

Interpersonal Variations in Gut Microbiota Profiles Supersedes the Effects of Differing Fecal Storage Conditions.

Caspar Bundgaard-Nielsen, Søren Hagstrøm, Suzette Sørensen

Supplementary images

Title: Interpersonal Variations in Gut Microbiota Profiles Supersedes the Effects of Differing Fecal Storage Conditions.

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Affiliations:

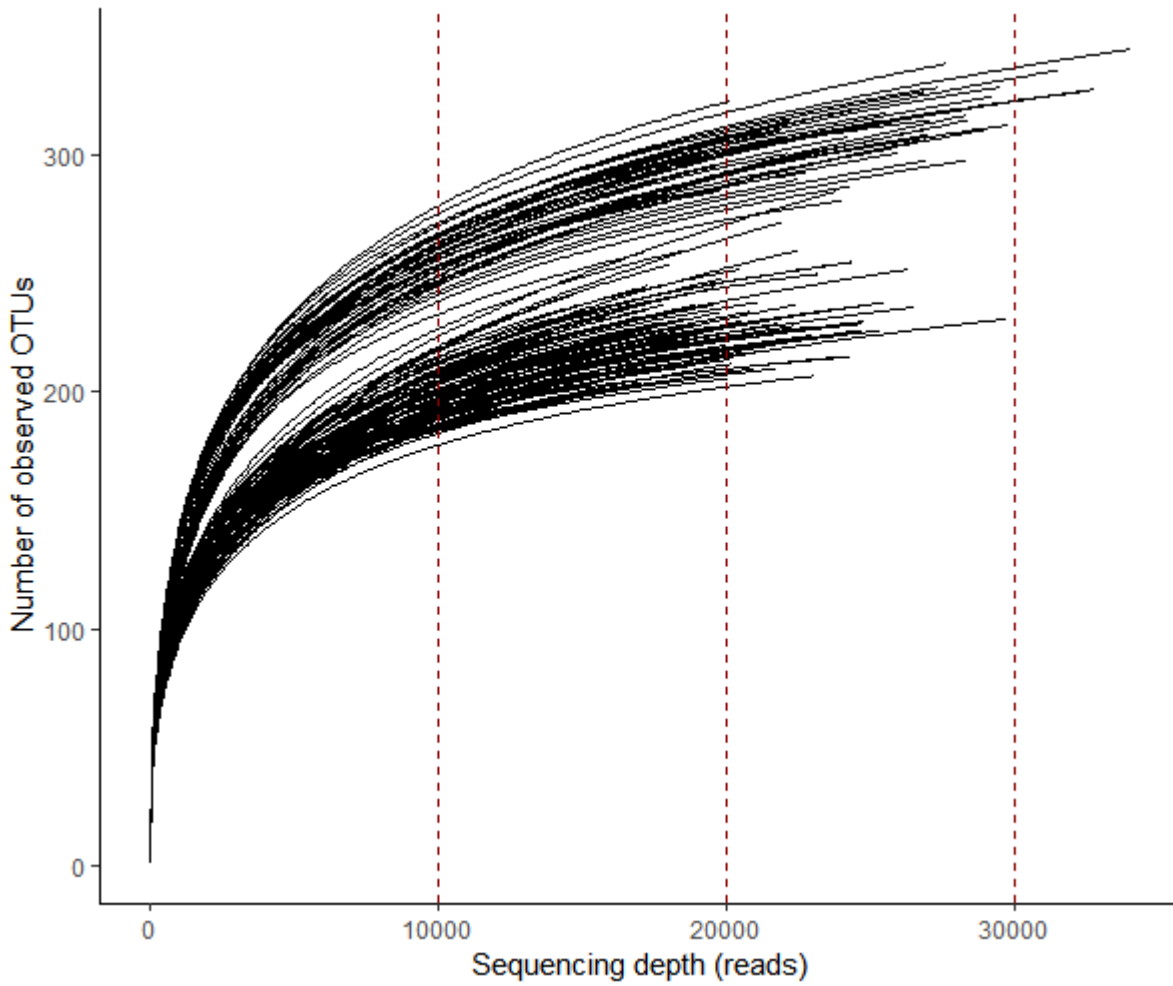
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1.1 Rarefaction curve



Generated in R through the Rstudio IDE, in ampvis2 package v.2.3.11. Note generation of new OTUs had started to plateau following end of sequencing. More than 10.000 reads were obtained in all samples.

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1.2 A comparison of triplicates and individual samples



For each donor, the heatmap is faceted by each setup, with the bacterial composition of each of the three triplicates being compared side-by-side. All triplicates are highly similar.

