Isopropyl wipe: 2 Pipettors (p2, p10), 02.mL rack(s), 1.5mL rack

Take out: tube of Big Dye
Primers (including C stretch primers if necessary)
Allow to warm up to room temperature

Label: 0.2mL tubes **per primer-** NC, RB, Q/K, HL60
dilution tubes for each region- Q/K, HL60
(Remember extra tubes for C stretches)

Make Cocktail: Dispense 8µL of Big Dye into each tube
Followed by 5µL of the appropriate primer
Lastly add 7µL* of sample in the order of NC's, RB's, Q/K, HL60

*NC's/ RB's take straight 7µL

Note: For known samples processed using the EZ1 in a batch and amplified in a set, the RB's volume will reflect the largest volume used for a sample in the set. If necessary, dH_20 will be added up to 7µL

*For Samples/ Positive target ~8ng
Formula: $\frac{\text{Target Sequencing Amount}}{\text{CE Concentration}} = \text{Amount of Sample Needed}$

Ex. CE concentration K1 HV1 = 42.20ng/µL

$\frac{8\text{ng}}{42.20\text{ng}/\mu\text{L}} = 0.18\mu\text{L} = 0.2\mu\text{L}$

Total amount= 7µL
0.2µL sample + 6.8µL dH_20

Dilution for both primers (2X)= 14µL
0.4µL sample + 13.6µL dH_20

Vortex dilution tubes
Add 7µL into A1 tube and 7µL into B1 tube
Repeat for K1 HV2

Lastly make your dilutions for HL60 and add to the appropriate labeled tubes

Spin down tubes and place into Thermal cycler

Determine total ng Sequenced:

Ex. 42.20ng/µL X 0.2µL = 8.44ng= 8.4ng

Remember:

With C-stretches- count up all peaks for CE Concentration

Knowns- A4, B4 for HV1 C-stretch
- D2 for HV2 C-stretch

*Extra dilution tubes, may need to add water to NC's/RB's for HV1

Q's- A4 for HV1B, B4 for HV1A
- Proceed as usual for HV2 C-stretch (D2 takes care of it, however C4 can additionally be used)

*Extra dilution tubes