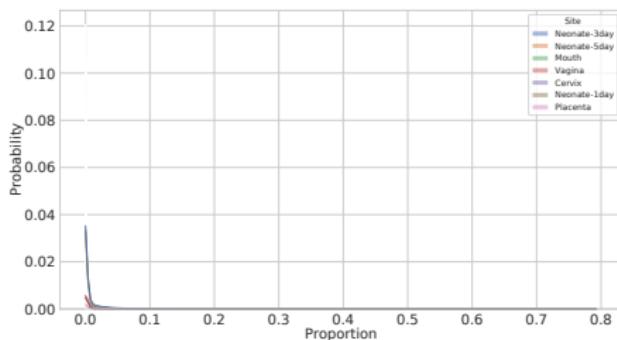


Figure: Proportion distribution

Microbial community with Proportion

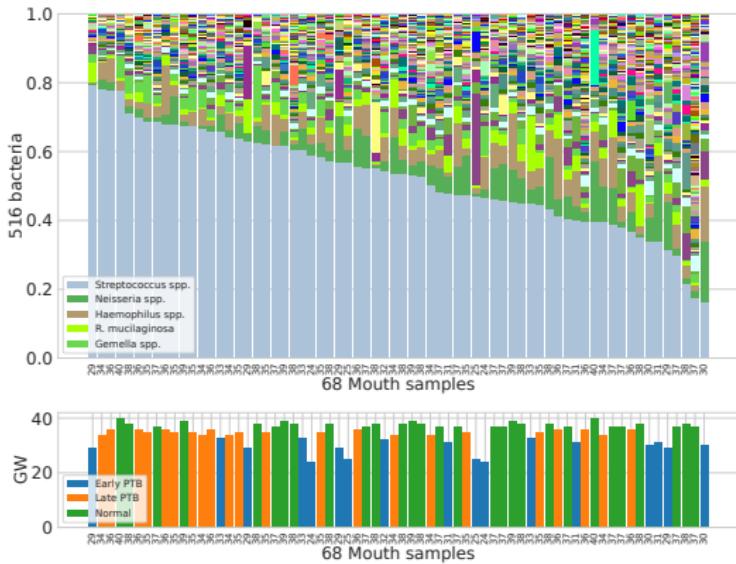
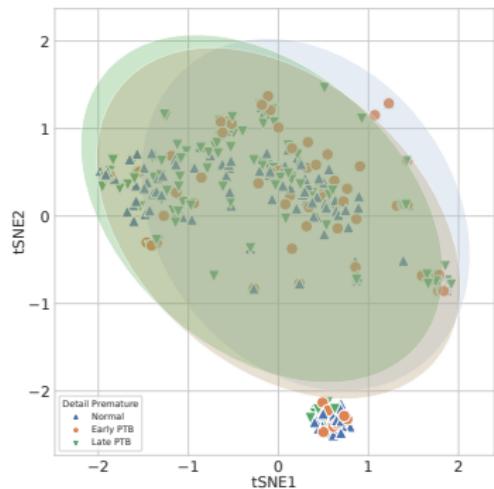
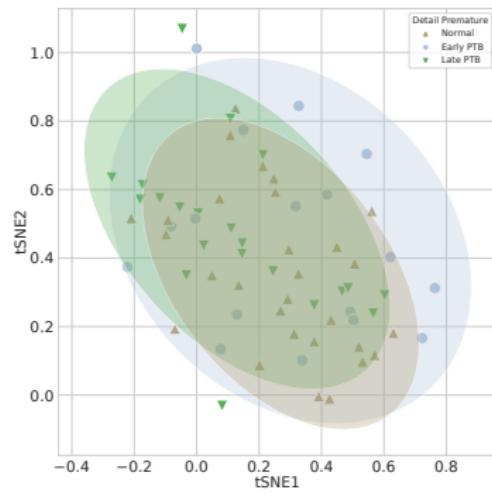


Figure: Microbial community with Proportion

t-SNE with Proportion



(a) All



(b) Mother Mouth

Figure: t-SNE plot of Proportion with PTB

Notable Taxa I

Streptococcus spp.

- *S. mutans*: pathogen of dental caries
- Membrane vesicles of Group B *Streptococcus* disrupt feto-maternal barrier (Surve et al., 2016).
∴ Leading to PTB

Neisseria spp.

- *N.* colonize the mucosal surfaces of many animals.
- Only two pathogens in 11 known species
 - *N. meningitidis*: Meningitis & Sepsis
 - *N. gonorrhoeae*: Gonorrhea (sexual transmitted disease)
- *N. gonorrhoeae* results adverse pregnancy outcomes (Heumann, Quilter, Eastment, Heffron, & Hawes, 2017).

Notable Taxa II

Haemophilus spp.

- *H.* inhabit the mucous membranes of upper respiratory tract, mouth, vagina, & intestinal tract.
- *H. influenzae*: A major cause of systemic infection
- PTB caused by *H. influenzae* (Hills et al., 2022) and *H. parainfluenzae* (Mendz, Petersen, Quinlivan, & Kaakoush, 2014).

R. mucilaginosa

- *Rhodotorula mucilaginosa*
- *R.* is a genus of pigmented yeasts.
- Catheter-related bloodstream infection due to *R. mucilaginosa* (Kitazawa, Ishigaki, Seo, Yoshino, & Ota, 2018).
- ∴ *Rhodotorula* bloodstream infections

Gemella spp.

- *G. bacteria* are primarily found in mucous membranes of human e.g. oral cavity & upper digestive tract.
- *G. haemolysans* causes endocarditis (Mosquera, Zabalza, Laniero, & Blanco, 2000).
- Women who have PTB also have significant lower level of *G.* (Li et al., 2021).

4. Results

4.2. Diversity Index

Diversity Index

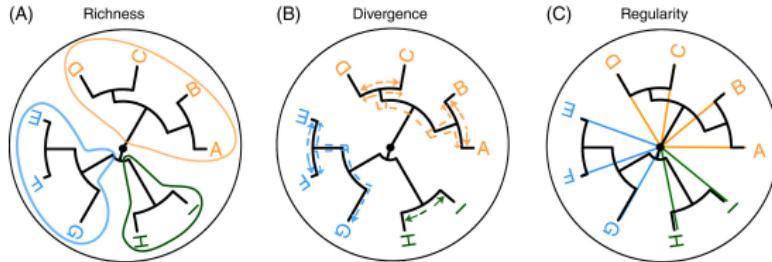


Figure: Three dimensions of phylogenetic information (C. M. Tucker et al., 2017)

