Degenerate Bases & Spiking Introduction

Degenerate base means more than one base possibility at a particular position, this is usually the case when a DNA sequence is derived from amino acid sequence with codon based sequence. An oligo sequence can be synthesized with multiple bases at the same position, this is termed as degenerate base also sometime referred as "wobble" position or "mixed base". IUB (International Union of Biochemistry) has established single letter codes for all possible degenerate possibilities. An example is "R" that is A+G at the same position with 50% of the oligo sequence will have an A at that position, and the other 50% have G. A degenerate base position may have any combination of two, three, or four bases.

For degenerate (mixed bases) positions use the following IUB codes.

- R=A+G
- Y=C+T
- M=A+C
- K=G+T
- S=G+C
- W=A+T
- H=A+T+C
- B=G+T+C
- D=G+A+T
- V=G+A+C
- N=A+C+G+T

Custom Spiking Internal & Custom Column (3' base spiking/mix)

Custom spiking is the addition of differing molar concentration of bases at a single position, this is different from degeneracy at a position based on codons. Codon based degeneracy is usually equimolar concentration of each base at the same position (done at no extra charge for all internal and 5' position, see order form for single letter IUB codes). Custom spiking (example, 10% A, 75% G, 5% C & 10% T) has to be specified as required on the order form.

Custom column has to be prepared when the degeneracy and custom spiking is at the 3' position. Customers who wish custom spiking at certain positions of their oligo must include the relevant specifics position and spiking composition) in the comments section of the on-line order form for that particular oligo. The prices listed below is for one 3' site or up to 8 internal sites in the same oligo.

Alternate Degenerate Base Modifications

Degenerate bases, in the context of modified bases, refers to their ability to form a reasonably stable base pair with more than one base, for example, with all pyrimidines (C and T) or purines (A and G). Examples of degenerate bases include deoxyinosine (dI) and 5-nitroindole, both of which can pair with all four naturally-occurring bases. Incorporation of degenerate base modifications is desirable in cases when either imprecise or random base-pairing is required, and the resulting "mismatched" complements need to be stable. Examples include reverse-translation of known protein sequence for oligo design (oligos to be used as primers or probes), development of an in vitro or in vivo oligo probe able to hybridize to related but distinct genes (such as viral sub-arrays or allelic variants (SNPs), indels, etc), in vitro site-directed mutagenesis and motif cloning (1).
Degenerate Bases & Spiking Design Protocols

Use of Degenerate Bases--Design Considerations

I. Inosine

The ability of inosine to act as a degenerate base makes it particularly useful as a way to reduce the overall degeneracy of degenerate PCR primer sets. Such sets are commonly used for DNA amplification of regions where only a gene’s protein sequence is known, or when the goal is to amplify similar genes from different species. Since inosine is capable of base-pairing with any natural nucleotide, it can be used to substitute for any "N" (A,C,G,T) degenerate position (see Designing Degenerate Primers and Degenerate primers). When using inosine in this manner, be aware that because this base does not base-pair with natural nucleotides with equal affinity (I-C>I-A>I-T~I-G), there will be some difference in priming efficiency between the members of the degenerate primer set. However, in most cases, the overall increase in priming efficiency afforded by the 4-fold reduction in degeneracy per inosine substitution outweighs this, as such substitution both increases the effective concentration of these primers in the pool and also reduces the amount required optimization of the reaction conditions.

II. 5-nitroindole

5-nitroindole functions as a non-hydrogen bonding universal base, and pairs indiscriminately with any natural nucleotide by base-stacking interactions. 5-nitroindole is particularly useful as a universal base in degenerate hybridization probes. Thus, it can sometimes serve as a useful alternative to inosine in cases where avoidance of bias in base-pairing is critical.
Degenerate Bases & Spiking Applications

The most commonly used degenerate modified base is deoxyinosine, which serves as a more-or-less “universal” base, as it is capable of pairing with all four natural nucleotides, though not with equal affinity (I-C > I-A > I-T ~ I-G > I-I). Even so, inosine continues to be successfully used in this role in a variety of applications requiring degeneracy at certain base positions of primers and probes, particularly at wobble positions, where degeneracy might be needed to permit annealing to many different, but closely related, sequences (2). For degenerate PCR, incorporating inosine instead of mixed bases into degenerate primers often yields superior amplification results due to inefficient hybridization of the mixed-base degenerate primers (3). When a guanine-rich PCR primer is needed, substitution of inosine for one or more guanines helps reduce undesirable G-quartet formation and primer-dimer artifacts (4). For DNA microarrays, inosine can be used to increase the stability of an oligo library without increasing the library’s diversity, at considerable cost savings (5). Other degenerate bases are useful for certain specialized applications. For example, 5-nitroindole base-pairs indiscriminately with any of the natural nucleotides, a consequence of the fact that it interacts via base-stacking, not hydrogen bonding (6). 5-nitroindole has been incorporated into nested sets of oligo probes to target regions of rRNA in different microorganisms in order to ensure equal probe specificity across them (7). However, its ability to act as a “universal” degenerate base is position-dependent, that is, on where it is located within a primer or probe. The potential uses of other degenerate bases, such as 2-amino purine, iso-dG, and 5-methyliso-dC, can be found in their respective tech sheets.
References