Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2´-5´ linked Oligos

Oligo Modifications

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

Codon-Based Mutagenesis Introduction

Directed molecular evolution and combinatorial methods are key strategies used for protein engineering research. These approaches commonly involves using partially randomized synthetic oligonucleotides to generate a partially randomized gene library, expressing it in an appropriate vector to generate the protein set encoded by the library, and then screening the expressed proteins for improved or modified characteristics. Examples of the latter include changes in protein-ligand binding affinity, enzyme selectivity, or protein stability. While a variety of site-directed mutagenesis methods can be employed to introduce randomization at specific base positions of a gene, such methods often introduce significant amounts of undesirable codon bias into the library, due to the mismatch between the base-by-base nature of classical oligonucleotide synthesis and the triplet nature of the codon (1). The problem of unwanted codon bias can be eliminated by synthesizing the random portion of the oligos codon-by-codon, with trimer phosphoramidites, instead of base-by-base. This codon-based mutagenesis system conveys the ability to effectively control codon bias in the system. The researcher can choose to completely randomize a particular amino acid position in the expressed protein, or flexibly tune the relative amounts of different amino acids at that position, as needs require (2). The combination of standard and custom Trimer Codon phosphoramidite mixes is a powerful research tool for anyone doing protein engineering research.

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Codon-Based Mutagenesis Design Protocols

Codon-Directed Mutagenesis--Design Considerations
I. <u>When are standard Trimer Codon Phosphoramidites suitable?</u>

Standard trimer codon phosphoramidite mixes are currently optimized around E. coli codon abundances, with the most abundant codon used to code for a given amino acid selected for inclusion. This optimization is reasonable, given that their expected use is for oligos slated for generation of E. coli-based libraries for protein engineering. Other organisms (e.g., yeast or Drosophila) have different codon abundances/usage patterns (Codon Usage in Different Organisms). Thus, when performing codon-directed mutagenesis in an organism other than E. coli, it is important to compare the codon usage pattern of that organism with that of E. coli to ascertain the suitability of the standard mixes. II. <u>Phage Display Library Construction for Protein Engineering: dat/ung method</u>

After synthesis of a mutagenic oligonucleotide using Trimer Phosphoramidites, the oligo will have a randomized trimer codon region with fixed sequences on each side. For protein engineering applications, such mutagenic oligos typically are cloned into E. coli to construct a phage display library. The dut/ung method is one such construction method that is highly efficient in this regard (6).

<u>Background</u>: Dut is a gene in E. coli that encodes for dUTPase, which degrades dUTP. A dut(-) strain lacks this enzyme, so an elevated concentration of dUTP accumulates, resulting in incorporation of U in place of T at some base positions during DNA replication. The ung gene encodes uracil N-glycosylase, which normally excises U from DNA. A ung(-) strain lacks this, so U bases are not excised from DNA in the bacterium. Thus, in a dut(-)/ung(-) double mutant, any U bases that are incorporated into DNA during replication are not repaired. However, because U and T have identical base-pairing properties, the T-to-U conversion is not mutagenic. <u>Basic steps of the method</u>

(1) A dut(-)/ung(-) strain is infected with M13 phage, and a circularized dU-containing M13 ssDNA template is obtained.

(2) The mutagenic oligonucleotide is annealed to this template in vitro via the fixed regions of the oligo (the mismatched randomized trimer codon region itself does not bind to the template).

(3) T7 DNA polymerase and T4 ligase are added, and the mutagenic oligo now functions as a primer for in vitro DNA replication, as the complementary strand is generated and the ends ligated together. The result is covalently closed circular dsDNA (CCC-ds DNA), with a dU-containing strand and a non-dU-containing strand (which contains the randomized trimer codon region).

(4) The CCC-ds-DNA is transformed into ung(+) E. coli. Uracil N-glycosylase excises U from the dU-containing strand, leaving apyrimidinic sites in it, which are recognized and cut by endonucleases. Thus the dU-containing strand is preferentially destroyed, and only the mutant strand is replicated, leading to its enrichment.

