Core B- Plan to minimize the impact of differences in gut microbiome as a source of experimental variability.

Over the past decade a growing body of literature has extensively documented the impact of the intestinal microbiome in health and disease(1-3). Although the studies proposed in this program project grant do not directly study the impact of the microbiome in trained immunity at the pulmonary mucosa we do recognize that variations in microbiome can affect the reproducibility of the experimental readouts to be studied (4). It has been documented that mice obtained from diverse commercial vendors vary significantly in their microbiome (5-7). Core B will be a critical asset to minimize experimental variability in the proposed studies by serving as the common source of mice for all studies to be performed. Moreover, we have taken specific steps to minimize variations in microbiome in the mouse strains to be maintained and expanded by Core B. The following steps and procedures were chosen based on published recommendations (4, 8) and will help minimize the impact of variations in microbiome as a source of experimental variability:

1) All stains will be maintained in the same room under equal housing conditions. Critical factors in housing that have been documented to affect the microbiome include: diet (9), water treatment (10), light cycles (11), bedding (12) and caging (13). Therefore, maintaining equal housing and husbandry conditions will be critical in ensuring microbiome stability in our colony and help minimize experimental variability(4, 8).
2) The primary source of microbiome in the pups will be the birth dam (4, 14). It has been documented that the microbiome remains stable for generations in established colonies (15, 16). Thus, establishing dedicated breeding cages for each study will facilitate the availability of experimental mice with a stable flora for the duration of a given study aim. The time frame of planned studies spans an average observational period of three months. Based on our experience we anticipate that a given dam will be productive for 8 months and give birth to an average of 6 litters. Thus, within a given experimental approach we will be able to provide mice with comparable microbiome for at least two independent studies.
3) All mouse strains with selected gene targeting in floxed-cre settings are maintained as heterozygous breeders for the Cre trait. Therefore, this breeding strategy will generate experimental mice as well as ideal control littermates that come from the same birth dam, are housed in equal conditions but do not carry the genetic alteration. All mice will be tagged upon weaning and typing. Careful records will be maintained to trace the birth dam. Experimental gene-deficient mice and control littermates will remain co-housed. For each experiment within a project, investigators will be given access to paired gene-deficient and control littermates and information on breeding cage origin. These data will be included as results are gathered and published.
4) For global knockout strains that are maintained by breeding of KO x KO we will co-house KO mice with WT controls of the same sex upon weaning and will remained cohoused for 3-4 weeks prior the start of an experiment.
5) Representative fecal pellets samples will be collected from breeding cages at monthly intervals and stored. Fecal pellet samples from weaned litters will be collected one week prior a scheduled experiment and stored. Samples will be coded to track breeder and respective litters. In the event that unexpected experimental variability starts arising over time we will perform microbiome analyses on stored fecal pellets to determine whether shifts in microbiome underlie the variability.

Altogether, Core B will provide a cohort of animal strains for the studies proposed in this PPG at a level of standardization and customization not available from commercial vendors or from each individual laboratory.

References

1. Honda K, Littman DR. The microbiome in infectious disease and inflammation. Annu Rev Immunol. 2012;30:759-95. Epub 2012/01/10. doi: 10.1146/annurev-immunol-020711-074937. PubMed PMID: 22224764; PMCID: PMC4426968.
2. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol. 2016;16(6):341-52. Epub 2016/05/28. doi: 10.1038/nri.2016.42. PubMed PMID: 27231050; PMCID: PMC5541232.
3. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9(5):313-23. Epub 2009/04/04. doi: 10.1038/nri2515. PubMed PMID: 19343057; PMCID: PMC4095778.
4. Franklin CL, Ericsson AC. Microbiota and reproducibility of rodent models. Lab Anim (NY). 2017;46(4):114-22. Epub 2017/03/23. doi: 10.1038/laban.1222. PubMed PMID: 28328896; PMCID: PMC5762113.
5. Denning TL, Norris BA, Medina-Contreras O, Manicassamy S, Geem D, Madan R, Karp CL, Pulendran B. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. J Immunol. 2011;187(2):733-47. Epub 2011/06/15. doi: 10.4049/jimmunol.1002701. PubMed PMID: 21666057; PMCID: PMC3131424.
6. Hufeldt MR, Nielsen DS, Vogensen FK, Midtvedt T, Hansen AK. Variation in the gut microbiota of laboratory mice is related to both genetic and environmental factors. Comp Med. 2010;60(5):336-47. Epub 2011/01/26. PubMed PMID: 21262117; PMCID: PMC2958200.
7. Ivanov, II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe. 2008;4(4):337-49. Epub 2008/10/16. doi: 10.1016/j.chom.2008.09.009. PubMed PMID: 18854238; PMCID: PMC2597589.
8. Laukens D, Brinkman BM, Raes J, De Vos M, Vandenabeele P. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. FEMS Microbiol Rev. 2016;40(1):117-32. Epub 2015/09/02. doi: 10.1093/femsre/fuv036. PubMed PMID: 26323480; PMCID: PMC4703068.
9. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe. 2008;3(4):213-23. Epub 2008/04/15. doi: 10.1016/j.chom.2008.02.015. PubMed PMID: 18407065; PMCID: PMC3687783.
10. Sofi MH, Gudi R, Karumuthil-Melethil S, Perez N, Johnson BM, Vasu C. pH of drinking water influences the composition of gut microbiome and type 1 diabetes incidence. Diabetes. 2014;63(2):632-44. Epub 2013/11/07. doi: 10.2337/db13-0981. PubMed PMID: 24194504; PMCID: PMC3900548.
11. Deaver JA, Eum SY, Toborek M. Circadian Disruption Changes Gut Microbiome Taxa and Functional Gene Composition. Front Microbiol. 2018;9:737. Epub 2018/05/01. doi: 10.3389/fmicb.2018.00737. PubMed PMID: 29706947; PMCID: PMC5909328.
12. Bidot WA, Ericsson AC, Franklin CL. Effects of water decontamination methods and bedding material on the gut microbiota. PLoS One. 2018;13(10):e0198305. Epub 2018/10/26. doi: 10.1371/journal.pone.0198305. PubMed PMID: 30359379; PMCID: PMC6201873.
13. Ericsson AC, Gagliardi J, Bouhan D, Spollen WG, Givan SA, Franklin CL. The influence of caging, bedding, and diet on the composition of the microbiota in different regions of the mouse gut. Sci Rep. 2018;8(1):4065. Epub 2018/03/08. doi: 10.1038/s41598-018-21986-7. PubMed PMID: 29511208; PMCID: PMC5840362.
14. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science. 2016;352(6285):539-44. Epub 2016/04/30. doi: 10.1126/science.aad9378. PubMed PMID: 27126036; PMCID: PMC5050524.

15. Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC, Villablanca EJ, Wang S, Mora JR, Umesaki Y, Mathis D, Benoist C, Relman DA, Kasper DL. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 2012;149(7):1578-93. Epub 2012/06/26. doi: 10.1016/j.cell.2012.04.037. PubMed PMID: 22726443; PMCID: PMC3442780.
16. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, Zarate Rodriguez JG, Rogers AB, Robine N, Loke P, Blaser MJ. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158(4):705-21. Epub 2014/08/16. doi: 10.1016/j.cell.2014.05.052. PubMed PMID: 25126780; PMCID: PMC4134513.
6)