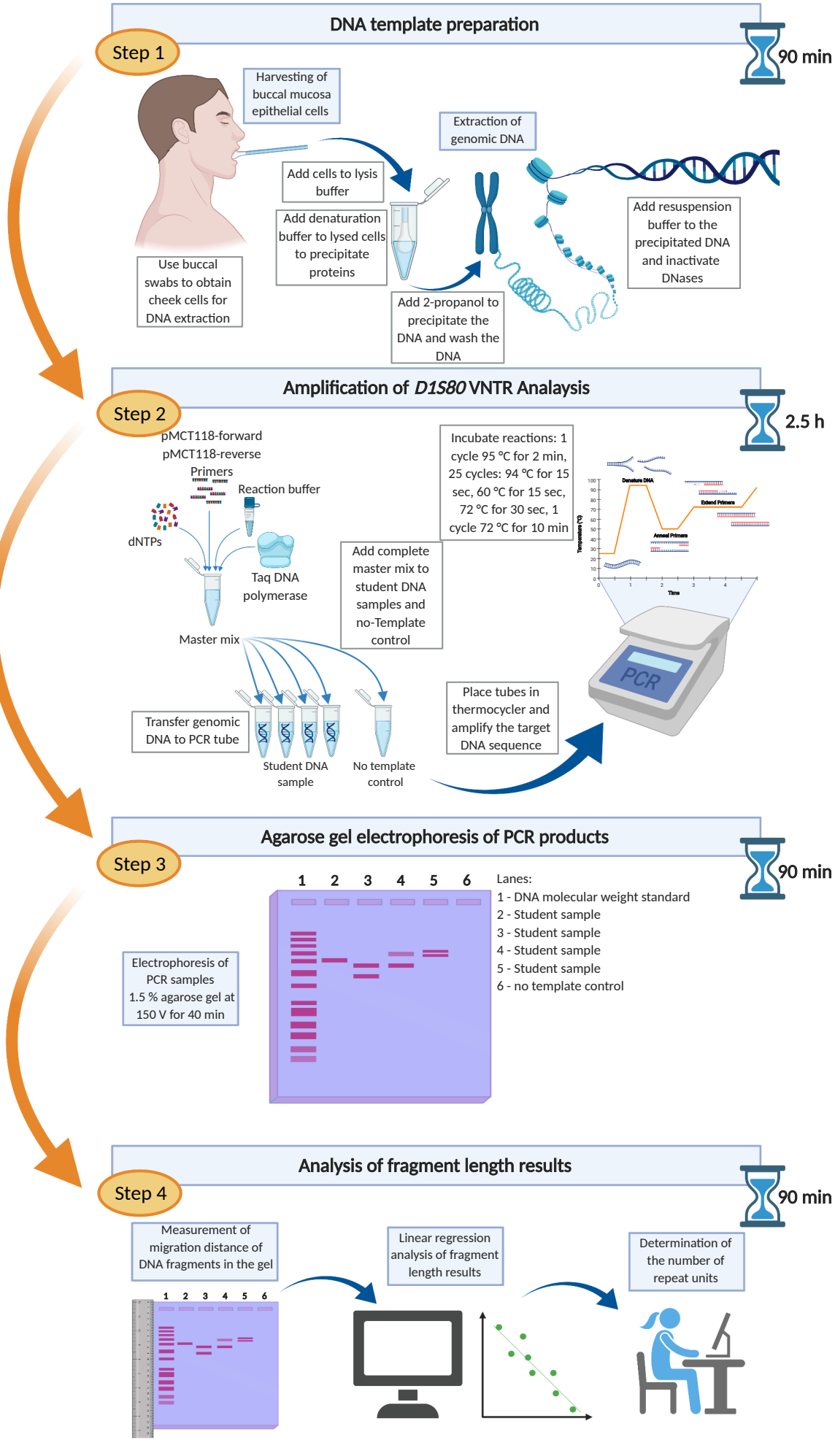
DNA template preparation
Step 1
90 min
Harvesting of buccal mucosa epithelial cells
Use buccal swabs to obtain cheek cells for DNA extraction
Add cells to lysis buffer
Add denaturation buffer to lysed cells to precipitate proteins
Add 2-propanol to precipitate the DNA and wash the DNA
Extraction of genomic DNA
Add resuspension buffer to the precipitated DNA and inactivate DNases
Amplification of D1S80 VNTR Analysis
Step 2
2.5 h
pMCT118-forward
pMCT118-reverse
Primers
Reaction buffer
dNTPs
Taq DNA polymerase
Master mix
Add complete master mix to student DNA samples and no-Template control
Incubate reactions: 1 cycle 95 °C for 2 min, 25 cycles: 94 °C for 15 sec, 60 °C for 15 sec, 72 °C for 30 sec, 1 cycle 72 °C for 10 min
Denature DNA
Anneal Primers
Extend Primers
Temperature (°C)
Time
PCR
Transfer genomic DNA to PCR tube
Student DNA sample
No template control
Place tubes in thermocycler and amplify the target DNA sequence
Agarose gel electrophoresis of PCR products
Step 3
90 min
1 2 3 4 5 6
Lanes:
1 - DNA molecular weight standard
2 - Student sample
3 - Student sample
4 - Student sample
5 - Student sample
6 - no template control
Electrophoresis of PCR samples
1.5 % agarose gel at 150 V for 40 min
Analysis of fragment length results
Step 4
90 min
Measurement of migration distance of DNA fragments in the gel
1 2 3 4 5 6
Linear regression analysis of fragment length results
Determination of the number of repeat units