FIREBIRD
BIOMOLECULAR SCIENCES, LLC

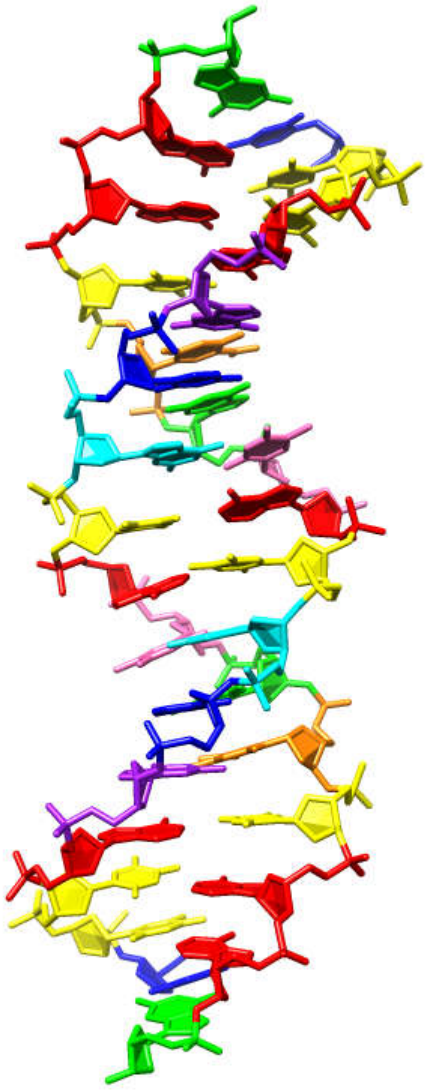
2021
Product Catalog

Nucleic acid analogs for:
- DNA sequencing
- Diagnostics
- SNP detection
- Synthetic Biology

www.firebirdbio.com

Contents

Firebird reagents and the origins of life



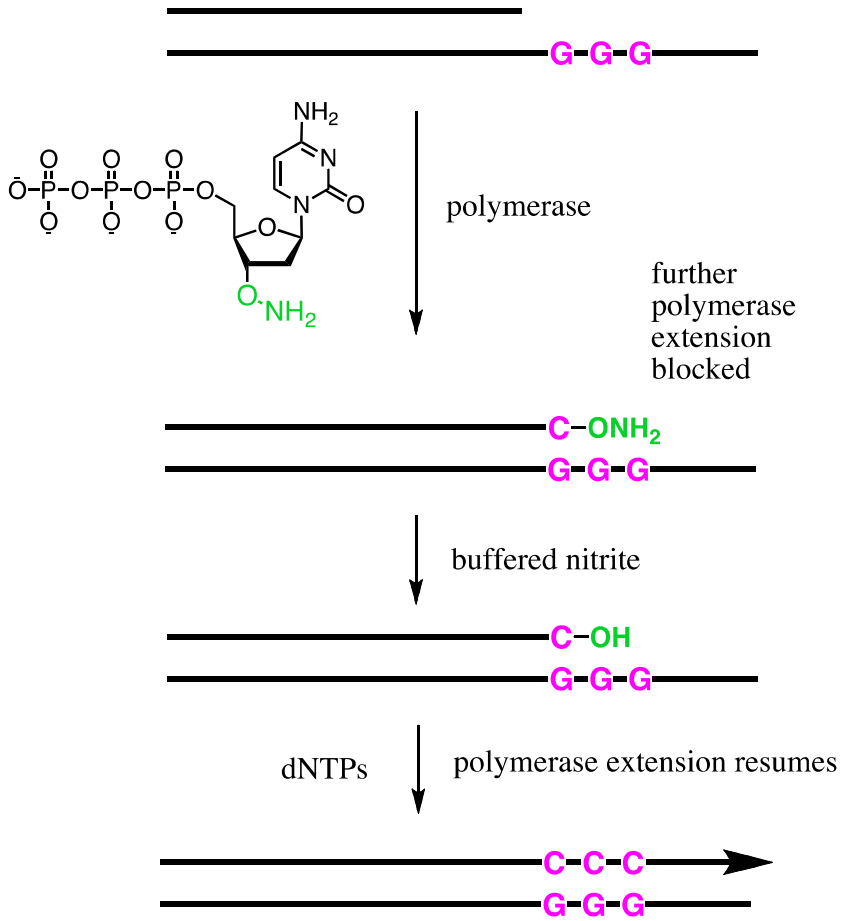
This catalog presents reagents and technologies to aid in the development of new biomedical innovations. Several of the products presented here were developed with the goal of better understanding the features of DNA and RNA that make them the carriers of genetic information for life on earth (Karalkar and Benner, 2018).

The figure shows the crystal structure of a double helix built from an expanded genetic alphabet of eight hachimoji building blocks, G (green), A (red), C (dark blue), T (yellow), B (aqua) P (blue), S (violet), and Z (orange). Adopted from Hoshika et al., 2019.

Reagents in this catalog are sold for research use only. Please inquire for IVD use.

