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.
Chemical synthesis of oligos using IUB degenerate bases is programmed and automated to deliver the percentage of each base for reaction at that specific base position; example for the letter "N", 25% of each base will be delivered for coupling. The delivery and coupling may not be 100% accurate and efficient for each base and thus approximately 10% deviation should be expected and considered in the final oligo sequence.

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-strains 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 do 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

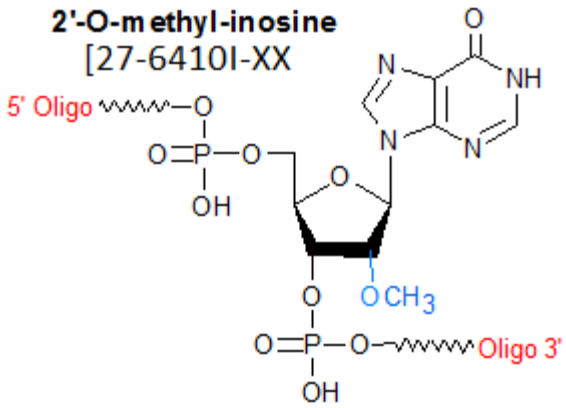
(1) Loakes, D. The applications of universal DNA base analogues.Nucleic Acids Res. (2001), 29: 2437-2447.
(2) Ohtsuka, E., Matsuki, S., Ikehara, M., Takahashi, Y., Matsubara, K. An alternative approach to deoxynucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions. J. Biol. Chem. (1985), 260: 2605-2608.
(3) Liu, H., Nichols, R. PCR amplification using deoxyinosine to replace entire codon and at ambiguous positions.Biotechniques. (1994), 16: 24-26.
(4) Cheng, A., Van Dyke, M.W. Oligodeoxyribonucleotide length and sequence effects on intramolecular and intermolecular G-quartet formation. Gene (1997), 197: 253-260.
(5) Watkins, N.E., SantaLucia, J. Nearest-neighbor thermodynamics of deoxyriboinosine pairs in DNA duplexes. Nucleic Acids Res. (2005), 33: 6258-6267.
(6) Loakes, D.; Brown, D.M. 5-Nitroindole as a universal base analogue. Nucleic Acids Res. (1994), 22: 4039-4043.
(7) Zheng, D.; Raskin, L. Quantification of Methanosaeta species in anaerobic bioreactors using genus- and species-specific hybridization probes. Microb. Ecol. (2000), 39: 246-262.

Modificaton Code List

Modification	Code	Catalog Number
2'-O methyl Inosine	[mI]	27-6410I
2-Amino Purine deoxyribose	[2-AP]	26-6505
2-Amino Purine ribose	[2-A-rP]	27-6505
5-methyl isodeoxycytosine (Me iso dC)	[5-Me-isodC]	26-6513
5-nitroindole	[5NitInd]	26-6476
Degenerate Base (Mixed Base)/ Custom Column	[CC]	26-6201
deoxyXanthosine	[dX]	26-6532
dK degenerate base	[dK]	26-6589
dP degenerate base	[dP]	26-6588
Inosine deoxy (dI)	[dI]	26-6403
Inosine ribo [rI]	[rI]	27-6421
iso deoxyguanosine dG (iso dG)	[iso dG]	26-6514
Spiking Custom	[CSXX]	26-CSXX
Spiking Custom-01	[CS01]	26-CS01

2'-O methyl Inosine

Category	Others
Modification Code	mI
Reference Catalog Number	27-6410I
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.22



Antisense Oligos (ODN) & siRNA Oligo Modifications
Click here for more information on antisense modifications, design & applications.

2'-O-Methyl inosine is classified as a 2'-O-Methyl RNA monomer. 2'-O-Methyl nucleotides are most commonly used **to confer nuclease resistance** to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate internucleotide linkages within the oligo.

2'-O-Methyl bases are classified as a 2'-O-Methyl RNA monomer. 2'-O-Methyl nucleotides are most commonly used **to confer nuclease resistance** to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate internucleotide linkages within the oligo.

The hydrogen bonding behavior of a 2'-O-Methyl RNA/RNA base pair is closer to that of an RNA/RNA base pair than a DNA/RNA base pair. Consequently, the presence of 2'-O-Methyl nucleotides **improves duplex stability**. Indeed, incorporation of a 2'-O-Methyl nucleotide into an anti-sense oligo (resulting in a 2'-O-Methyl RNA/DNA chimeric), lead to a **increase** in the Tm of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, **of 1.3^{O}C per 2'-O-Methyl RNA residue added** (2). Moreover, from a synthesis standpoint, the coupling efficiency of 2'-O-Methyl phosphoramidites are higher than those of RNA monomers, resulting in higher yield of full-length oligos.
Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [Tm Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.

8C per substitution] Increased C-5 propynyl-U Increased [1.7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased Click here for complete list of duplex stability modifications

ASO's and siRNA Modifications.
Click this link to view ASO's and siRNA Modifications.

