PCR using KAPA

- Dilute DNA template:
    1. 1 ng/µl if template is a PCR fragment
    2. 10 ng/µl if template is a plasmid
    3. 100 ng/µl if template is either a bacmid or genomic DNA
- Dilute primers to a final concentration in the reaction of 0.3 µM:
    1. Take 1.5 µl from each primer and add 12 µl water – this gets you 10 µM
    2. From the 10 µM tube take 1.5 µl into 50 µl reaction
- Set up the reaction (for 50 µl reaction):
    1. 1 µl DNA template
    2. 1.5 µl primer mix
    3. 22.5 µl water
    4. 25 µl 2X Kapa HiFi HotStart ReadyMix
- Thermocycler:
    1. 3 minutes at 95 °C
    2. 10-15 seconds at 98 °C
    3. 15-20 seconds at 60 °C (This is for annealing. Don't go below 60 °C . You can increase the temperature or use a gradient if you get non-specific amplification)
    4. 1 minute/kb template at 72 °C
    5. Repeat steps 2-4 for 29-34 cycles
    6. 5-10 minutes at 72 °C
    7. Keep at 12 °C