Reversible terminators

Firebird has introduced the 3'-ONH_2 reversible terminator as an alternative to the larger 3'-OCH_2N_3 group (Hutter et al., 2010). The 3'-ONH_2 group is accepted by a variety of polymerases. After incorporation, further primer extension is blocked. The 3'-ONH_2 group is cleaved with buffered aqueous sodium nitrite to regenerate a standard 3'-OH.



3'-ONH_2 reversible terminators can be used in DNA sequencing (Hutter et al., 2010), Oligonucleotide synthesis (Jensen and Davis, 2018, Sarac and Hollenstein, 2019) and SNP analysis (Chen et al., 2010).

Polymerase variants have been developed that incorporate these terminators with improved efficiency over standard Taq DNA polymerase (Chen et al., 2010):

POL475-400	400 units	$110.00
POL475-1000	1000 units	$250.00

Reversible terminators: Which one?

As shown on the following pages, Firebird offers reversible terminators in several different formats:

The ONH2-170 series: Ready to use, untagged
The ONH2-100 series: Oxime blocked, untagged
The ONH2-DT series: Diol-linked tags
The ONH2-DS series: Disulfide-linked tags

The 170 series ("ready to use") are crude products from a solid phase synthesis procedure that results in approximately 90% pure terminator triphosphates, which should be sufficient for most purposes. This series carries a free 3'-ONH_2 group and can be incorporated by various enzymes without further modification. The shelf life of this series is about one year (if stored properly, at or below -20°C).

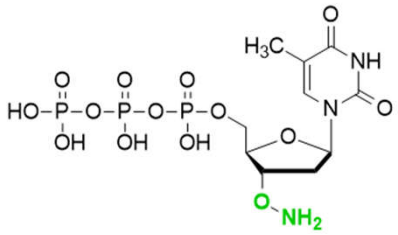
The 100 series ("oxime blocked") are the acetoxime form of the 170 series and has a longer shelf life (many years, if stored properly). This series is HPLC purified to remove most of the impurities, albeit at an increased cost. But these oximes will not be incorporated by enzymes. For enzymatic incorporation the free 3'-ONH_2 must be regenerated before use, which can be achieved in situ with buffered aqueous methoxylamine.

The DT and DS series are reversible terminators carrying a cleavable linker attached to the nucleobase with a free amino group, allowing for the attachment of a tag (e.g. fluorescent dye or biotin). As in the 100 series, the 3'-ONH_2 group is protected as the acetoxime, for attachment of the tag and subsequent purification as well as increased shelf life. And as with the 100 series, this oxime needs to be deprotected with buffered aqueous methoxylamine before use. Upon incorporation of the terminator into an oligonucleotide the linker can be cleaved under mild conditions (dilute aqueous periodate for the DT series, phosphine or thiol reagents for the DS series) to remove the tag.

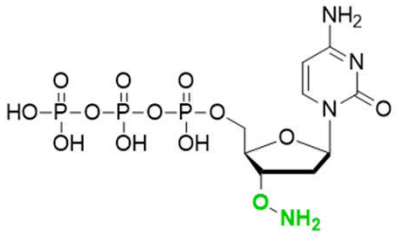
For a fee, Firebird scientists can attach a customer specified tag onto the primary amine; please inquire for pricing.

Reversible terminators:
Ready to use, untagged

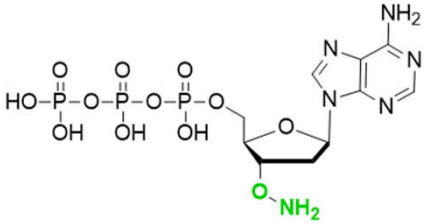
Reversible terminators with a free 3'-ONH_2 are ready to use and do not require deprotection or further treatment before use.



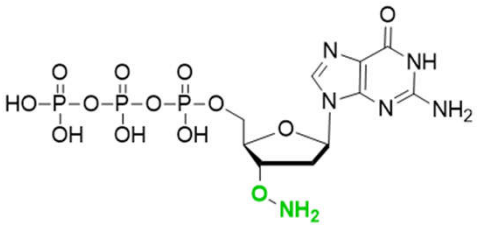
Thymine
TONH2-171
5 µmoles $600.00
50 µmoles $ 4200.00



Cytosine
CONH2-172
5 µmoles $750.00
50 µmoles $5250.00



Adenine
AONH2-173
5 µmoles $750.00
50 µmoles $5250.00

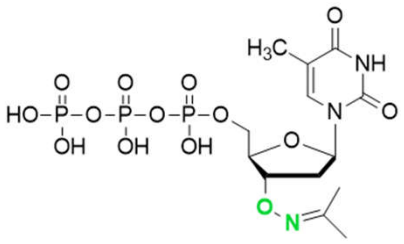


Guanine
GONH2-174
5 µmoles $750.00
50 µmoles $5250.00

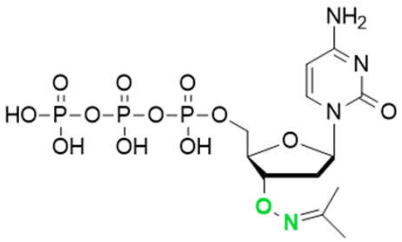
Please inquire for bulk pricing.