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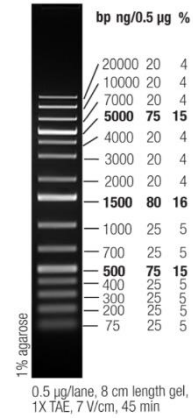
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1.3 Gel electrophoresis

DNA integrity of all fecal samples, following DNA extraction, were evaluated using agarose gel electrophoresis. The following characteristics for the electrophoresis were used:

- 1 % agarose gel in TBE buffer
- 1:10,000 concentration of Gelred Nucleic Acid Gel stain (Biotium)
- 100 V for 3 hours and 40 minutes
- GeneRule 1kb plus DNA Ladder (Thermo Scientific, see the image to the right).

GeneRuler 1 kb Plus DNA Ladder, ready-to-use



For each replicate, for each separate donor, the following setup was used for load the gel:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ladder	-80 °C	-20 °C - 24h	-20 °C - 72h	PSP, RT - 24h	PSP, RT - 72h	PSP, 4 °C - 24h	PSP, 4 °C - 72h	DNA/RNA shield, RT - 24h	DNA/RNA shield, RT - 72h	DNA/RNA shield, 4 °C - 24h	DNA/RNA shield, 4 °C - 72h	RNA-later, RT - 24h	RNA-later, RT - 72h	RNA-later, 4 °C - 24h	RNA-later, 4 °C - 72h

Following electrophoresis, the gels were visualized using a SynGene G-boks with automatic exposure. A subsequent manual overexposure of 3 seconds was used, to improve visualizing DNA integrity.

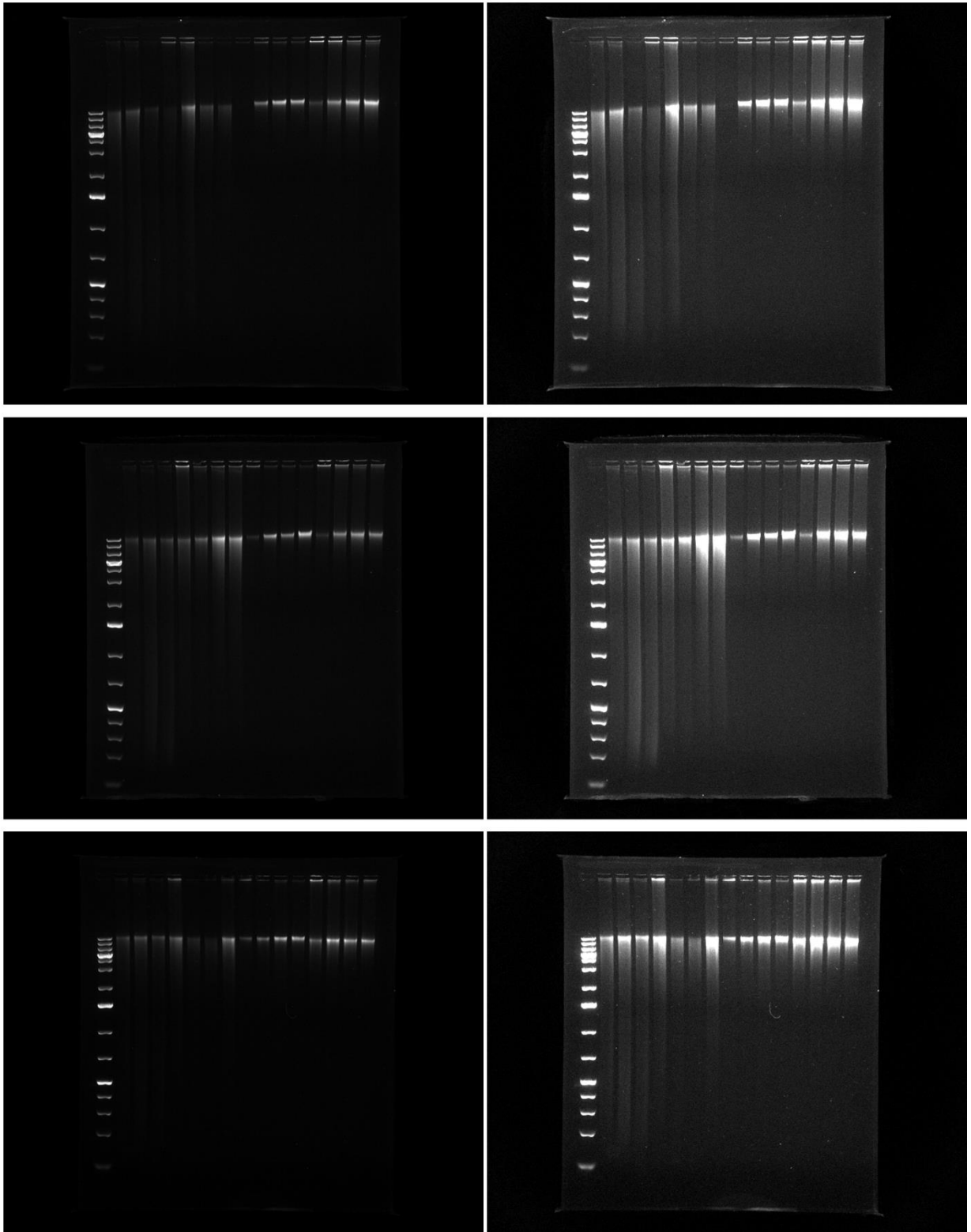
The resulting gels can be seen on the next three pages.

