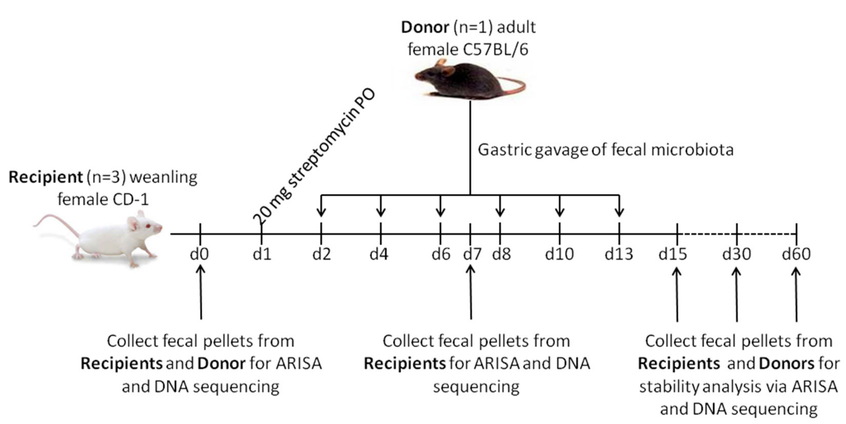
Murine Microbiota Transfer and Stability Analysis

K Chesney; C Franklin, DVM, PhD; and A Ericsson, DVM, PhD
Department of Veterinary Pathobiology, College of Veterinary Medicine
Univ. of Missouri, Columbia, MO 65211

BACKGROUND

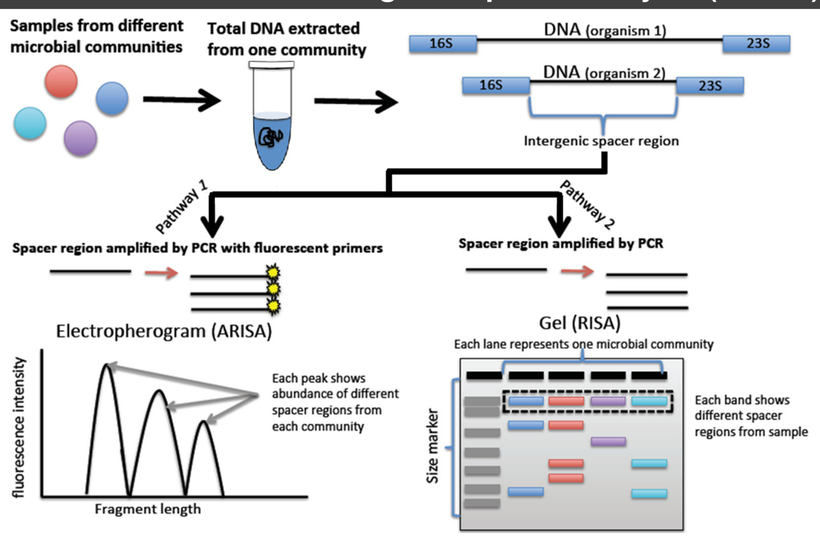
Recent data suggest that composition of intestinal microbiota can influence a number of physiologic processes (Hentges et. Al., 1985; Steffen and Berg, 1983). In murine models, variable compositions can result in differences in model phenotypes; thus, microbiota represents a potential study variable. Cryo-preserved mutant mouse strains maintained as germplasm are resuscitated using an outbred surrogate dam, resulting in colonization of pups with differing microbiota than the original line. As a result, model phenotypes may inadvertently change. To address this, our long term goal is to better define microbiota "enterotypes" of mice and take these into consideration when resuscitating mice from cryo-preserved germplasm. Pragmatically, this paradigm will require creation of surrogate dams with differing microbiota. We anticipate these will be generated through microbiota transfer and performed the following study to optimize such transfers and assess their long term stability.

METHOD



Identical experiment with **Donor CD-1** and **Recipient C57BL/6** mice performed in parallel

Automated Ribosomal Intergenic Spacer Analysis (ARISA)



INITIAL STUDIES

- Microbiota of C57BL/6 and CD-1 mice was followed from weanling through early adulthood. In both strains, microbiota stabilized to a ARISA pattern that was similar among mice from the same strain, but different between the two strains.
- Two fecal transfers spaced 24 hours apart, a method used previously to change the microbiota composition (Markle et. al., 2013), was shown to be unsuccessful.
- Five fecal transfers spaced 24 hours apart each was shown to be inconclusive, as the initial microbiota composition of recipients was not varied enough from donor composition.

QUESTIONS & OBJECTIVES

- **Can one murine microbiota composition be transferred to a mouse with a different composition?**
  - *Oral gavage of CD-1 or C57BL/6 weanling mice with fecal samples from adult C57BL/6 or CD-1 mice, respectively, with different microbiota patterns as shown via ARISA.*
  - *Record microbiota composition of weanling mice post-gavage to determine success of fecal transfer.*
- **How stable is a microbiota transfer between individuals?**
  - *Samples will be taken 30 and 60 days post-initial gavage to determine stability of transfer.*

RESULTS

Oral Gavage of C57BL/6 Weanling Mice with Fecal Samples from Adult CD1 Donor Mouse