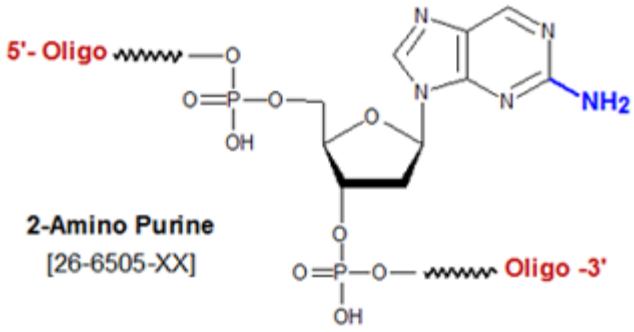
ASO's and siRNA Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane. Cholesterol TEG, alpha-Tocopherol TEG (a natural isomer of vitamin E), stearyl and GalNAc modifications have been shown to effective for delivery of ASO's and siRNA in addition to cell penetrating peptides.
Click this link to view these modifications.

References

1. Cotton, M.; Oberhauser, B.; Burnar, H. *et al.* 2'O methyl and 2'O ethyl oligoribonucleotides as inhibitors of the in vitro U7 snRNP-dependent messenger-RNA processing event. *Nucleic Acids Res.* (1991) , **19**:2629-2635.
2. Kawasaki, A.M. *et al.*, Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease resistant antisense compounds with high affinity and specificity for RNA targets, *Journal of Medicinal Chemistry* (1993), **36**: 831-841.

2-Amino Purine

Category	Others
Modification Code	2-AP
Reference Catalog Number	26-6505
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	313.21

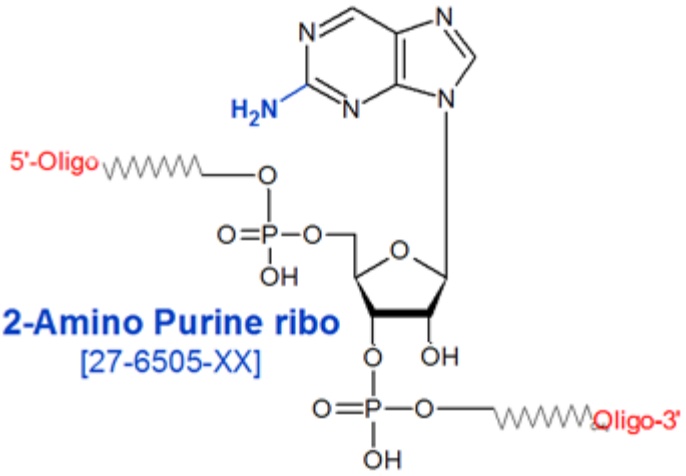


Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

2-Amino Purine ribose

Category	RNA Oligo Synthesis
Modification Code	2-A-rP
Reference Catalog Number	27-6505
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Deletion of the O6 carbonyl group of guanosine results in 2-aminopurine riboside (2-AP). The hydrogen bonding pattern of the 2-aminopurine nucleobase (N1 acceptor, H-N2 donor) is isomeric with that of adenosine (N1 acceptor, H-N6 donor). 2-Amino Purine (2-AP) is a fluorescent molecule that is classified as an adenine and guanine analog, and thus can pair with both thymine and cytosine bases (1). It is an attractive choice for use as a probe in nucleic acid secondary structural studies, both because its fluorescence is highly sensitive to the nature of the local environment, and because it usually does not significantly affect duplex stability (2). Examples include the hairpin-loop structure of the (CAG)8 repeat, involved in several neurodegenerative disorders—2AP substituted for A (3), the G-quadruplex telomeric structure [AGGG(TTAGGG)3]—2AP substitute for A (4). 2-AP also has been used to characterize the effects of DNA mismatch repair on mutagenesis induced by several different nucleoside analogs (5).

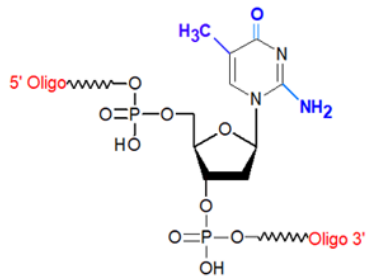
2-Amino purine nucleoside allows the study of the role of exocyclic functional groups, base stacking, and hydrogen bonding patterns in purine-containing nucleic acids. For example, replacement of guanosine residues with 2-AP in the core region of hammerhead ribozymes was useful in determining their role in stabilizing the transition state of ribozyme cleavage (6). The nature of hydrogen-bonding between G-A mismatches in RNA internal loops was studied with 2-AP (7). The role of hydrogen-bonding and stacking interactions in the stability of GNRA loops was probed using 2-AP substitutions (8). The thermodynamic parameters for RNA loops of the type (A)n were determined using time-resolved spectrofluorimetry on RNAs bearing 2-AP residues in place of A residues, since 2-AP is blue fluorescent and was found to have properties in the (A)n region that were otherwise very similar to adenosine (9). In this sense, 2-AP can be used as a non-invasive conformational probe in RNA studies. Of the different phosphoramidites that have been used for 2-aminopurine riboside incorporation into RNA oligonucleotides (6-10), we have chosen to offer 2-Aminopurine riboside CEP in the particular form shown (6,9) which appears to offer the best results in RNA synthesis yield and purity. **References**

Jean JM, Hall KB (2001). "2-Aminopurine fluorescence quenching and lifetimes: role of base stacking".

