

mtDNA WI-06 Amplification

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Approved by Director: Dr. Guy Vallaro

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

Master Mix (1 sample):

dH ₂ O	6μL
10X Buffer	2.5μL
BSA	2.5μL
*dNTP's	2μL
+Primer1	0.5μL
+Primer2	0.5μL
Taq Gold	1μL
Total:	15μL

In general, master mix will be made as an n+1 batch, where n = the number of samples and controls to be amplified.

*Alternatively, individual dNTPs (dATP, dCTP, dGTP, and dTTP) may be added at a volume of 0.5 μL (each dNTP) per 1X master mix.

+Primer Sets to be used:

Question/Low Template Samples

HV1A, HV1B, HV2A, HV2B

HV1A: A1/B2

HV1B: A2/B1

HV2A: C1/D1

HV2B: C2/D1

Known /High template Samples

HV1, HV2

HV1: A1/B1

HV2: C1/D1

Vortex, spin down each tube

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

For Question/Low Template samples extracted using PCIA:

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

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

Make Sample: Add 10µL of sample into the sample tube

Make Positive: Add 10µL of HL60 into the positive tube

For known samples extracted using PCIA:

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

Make Reagent Blank: Dilute RB 1:10 and add 10µL of reagent blank into the RB tube

Make Sample (Buccal): Dilute sample 1:50 and add 10µL of sample into the sample tube

Make Sample (Blood): Dilute sample 1:10 and add 10µL of sample into the sample tube

Make Positive: Add 10µL of HL60 into the positive tube

For known samples extracted using EZ1:

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

Make Reagent Blank: Add 1µL of reagent blank and 9µL of dH₂O into the RB tube*

Make Sample: Add 1µL of sample and 9µL of dH₂O into the sample tube*

Make Positive: Add 10µL of HL60 into the positive tube

Note: EZ1 batches contain only one RB. The batches may be broken down into sets for ease of processing. For each amplification set: The RB (from the batch), Negative Control and POS will be amplified.

*If necessary amount of sample used can be adjusted up to 10µL. RB must reflect largest volume used in amplification set.

Spin down tubes and place into thermal cycler

Questioned/Low template Samples
- 36 cycles

Known /High template Samples
- 32 cycles