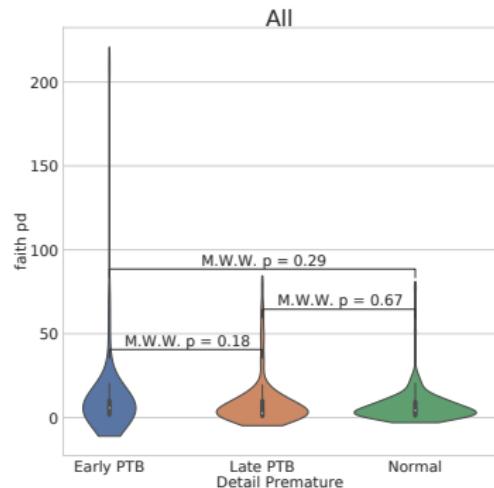
- A quantitative measure that shows richness, divergence, and regularity (C. M. Tucker et al., 2017)
- Alpha diversity: the richness of taxa **at a single community**
- Beta diversity: taxonomy differentiation **between communities**

4. Results

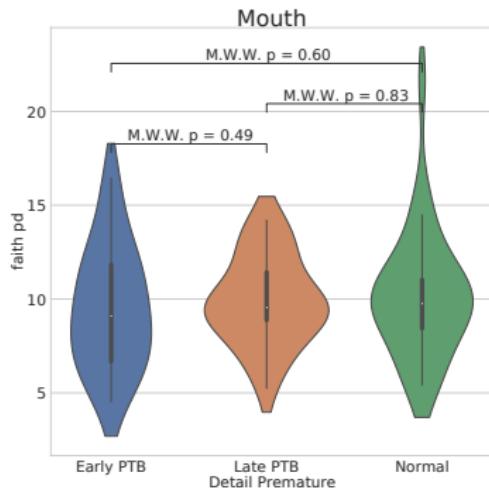
4.2. Diversity Index

4.2.1. Alpha-diversity

Violin Plot with Alpha-diversity I



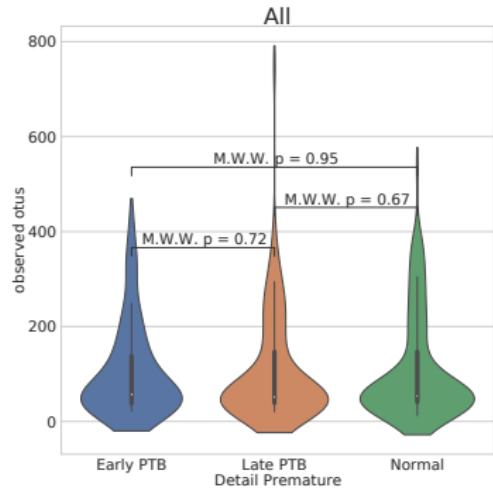
(a) All



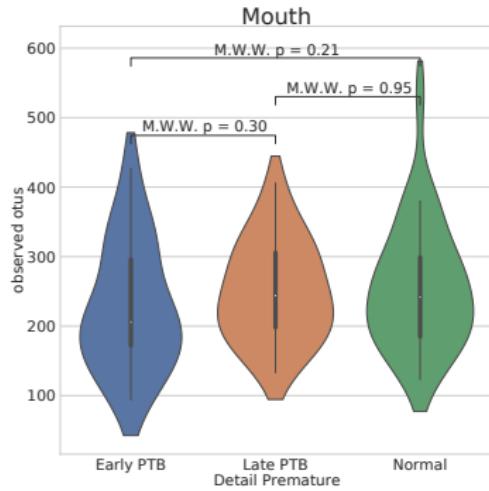
(b) Mother Mouth

Figure: Detail premature & Faith's PD

Violin Plot with Alpha-diversity II



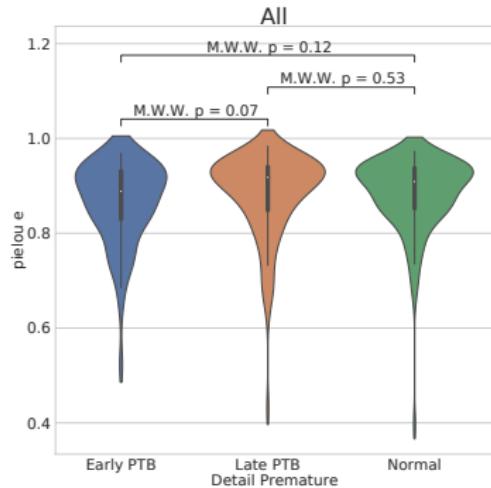
(a) All



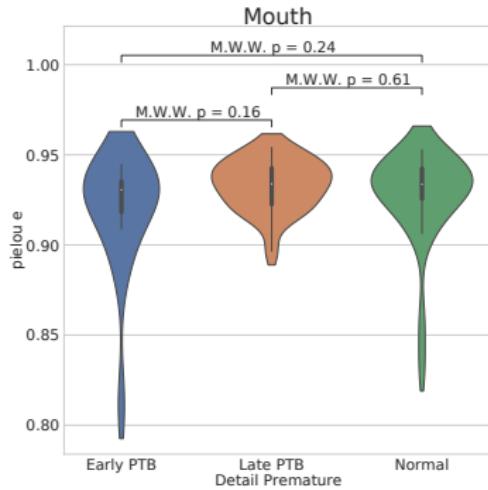
(b) Mother Mouth

Figure: Detail premature & Observed OTUs

Violin Plot with Alpha-diversity III



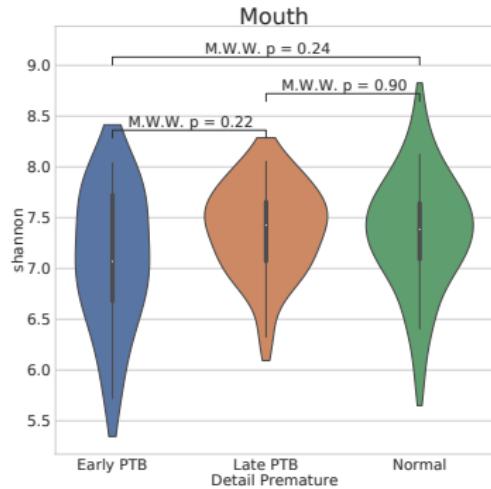
(a) All



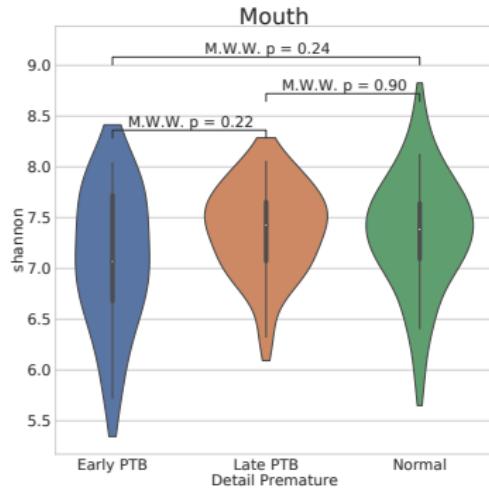
(b) Mother Mouth

Figure: Detail premature & Pielou Evenness

Violin Plot with Alpha-diversity IV



(a) All



(b) Mother Mouth

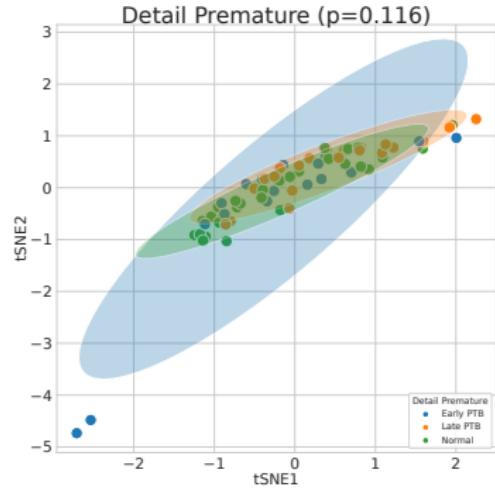
Figure: Detail premature & Shannon Entropy

