## Figure S1

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

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

				S	pecify the rar	nge of the forward prir
					A	
PCR Template	Reset page Save search parameters	Retrieve recent resul	ts Publication Tips	for finding specific	primers	
Enter accession, gi, or FA	STA sequence (A refseq record is preferred)	6 <u>Clear</u>	Range			
		Forwa	From ard primer	То	1	
		Rever	rse primer		<u>ilear</u>	
Or, upload NASTA file	Browse No file selected.					
Pasí	te vour sequence here		Y			
	··· ) · ··· · · · · · · · · · · · · · ·		Specify the I	range of the rev	erse primer	

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.

	Min	Max				
PCR product size	250	500				Product size between 250-500 (optimal), maximum 700 bp
# of primers to return	10					
	Min	Opt	Max		Max Tm difference	
Primer melting temperatures (Tm)	57.0	60.0	63.0	3	0	→ Primer melting (Tm) between 57-63 °C

Primer Pair Specif	city Checking Parameters					
Specificity check	Enable search for primer pairs specific to the intended PCR template					
Search mode	Automatic O Database Genomes for selected organisms (primary reference assembly only) - O					
Database	Refseq mRNA					
Exclusion	🗆 Exclude predicted Re(seq transcripts (accession with XM, XR prefix) 🗌 Exclude uncultured/environmental sample sequences 😣					
Organism	Homo sapiens					
	Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.					
	Ade more organisms					
	$\downarrow$ $\setminus$ $\downarrow$					
	Choose your organism Choose database specificity: RefSeq mRNA for mRNA/cDNA and gDN Genome of reference for gDNA primers					

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!

Advanced parameters	<u>s</u>		<ul> <li>Press here</li> </ul>	to change Advanced Parameters				
Primer Size	Min 19	Opt 22	Max 25					
Change these values to 19 (minimum), 22 (optimal) and 25 (maximum)								
Get Primers Show results in a new window Vuse new graphic view								
■ Click get prime	rsl							

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: <u>http://dx.doi.org/10.17632/k92cnf2fph.1</u>