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Codon-Based Mutagenesis Applications

As described in the Introduction, the main use of trimer phosphoramidites is for developing partially randomized gene libraries based on the concept of codon-based mutagenesis. Because trimer codons cover all 20 natural amino acids, their use avoids problems associated with random point, or saturating, mutagenesis. In a random point mutagenesis approach, because the use of single base mutations generated by error-prone PCR produces only about six amino acid substitutions on average, concurrent mutation of adjacent nucleotides must occur; this requirement often makes it very difficult to find a clone with the desired amino acid change. For example, in a 100 aa protein, the likelihood of converting Tyr (TAC) to Asn (AAC) is only 1/900, the likelihood of converting Tyr (TAC) to Met (ATG) is 1.37 x 10E-9 (3). In saturating mutagenesis, pools of degenerate oligos are used with N's at the site(s) of the codon(s) to be mutated. However, because some amino acids are represented by higher numbers of codons than others (for example, six for Arg, one for Trp), there will be large differences in the relative amounts of oligos containing particular amino acid mutations, leading to substantial undesired codon bias. Also, about 5% of the oligos will contain stop codons that will cause improper chain termination (3). By contrast, use of trimer phosphoramidites allows the incorporation of an equimolar mix of all 20 natural amino acids, or any subset, into any position of a sequence. This advantage eliminates codon bias, presence of unwanted stop codons or frameshift mutations, and makes searching a clonal library for desired mutants much more efficient. Multiple amino acids within a protein can be modified at the same time while the overall tertiary structure is maintained. Trimer phosphoramidites have been used to develop phage display libraries with greater diversity (as much as 10x greater) than by traditional methods, with a high degree of amino acid uniformity (4). Trimer phosphoramidites were also used to maximize the diversity of a partially randomized library for use in developing streptavidin variants with altered specificities for desthiobiotin, a biotin analog, by directed evolution (5).

References

(1) Neylon, C. Chemical and biochemical strategies for the randomization of protein encoding DNA sequences: library construction methods for directed evolution. Nucleic Acids Res. (2004), 32: 1448-1459.

(2) Kayushin, A., Korosteleva, M., Miroshnikov, A. Large-scale solid-phase preparation of 3'-unprotected trinucleotide phosphotriesters-precursors for synthesis of trinucleotide phosphoramidites. Nucleosides Nucleotides Nucleic Acids (2000), 19: 1967-1976.

(3) Randolph, J., Yagodkin, A., Azhayev, A., Mackie, H. Codon-based Mutagenesis. Nucleic Acids Symposium Series (2008), 52: 479.

(4) Krumpe, L.R.H., Schumacher, K.M., McMahon, J.B., Makowski, L. Mori, T. Trinucleotide cassettes increase diversity of T7 phage-displayed peptide library. BMC Biotechol. (2007), 7: 65-72.

(5) Levy, M., Ellington, A.D. Directed Evolution of Streptavidin Variants Using IVC. Chem. Biol. (2008), 15: 979-989.

(6) Sidhu, S.S., Lowman, H.B., Cunningham, B.C., Wells, J.A. Phage display for selection of novel binding peptides. Methods Enzymol, 2000, 328, 333-363.

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Modificaton Code List

Modification	Code	Catalog Number
Spiking Custom	[CSXX]	26-CSXX
Trimer Codon Custom Mix	[TCX]	26-6542
Trimer Codon Mix 1 Sense (Mix of 20 Sense codons)	[TC1s]	26-6543
Trimer Codon Mix 2 Sense (Mix of 19 codons without Cys)	[TC2s]	26-6544

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Product Specifications

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Oligo Modifications

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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

Spiking Custom
[26-6200-XX]
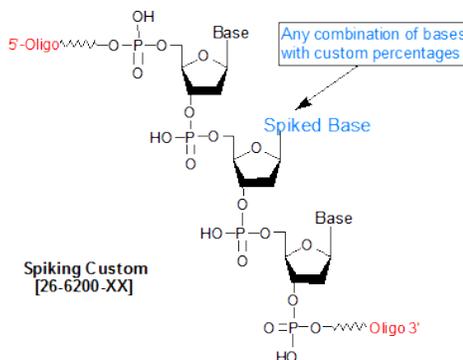
DNA & RNA Oligo Custom Spiking. The prices listed is for one custom spiking mix setup, additional standard per base charges apply.

