March 25, 2013
Video conference on Surface Sampling.

Participants:
Oregon: Adam Altrichter, James Meadow, Ashley Bateman
Berkeley: Brandon Bubba Brooks and Rachel Adams
Cornell: Denina Hospodsky
UT Austin: Juan Pedro Maestre Wic, Alexandra Caya, Chloe Wooldridge

On the issue of cotton vs. nylon swabs:
There does not appear at this time to be a clear indication of which material is best for subsequent extraction. J. Meadow cites Probst et al 2010 validating nylon-flocked swabs. Other work (e.g. Brownlow et al 2012) claims cotton in better. B. Brooks has collected some of these references and will start a Mendeley group. They will be tagged with the punchline as to which material performed better.

On the issue of negatives:
It appears more common to get amplification in the negative when targeting fungi than targeting bacteria. R. Adams bioinformatically removes OTUs detected in negative controls from other samples. B. Brooks notes that M. Miletto has shown that PCR reagents and polymerase can affect amplification of bacteria negatives. D. Hospodsky noted that dilution of master mix can avoid the E. coli trace in bacteria amplification (see Spangler et al: "Optimizing Taq polymerase concentration for improved signal-to-noise in the broad range detection of low abundance bacteria" in PlosOne 2009).

	Environment	Sampling Device & Buffer	Extraction	Notes
Oregon	Classroom	Swab 17x17cm sampling square with a Nylon-flocked-swab moistened in a tween-salt solution	Cut tip into tube, bead-beat, MoBio PowerWater DNA kit	Use the red (552C) Nylon-flocked-swab as it is sturdier than the green (551C)
	Chamber	Passive collection on large petri dish sitting on surface, cotton swab moistened in a tween-salt solution wiped along dishes to collect dust.	Cut tip into tube with 4ml PBS and vortex 10 min. Concentrate soln to ~500ul with pink Amicon 30K Centrifuge Filter Units, use ~half with MoBio Power Soil	We now have confirmation that this method worked successfully, petri dishes only in chamber for 2 hrs.

			Kit	
Berkeley	Hospital	Foam swab	Cut tip into tube, MoBio Power Soil kit	
	Homes	Cotton-tipped applicator wiped along surface, moistened with water	Followed Fierer et al 2010: Tip cut into provided MoBio Power Soil Kit, incubated at 65C for 10 minutes before horizontal vortexing for 10 minutes.	Drains showed less efficiency than dry surfaces with this extraction method
Cornell	Homes	Nylon-flocked-swab moistened with PBST		Notes importance of standardizing time and area of swab
	Homes	Wipes: 9 x 9in, used to swab a square meter of floor (compare to Yamamoto et al: Assessing allergenic fungi in house dust by floor wipe sampling and quantitative PCR, Indoor Air 2011)	Wipes shaken overnight in PBST, isolated onto 0.2um filter using a filter gallery/ vacuum filtration	Can elute in 20ul to concentrate DNA in DNA elution step (using thinner silica filters in spin filter step such as Qiagen kits); often uses 1:10 dilution of genomic DNA for subsequent amplification due to inhibitors
UT Austin	Homes – shower drains	Foam swabs (VWR) moistened and stored in PBS	Trying different methods and showing manual is often better than kits: PowerSoil had lower yield than mercaptoethanol for fungi, for example.	Note that there may be better extractions protocol for targeting bacteria vs. fungi