4. Results

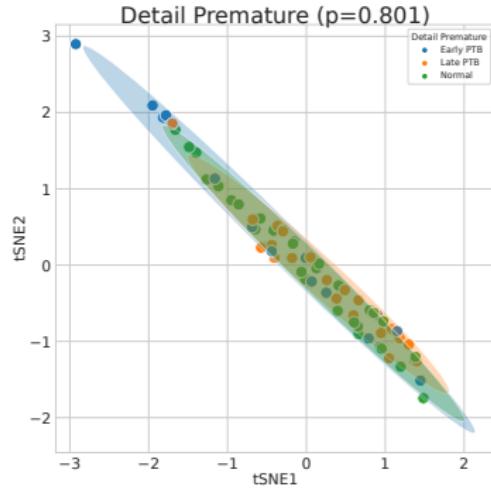
4.2. Diversity Index

4.2.2. Beta-diversity

Beta-diversity t-SNE plots I



(a) Bray-Curtis



(b) Hamming

Figure: Beta-diversity t-SNE plots

4. Results

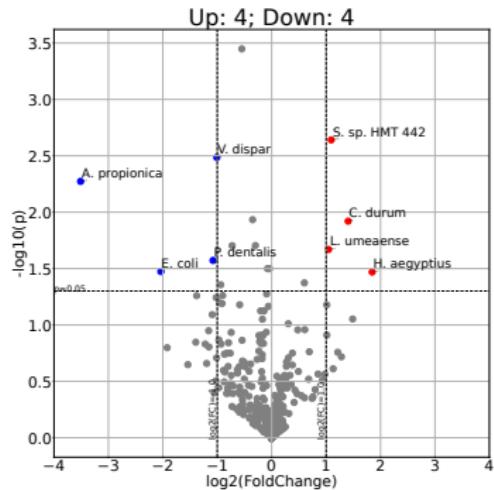
4.3. Taxonomy Analyses

4. Results

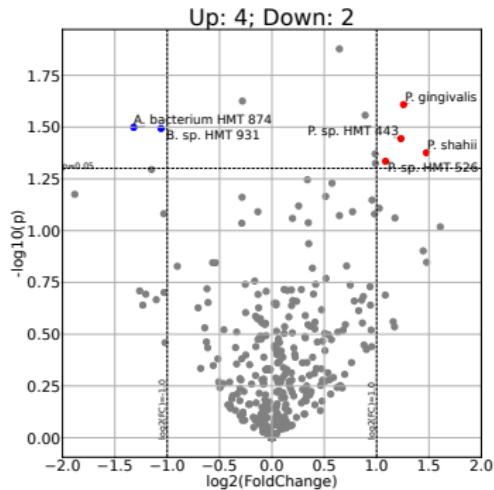
4.3. Taxonomy Analyses

4.3.1. Differentially Abundant Taxa

Volcano plots I



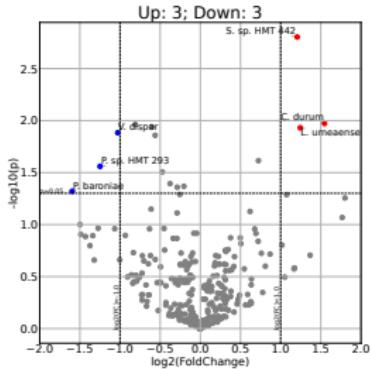
(a) Early PTB vs. other



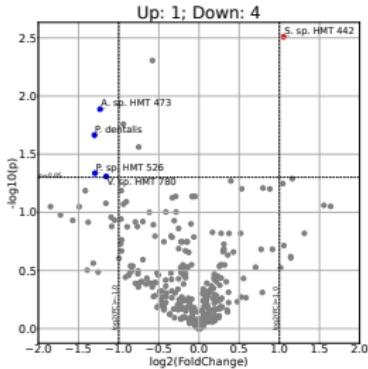
(b) PTB vs. Normal

Figure: DAT in Mouth

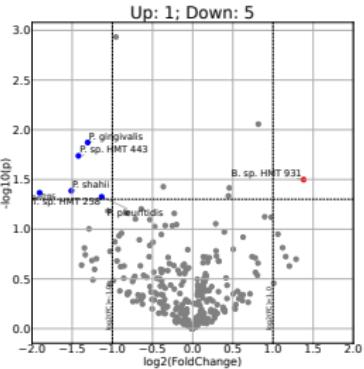
Volcano plots II



(a) Early vs. Late



(b) Early vs. Normal



(c) Late vs. Normal

Figure: DAT in Mouth

Venn Diagrams

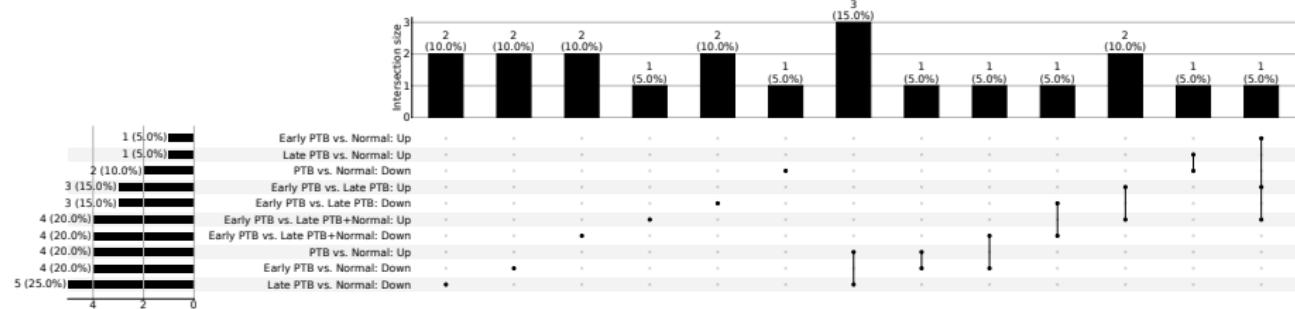
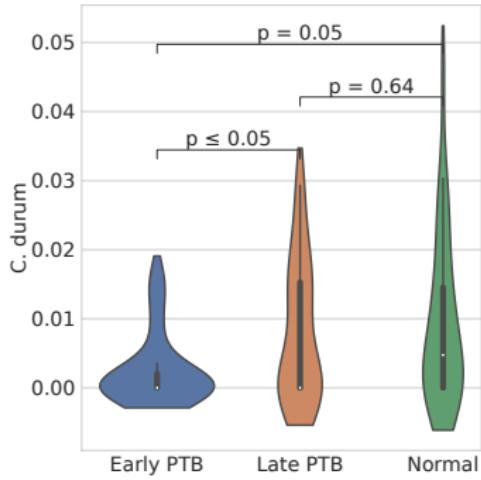


Figure: DAT Upset plot

Union of DAT

A total of 20 taxa selected as DAT.