Proc. Natl. Acad. Sci. U.S.A. 98 (1): 37-41. doi:10.1073/pnas.011442198.
1. Negishi, K.; Bessho, T.; Hayatsu, H. Nucleoside and nucleobase analog mutagens. *Mutat. Res.* (1994), **318**: 227-238.
2. Ballin, J.D., et al. Local RNA Conformational Dynamics Revealed by 2-Aminopurine Solvent Accessibility. *Biochemistry* (2008), **47**: 7043-7052.
3. Degtyareva, N.N.; Reddish, M.J.; Sengupta, B.; Petty, J.T. Structural Studies of a Trinucleotide Repeat Sequence Using 2-Aminopurine. *Biochemistry* (2009), **48**: 2340-2346.
4. Kimura, T.; Kawai, K.; Fujitsuka, M.; Tetsuro, M. Monitoring G-quadruplex structures and G-quadruplex-ligand complex using 2-aminopurine modified oligonucleotides. *Tetrahedron* (2007), **63**: 3585-3590.
5. Negishi, K.; et al. Binding specificities of the mismatch binding protein, MutS, to oligonucleotides containing modified bases. *Nucleic Acids Res. Supplement No. 1* (2001), 221-222.
6. Tuschl, T.; Ng, M. M. P.; Pieken, W.; Benseler, F.; Eckstein, F. Biochemistry 1993, 32, 11658-11668.
7. SantaLucia, J., Jr.; Kierzek, R.; Turner, D. H. J. Am. Chem. Soc. 1991, 113, 4313-4322.
8. Wörner, K.; Strube, T.; Engels, J. W. Helv. Chim. Acta 1999, 82, 2094-2104.
9. Zagorowska, I.; Adamiak, R. W. Biochemie 1996, 78, 123-130.
10. Doudna, J. A.; Szostak, J. W.; Rich, A.; Usman, N. J. Org. Chem. 1990, 55, 5547-5549.

5-Me iso dC

Category	Minor Bases
Modification Code	5-Me-isodC
Reference Catalog Number	26-6513
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



5-methyl isodeoxycytosine (Me isodC)
[26-6513-XX]

5-methyl iso-deoxycytosine (5-Me-iso-dC) forms a Watson-Crick base pair with iso-dG, but has a different type of hydrogen bonding pattern than those observed for the natural base pairs A:T and C:G. Substitution of a 5-Me-iso-dC:iso-dG base pair for a C:G pair increases the Tm of the resulting duplex by ~2degC per base pair substitution (1,2). Furthermore, since 5-Me-iso-dC does not pair with dG, 5-Me-iso-dC:iso-dG can function as a stable unnatural base pair that can be used to expand the genetic code. The combination of 5-Me-iso-dC's high selectivity for iso-dG, and the resulting base pair's high thermodynamic stability, make this modified base particular attractive in the following applications:

(a) Molecular recognition: The 5-Me-iso-dC:iso-dG base pair has been incorporated into hybridization assays to enhance probe-target specificity and reduce spurious hybridization to non-target sequences. For example, Collins and co-workers significantly improved the sensitivity of a branched DNA quantitative hybridization assay for detecting the HIV POL sequence by incorporating ~30% 5-Me-iso-dC and iso-dG into the pre-amplifier, branched DNA (bDNA) amplifier and alkaline phosphate probe sequences used in the assay (3). Use of this strategy resulted in a significant reduction in non-specific hybridization of the above three sequence types to non-target nucleic acid sequences, and thus less amplification of background. The limits of detection of the assay were improved 10-fold, from < 500 HIV molecules/mL to < 50 molecules/mL.

(b) qPCR and artificially expanded genetic systems: A number of research groups have been working on optimizing PCR amplification on templates containing 5-Me-iso-dC. Such optimization is necessary to enable the full development of artificially expanded genetic systems utilizing an expanded genetic code, thereby allowing for the site-specific incorporation of novel functional components (such as unnatural amino acids) into proteins. In 2004, Johnson and co-workers observed that, by using the Klenow fragment of Taq polymerase (KF-Taq) in PCR, the fidelity of the 5-Me-iso-dC:iso-dG base pair was about 96% per amplification cycle (4). The limit in fidelity is chiefly due to the ability of iso-dG's 1,2 tautomer to mis-pair with dT. More recently, Sismour and Benner solved this problem by using 2-thio-dT (dT*) in place of dT. dT*pairs with dA, but not with iso-dG (5). Using this artificial base pair system (5-Me-iso-dC:iso-dG, dA:dT*, dC:dG) with KF-Taq, the fidelity in PCR was increased to about 98% per amplification cycle.

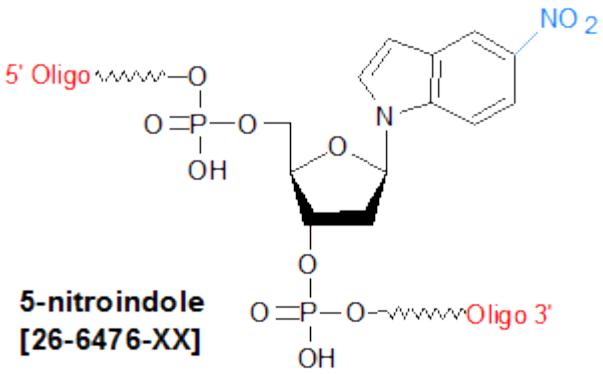
The achievement of high fidelity PCR with the 5-Me-iso-dC:iso-dG base pair opens the door to developing both artificially expanded genetic systems (6) and novel qPCR systems (for example, Promega's Plexor technology) based on this approach.

