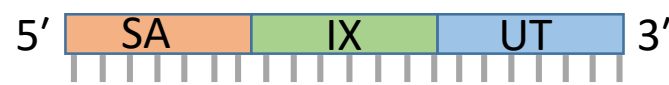
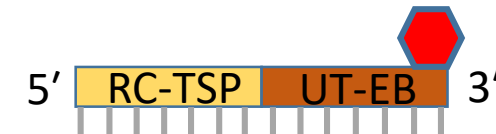
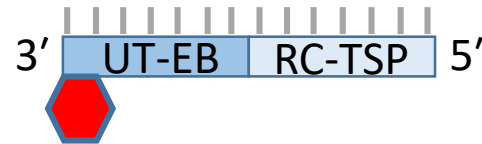


Reverse complement target specific probes with universal tail

(Universal)  
index primer (UIP)

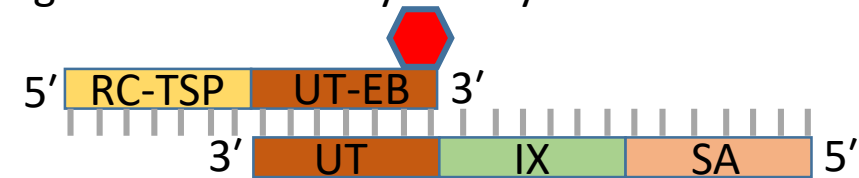
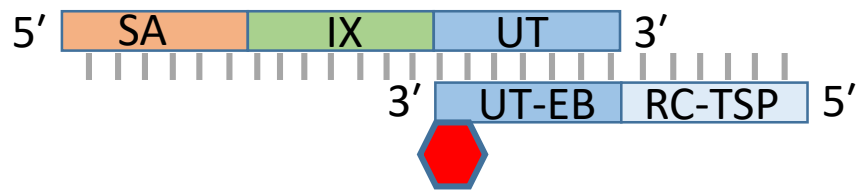


Reverse complement  
(RC) probe

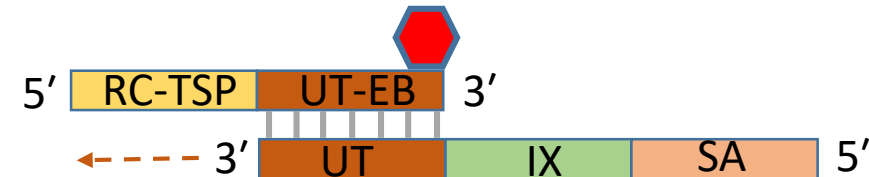
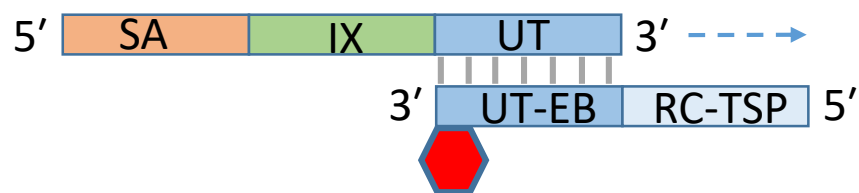


1a)

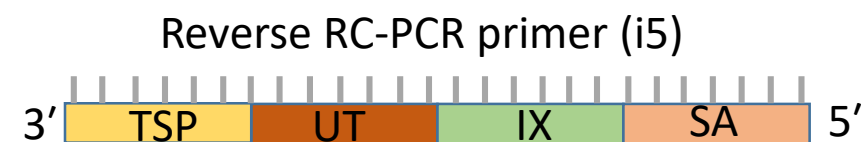
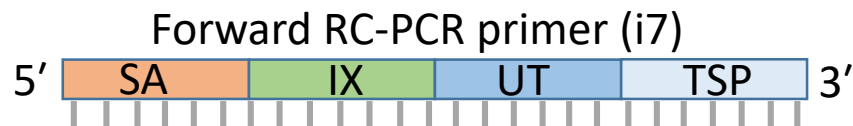
Functional targeted index primers are generated in every PCR cycle



