Inosine (dI & rl) is classified as a nucleotide base analog. It is structurally similar to guanosine, but missing the 2-amino group. Because it is able to form two hydrogen bonds with each of the four natural nucleotide bases (1), it is commonly used by researchers as a “universal” base—meaning that it can base pair with all the naturally-occurring bases—in synthetic oligos. Inosine typically is substituted for the nucleoside at the third (“wobble”) position of codons, in order to reduce the complexity of mixed oligo PCR primers/hybridization probes needed to deal with degenerate codons in the target DNA (2, 3). However, it is important to remember that I does not base pair equally well with the naturally-occurring bases, with the order of thermodynamic stability being I-C > I-A > I-G ~ I-T. Thermodynamic stability of inosine-containing duplexes is also affected by neighboring bases (4). Consequently, when using inosine as an alternative to mixed-base degeneracy at a particular oligo position, keep in mind that the above base-pairing bias may lead to differences in the oligo’s priming or hybridization efficiency in the corresponding degenerate regions of the target. Because the effect could be more pronounced when dI is at the 3’-position, it may be advisable to use primers with and without I at the 3’-end, in order to maximize diversity of PCR products (5).

**References**