**MetaPolyZyme (MPZ) Extreme Microbiome Project Protocol**

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**Important before starting:**

* All samples must be free of inhibitors such as heavy metals, humic acids, polyphenols, tannins, and heavy polysaccharides, etc.
* All samples must be resuspended in pH 7.5 PBS+- 0.2, in order for maximum enzymatic digestion. The final sample suspension pH should be checked by pipetting 5 ul onto litmus paper( EMD ColorPast 6.5-10, Cat# 9583)
* Absolutely no EDTA or proteinase K can be present during incubation with MetaPolyzyme (MPZ) treatment. EDTA will kill the enzymatic activity and ProK will destroy the MPZ.

**Protocol:**

1. Start with enough sample that can be thoroughly mixed (≤ ~50 mg).
   1. Amount of sample used is experiment and PI specific.
   2. OPTIONAL:
      1. If salts, heavy metals, sulfur compounds, acids, or etc. are present in the sample, wash the sample by simple pellet centrifugation and resuspending in 1X PBS, pH 7.5.
      2. If the sample is in water or has significant volume, add enough 10X PBS, pH 7.5 to equal 1-2X PBS final. Double check the pH by pipetting 5 ul on to litmus paper. A good total working volume is 100-200 ul.
      3. Add Sodium Azide to a final concentration of 0.2- 0.5% to inhibit bacterial growth during the MetaPolyzyme (MPZ) incubation.
2. Add 2-20 ul of MPZ per 100 ul of total sample volume (see graph below).
   1. OPTIONAL: Add an equal amount of DNA-free lysozyme to the sample to boost digestion.
3. Incubate at 35°C from 2 - 6 hrs (can incubate up to 12 hrs).
   1. If the end goal is RNA, the addition of RNase inhibitor is required (ie RiboLock).
   2. It is also better to do a shorter incubation time when trying to recover RNA.
4. After incubation, the samples can be processed using any favorite DNA kit.
   1. OPTIONAL:
      1. Heat samples to 90°C for 10 min to kill the enzyme, followed by snap freeze at -80°C and then heat again to 90°C to "pop" the cells. This optional freeze-thaw step is performed rapidly. Fianlly add Proteinase K and move on to favorite kit.
      2. If RNA is the end goal, omit the optional freeze thaw step above and go straight to Trizol or RLT and follow the standard protocol for the specified volume.