

mtDNA WI-06 Amplification

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Take out box of reagents (stored in freezer)

Turn on thermal cycler

Clean Laminar Flow Hood: Fresh 20% (in house) Bleach or 10% stabilized bleach

Isopropyl wipe: 3 pipettors (p10, p20, p200), 1.5mL rack,

Falcon tube rack

UV for at least 15 minutes

Stratalink: Labeled 0.2mL PCR tubes

Amp rack

Tube of dH₂O

Labeled 0.5mL tubes for Master Mixes

Stratalink for at least 15 minutes

| | | |
|-----------------------|-------------------|--------|
| Make 5X Master Mixes: | dH ₂ O | 30μL |
| | 10X Buffer | 12.5μL |
| | BSA | 12.5μL |
| | dNTP's | 10μL |
| | Primer1 | 2.5μL |
| | Primer2 | 2.5μL |
| | Taq Gold | 5μL |

Vortex, spin down each tube

Aliquot 15μL of each Mix into the appropriate labeled 0.2mL tubes

Make Negative: Add 10μL of dH₂O into each NC tube

Make Reagent Blank: Add 10μL* of reagent blank into each RB tube

Make Sample ; Add 10μL* of sample into each Q/K tube

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Make Positive: Add 10µL of HL60 into each positive tube
Spin down tubes and place into thermal cycler

***Questioned/Low template Samples**

- HV1A, HV1B, HV2A, HV2B
- A1/B2 for HV1A, A2/B1 for HV1B
- C1/D2 for HV2A, C2/D1 for HV2B
- Do not dilute RB's
- 36 cycles

***Known /High template Samples**

- HV1, HV2
- A1/B1 for HV1, C1/D1 for HV2
- Blood 1:10, Buccal 1:50
- Dilute RB's 1:10
- 32 cycles

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