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.

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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

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Product Specifications

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Oligo Modifications

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Trimer Codon Custom Mix

Category	Others
Modification Code	TCX
Reference Catalog Number	26-6542
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	930

Trimer Codon Mix
[26-6542-XX, 26-6543-XX, 26-6544-XX]
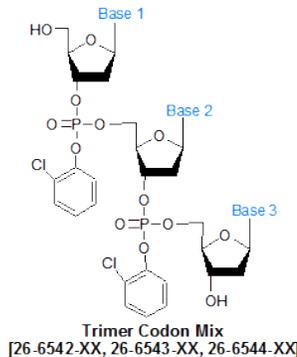
Custom Trimer Codon Sense Mix Percentage Ordering Template Download Sense Trimer Mix Excel Worksheet Template
Custom Trimer Codon Antisense Mix Percentage Ordering Template Download Antisense Trimer Mix Excel Worksheet Template
Custom Trimer Codon Combined Mix Percentage Ordering Template Download Custom Trimer Mix Excel Worksheet Template

Product Note Different combination in percentage of the individual 20 amino acid mix codons can be ordered to be synthesized at the same codon position. Similarly there is no limit to the number of different sites that can be such modified. At Gene Link we term these sites as 'trimer codon spiked'. Thus at a single codon position we can trimer codon spike it with a customer desired percentage of a particular codon or number of amino acid codon. The maximum length of the oligo is 250mer.

Trimer Mix Codon Usage Table & Reaction Factor Trimer Mix Codon Usage Table.

Description Directed molecular evolution and combinatorial methods are key strategies used for protein engineering research. These approaches commonly involve using partially randomized synthetic oligonucleotides to generate a partially randomized gene library, expressing it in an appropriate vector to generate the protein set encoded by the library, and then screening the expressed proteins for improved or modified characteristics. Examples of the latter include changes in protein-ligand binding affinity, enzyme selectivity, or protein stability. While a variety of site-directed mutagenesis methods can be employed to introduce randomization at specific base positions of a gene, such methods often introduce significant amounts of undesirable codon bias, frame-shift mutations, and stop codons into the library, due to the mismatch between the base-by-base nature of classical oligonucleotide synthesis and the triplet nature of the codon (1,2).

The problem of unwanted codon bias can be eliminated by synthesizing the random portion of the oligos codon-by-codon, with trimer phosphoramidites, instead of base-by-base.

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This codon-based mutagenesis system conveys the ability to effectively control codon bias in the system, as well as unwanted stop codons and frame-shift mutations. The researcher can choose to completely randomize a particular amino acid position in the expressed protein, or flexibly tune the relative amounts of different amino acids at that position, as needs require (3). Trimer phosphoramidites have been used to develop phage display libraries with greater diversity (as much as 10x greater) than by traditional methods, with a high degree of amino acid uniformity (4). Trimer phosphoramidites were also used to maximize the diversity of a partially randomized library for use in developing streptavidin variants with altered specificities for desthiobiotin, a biotin analog, by directed evolution (5).The combination of standard and custom Trimer Codon phosphoramidite mixes is a powerful research tool for anyone doing protein engineering research.