Reversible terminators:
Oxime blocked, untagged

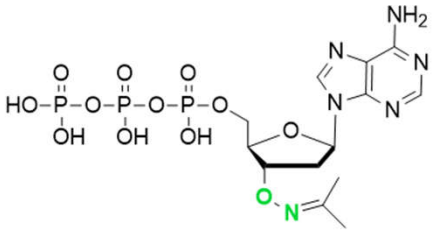
Reversible terminators are also available with the 3'-ONH_2 protected as the acetoxime, which are more suitable for long term storage than our ready-to-use reversible terminators. For enzymatic incorporation, the oxime must be deprotected to the free 3'-ONH_2 before use, which can be achieved in situ with buffered aqueous methoxylamine.



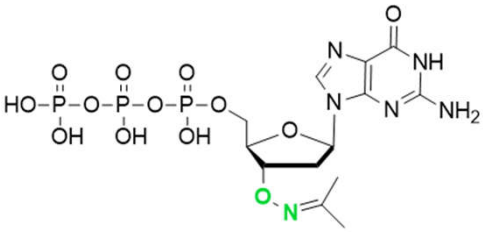
Thymine
TONH2-101
5 µmoles $900.00



Cytosine
CONH2-102
5 µmoles $1050.00



Adenine
AONH2-103
5 µmoles $1050.00

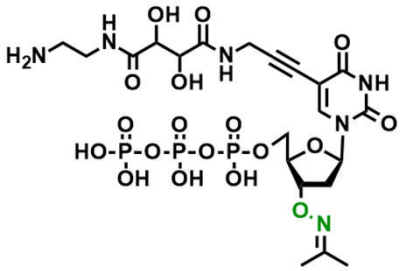


Guanine
GONH2-104
5 µmoles $1050.00

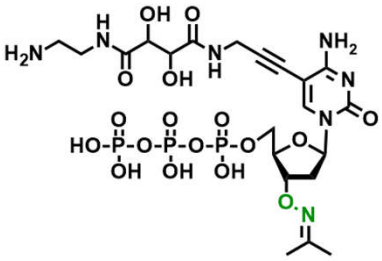
Please inquire for bulk pricing and availability.

Reversible terminators: diol-linked tags

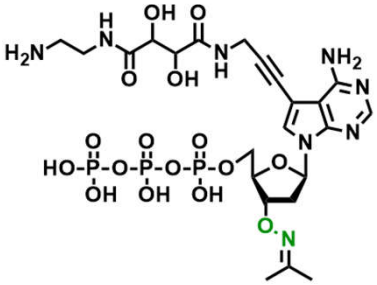
Firebird offers reversible terminators with a 3'-ONH_2 (protected as the acetoxime) and a diol linker carrying a free amino group, to which a tag (fluorescent dye or other moiety) can be attached. The diol can be rapidly cleaved with dilute aqueous periodate.



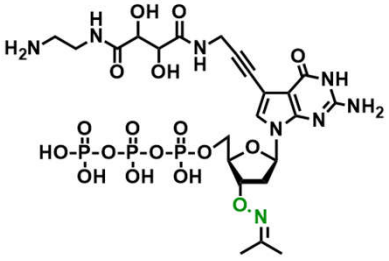
Thymine
TONH2-DT
1 µmole $1750.00



Cytosine
CONH2-DT
1 µmole $1900.00



Adenine
AONH2-DT
1 µmole $2200.00



Guanine
GONH2-DT
1 µmole $2300.00

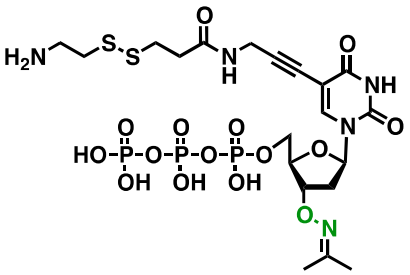
For enzymatic incorporation, the oxime must be deprotected to the free 3'-ONH_2 before use, which can be achieved in situ with buffered aqueous methoxylamine.

Please inquire for bulk pricing.

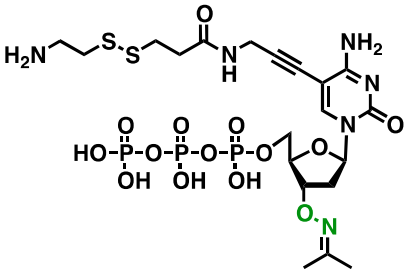
Not available for sequencing applications on certain machines and in certain jurisdictions. Please inquire.

Reversible terminators: disulfide-linked tags

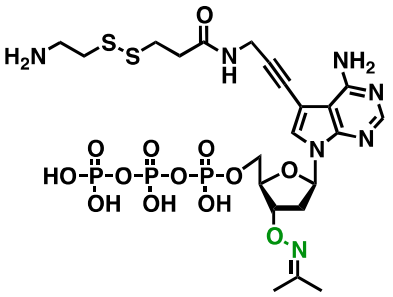
Firebird offers reversible terminators with a 3'-ONH_2 (protected as the acetoxime) and a disulfide linker carrying a free amino group, to which a tag (fluorescent dye or other moiety) can be attached. The disulfide can be rapidly cleaved with phosphine or thiol reagents.



