Contributions of non-corresponding authors

Octavio Mondragon-Palomino

1. **Idea generation.** Conceived the project with RFI. Contributed financially to the project.
2. **Preliminary experimental work.** Demonstrated the feasibility of the project in preliminary experiments. Learned necessary technologies that were originally unavailable in the lab. Literature search and *in silico* testing of probes. Tested multiple tissue sample preservation strategies.
3. **Method development.** Conceived and developed the method's workflow. Conceived and supervised the development of *in vitro* hybridization assays in shallow gels. Conceived lysozyme treatment optimization in gels.
4. **Data accumulation.** Planned and performed all tissue sample preparation and *in situ* microscopy. Trained and advised RP and JG in microscopy for *in vitro* hybridization assays. Performed and analyzed controls for *in situ* HCR. Obtained spectral references and executed spectral imaging strategy. Collected samples for sequencing with RP. Planned and performed ciprofloxacin challenge experiments.
5. **Data analysis.** Processed all *in situ* imaging, extracted all data from *in situ* imaging, conceived and executed spatial analysis in crypts, conceived statistical analysis across crypts (HCA) with AL, calculated population correlations across crypts. Made measurements of mucus layers of the proximal colon. Analyzed in situ HCR controls. Conceived and supervised data analysis for *in vitro* hybridization assays in shallow gels. Planned and performed all data analysis for ciprofloxacin challenge experiments.
6. **Outline writing.** Conceived and wrote outlines.
7. **Figure generation.** Created the figures for the main text except Fig. 2c and Fig. 6. Created early versions of Fig. 5b with data by JG. Created Supplementary Figs. S5-S6, and created Supplementary Figs. S3-S4 with materials provided by JG and RP. Created Supplementary Table S2. Created Supplementary Table S1 with material provided by JG. Created Supplementary Videos.
8. **Manuscript writing.** Wrote the manuscript.
9. **Addressed all reviewer and editorial requests.**

Roberta Poceviciute

1. **Method development.** Major contributor to the development of the sample preparation method. Developed the assay for the optimization of lysozyme treatment.
2. **Data accumulation.** Collected all data of lysozyme treatment optimization. Collected samples for sequencing with OMP.
3. **Data analysis.** Analyzed all data of lysozyme treatment optimization experiments in coordination with OMP. Contributed to the analysis of data from *in vitro* hybridization assays in shallow gels.
4. **Figure generation.** Created Fig. 2c and Supplementary Figs. S1-S2. Supplied plots for Supplementary Fig. S4, and color intensity plot for final version of Fig. 5b.
5. **Manuscript writing.** Wrote methods and results/discussion of lysozyme treatment optimization.

Antti Lignell

1. **Idea generation.** Conceived quantitative multiplexing of bacteria by HCR staining together with OMP. Conceived the idea of hierarchical clustering analysis (HCA) approach to describe bacterial colonization patterns in strong collaboration with OMP. Contributed financially to the project.
2. **Preliminary experimental work.** Participated in preliminary experiments regarding HCR staining of bacteria.
3. **Method development.** Developed the HCR staining protocol together with OMP. Advised on the development of sample mounting protocol.
4. **Data analysis.** Developed the code and performed the HCA and tSNE analyses, as well as mapping crypt states to the physical space.
5. **Figure generation.** Generated plots for Fig. 6 and Supplementary Fig. S7.

6. **Manuscript writing.** Contributed to main text related to HCA and tSNE analyses. Edited late versions of the text.

Jessica Griffiths

1. **Idea generation.** Contributed to the conception of *in vitro* hybridization assays in shallow gels.
2. **Preliminary experimental work.** Researched and selected bacterial species for positive controls of clept1240 detection sequence.
3. **Method development.** Developed and troubleshot *in vitro* hybridization assays in shallow gels.
4. **Data accumulation.** Performed all microscopy for *in vitro* hybridization assays in shallow gels (Fig. 5b and Supplementary Fig. S3-S4).
5. **Data analysis.** Developed a computational image processing pipeline in commercial software (Imaris) for the quantification of *in vitro* hybridization assays in shallow gels. Analyzed the resulting data (Supplementary Fig. S4). Analyzed data for Fig. 5b.
6. **Figure generation.** Provided early versions of Fig. 5b, Supplementary Fig. S3-S4.
7. **Manuscript writing.** Provided written summary of the methods, data collection and data analysis for *in vitro* hybridization assays in shallow gels.

Heli Takko

1. **Method optimization**. Preliminary/exploratory optimization of HCR v2.0 conditions
    - Troubleshooting of CFB560 cross-reactivity against E. coli in vitro gels
    - Formamide curve generation for MUC1437 probe against A. muciniphila in vitro gels (true target)

2. Data analysis. Preliminary/exploratory image segmentation in Ilastik.