# A rapid and versatile method to synthesize internal standards for competitive PCR

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Competitive PCR is a quantitative adaptation of the PCR method in which a known number of copies of a synthetic mutated internal standard is introduced with the sample into the PCR reaction mixture (1, 2). The internal standard is amplified with the same primers (and thus presumably with the same efficiency) as the endogenous target sequence, and is distinguished from the product derived from the endogenous sequence by either size, restriction endonuclease cleavage, or specific hybridization. Absolute quantitation may be achieved by comparing the relative amounts of the two products. Since the design, synthesis and quantitation of the internal standard often involves multiple steps, competitive PCR may be very cumbersome. We now report a rapid and highly versatile one-step method to synthesize internal standards in a single day.

First, two primers are synthesized. One primer (primer $a_{20}$ in Figure 1, panel A) is a conventional PCR primer approximately 20 nucleotides in length that corresponds to the target sequence. The second primer (primer $b_{20}c_{20}$ in Figure 1, panel A) is approximately 40 nucleotides in length in which 20 nucleotides at the 3′ end (segment $b_{20}$) correspond to the opposite strand of the target sequence a predetermined distance from primer $a_{20}$, and 20 nucleotides at the 5′ end (segment $c_{20}$) that correspond to the target sequence n nucleotides upstream from segment $b_{20}$. Amplification with these primers from genomic DNA or cDNA results in a PCR product that may be freed from excess primers and dNTPs by ultrafiltration (Millipore, 100,000 M.W. cut off; Bedford, MA), or gel purification, and quantitated using conventional methods. (Note that during PCR, the $c_{20}$ portion of primer $b_{20}c_{20}$ can anneal to its complementary sequence on the target DNA, but can not be extended by Taq polymerase.)

A known number of copies of the internal standard may be introduced into the samples and amplified with primers $a_{20}$ and $c_{20}$ ($c_{20}$ is a primer 20 nucleotides in length whose sequence is identical to segment $c_{20}$ of primer $b_{20}c_{20}$). With this approach, two products are generated, one derived from the endogenous template, and another, *n* base pairs smaller derived from the internal standard. If an RNA internal standard is desired, an SP6 or T7 RNA polymerase recognition sequence may be incorporated onto the 5′ end of primer $a_{20}$ for subsequent *in vitro* transcription (3), or the PCR product may be subcloned into an appropriate vector that contains an RNA polymerase recognition sequence.

To illustrate the versatility of this method, we co-amplified varying amounts of human genomic DNA ($1.5-24\times10^3$ copies) with a fixed quantity of internal standard ($6\times10^3$ copies) corresponding to a portion of the insulin receptor gene (Figure 1, panel B). As expected, two products of the predicted size and relative amounts were generated. We conclude that the strategy described is a very rapid and versatile method to generate internal standards for competitive PCR in a single day.

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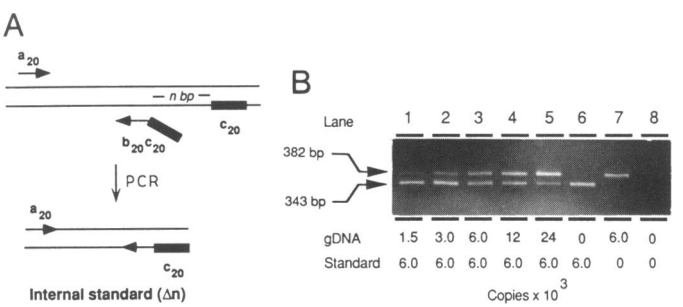


**Figure 1. Panel A.** Schematic of procedure. **Panel B.** Lanes 1–5; co-amplification of increasing quantities of human genomic DNA ($1.5-24\times10^3$ copies) with a fixed quantity ($6\times10^3$ copies) of a human insulin receptor gene internal standard synthesized according to the method described in the text. Lane 6: genomic DNA alone ($6\times10^3$ copies). Lane 7: Internal standard DNA alone ($6\times10^3$ copies). Lane 8: no DNA template.

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