Human, Bacterial and Fungal Amplicon Collection and Processing for Sequencing

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Summary of Q&A (last updated on 9/19/2016)

During the experiment

Q#1. *What is the rationale that you only start with 100 µl for the extraction, which is rather small volume to moisten the Catch All swab?*

A. Because of the low biomass in the skin, we try to keep the volumes low. This amount is adequate to moisten the swab for sampling a 4cm2 area. If you are planning on sampling a larger area, it'd be worth experimenting with larger volumes.

Q#2. *Does the MasterPure kit, which is designed for yeast DNA extraction, affect the efficiency on extracting bacterial DNA, since I did not detect correct size band after PCR? Should I lyse the cells longer in the bead beater to improve the lysis?*

A. We've had good experience co-extracting bacterial DNA using this protocol (we use the same eluate for both 16S and ITS sequencing). Depending on the skin site, we may or may not see a band on the gel. High yield sites (e.g., inner nares) can have a band. Did you do a positive control? You might run a positive extraction control, like the nares. In our experience, the combination of the lysis buffer + bead beating is sufficient to lyse both bacterial and fungal cells.

Q#3. *How to measure the concentration of the DNA after extraction other than using NanoDrop? How much DNA you normally get using this protocol to isolate skin microbiome in your experience?*

A. Try the high-sensitivity (not broad range) Qubit kit. We find that NanoDrop is not accurate at low concentrations.

Yield varies very significantly by skin site. For example, for the inner nostril, you might expect 50 ng/uL; for a low biomass sites like the forearm or inner elbow you might achieve 0.5 ng/uL. Run your negative control in your PCR (also include a negative control for the PCR only), and see if you get a detectable band with yield--that will be informative as to potential contamination in your collection method.