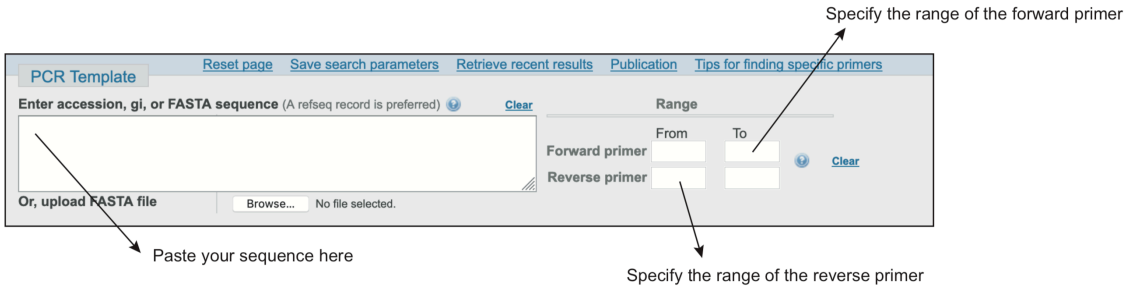
Figure S1

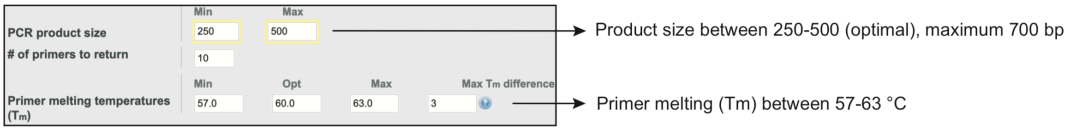
We recommend using PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to perform primer design.

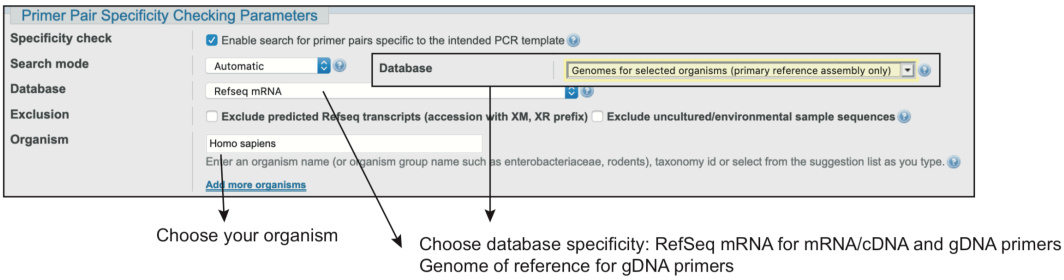
1. First, introduce your target sequence (surrounding the mutation of interest) in the PCR template section as outlined below:



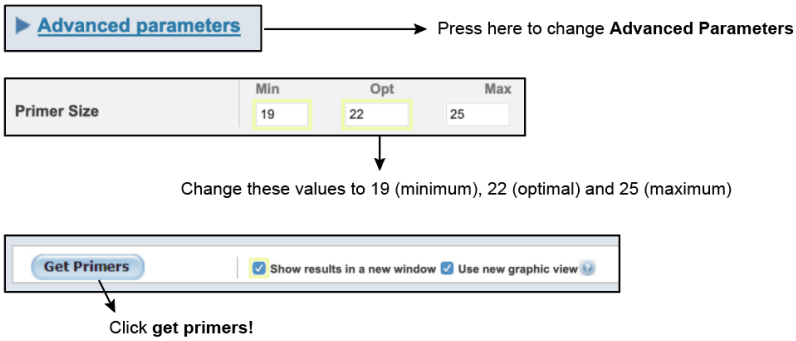
You can also specify the range where forward and reverse primers should be located, which should be intronic regions for gDNA primers and previous and following exons for mRNA primers outlined in Figure 1.

2. Then introduce the desired PCR product size (usually between 250-500 bp, and no more than 700 bp) and the primer melting temperature, which should be set to 57-63 °C. Then select the database to check primer specificity against (RefSeq in the case of mRNA/cDNA primers, and both RefSeq and genome databases for gDNA primers), as well as the organism. Keep the rest of parameters with default settings.





3. Finally, click in “Advanced parameters”, change the desired primer length to 19 bp (minimum), 22 bp (optimal) and 25 bp (maximum) and click Get Primers!



Once the analysis is finished, a window will open with different primer designs (up to 10). Pick only those which are specific to your target region of interest, minimizing the self-complementarity and self 3’-complementarity score. Avoid primers with >2 purine bases (adenine, A; thymine, T) in the 3’end.

Figure S1, related to Before you start: Optimization 2 section. Step by step primer design using PrimerBlast.

Figure S2

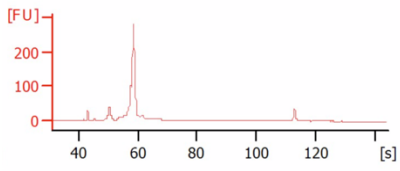


Figure S2, related to Troubleshooting section. Representative traces of a blank library with high concentration of primer dimers at low molecular weight.

Figure S3

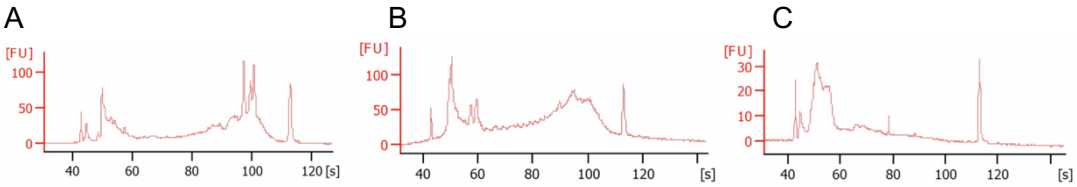


Figure S3, related to Troubleshooting section. Representative traces of poor quality libraries showing RNA degradation.

Figure S4

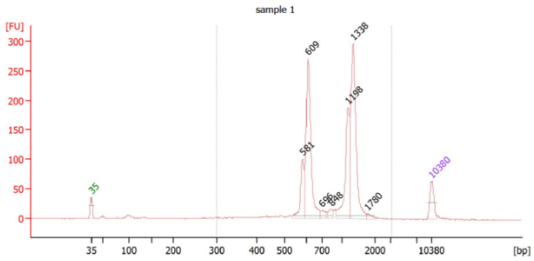


Figure S4, related to Troubleshooting section. Representative traces of poor quality libraries showing high amplification of ERCC molecules but not full-length cDNA.

Supplemental Methods

Available in Mendeley Data: http://dx.doi.org/10.17632/k92cnf2fph.1