KAPA HiFi PCR protocol

Protocol for the amplification of DNA fragments > 1500 bp in size or for preparation of genomic libraries.

1. Get Primers, dNTPs, and KAPA HiFi Fidelity Buffer (5X) from freezer and thaw.
2. Label 8-well PCR tube strips with sample IDs, date, project name, and locus name.
3. Vortex all thawed reagents and microcentrifuge briefly to collect the solution in the bottom of tube.
4. Using **non-aerosol pipette tips**, prepare PCR master mix in a 1.5 mL microcentrifuge tube placed in ice block:

Component	Volume per sample
Nucelease Free Water	16.25 µL
5X Fidelity Buffer	5.0 µL
10 mM KAPA dNTP Mix	0.75 µL
10 µL Primer 1	0.75 µL
10 µL Primer 2	0.75 µL
1 U/µL KAPA HiFi Polymerase	0.5 µL

5. Vortex master mix, microcentrifuge briefly to collect solution in the bottom of tube, and return to ice block.
6. Place labeled PCR tube strips in ice block.
7. Reusing the **same non-aerosol pipette tip**, pipette **24 µL** of master mix to each of the strip tube wells.
8. Using a **different aerosol pipette tip** for each DNA sample, pipette **1 µL** of each DNA sample into labeled PCR tube strips.
9. Place lids on PCR tube strips, flick tubes to mix, microcentrifuge briefly to collect solution in bottom of tube and return to ice block.

10. Start protocol on thermocycler, wait for block temperature to pass **70°C**, then move the PCR tube strips onto the thermocycler block and close and tighten lid.

11. Label a 96 well plate and lid with the date, project name, and locus name and leave next to thermocylcer.

12. When thermocylcer protocol is complete place PCR tube strips onto labelled 96 well plate and move to 4°C refrigerator.