Donor A

Normal exposure

3 sec. overexposure

Triplicates

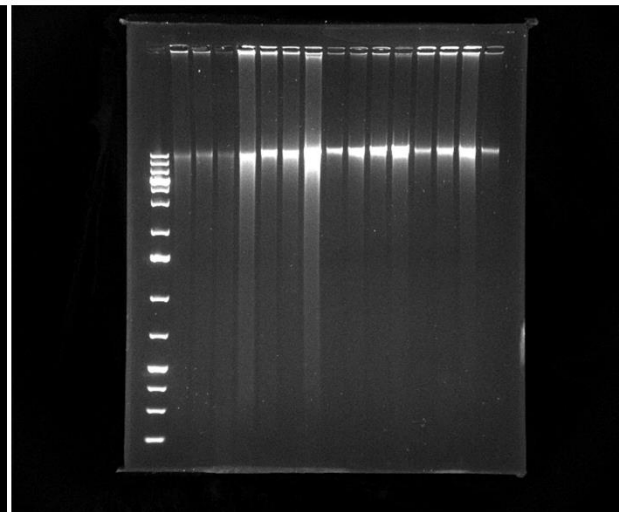
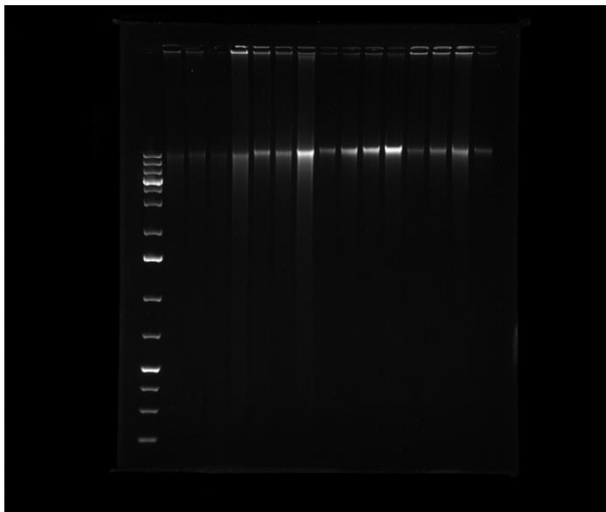
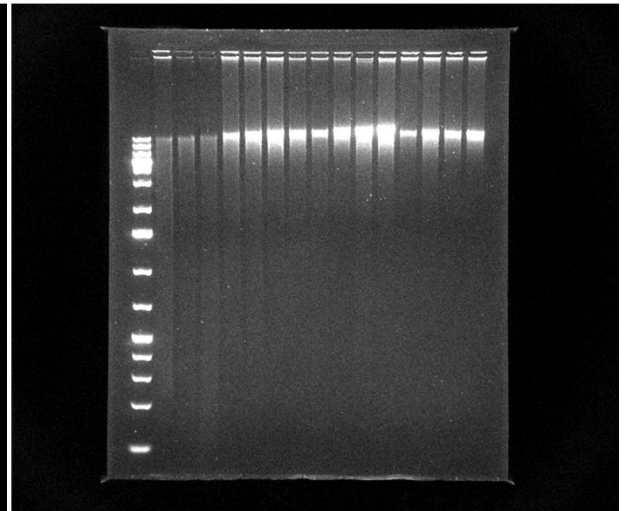
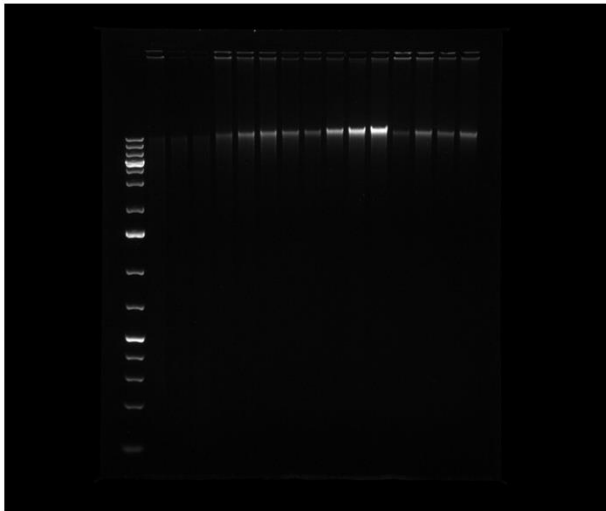
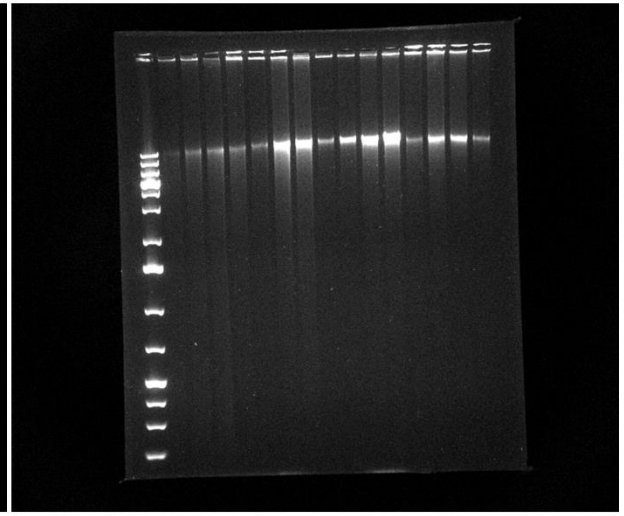
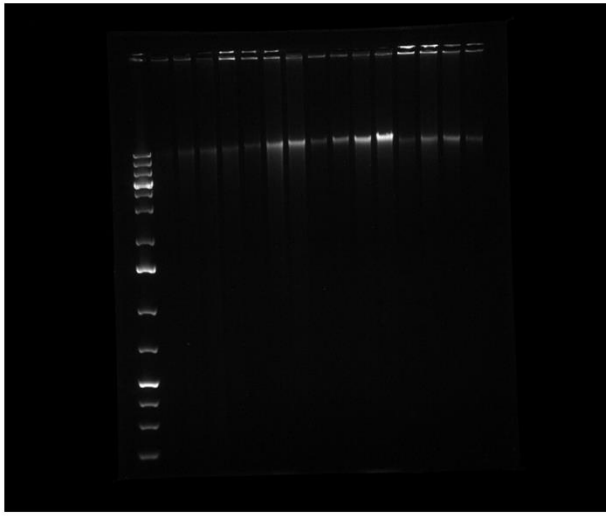


