SETTING UP QPCR ASSAYS: IT'S ALL IN THE DETAILS

qPCR is one of the most commonly used techniques in biological laboratories. Whether you're a novice or a weathered veteran, check out our tips to ensure reliable data, every time.

START (PRIMER) STRONG

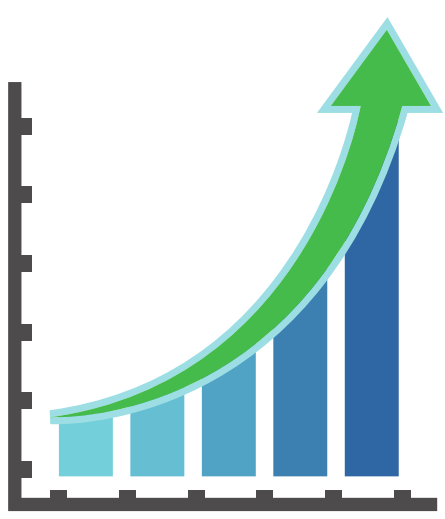
If primers or assays for your gene have already been developed or validated, don't reinvent the wheel!

If you do need to design your own primers, look up the consensus cDNA sequence for your gene of interest, and then use existing software to evaluate and pick primers based on the specifications of your experiment.

DOUBLE VISION

The concentration of DNA should double after each PCR cycle

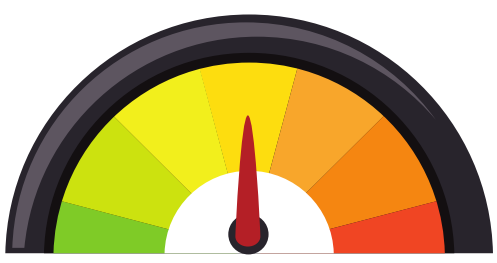
Check using either: (1) a standard curve experiment with varying amounts of template (i.e., 20ng, 40ng, 80ng) and evaluate the Ct values; or (2) serially dilute the template and plot Ct vs log of template concentration for efficiency of PCR



HOT AND COLD

Optimize annealing temperature

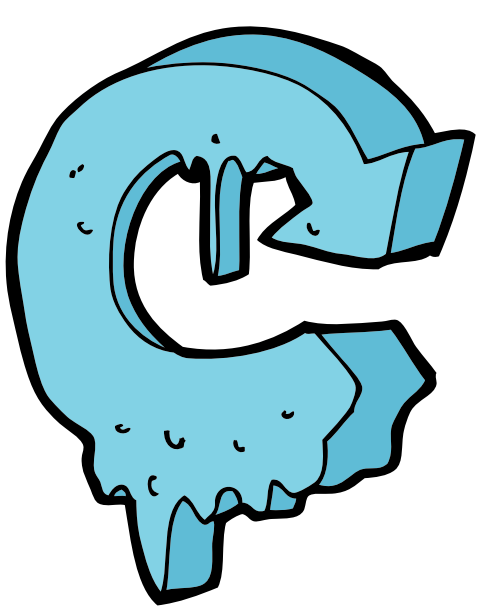
Using standard PCR, compare reactions at different annealing temperatures. This will allow you to determine which temperature will result in the maximum fluorescence detection (a.k.a. the strongest, single product band).



CONFIDENCE IS KEY

Make sure your assay results in a single product

Before you perform qPCR, take an aliquot of the cDNA template, add it to your assay, and run the product out on a gel. If you spot multiple bands or smears, you probably have non-specific amplification.



TAKE IT SLOW

A melt curve at the end of your PCR experiment can help identify non-specific amplification

Slowly increasing the temperature leads to the denaturing of dsDNA and dissociation of fluorescent dye, thus decreasing fluorescence. This data is visualized as peaks — one peak per amplicon. Multiple peaks indicates non-specific amplification.