References

1. Switzer, C.; Moroney, S.E.; Benner, S.A. Enzymatic incorporation of a new base pair into DNA and RNA. *J. Am Chem. Soc.* (1989), **111**: 8322-8323.
2. Horn, T.; Chang C-A.; Collins, M.L. Hybridization properties of the 5-methy-isocytidine/isoguanosine base pair in synthetic oligodeoxynucleotides. *Tetrahedron Lett.* (1995), **36**: 2033-2036.
3. Collins, M.L.; Irvine, B.; Tyner, D.; Fine, E.; et al. A branched DNA signal amplification assay for quantification of nucleic acid targets below 100 molecules/ml.*Nucleic Acids Res.* (1997), **25**: 2979-2984.
4. Johnson, S.C.; Sherrill, C.B.; Marshall, D.J.; Moser, M.J.; Prudent, J.R. A third base pair for the polymerase chain reaction: inserting isoC and isoG. *Nucleic Acids Resl.* (2004), **32**: 1937-1941.
5. Sismour, A.M.; Benner, S.A. The use of thymidine analogs to improve the replication of an extra DNA base pair: a synthetic biological system. *Nucleic Acids Resl.* (2005), **33**: 5640-5646.
6. Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A.M.; Benner, S.A. Artificially expanded genetic information system: a new base pair with an alternative hydrogen bonding pattern, *Nucleic Acids Res.* (2006), **34**: 6095-6101.

5-nitroindole

Category	Duplex Stability
Modification Code	5NitInd
Reference Catalog Number	26-6476
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	340.23



Click here for a complete list of Degenerate Bases & Spiking Oligo Modifications

5-Nitroindole is a hydrophobic aromatic compound, and can be used as a **universal base analog** in oligonucleotides. The term "universal base" refers to a base with the ability to replace any of the four natural bases without significantly destabilizing either neighboring base-pair interactions or disrupting the expected functional capability of the resulting modified oligonucleotide. Incorporation of universal bases into oligos is desirable in cases when either imprecise or random base-pairing is required, and the resulting "mis-matched" complements need to be stable. Examples of such situations 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 (for example, viral sub-strains or allelic variants--SNPs, indels, etc.), *in vitro* mutagenesis and motif cloning.

5-Nitroindole does not discriminate between the four natural nucleotide bases in duplex formation. This is in contrast to deoxyinosine (dI), which, although often used as a "universal" base, actually shows fairly strong base-pair bias. 5-Nitroindole's lack of discrimination ("universality") is a consequence of the fact that it does not form hydrogen bonds with natural bases. Instead, 5-nitroindole stabilizes the duplex via base-stacking interactions, and is superior in this regard than 3-nitropyrrole, which also has been used as a universal base (1,2). Much research work on 5-nitroindole has focused on its potential as an alternative to the used of mixed bases in PCR or Sanger sequencing primers. The ability of 5-nitroindole to function in this way, however, is strongly dependent on where it is located in the primer. Based on the results of several research studies (reviewed in 3), the following guidelines may be useful in optimizing the utility of this base analog for these applications:

1. Substitution of 5-nitroindole as a universal base is less destabilizing towards the ends of oligos than towards the center.
2. Grouped substitutions are more easily tolerated than spaced ones, that is, contiguous rather than codon third substitutions.
3. If more than two codon third substitutions are incorporated into a PCR/sequencing primer, priming generally is poor.

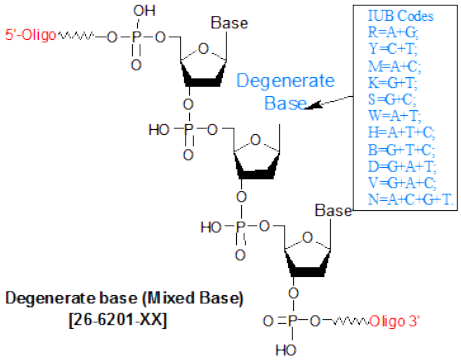
4. Up to four contiguous substitutions in the middle or 5'-end of a PCR/sequencing primer generally yield acceptable amounts of PCR product or sequencing ladder. Above that priming is poor, due to the run of 5-nitroindoles forming undesirable secondary structures in the primer.
5. Substitutions at, or within eight bases of, the 3'-end produce primers that generally are ineffective at priming.

Beyond primers, nested sets of universal oligonucleotide probes containing 5-nitroindole have been successfully used to target regions of rRNA in a variety of microorganism species, in order to ensure equal probe specificity in all target organisms (4). 5-nitroindole has also been used to examine protein-DNA interactions relevant to nucleotide excision repair (NER) (5) and RNA polymerase binding (6). In addition, the potential of 5-nitroindole to enhance the stability of duplexes formed between a target DNA and octa-/decanucleotide probes immobilized on DNA microarrays has been investigated (7). Finally, 5-nitroindole often has been used to stabilize DNA structures of different types (3). **References**