Violin Plots I

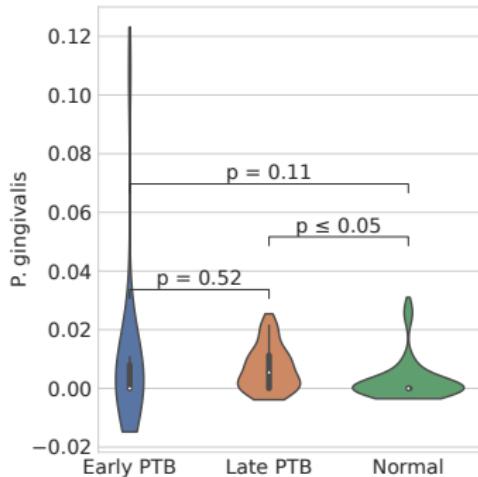


C. durum

- *Corynebacterium durum*
- Poly-microbial interactions
⇒ Oral mucosal & Gingival cells
(Redanz et al., 2021).
- Synergism between *C.* & *Streptococcus* in oral commensals (Treerat et al., 2020).

Figure: *C. durum*

Violin Plots II



P. gingivalis

- *Porphyromonas gingivalis*
- *P. gingivalis* is well-known periodontitis pathogen.
- *P. gingivalis* is associated with bacterial vaginosis (Africa, Nel, & Stemmet, 2014).
- *P. gingivalis* ⇒ Adverse pregnancy outcomes (Miyauchi et al., 2018).

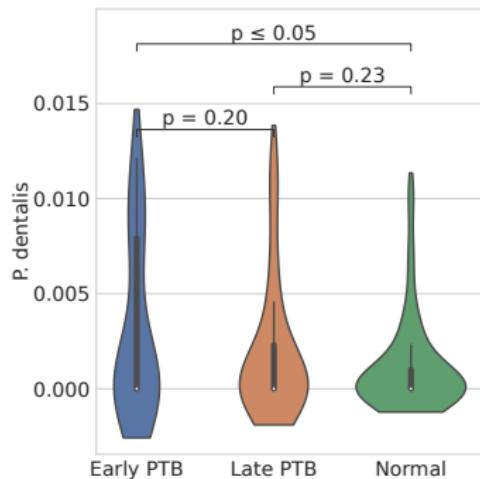
Figure: *P. gingivalis*

Violin Plots III

Prevotella spp.

- *P.* interactions with human health (Tett, Pasolli, Masetti, Ercolini, & Segata, 2021).
- *P.* spp. might have different role in PTB (Freitas, Bocking, Hill, & Money, 2018).
- *P.* spp. are associated with human infections (Sharma, Garg, Hasan, & Shirodkar, 2022).

Violin Plots IV



P. dentalis

- *Prevotella dentalis*
- *P. dentalis* is observed as colorectal cancer-promoting bacterium (Shi et al., 2022).

Figure: *P. dentalis*

Violin Plots V

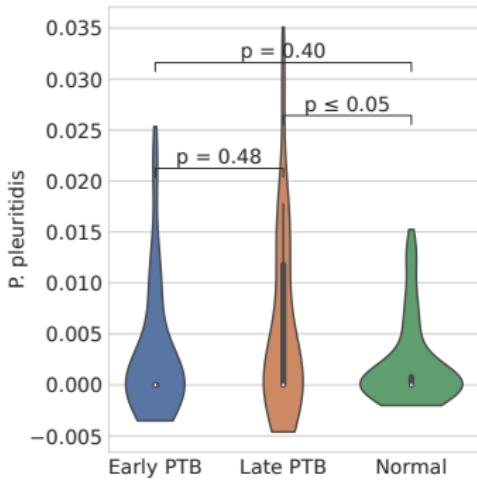


Figure: *P. pleuritidis*

P. pleuritidis

- *Prevotella pleuritidis*
- *P. pleuritidis* causes lung abscess (Asif, Roy, & Ahmad, 2020).
- *P. pleuritidis* is abundant in oral microbiome of smokers (Al Bataineh et al., 2020).
- *P. pleuritidis* associated with gastric cancer (Yang et al., 2022).

4. Results

4.4. Machine Learning

ML algorithm comparison

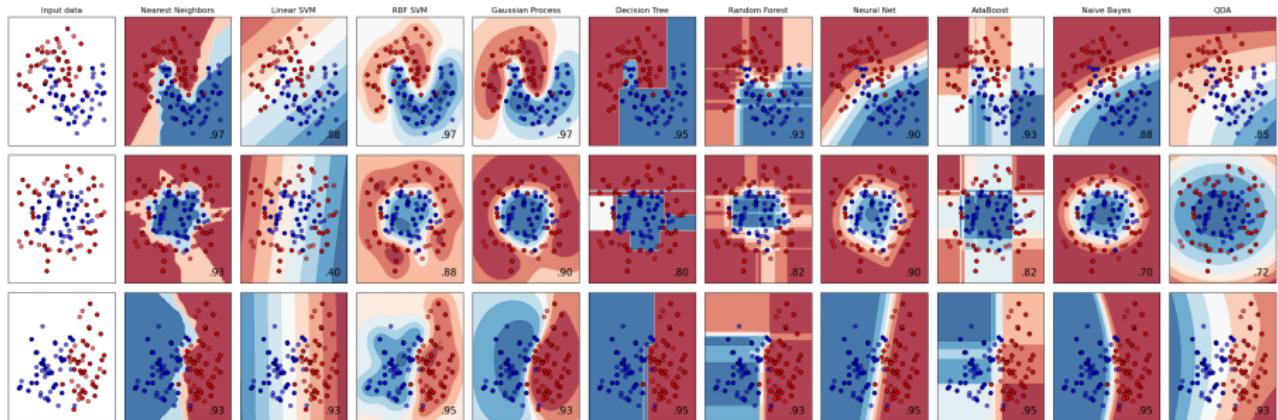


Figure: Classification Comparison (Pedregosa et al., 2011)

Oversampling

SMOTE (Chawla, Bowyer, Hall, & Kegelmeyer, 2002)

- Synthetic Minority Oversampling Technique
- An algorithm that makes pseudo-sample
- Using K -Nearest Neighbor algorithm