Donor B

Normal exposure

3 sec. overexposure

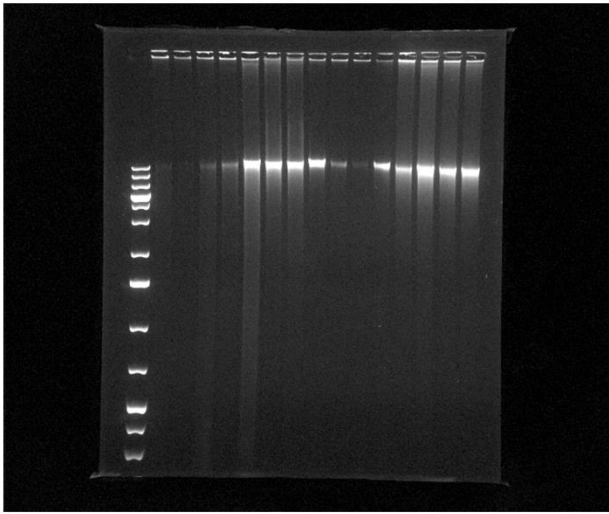
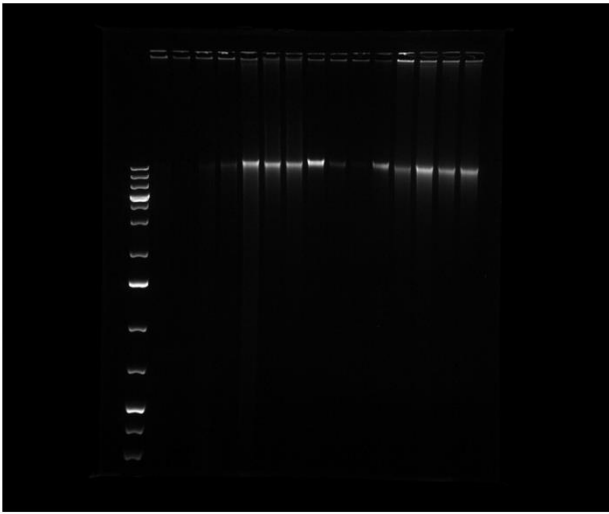
Triplicates



Donor C

Normal exposure

3 sec. overexposure



Triplicates