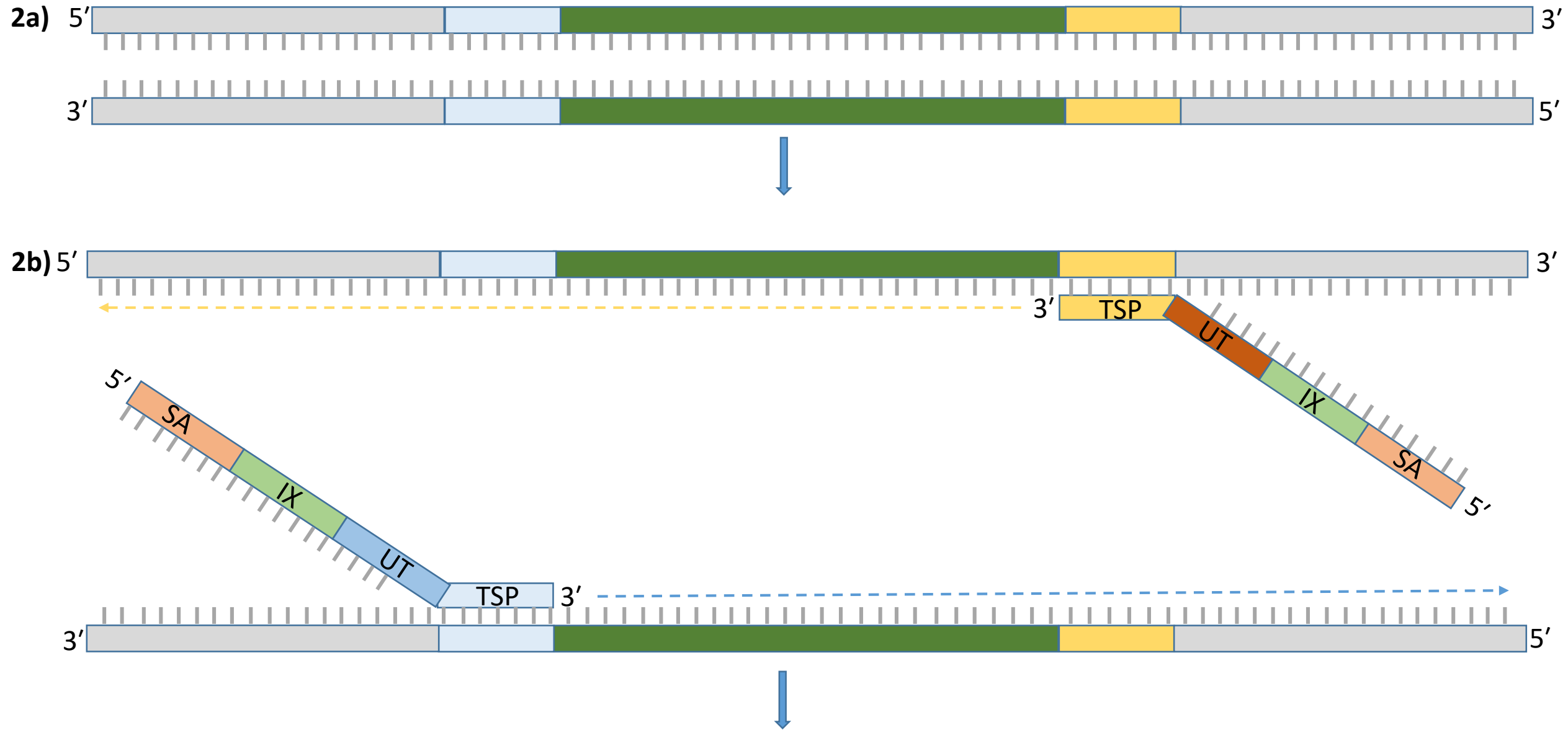
1b)