NOTE: Trimer Codon Mix 2 omits the codon for cysteine. This option is for those researchers who specifically want to exclude cysteine from their protein/peptide libraries. Such omission is often done to avoid complications resulting from intrachain and/or interchain crosslinking between cysteines, via their thiol groups, to form disulfide bridges. **References**

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4. Krumpe, L.R.H., Schumacher, K.M., McMahon, J.B., Makowski, L. Mori, T. Trinucleotide cassettes increase diversity of T7 phage-displayed peptide library. *BMC Biotechol.* (2007), **7**: 65-72.
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Product Specifications

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Oligo Modifications

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Trimer Codon Mix 1 Sense

Category	Others
Modification Code	TC1s
Reference Catalog Number	26-6543
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	930
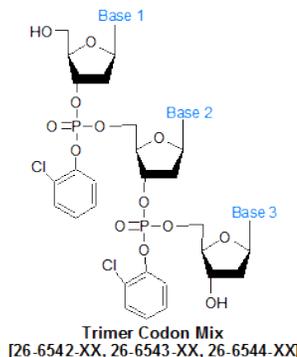
Trimer Codon Mix
[26-6542-XX, 26-6543-XX, 26-6544-XX]

Product Note Different combination in percentage of the individual 20 amino acid mix codons can be ordered to be synthesized at the same codon position. Similarly there is no limit to the number of different sites that can be such modified. At Gene Link we term these sites as 'spiked'. Thus at a single codon position we can spike it with a customer desired percentage of a particular codon or number of amino acid codon. The maximum length of the oligo is 250mer.

Trimer Mix Codon Usage Table & Reaction Factor Trimer Mix Codon Usage Table.

Description Trimer Codon Mix 1 Sense is a mix of all 20 Amino Acid Sense codons.

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Trimer phosphoramidites were also used to maximize the diversity of a partially randomized library for use in developing streptavidin variants with altered specificities for desthiobiotin, a biotin analog, by directed evolution (5).The combination of standard and custom Trimer Codon phosphoramidite mixes is a powerful research tool for anyone doing protein engineering research. **References**

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Oligo Modifications

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Trimer Codon Mix 2 Sense

Category	Others
Modification Code	TC2s
Reference Catalog Number	26-6544
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	930

Trimer Codon Mix
[26-6542-XX, 26-6543-XX, 26-6544-XX]
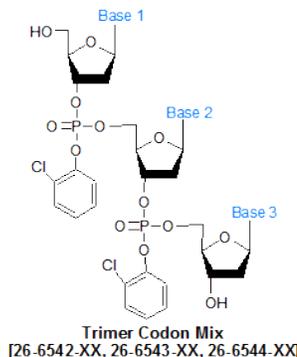
Trimer Codon Mix 2 (Mix of 19 codons without Cys) Product Note

Trimer Codon Mix 2 omits the codon for cysteine and contains codons for the other 19 amino acids. This option is for those researchers who specifically want to exclude cysteine from their protein/peptide libraries. Such omission is often done to avoid complications resulting from intrachain and/or interchain crosslinking between cysteines, via their thiol groups, to form disulfide bridges.

Custom Trimer Mix Product Note

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Trimer Mix Codon Usage Table & Reaction Factor Trimer Mix Codon Usage Table.

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The researcher can choose to completely randomize a particular amino acid position in the expressed protein, or flexibly tune the relative amounts of different amino acids at that position, as needs require (3). Trimer phosphoramidites have been used to develop phage display libraries with greater diversity (as much as 10x greater) than by traditional methods, with a high degree of amino acid uniformity (4). Trimer phosphoramidites were also used to maximize the diversity of a partially randomized library for use in developing streptavidin variants with altered specificities for desthiobiotin, a biotin analog, by directed evolution (5).The combination of standard and custom Trimer Codon phosphoramidite mixes is a powerful research tool for anyone doing protein engineering research.

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3. Kayushin, A., Korosteleva, M., Miroshnikov, A. Large-scale solid-phase preparation of 3'-unprotected trinucleotide phosphotriesters-precursors for synthesis of trinucleotide phosphoramidites. *Nucleosides Nucleotides Nucleic Acids* (2000), **19**: 1967-1976.

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