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 dH_20
Labeled 0.5mL tubes for Master Mixes
Stratalink for at least 15 minutes

Make 5X Master Mixes:

dH_20	30µL
10X Buffer	12.5µL
BSA	12.5µL
*dNTP's	10µL
Primer1	2.5µL
Primer2	2.5µL
Taq Gold	5µL

*Alternatively, individual dNTPs (dATP, dCTP, dGTP, and dTTP) may be added at a volume of 2.5 µL (each dNTP) per 5X master mix.

Vortex, spin down each tube
Aliquot 15µL of each Mix into the appropriate labeled 0.2mL tubes

Make Negative: Add 10µL of dH_20 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

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