PROTOCOL: Direct PCR from Nail Samples

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Sample:

- Nail clippings
    - Nails should be cut into small pieces to maximize exposed surface area

Materials:

- Entopsis Inc.:
    - PCR*opsis*™ Reagent RVD with RVD Enhancer (see product IFU)
    - PCR*opsis*™ Lysis Beads (see product IFU)
- PCR master mix
- Primers
- Test sample
- 2 mL round-bottom tubes
- Thin-walled PCR tubes
- qPCR Thermocycler

Methods:

1. Cut nail samples into small pieces measuring less than 0.5 cm in length to maximized exposed surface area
2. Add roughly 0.25 grams of Lysis Beads to a 1.5 ~ 2 mL vial or well in a deep, 96-well plate
    1. One full PCR*opsis*™ Lysis Bead Scoop holds ~0.3 grams of beads
3. Place nail pieces into a vial or well with Lysis Beads
    1. A 2 mL, round-bottom tubes works best
4. Add ~200 µL of nuclease-free water to the vial or well with nail pieces and Lysis Beads
5. Cap tube or place a plate sealer on the deep well plate
6. Vortex on high for ~5 minutes to lyse microorganisms
7. Mix 20 µL PCR*opsis*™ Reagent RVD with RVD Enhancer with 20 µL of the sample pre-processed with PCR*opsis*™ Lysis Beads in a thin-walled tube or plate (0.2 ~ 0.6 mL)
    1. **For optimal results, the reagent needs to be added first to the tube/well before the sample is added.**
    2. Ratio of sample to PCR*opsis*™ reagent will remain 1:1, but volume can be increased if needed (example: 30 µl : 30 µl and so forth)
8. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
9. Heat diluted sample at 95˚C for 15~20 minutes in a thermal cycler or controlled heat block with a lid, and let cool at room temperature for ~10 seconds before continuing
    1. Heating for a slightly longer period (extra 2~5 minutes) of time does not negatively affect results
    2. Make sure the heating device has reached the desired temperature before applying sample.

3. You may need to increase the heating time if increasing the volume of sample and reagent past 100 µl of each
4. Sample heating can be performed using a controlled heating block or thermal cycler; however a device lid is highly recommended to minimize popping of tube caps or unpeeling of the plate sealer

10. Mix heated sample and use lysed / stabilized sample in your desired PCR procedure
    1. Lysed / stabilized sample should represent 15% ~ 30% of your final PCR mixture (i.e., 3~6 µL sample into a total volume of 20 µL) depending on the polymerase used
    2. You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample

Suggested PCR conditions:

1. Add ~5 µL of heated sample to 15 µL of qPCR mixture.
    a. qPCR mixture:
        i. 5x PCR Master Mix: 4 µl
        ii. Forward primer (10 pmol/ µl): 1 µl
        iii. Reverse primer (10 pmol/ µl): 1 µl
        iv. Probe (10 pmol/ µl): 0.5 µl
        v. Nuclease-Free Water: 8.5 µl
2. Run samples on qPCR Thermocycler for 45 cycles.
    a. DNA Amplification:
        i. 95˚C 5 minutes (initial denaturation)
            1. 95˚C 30 seconds
            2. 55˚C 30 seconds
            3. 72˚C 30 seconds
        ii. 72˚C 60 seconds (final extension)
        iii. 4˚C hold