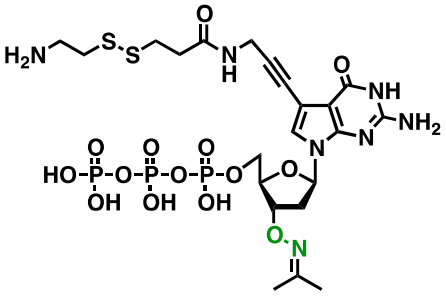
Thymine
TONH2-ST
1 µmole $ 1750.00



Cytosine
CONH2-ST
1 µmole $ 2000.00



Adenine
AONH2-ST
1 µmole $ 2400.00



Guanine
GONH2-ST
1 µmole $ 2500.00

For enzymatic incorporation, the oxime must be deprotected to the free 3'-ONH_2 before use, which can be achieved in situ with buffered aqueous methoxylamine.

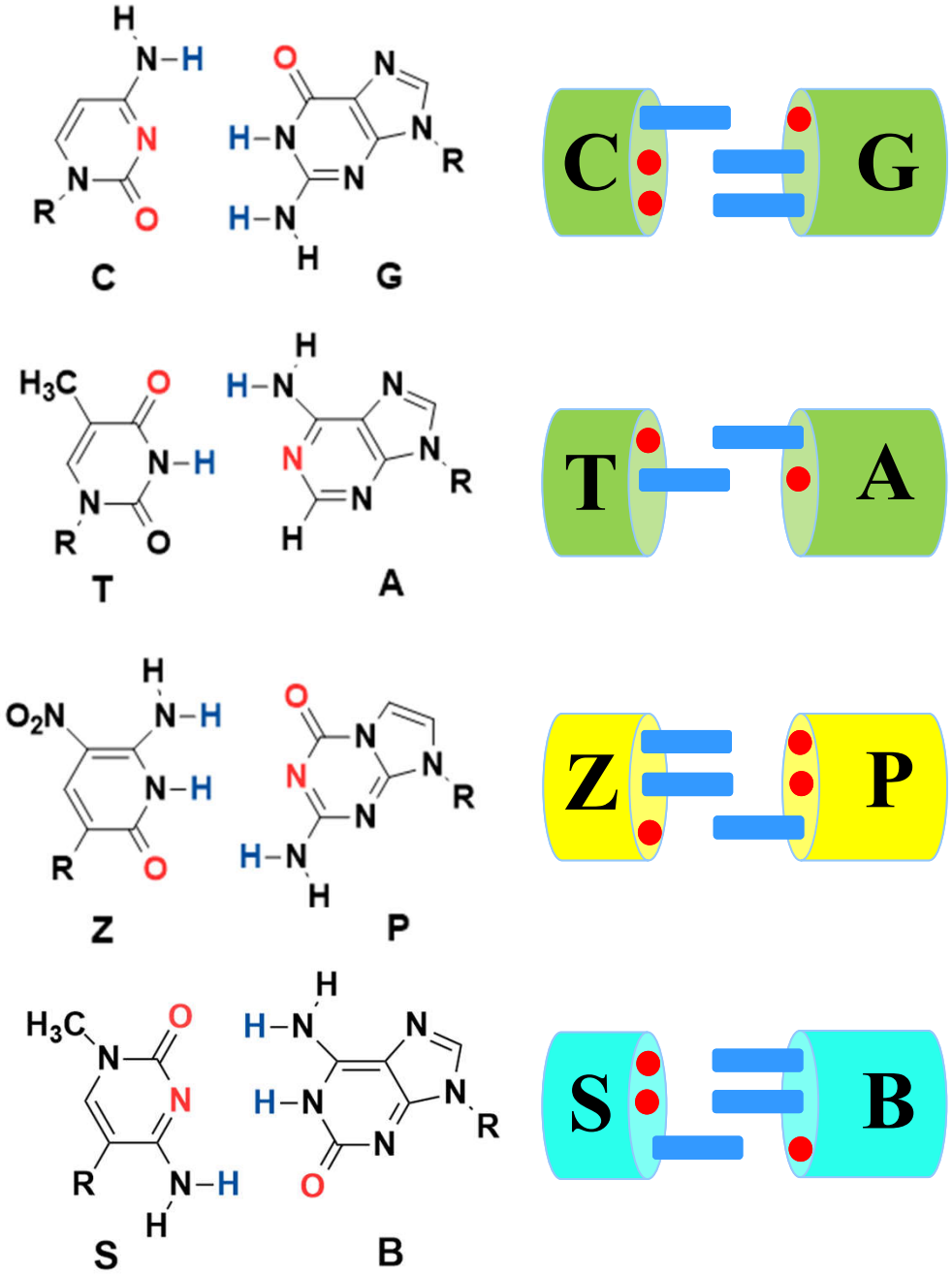
Please inquire for bulk pricing.

Not available for sequencing applications on certain machines and in certain jurisdictions. Please inquire.

