



PROTOCOL: Direct PCR from Urine Samples

v. 20220510

Sample:

- Urine sample
 - bacterial cells were pelleted at $>1,400xg$ for ~10 minutes
 - most of the urine supernatant was removed following centrifugation, leaving only 100 ~ 500 μL of urine over the pellet

Materials:

- Entopsis:
 - PCRopsis™ Reagent RVD with RVD Enhancer (see product IFU)
 - PCRopsis™ Support (see product IFU)
 - PCRopsis™ Lysis Beads (see product IFU)
- PCR master mix
- Primers
- Test sample
- Thin-walled PCR tubes
- qPCR Thermocycler

Methods:

OPTIONAL: PCRopsis™ Lysis Beads are recommended when amplifying gene targets from difficult to lyse bacteria and yeast / fungi.

- A. Add 200 μL of sample to a vial or well with Lysis Beads
 - B. Cap tube or place a plate sealer on the deep well plate
 - C. Vortex on high for ~5 minutes to lyse microorganisms
1. Add 2 μL of PCRopsis™ Support to 1 mL PCRopsis™ Reagent RVD with RVD Enhancer, and mix thoroughly
 - a. This mixture is stable for 24 hours
 2. Thoroughly mix 20 μL PCRopsis™ Reagent RVD with RVD Enhancer with PCRopsis™ Support + 20 μL of your sample in a thin-walled tube (0.2 ~ 0.6 mL) and cap tube
 - a. **For optimal results, the reagent needs to be added first to the tube/well before the sample is added.**
 - b. Ratio of sample to PCRopsis™ reagent will remain 1:1, but volume can be increased if needed (example: 30 μL : 30 μL and so forth)
 3. Heat mixture of reagent + urine sample for 10 ~ 20 minutes at 95°C and let cool at room temperature for ~10 seconds before continuing
 4. Mix thoroughly
 5. Add 3~6 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/ μ l): 0.5 μ l
- v. Nuclease-Free Water: 8.5 μ l
- 6. 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