1. Loakes, D.; Brown, D.M. 5-Nitroindole as a universal base analogue. *Nucleic Acids Res.* (1994), **22**: 4039-4043.
2. Loakes, D.; Hill, F.; Brown, D.M.; Salisbury, S.A. Stability and structure of DNA oligonucleotides containing non-specific base analogues. *J. Mol. Biol.* (1997), **270**: 426-435.
3. Loakes, D. The applications of universal DNA base analogues.*Nucleic Acids Res.* (2001), **29**: 2437-2447.
4. Zheng, D.; Raskin, L. Quantification of *Methanosaeta* species in anaerobic bioreactors using genus- and species-specific hybridization probes. *Microb. Ecol.* (2000), **39**: 246-262.
5. Buschta-Hedayat, N.; Buterin, T.; Hess, M.T.; Missura, M.; Naegeli, H.Recognition of non-hybridizing base pairs during nucleotide excision repair of DNA. *Proc. Natl Acad. Sci. USA* (1999), **96**: 6090-6095. 6. Matlock, D.L.; Heyduk, T. Sequence determinants for the recognition of the fork junction DNA containing the -10 region of promoter DNA by E. coli *RNA* polymerase *Biochemistry* (2000), **39**: 12274-12283. 7. Parinov, S.; Barsky, V.; Yershov, G.; Kirillov, E.; Timofeev, E.; Belgovskiy, A.; Mirzabekov, A. DNA sequencing by hybridization to microchip octa- and decanucleotides extended by stacked pentanucleotides. *Nucleic Acids Resl.* (1996), **24**: 2998-3004.

Custom Column 3'

Category	DNA Oligo Synthesis
Modification Code	CC
Reference Catalog Number	26-6201
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308



There is no additional charge if the degenerate mixed base is at an internal position or at the 5' end. Degenerate mixed base charges are only for 3' end positions. A custom column charge is applied.

Custom column (3') is a set of special controlled pore glass (CPG) synthesis solid supports that are used for synthesizing oligonucleotides with degenerate (mixed-base) nucleotides at the 3'-end.

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

Alternate Oligo Design Strategies The use of degenerate bases leads to complexity of oligo sequence and thus reduction in the percentage of the unique sequence. Consider the use of modifications such as Inosine that hybridizes to all four bases. See the listing of modifications as substitutes to reduce complexity if degenerate bases are used. Degenerate Base Modifications

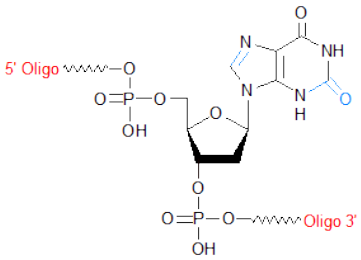
Custom Spiking & Custom Column(3' base spiking/mix)
Custom spiking is the addition of differing molar concentration of bases at a single position, this is different from degeneracy at a position based on codons. Codon based degeneracy is usually equimolar concentration of each base at the same position (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. See the following link

genelink.com/newsite/products/custspike.asp" target="modwindow">http://www.genelink.com/newsite/products/custspike.asp
Spiking Custom
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.

deoxyXanthosine

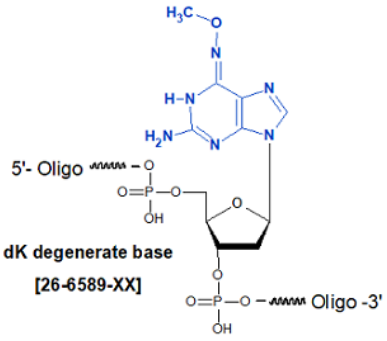
Category	Structural Studies
Modification Code	dX
Reference Catalog Number	26-6532
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	330.19



deoxyXanthosine (dX)
[26-6532-XX]

dK degenerate base

Category	Duplex Stability
Modification Code	dK
Reference Catalog Number	26-6589
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	358.25



Click here for a complete list of Degenerate Bases & Spiking Oligo Modifications

The degeneracy of the genetic code complicates the design of primers in the absence of unique sequence information. Classically inosine has been used at degenerate position as it is able to base pair with all four bases (1) and function as a universal base. Similarly 5-nitroindole was determined to be an effective universal base (2). The modified bases designated P and K show considerable promise as degenerate bases. The pyrimidine derivative P, when introduced into oligonucleotides, base pairs with either A or G, while the purine derivative K base pairs with either C or T. A dP+dK mix also can serve as a mixed base with much less degeneracy than dA+dC+dG+dT (N) (3, 4).

The degenerate bases P and K are copied as either pyrimidine (C or T) or either purine (A or G), respectively. They can be used together in oligomers to prime DNA synthesis in PCR and in sequencing reactions. In a direct comparison, they were more effective in primers than dI.

Fully degenerate sites may be formed in an oligonucleotide using an A/C/G/T mix but the complexity of the mixture of oligonucleotides formed in this way obviously rises with each insertion and limits the usefulness of this technique. The pyrimidine derivative P, when introduced into oligonucleotides, base pairs with either A or G , while the purine derivative K base pairs with either C or T. Oligonucleotides containing one or more P substitutions were found to form duplexes of stability equivalent to the parent sequence and exhibited sharp transitions on melting. Substitution with one or more K residues led to duplexes of reduced but still effective stability (3, 4).

Recommended Further Reading

Glen Report 8.1: New Universal and Degenerate Bases.

Glen Report 9.1: Properties of Oligonucleotides Containing the Bases P and K. **References:**

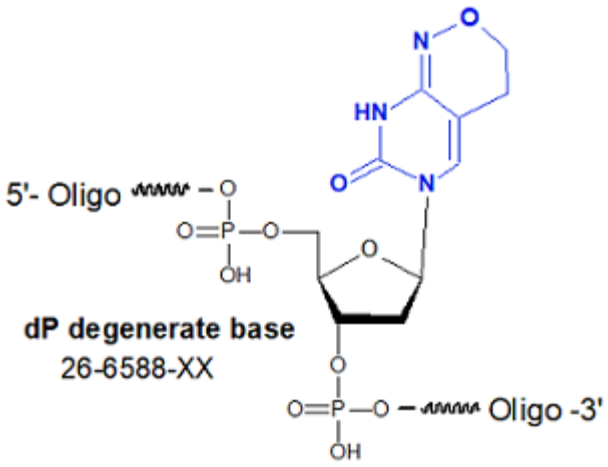
1. Liu, H. and Nichols, R. PCR amplification using deoxyinosine to replace entire codon and at ambiguous positions.*Biotechniques.* (1994), **16**: 24-26.