AEGIS™

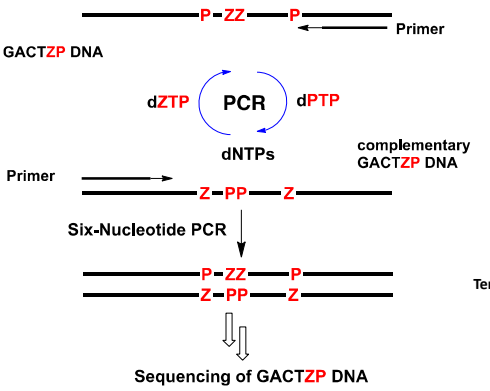
Artificially Expanded Genetic Information Systems

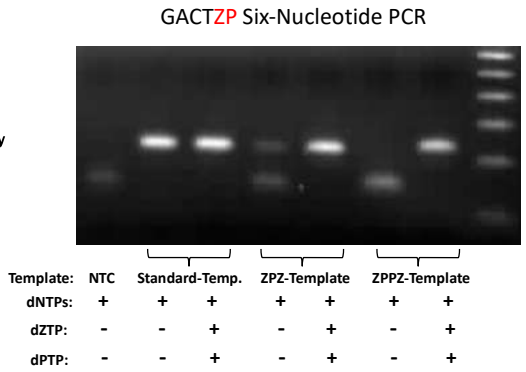
Firebird has created different heterocycles in order to implement additional hydrogen bonding patterns for the Z:P and S:B base pairs as shown below. These base pairs are "orthogonal" to C:G and A:T (Benner, 2004; Hoshika et al., 2019; Sefah et al., 2014; Yang et al., 2006).



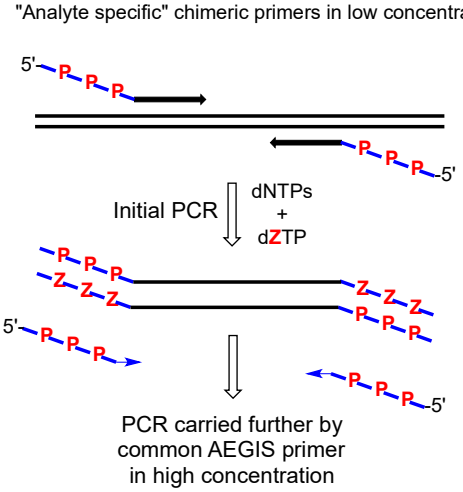
Using AEGIS:
For ultra-clean nested PCR

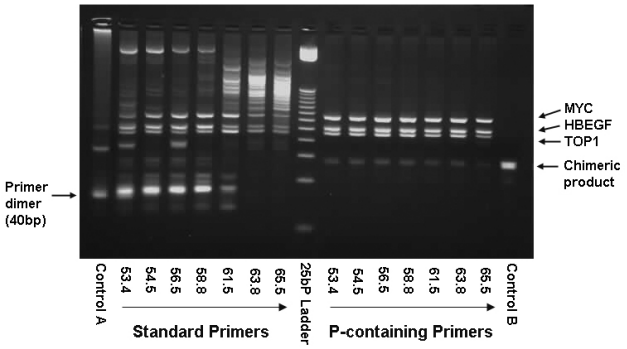
The Z:P pair is retained during PCR amplification, even when present as consecutive base pairs (Yang et al., 2011).





AEGIS-nested primers suppress noise in multiplex PCR (Yang et al., 2010) and support 22-plex PCR to detect RNA viruses (Glushakova et al., 2015a).