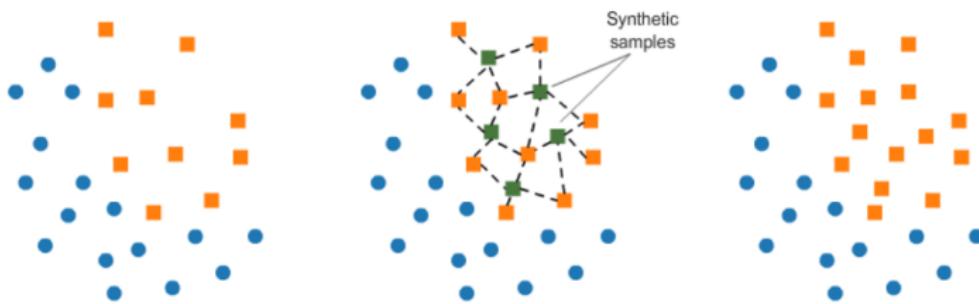


Figure: Workflow of SMOTE

4. Results

4.4. Machine Learning

4.4.1. Random Forest Classifier on Proportion

Random Forest with (Early vs. Late vs. Normal) I

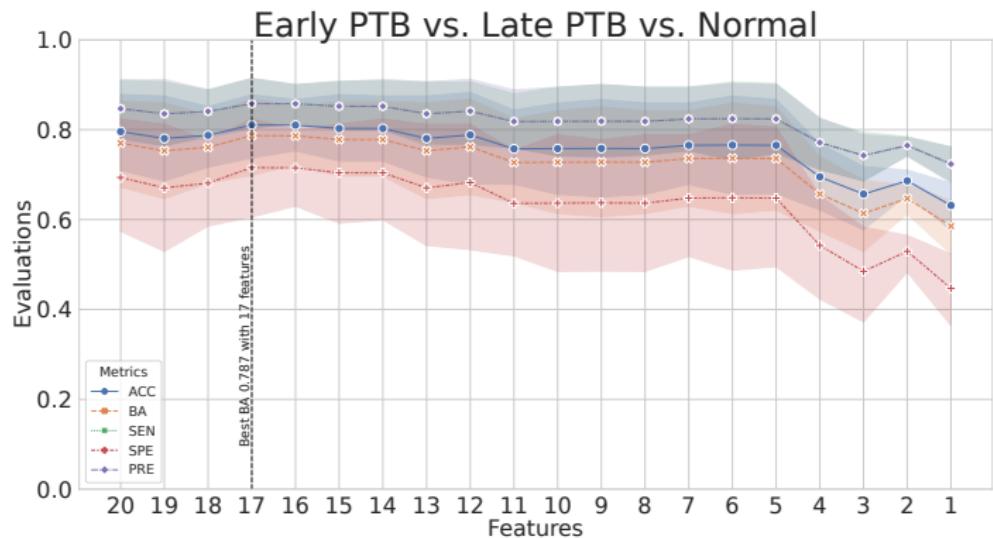


Figure: RF evaluations with feature counts

Random Forest with (Early vs. Late vs. Normal) II

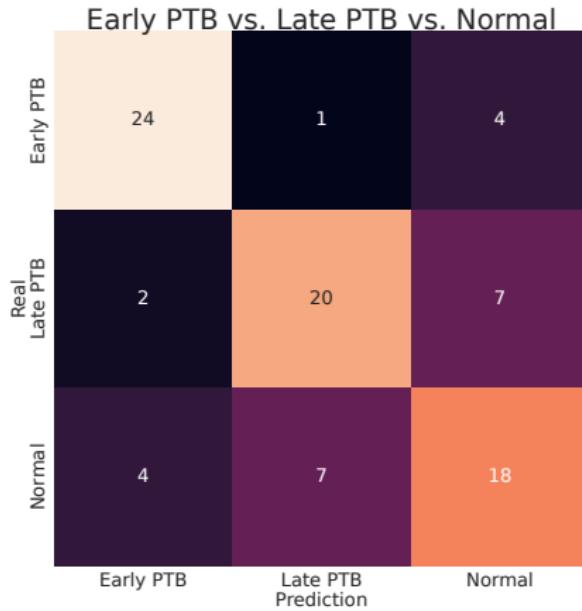


Figure: RF confusion matrix

Random Forest with (Early vs. Late vs. Normal) III

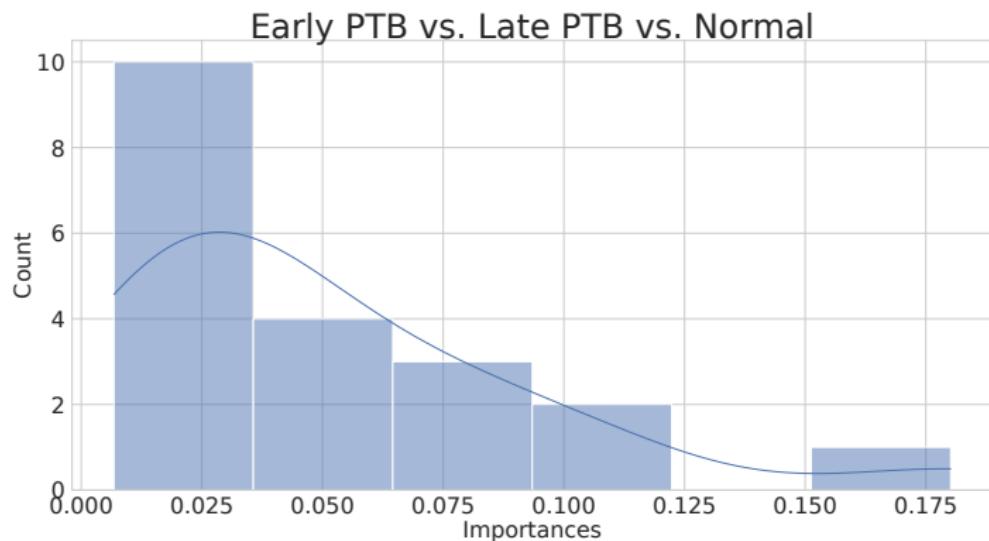


Figure: RF importances

Highest Importances

- ① *L. umeaense*
- ② *S. sp. HMT 442*
- ③ *V. dispar*

Random Forest with (Early vs. Late + Normal) I

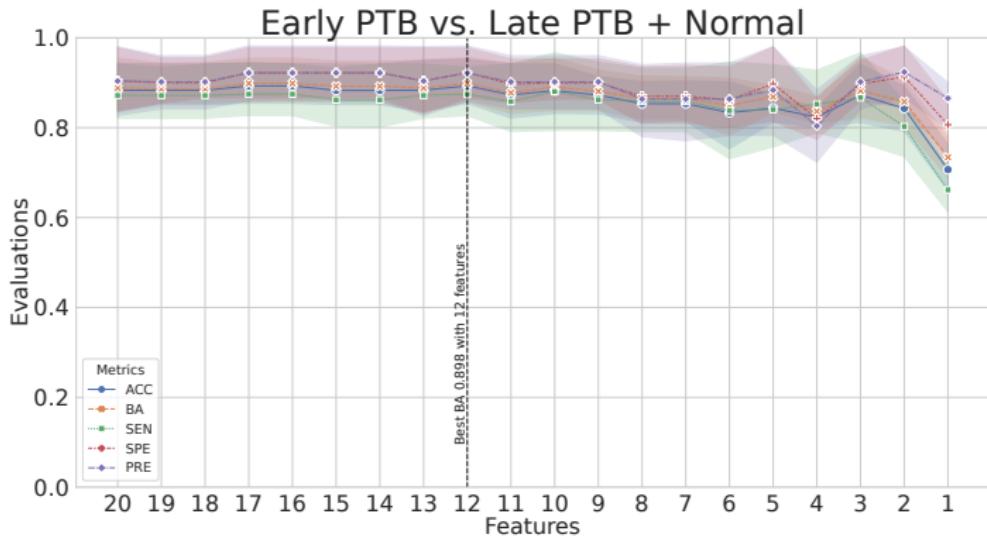


Figure: RF evaluations with feature counts

Random Forest with (Early vs. Late + Normal) II

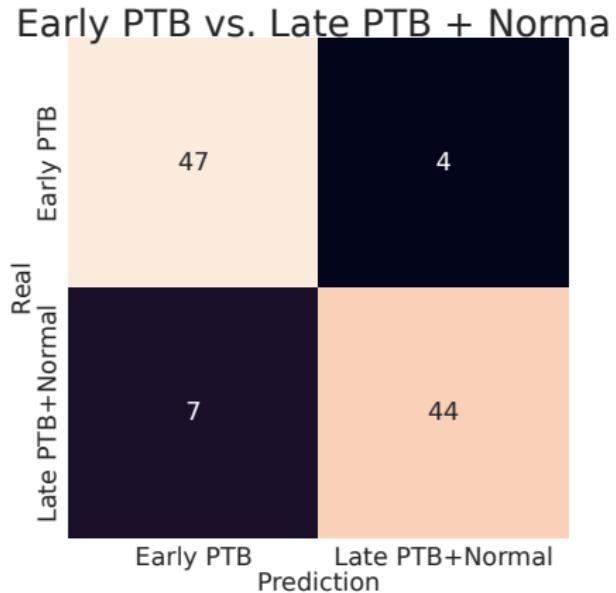


Figure: RF confusion matrix

Random Forest with (Early vs. Late + Normal) III

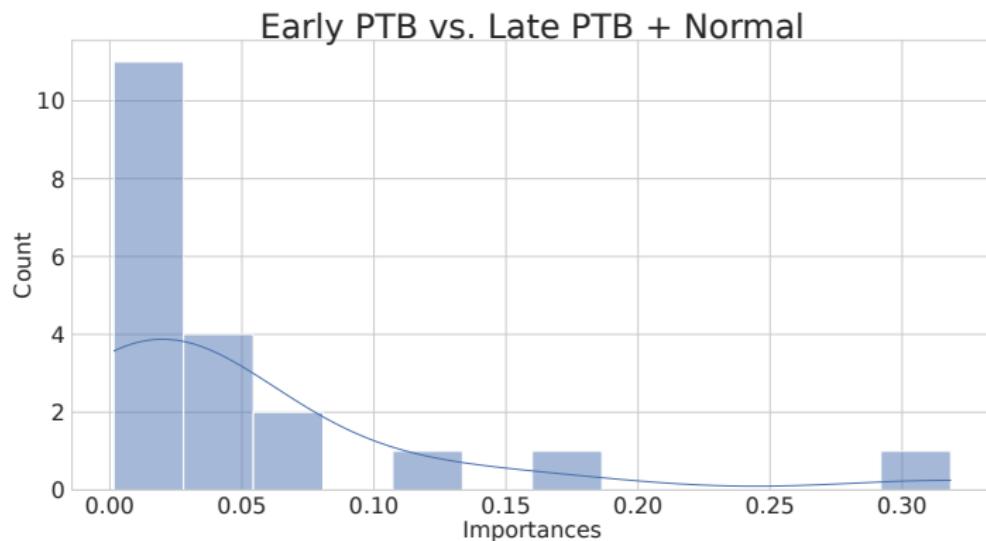


Figure: RF importances

Highest Importances

- ① *L. umeaense*
- ② *S. sp. HMT 442*
- ③ *C. durum*

