PROTOCOL: Direct PCR from Urine Samples

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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:
  - PCR*opsis*™ Support
  - PCR*opsis*™ Reagent RVD with RVD Enhancer
- PCR master mix
- Primers
- Test sample
- Thin-walled PCR tubes
- qPCR Thermocycler

Methods:

1. Add 2 µL of PCR*opsis*™ Support to 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer, and mix thoroughly
   a. This mixture is stable for 24 hours
2. Thoroughly mix 20 µL of your sample + 20 µL PCR*opsis*™ Reagent RVD with RVD Enhancer with PCR*opsis*™ Support in a thin-walled tube (0.2 ~ 0.6 mL) and cap tube
3. Heat mixture of reagent + urine sample for 10 minutes at 95˚C and let cool at room temperature for ~10 seconds before continuing
4. Mix thoroughly
5. 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
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