Background
Tumor-resident bacteria are an emerging component of the tumor microenvironment. Recent studies have shown their presence over multiple cancer types. Lately, mechanistic evidence in a murine breast cancer model indicates that these bacteria promote the metastatic process. However, the presence and configuration of the microbiome of human metastatic breast tumors have not been determined. Here, we characterized tumor-resident bacteria in a cohort of metastatic hormone receptor-positive breast cancer (MHRBC) patients with matched primary tumors.

Methods
We performed 16S rRNA sequencing targeting seven hypervariable regions (V2, V3, V4, V6-7, V8 and V9) in FFPE tissues from 40 patients with MHRBC and their matched primary tumors, all primary tumors had positive hormone receptors (estrogen and progesterone). Sequencing was performed using the Ion Torrent 16S Metagenomics kit and the IonPGM instrument. A machine learning classifier using 5 unique amplicons was generated to predict the metastatic site from selected amplicons. All analyzed reads showed a quality score of Q30.

Sequence data were processed using high resolution sample inference with DADA2. Controls included normal breast tissue, paraffin from all blocks and a simulated bacterial community. Taxonomy was assigned using the SILVA database v138. Feature selection was used to determine amplicon sequences in primary tumors related to their metastasis. A machine learning classifier was generated to predict the metastatic site from selected amplicons. All analyzed reads showed a quality score of Q30.

Conclusions
- We identified and categorized the tumor-resident bacteria of MHRBC.
- α-diversity was similar among sample types.
- β-diversity showed segregation between metastatic and primary tumors.
- Alphaproteobacteria, Gammaproteobacteria and Bacilli had increased relative abundance across metastatic and primary tumors.
- Differential abundance of Proteobacteria and Firmicutes species was identified in metastatic tumors.
- A machine learning classifier using 5 unique amplicons in primary tumors was capable of 100% precision and high recall for prediction of bone and liver metastatic site, but not lung metastases.

Acknowledgements
This study was supported by Chilean National Agency for Research and Development (ANID). Proyecto Fondecyt Regular Nº 1191743 (DC). Beca Doctoral ANID Nº 21201589 (CA).