Random Forest with (PTB vs. Normal) I

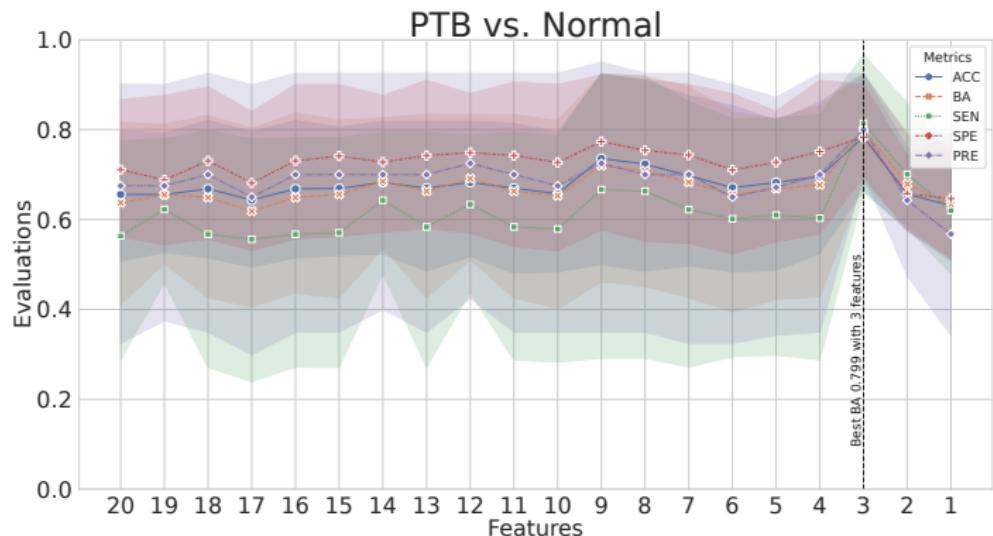


Figure: RF evaluations with feature counts

Random Forest with (PTB vs. Normal) II

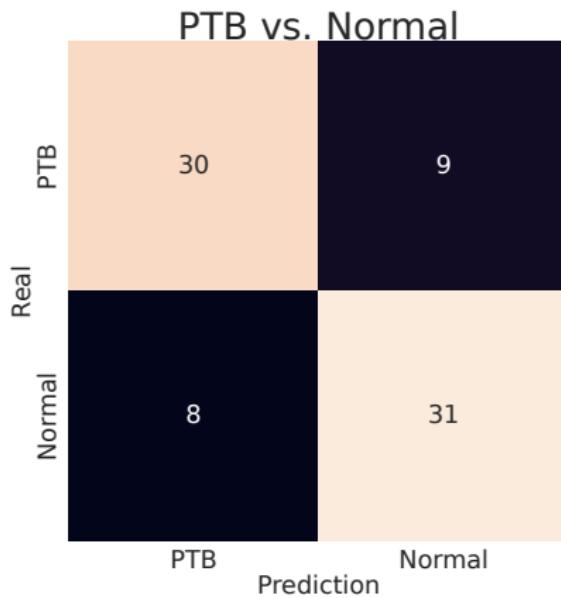


Figure: RF confusion matrix

Random Forest with (PTB vs. Normal) III

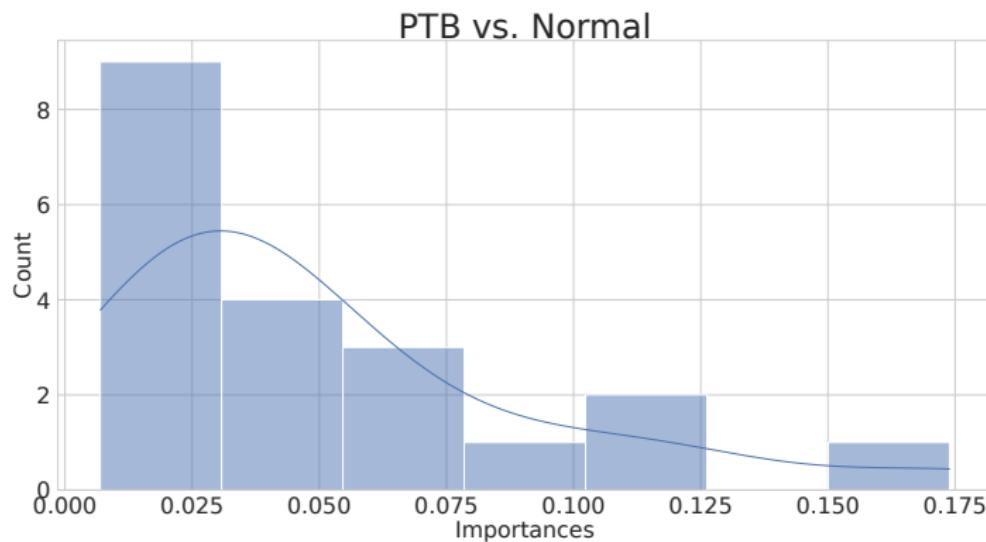


Figure: RF importances

Highest Importances

- ① *L. umeaense*
- ② *P. gingivalis*
- ③ *B. sp. HMT 931*

4. Results

4.5. Pathway Enrichment Prediction

PICRUSt2

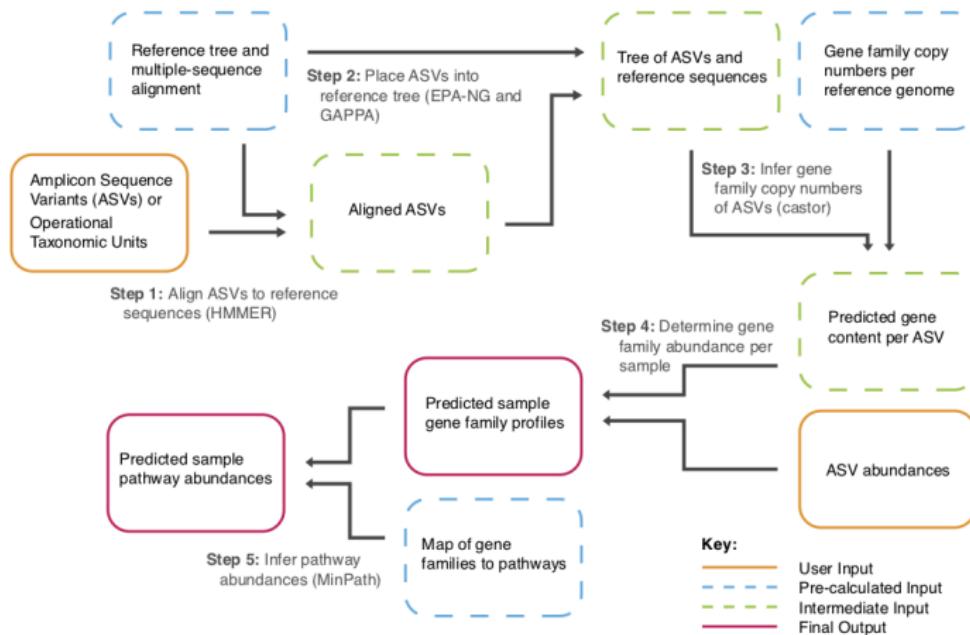


Figure: PICRUSt2 flowchart (Douglas et al., 2020)

Pathway Predictions: Early PTB vs. Late PTB I

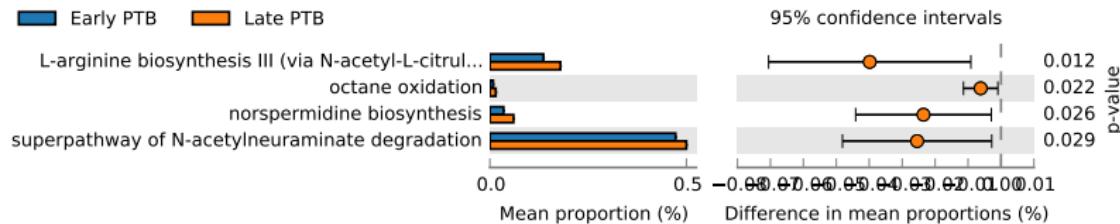


Figure: Early PTB vs. Late PTB

Pathway Predictions: Early PTB vs. Late PTB II

L-arginine biosynthesis III (via N-acetyl-L-citrulline) (PWY-5154)

- PWY-5154 is enriched in late PTB than early PTB.
- PWY-5154 is enriched in normal than early PTB.
- PWY-5154 is enriched in other than early PTB.
- PWY-5154 is increased with age in rats (Yang, Chen, Zhang, & Wang, 2021).