2. Loakes, D. and Brown, D.M. 5-Nitroindole as a universal base analogue. Nucleic Acids Res.

(1994), 22: 4039-4043.
3. Lin, P.K. and Brown, D.M. Synthesis and duplex stability of oligonucleotides containing cytosine-thymine analogues. Nucleic Acids Res. 1989 Dec 25;17(24):10373–10383
4. Lin, P.K. and Brown, D.M. Synthesis of oligodeoxyribonucleotides containing degenerate bases and their use as primers in the polymerase chain reaction. Nucleic Acids Res., (1992) 20: 5149-5152.

dP degenerate base

Category	Duplex Stability
Modification Code	dP
Reference Catalog Number	26-6588
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	330.23



Click here for a complete list of Degenerate Bases & Spiking Oligo Modifications

The degeneracy of the genetic code complicates the design of primers in the absence of unique sequence information. Classically inosine has been used at degenerate position as it is able to base pair with all four bases (1) and function as a universal base. Similarly 5-nitroindole was determined to be an effective universal base (2). The modified bases designated P and K show considerable promise as degenerate bases. The pyrimidine derivative P, when introduced into oligonucleotides, base pairs with either A or G, while the purine derivative K base pairs with either C or T. A dP+dK mix also can serve as a mixed base with much less degeneracy than dA+dC+dG+dT (N) (3, 4).

The degenerate bases P and K are copied as either pyrimidine (C or T) or either purine (A or G), respectively. They can be used together in oligos to prime DNA synthesis in PCR and in sequencing reactions. In a direct comparison, they were more effective in primers than dI.

Fully degenerate sites may be formed in an oligonucleotide using an A/C/G/T mix but the complexity of the mixture of oligonucleotides formed in this way obviously rises with each insertion and limits the usefulness of this technique. The pyrimidine derivative P, when introduced into oligonucleotides, base pairs with either A or G , while the purine derivative K base pairs with either C or T. Oligonucleotides containing one or more P substitutions were found to form duplexes of stability equivalent to the parent sequence and exhibited sharp transitions on melting. Substitution with one or more K residues led to duplexes of reduced but still effective stability (3, 4).

Recommended Further Reading

Glen Report 8.1: New Universal and Degenerate Bases.

Glen Report 9.1: Properties of Oligonucleotides Containing the Bases P and K. **References:**

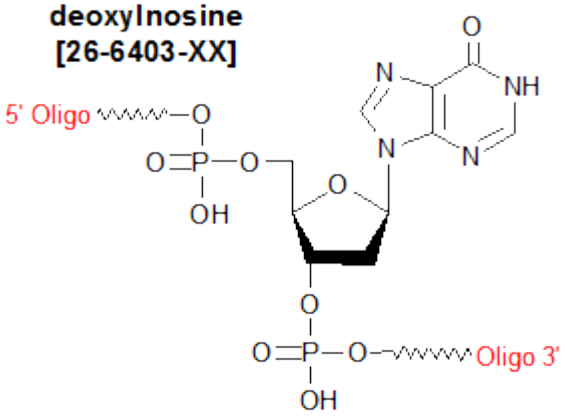
1. Liu, H. and Nichols, R. PCR amplification using deoxyinosine to replace entire codon and at ambiguous positions.*Biotechniques.* (1994), **16**: 24-26.

2. Loakes, D. and Brown, D.M. 5-Nitroindole as a universal base analogue. Nucleic Acids Res.

(1994), 22: 4039-4043.
3. Lin, P.K. and Brown, D.M. Synthesis and duplex stability of oligonucleotides containing cytosine-thymine analogues. Nucleic Acids Res. 1989 Dec 25;17(24):10373–10383
4. Lin, P.K. and Brown, D.M. Synthesis of oligodeoxyribonucleotides containing degenerate bases and their use as primers in the polymerase chain reaction. Nucleic Acids Res., (1992) 20: 5149-5152.

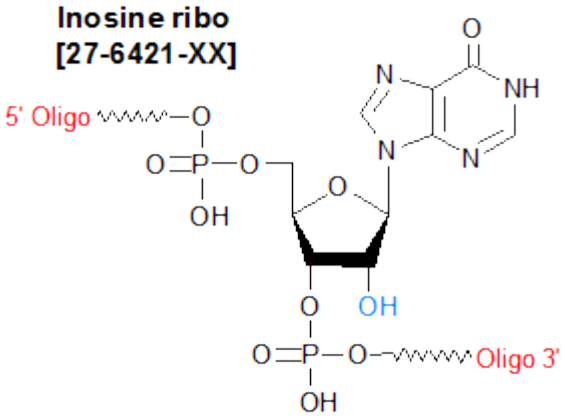
Inosine deoxy

Category	Duplex Stability
Modification Code	dI
Reference Catalog Number	26-6403
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	314.19



Inosine ribo [rI]