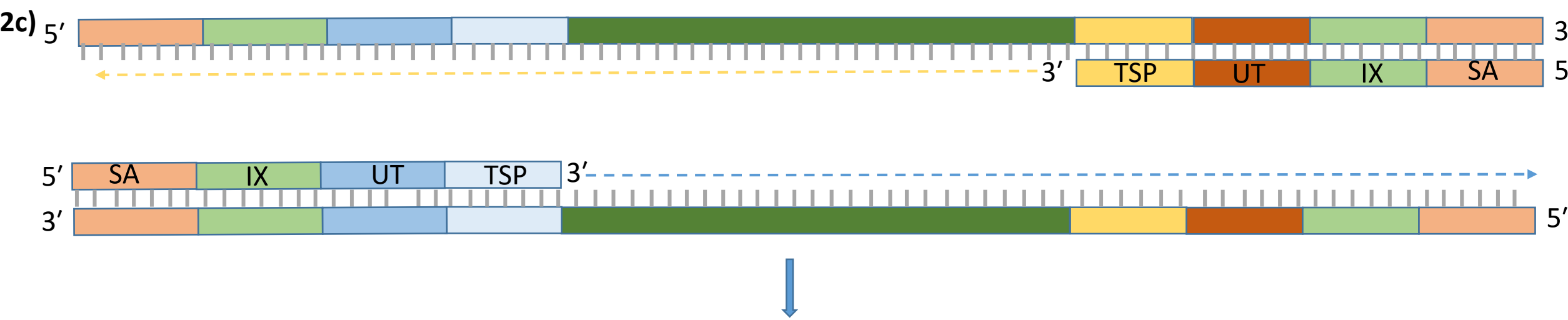


1c)

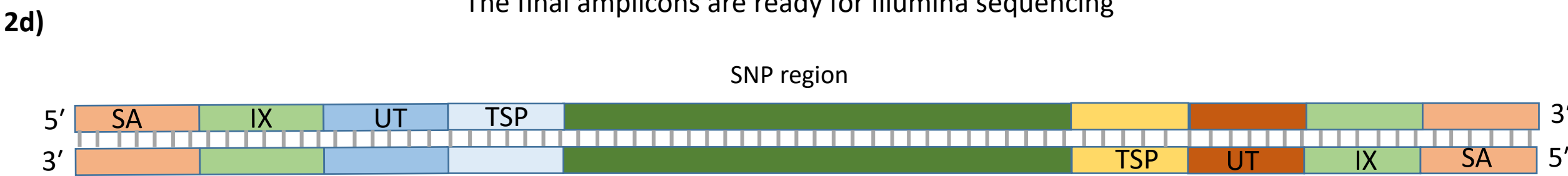


# Standard PCR with the SNP specific amplicons created





The final amplicons are ready for Illumina sequencing



- Legend:**
- UT – universal tail
  - IX – index (single or unique dual index possible)
  - SA – sequence adapter
  - UT-EB – extension blocker
  - RC-TSP – reverse complement target specific primer
  - TSP – target specific primer

**Graph 1.** The graphical presentation of the RC-PCR technology. The (universal) index primer (UIP) contains a unique dual index i7/i5 (single indexing also possible), sequence adapter, and universal tail. The RC probe includes an extension blocker with a universal sequence and the reverse complement of the SNP target-specific region (F/R). The indexing and multiplex PCR amplification are performed at the same time in one closed tube. The RC-PCR consists of 2 major steps. **1a – 1c)** The universal tail sequences of UIPs hybridize with the corresponding forward/reverse RC probe, and the target specific index primers are generated. In each PCR cycle, new target specific index primers are generated by the *Taq* polymerase that copies the sequence of the RC probe. **2a – 2d)** The PCR amplification of SNP-specific amplicons. DNA samples are now tagged with a sample-specific index and Illumina sequence adapter. Now samples can be pooled, purified, and sequenced.