- Arginine deficiency ⇒ PTB (SNYDERMAN, HOLT JR, NORTON, & PHANSALKAR, 1970; Zamora et al., 1997; Wu, Jaeger, Bazer, & Rhoads, 2004).

Octane oxidation (P221-PWY)

- P221-PWY is enriched in late PTB than early PTB.
- P221-PWY is enriched in normal than early PTB.
- P221-PWY is enriched in other than early PTB.
- P221-PWY is important for classifying the PTB (Deutsch et al., 2022):
higher at PTB than normal ?

Norspermidine biosynthesis (PWY-6562)

- PWY-6562 is enriched in late PTB than early PTB.
- PWY-6562 is enriched in other than early PTB.
- Norspermidine has antitumor activity via regulating DNA (Nishio et al., 2019).
- Norspermidine is used as a biofilm disassembly trigger (Böttcher, Kolodkin-Gal, Kolter, Losick, & Clardy, 2013).

Superpathway of N-acetylneuraminate degradation (P441-PWY)

- P441-PWY is enriched in late PTB than early PTB.
- P441-PWY is enriched in late PTB than normal.
- NPL gene ⇒ N-acetylneuraminate ↑
∴ Viral pathogenicity ↑ (Bomba et al., 2022).
- N-acetylneuraminate level increased at 24-28 GW in PTB women
(Ghartey, Bastek, Brown, Anglim, & Elovitz, 2015) ??