Category	Others
Modification Code	rI
Reference Catalog Number	27-6421
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	330.19

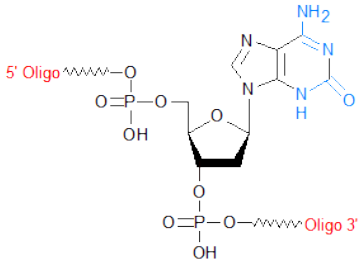


Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

iso dG

Category	Minor Bases
Modification Code	iso dG
Reference Catalog Number	26-6514
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



iso deoxyguanosine dG
[26-6514-XX]

iso dC and 5-methyl iso-deoxycytosine (5-Me-iso-dC) forms a Watson-Crick base pair with iso-dG, but has a different type of hydrogen bonding pattern than those observed for the natural base pairs A:T and C:G. Substitution of a 5-me iso-dC:iso-dG base pair for a C:G pair increases the Tm of the resulting duplex by ~2degC per base pair substitution (1,2). Furthermore, since iso dC and 5-Me-iso-dC does not pair with dG, iso dC and 5-Me-iso-dC:iso-dG can function as a stable unnatural base pair that can be used to expand the genetic code.

The combination of 5-Me-iso-dC's high selectivity for iso-dG, and the resulting base pair's high thermodynamic stability, make this modified base particular attractive in the following applications:

(a) Molecular recognition: The 5-Me-iso-dC:iso-dG base pair has been incorporated into hybridization assays to enhance probe-target specificity and reduce spurious hybridization to non-target sequences. For example, Collins and co-workers significantly improved the sensitivity of a branched DNA quantitative hybridization assay for detecting the HIV POL sequence by incorporating ~30% 5-Me-iso-dC and iso-dG into the pre-amplifier, branched DNA (bDNA) amplifier and alkaline phosphate probe sequences used in the assay (3). Use of this strategy resulted in a significant reduction in non-specific hybridization of the above three sequence types to non-target nucleic acid sequences, and thus less amplification of background. The limits of detection of the assay were improved 10-fold, from < 500 HIV molecules/mL to < 50 molecules/mL.

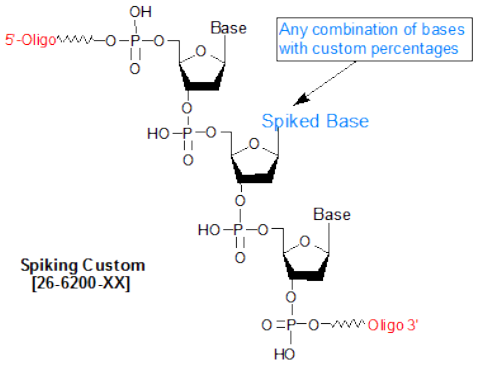
(b) qPCR and artificially expanded genetic systems: A number of research groups have been working on optimizing PCR amplification on templates containing 5-Me-iso-dC. Such optimization is necessary to enable the full development of artificially expanded genetic systems utilizing an expanded genetic code, thereby allowing for the site-specific incorporation of novel functional components (such as unnatural amino acids) into proteins. In 2004, Johnson and co-workers observed that, by using the Klenow fragment of Taq polymerase (KF-Taq) in PCR, the fidelity of the 5-Me-iso-dC:iso-dG base pair was about 96% per amplification cycle (4). The limit in fidelity is chiefly due to the ability of iso-dG's 1,2 tautomer to mis-pair with dT. More recently, Sismour and Benner solved this problem by using 2-thio-dT (dT*) in place of dT.

dT*pairs with dA, but not with iso-dG (5). Using this artificial base pair system (5-Me-iso-dC:iso-dG, dA:dT*, dC:dG) with KF-Taq, the fidelity in PCR was increased to about 98% per amplification cycle. The achievement of high fidelity PCR with the 5-Me-iso-dC:iso-dG base pair opens the door to developing both artificially expanded genetic systems (6) and novel qPCR systems (for example, Promega's Plexor technology) based on this approach. **References**

1. Switzer, C.; Moroney, S.E.; Benner, S.A. Enzymatic incorporation of a new base pair into DNA and RNA. *J. Am Chem. Soc.* (1989), **111**: 8322-8323.
2. Horn, T.; Chang C-A.; Collins, M.L. Hybridization properties of the 5-methy-isocytidine/isoguanosine base pair in synthetic oligodeoxynucleotides. *Tetrahedron Lett.* (1995), **36**: 2033-2036.
3. Collins, M.L.; Irvine, B.; Tyner, D.; Fine, E.; et al. A branched DNA signal amplification assay for quantification of nucleic acid targets below 100 molecules/ml.*Nucleic Acids Res.* (1997), **25**: 2979-2984.
4. Johnson, S.C.; Sherrill, C.B.; Marshall, D.J.; Moser, M.J.; Prudent, J.R. A third base pair for the polymerase chain reaction: inserting isoC and isoG. *Nucleic Acids Resl.* (2004), **32**: 1937-1941.
5. Sismour, A.M.; Benner, S.A. The use of thymidine analogs to improve the replication of an extra DNA base pair: a synthetic biological system. *Nucleic Acids Resl.* (2005), **33**: 5640-5646.
6. Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A.M.; Benner, S.A. Artificially expanded genetic information system: a new base pair with an alternative hydrogen bonding pattern, *Nucleic Acids Res.* (2006), **34**: 6095-6101.