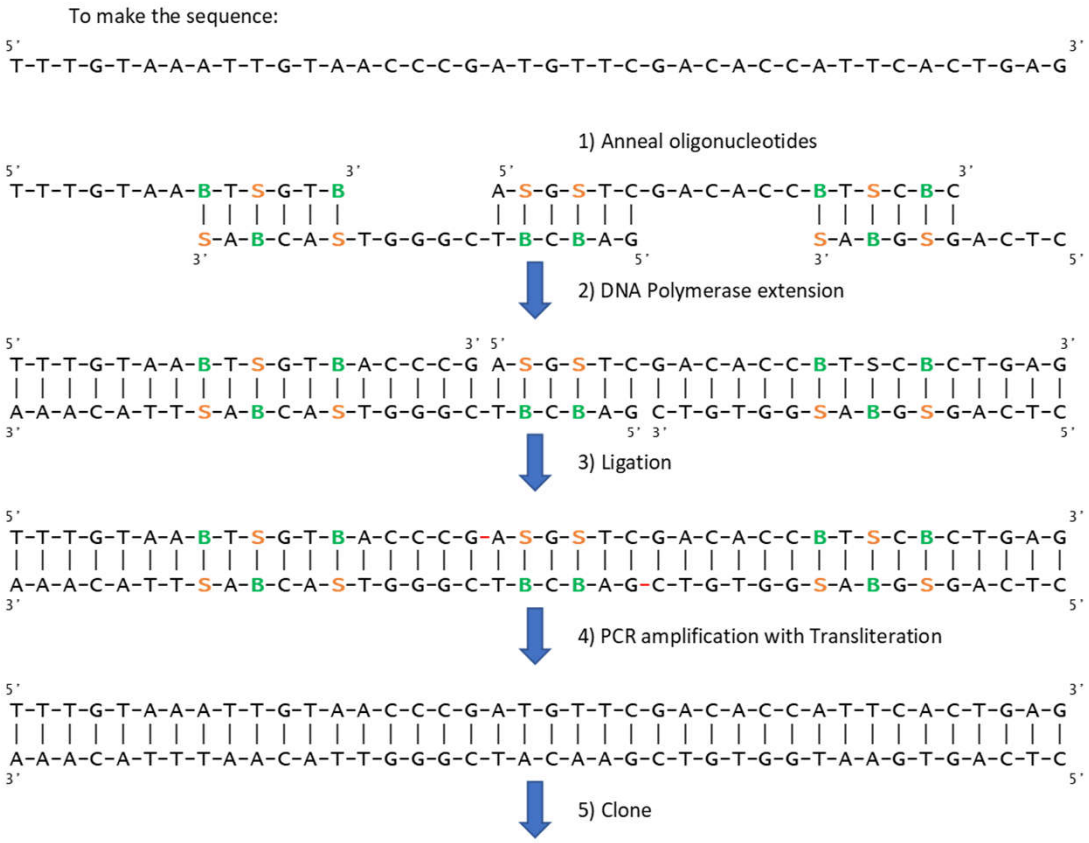




Using AEGIS:
Assembling long DNA constructs

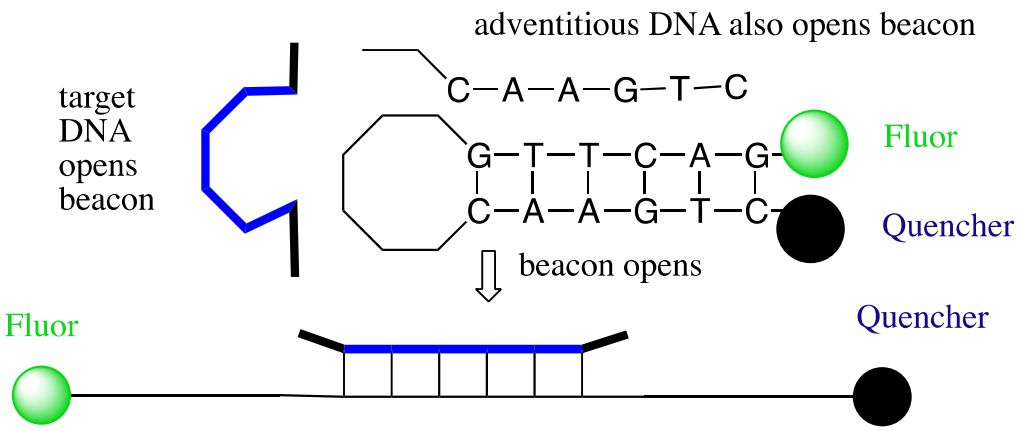
Adding AEGIS nucleotides to single-stranded oligos increases their information density. This allows clean and rapid hybridization of single stranded DNA unobstructed by hairpins, wandering strands, and non-canonical interactions. Then, using Firebird's "transliteration" technology, the AEGIS nucleotides are cleanly replaced by standard nucleotides giving an entirely natural gene (Bradley and Benner, 2014; Merritt et al., 2014).

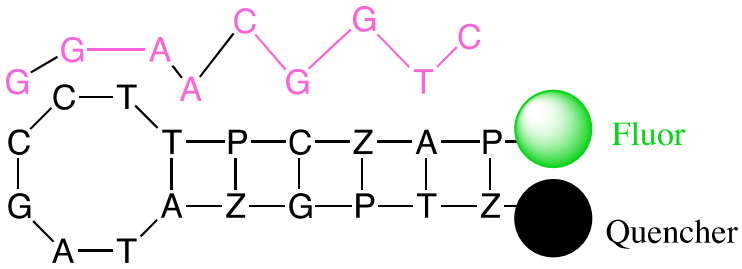
An example of this assembly is shown below: S and B residues in the tails of the primers guide specific alignment of the unique sequences at the ends of the primers. The SB basepair is converted to a TA basepair during PCR amplification.



Using AEGIS:
Molecular beacons

Adding AEGIS nucleotides to the stems of molecular beacons prevents their being opened by adventitious DNA and RNA in a complex biological sample. This allows beacons to deliver signals in complex biological samples with significantly lower noise (Sheng et al., 2008).