Pathway Predictions: Early PTB vs. Normal I

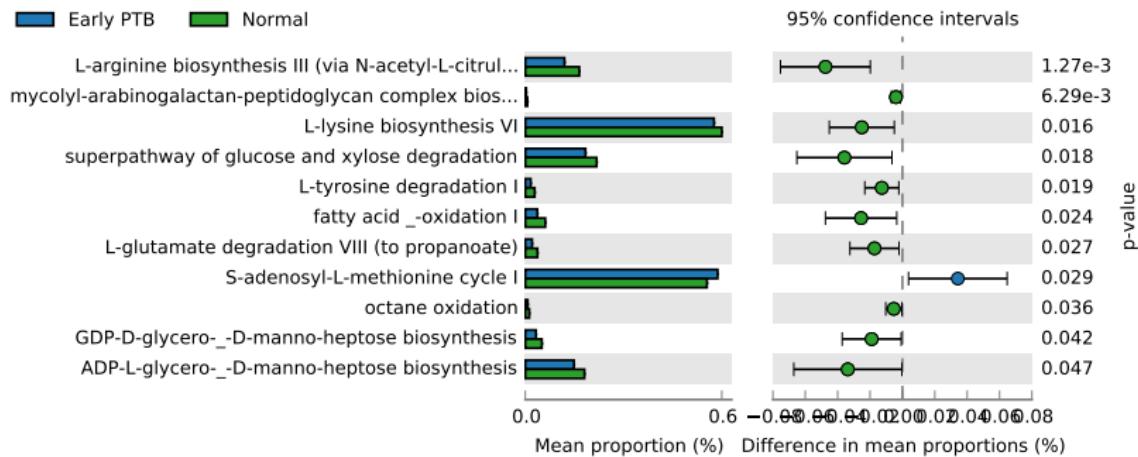


Figure: Early PTB vs. Normal

Pathway Predictions: Early PTB vs. Normal II

S-adenosyl-L-methionine cycle (PWY-6151)

- PWY-6151 is enriched in early PTB than normal.
- PWY-6151 is enriched in early PTB than other.

L-lysine biosynthesis VI (PWY-5097)

- PWY-5097 is enriched in normal than early PTB.
- PWY-5097 is enriched in normal than late PTB.
- PWY-5097 negatively associated with severity of multiple sclerosis (Zhou et al., 2022).
- Lysine level in serum was higher in term deliveries with PTB symptoms than without PTB symptoms (Lizewska et al., 2018).
- Lysine level in the first trimester are associated with adverse birth outcomes in Rhea cohort (Maitre et al., 2014).

Pathway Predictions: Early PTB vs. Normal III

Superpathway of glucose and xylose degradation (PWY-6901)

- PWY-6901 is enriched in normal than early PTB.
- PWY-6901 is enriched in other than early PTB.
- PWY-6901 associated with *zonulin* level (Kaczmarczyk et al., 2021)
 \Leftarrow a marker of small intestinal paracellular permeability
∴ Development of immunity during early life.

L-tyrosine degradation I (TYRFUMCAT-PWY)

- TYRFUMCAT-PWY is enriched in normal than early PTB.
- Oral antibiotics ⇒ microbiome alteration
∴ Promote oral squamous cell carcinoma (Wei et al., 2022).
- TYRFUMCAT-PWY was depleted in moderate & high activity rheumatoid arthritis patients (Ruiz-Limón et al., 2022).
- A tyrosine-related gene mutation impresses to PTB (Gómez et al., 2010).
∴ Alteration in inflammation response

Fatty acid β -oxidation I (FAO-PWY)

- FAO-PWY is enriched in normal than early PTB.
- FAO-PWY is enriched in other than early PTB.
- FAO-PWY is associated with fecal calprotectin level and IL12 (Davrandi, Harris, Smith, Murray, & Lowe, 2022).
- Ω -3 fatty acid \Rightarrow Prevent recurrent PTB (Harper et al., 2010).

L-glutamate degradation VIII (to propanoate) (PWY-5088)

- PWY-5088 is enriched in normal than early PTB.
- PWY-5088 is enriched in other than early PTB.
- PWY-5088 is enriched in gut microbiome of Parkinson's disease (Wallen et al., 2022).
- GABA & glutamate play a major role in fetal neonatal brain development (Basu, Pradhan, du Plessis, Ben-Ari, & Limperopoulos, 2021).

Pathway Predictions: Early PTB vs. Other I

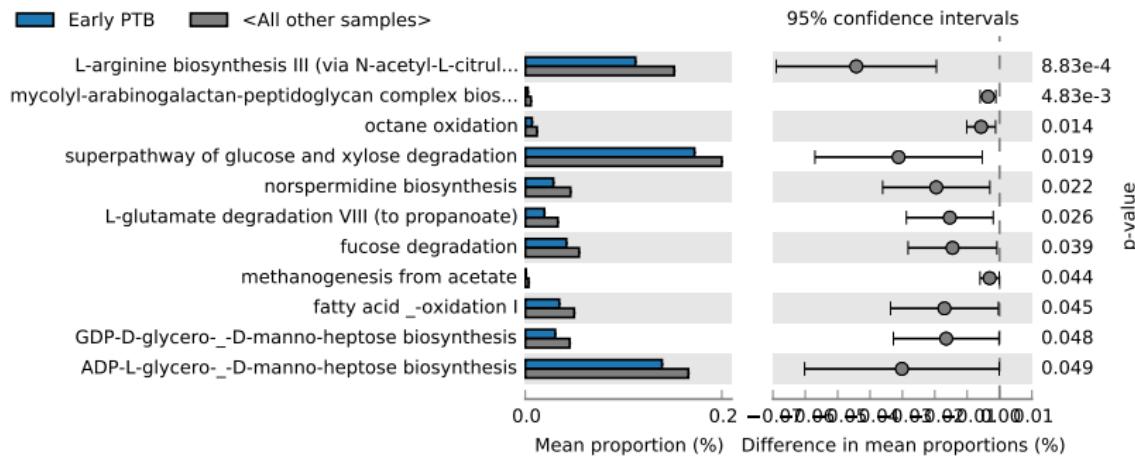


Figure: Early PTB vs. Late PTB + Normal

Fucose degradation (FUCCAT-PWY)

- FUCCAT-PWY is enriched in other than early PTB.
- Fucose have a linkage with intestinal health & disease (Garber, Hennet, & Szymanski, 2021).
- FUCCAT-PWY is enriched in upper respiratory microbiome of pregnant women (Solazzo et al., 2022).
- Porous cervical mucus plug \Rightarrow Vaginal infection \Rightarrow Fucose pathway
 \therefore PTB in mice (Lacroix, Gouyer, Rocher, Gottrand, & Desseyn, 2022).

Methanogenesis from acetate (METH-ACETATE-PWY)

- METH-ACETATE-PWY is enriched in other than early PTB.
- METH-ACETATE-PWY is enriched in upper respiratory microbiome of pregnant women (Solazzo et al., 2022).
- Methanogenesis have a major role in preventing acid accumulation (Abou Chacra & Fenollar, 2021).

5. Discussion

6. References

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