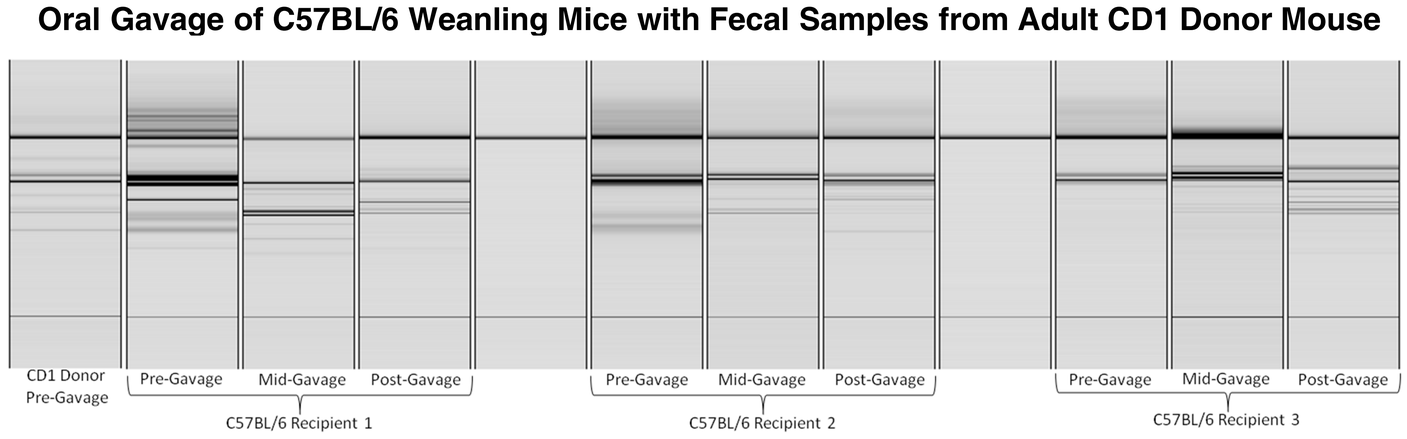


Figure 1. ***Change in microbiota of weanling recipient C57BL/6 mice after gavage of fecal samples from adult CD-1 donor mouse.*** Samples where taken pre-gavage (day 0), mid-gavage (day 7) and post-gavage (day 15) of the transfer to determine time course of microbiota changes. Samples are compared to CD-1 Donor (lane 1) to assess qualitative success of transfer.

Oral Gavage of CD1 Weanling Mice with Fecal Samples from Adult C57BL/6 Donor Mouse

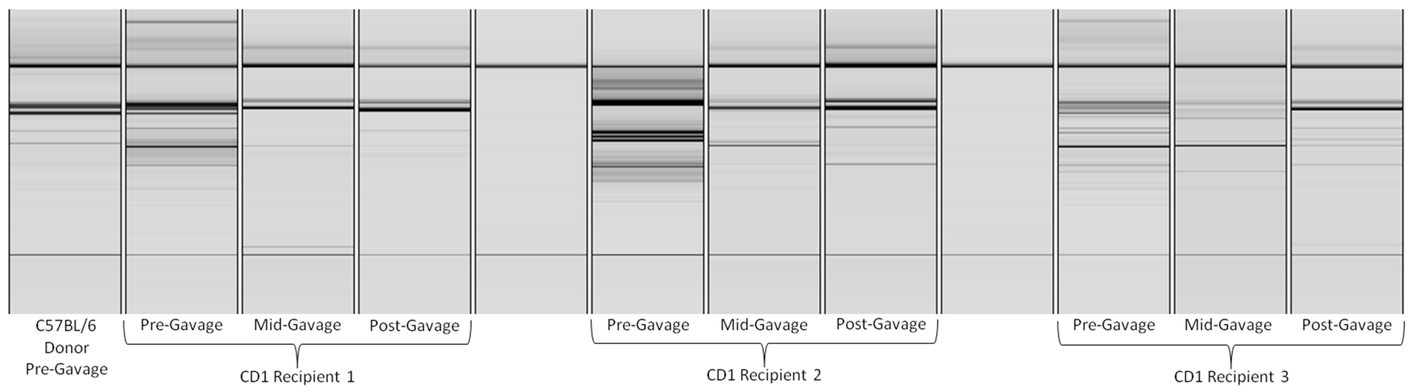


Figure 2. ***Change in microbiota of weanling recipient CD-1 mice after gavage of fecal samples from adult C57BL/6 donor mouse.*** Samples where taken pre-gavage (day 0), mid-gavage (day 7) and post-gavage (day 15) of the transfer to determine time course of microbiota changes. Samples are compared to C57BL/6 Donor (lane 1) to assess qualitative success of transfer.

CONCLUSION

- **Qualitative analysis of ARISA data shows a shift in recipient microbiota to resemble donor composition post-Streptomycin dosage and 6-dose fecal transfer**
  - *ARISA shows a gradual shift in microbiota composition, suggesting the requirement of multiple transfers to obtain results*
  - *Further analysis via sequencing will provide quantitative data of phyla composition*

FUTURE INVESTIGATION

- Quantitative analysis of microbiota transfer to obtain percentages of Bacteroidetes and Firmicutes pre- and post- gavage of weanling recipients.
- Identification of potential enterotypes in the murine species.
- Assess whether transfer of cryo-preserved microbiota is equivalent to transfer of fresh microbiota.
- Assess whether microbiota transfer recipients transfer their modified microbiota to embryo transfer pups

ACKNOWLEDGMENTS

The research team would like to thank the following sponsors for their generous contributions to funding of this project:

American Society of Laboratory Animal Practitioners
IDEXX-RADIL
NIH U42 OD010918