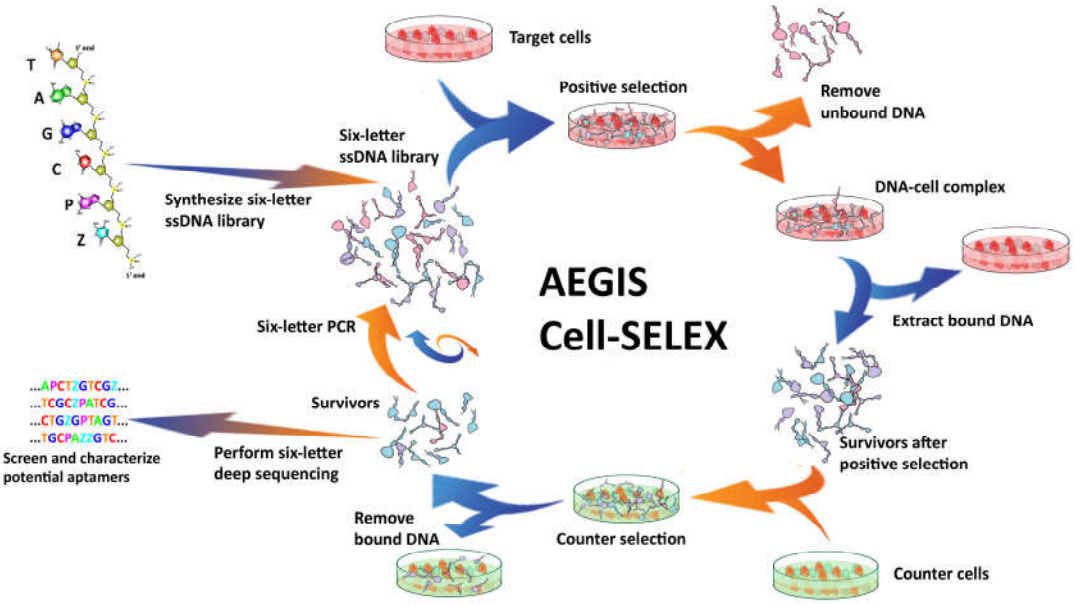
No adventitious DNA can open a beacon that has an AEGIS stem

Firebird offers custom-synthesized beacons with a wide range of fluorescent dyes and quenchers. Please inquire.

AEGIS:
Increasing Aptamer diversity

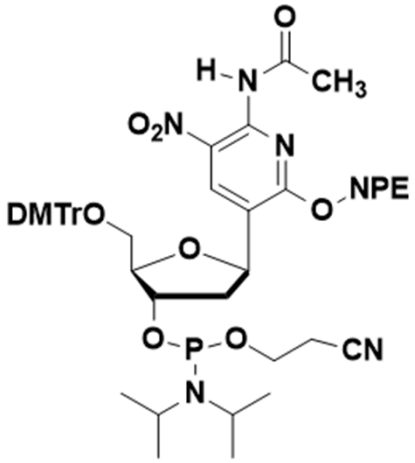
The dP and dZ bases increase the chemical repertoire of nuleic acids for aptamer selections (Biondi et al., 2016; Sefah et al., 2014; Zhang et al., 2015, 2016). Of special note is the NO_2 group present on dZ.

The figure below shows one application of AEGIS for the selection of aptamers specific to a cell surface protein on a cancer cell (Zhang et al., 2016).



AEGIS:
dZ and dP Phosphoramidites

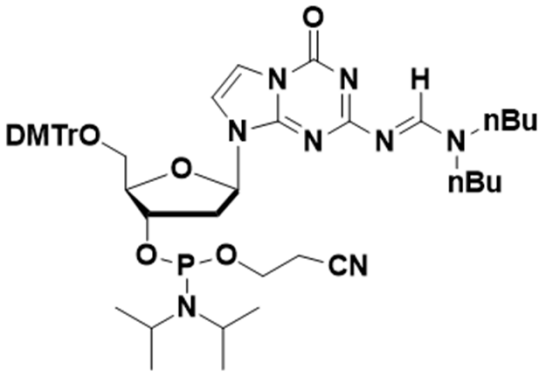
dZ Phosphoramidite



Hydrogen bonding pattern: Z
Sugar: 2'-Deoxyribose
Heterocycle: Nitropyridine
Linear Formula $C_{50}H_{57}N_6O_{12}P$
Mol Weight 964.99

Cat. No. dZ-PA-101
100 mg $ 720.00
1 gram $5760.00

dP Phosphoramidite



Hydrogen bonding pattern: P
Sugar: 2'-Deoxyribose
Heterocycle: Imidazotriazine
Linear Formula $C_{49}H_{65}N_8O_7P$
Mol Weight 909.08

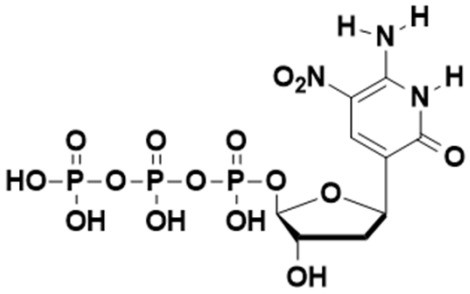
Cat. No. dP-PA-102
100 mg $ 850.00
1 gram $6800.00

(Yang et al., 2006)
Please inquire for bulk pricing

Oligonucleotides are available that incorporate dZ and dP.
Please inquire for availability of ribonucleoside derivatives.

