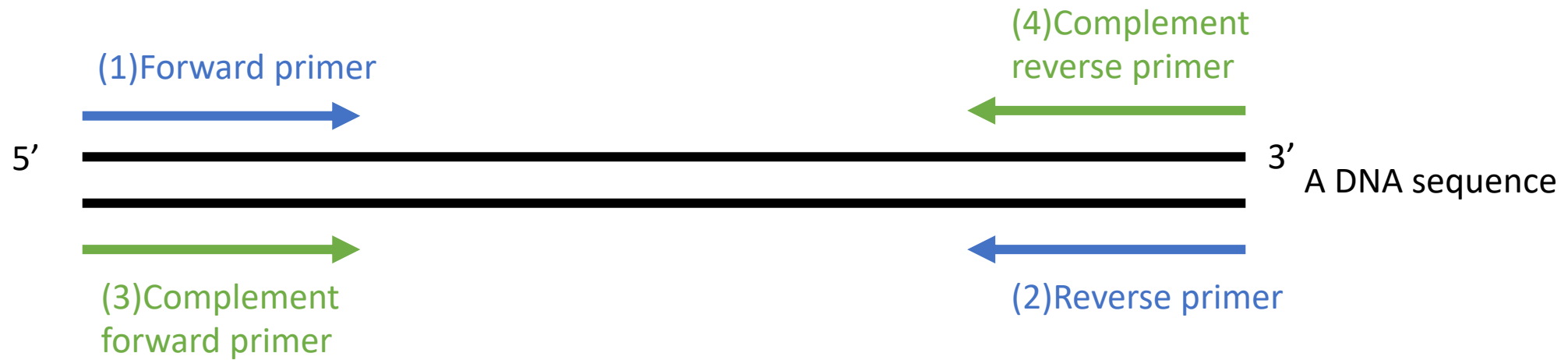


Graphical abstract



To perform PCR, primer pairs are needed. For a given double-stranded DNA sequence, two combinations of primer pairs are available:

a. (1)forward primer + (2)reverse primer; b. (3)complement forward primer + (4)complement reverse primer;

Our code generates all suitable primers and complement primers at one given position. To generate primer pairs, the users simply run the code twice, each time with a different position number, and combine.

For example, if 1680bp – 1900bp is the DNA sequence of interest:

First, users input b <- 1680bp, (1)forward primers and (3)complement forward primers are generated by code; Second, users input b<- 1900bp, (2)reverse primers and (4)complement reverse primers are generated by code; Finally, users have (1)(2) (3)(4) four data frames, and can decide which combinations to pick.

Note: all primers generated are already 5' to 3', ready for production, so no further reverse complement is needed.