Spiking Custom

Category	DNA Oligo Synthesis
Modification Code	CSXX
Reference Catalog Number	26-CSXX
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308



DNA & RNA Oligo Custom Spiking. The prices listed is for one custom spiking mix setup, additional standard per base charges apply.

Click here for a complete list of Degenerate Bases & Spiking Oligo Modifications
Custom spiking [CSXX] 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% C, 5% G & 10% T or U) has to be specified as required in the comments section of the sequence. Sequence Entry Example
Example of denoting custom spiking [CSXX] is to use code [CS01] or [CS02] etc.and write in the comment section your calculated percentage of spiking, example [CS01]=10% A, 75% C, 5% G & 10% T or U. Similarly, [CS02]= 15% A, 70% G, 5% C & 10% T or U and [CS03] and onward for more spiked positions.
Custom Spiking Base Percentage Codes and Sequence Entry

Code for Sequence Entry

Percentage Spiked Bases

Short Notation [CS01] 10% A, 75% C, 5% G & 10% T or U 10750510 [CS02] 15% A, 70% C, 5% G & 10% T or U 15700510
Please write the exact spiking code , percentage composition of each site in the comments section of the oligo sequence submission field.
Sequence: ACGTGTGCAGT[CS01]ACCCGTACNBR[CS02]AGCATCATC[CS01][CS01][CS02]ACGTCGC

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 online order form for that particular oligo.

Degenerate Base

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.

Chemical synthesis of oligos using IUB degenerate bases is programmed and automated to deliver the percentage of each base for reaction at that specific base position; example for the letter "N", 25% of each base will be delivered for coupling. The delivery and coupling may not be 100% accurate and efficient for each base and thus approximately 10% deviation should be expected and considered in the final oligo sequence.

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

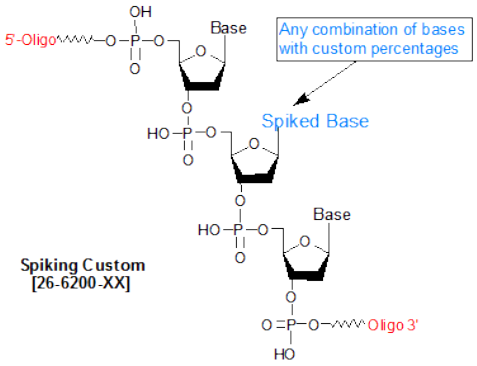
R=A+G
Y=C+T
M=A+C
K=G+T
S=G+C
W=A+T
H=A+T+C
B=G+T+C
D=G+A+T
V=G+A+C
N=A+C+G+T

There is no additional charge if the degenerate mixed base is at an internal position or at the 5' end. Degenerate mixed base charges are only for 3' end positions. A custom column charge is applied. See the following link for details. http://www.genelink.com/newsite/products/custspike.asp

Alternate Oligo Design Strategies The use of degenerate bases leads to complexity of oligo sequence and thus reduction in the percentage of the unique sequence. Consider the use of modifications such as Inosine that hybridizes to all four bases. See the listing of modifications as substitutes to reduce complexity if degenerate bases are used. Degenerate Base Modifications

Spiking-01

Category	DNA Oligo Synthesis
Modification Code	CS01
Reference Catalog Number	26-CS01
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308



DNA & RNA Oligo Custom Spiking. The prices listed is for one custom spiking mix setup, additional standard per base charges apply.

Click here for a complete list of Degenerate Bases & Spiking Oligo Modifications
Custom spiking [CSXX] 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% C, 5% G & 10% T or U) has to be specified as required in the comments section of the sequence. Sequence Entry Example
Example of denoting custom spiking [CSXX] is to use code [CS01] or [CS02] etc.and write in the comment section your calculated percentage of spiking, example [CS01]=10% A, 75% C, 5% G & 10% T or U. Similarly, [CS02]= 15% A, 70% G, 5% C & 10% T or U and [CS03] and onward for more spiked positions.
Custom Spiking Base Percentage Codes and Sequence Entry

Code for Sequence Entry

Percentage Spiked Bases

Short Notation [CS01] 10% A, 75% C, 5% G & 10% T or U 10750510 [CS02] 15% A, 70% C, 5% G & 10% T or U 15700510
Please write the exact spiking code , percentage composition of each site in the comments section of the oligo sequence submission field.
Sequence: ACGTGTGCAGT[CS01]ACCCGTACNBR[CS02]AGCATCATC[CS01][CS01][CS02]ACGTCGC

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 online order form for that particular oligo.

Degenerate Base

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.

Chemical synthesis of oligos using IUB degenerate bases is programmed and automated to deliver the percentage of each base for reaction at that specific base position; example for the letter "N", 25% of each base will be delivered for coupling. The delivery and coupling may not be 100% accurate and efficient for each base and thus approximately 10% deviation should be expected and considered in the final oligo sequence.

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

There is no additional charge if the degenerate mixed base is at an internal position or at the 5' end. Degenerate mixed base charges are only for 3' end positions. A custom column charge is applied. See the following link for details. http://www.genelink.com/newsite/products/custspike.asp

Alternate Oligo Design Strategies The use of degenerate bases leads to complexity of oligo sequence and thus reduction in the percentage of the unique sequence. Consider the use of modifications such as Inosine that hybridizes to all four bases. See the listing of modifications as substitutes to reduce complexity if degenerate bases are used. Degenerate Base Modifications