AEGIS:
dZ and dP Triphosphates

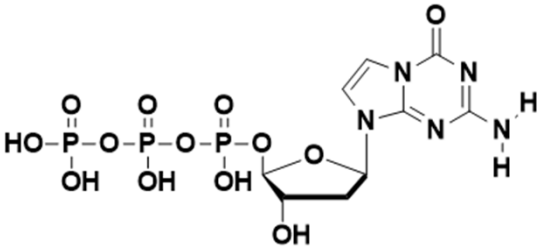
dZ Triphosphate



Hydrogen bonding pattern: Z
Sugar: 2'-deoxyribose
Heterocycle: Nitropyridine
Linear Formula $C_{10}H_{16}N_3O_{15}P_3$
Mol Weight 511.17

Cat. No. dZTP-101
1 µmole $ 400.00
5 µmoles $ 1600.00

dP Triphosphate



Hydrogen bonding pattern: P
Sugar: 2'-deoxyribose
Heterocycle: Imidazotriazine
Linear Formula $C_{10}H_{16}N_5O_{13}P_3$
Mol Weight 493.15

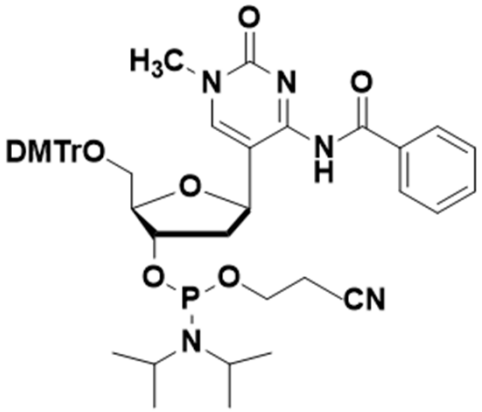
Cat. No. dPTP-201
1 µmole $ 420.00
5 µmoles $ 1680.00

(Yang et al., 2007)
Please inquire for bulk pricing.

Please inquire for availability of ribonucleoside derivatives.

AEGIS:
dS and dB Phosphoramidites

dS Phosphoramidite

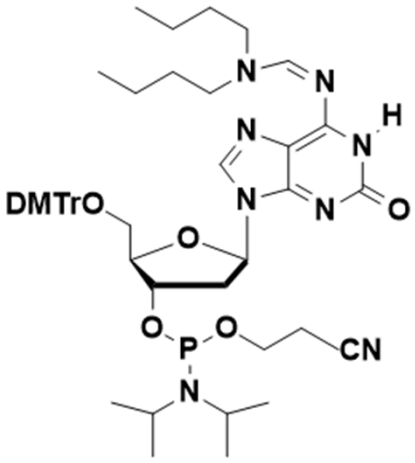


Hydrogen bonding pattern: S
Sugar: 2'-Deoxyribose
Heterocycle: Pseudo-C
Linear Formula $C_{47}H_{54}N_5O_8P$
Mol Weight 847.93

Cat. No. dS-PA-104S

100 mg	$ 720.00
1 gram	$5760.00

dB Phosphoramidite



Hydrogen bonding pattern: B
Sugar: 2'-Deoxyribose
Heterocycle: Purine
Linear Formula $C_{49}H_{68}N_7O_7P$
Mol Weight 898.08

Cat. No. dB-PA-103P

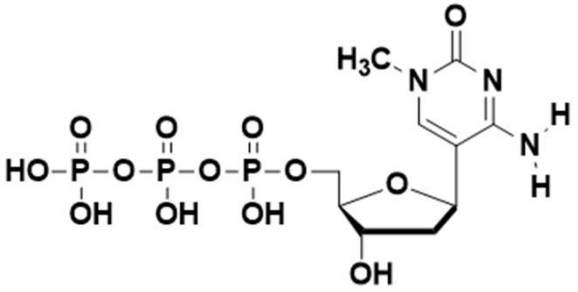
100 mg	$ 600.00
1 gram	$4800.00

Please inquire for bulk pricing.

Oligonucleotides are available that incorporate dS and dB.
Please inquire for availability of ribonucleoside derivatives.

AEGIS:
dS and dB Triphosphates

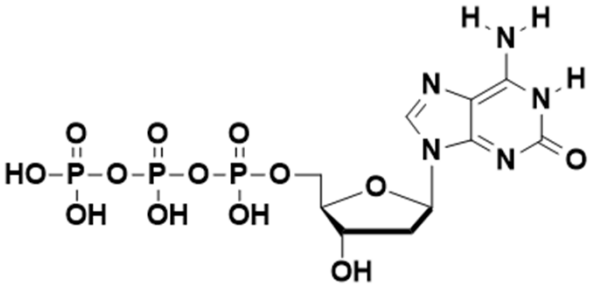
dS Triphosphate



Hydrogen bonding pattern: S
Sugar: 2'-deoxyribose
Heterocycle: Pseudo-C
Linear Formula $C_{10}H_{18}N_3O_{13}P_3$
Mol Weight 481.18

Cat. No. dSTP-401S
1 µmole $ 400.00
5 µmoles $ 1600.00

dB Triphosphate



Hydrogen bonding pattern: B
Sugar: 2'-deoxyribose
Heterocycle: Purine
Linear Formula $C_{10}H_{16}N_5O_{13}P_3$
Mol Weight 507.18

Cat. No. dBTP-301P
1 µmole $ 400.00
5 µmoles $ 1600.00

Please inquire for bulk pricing.

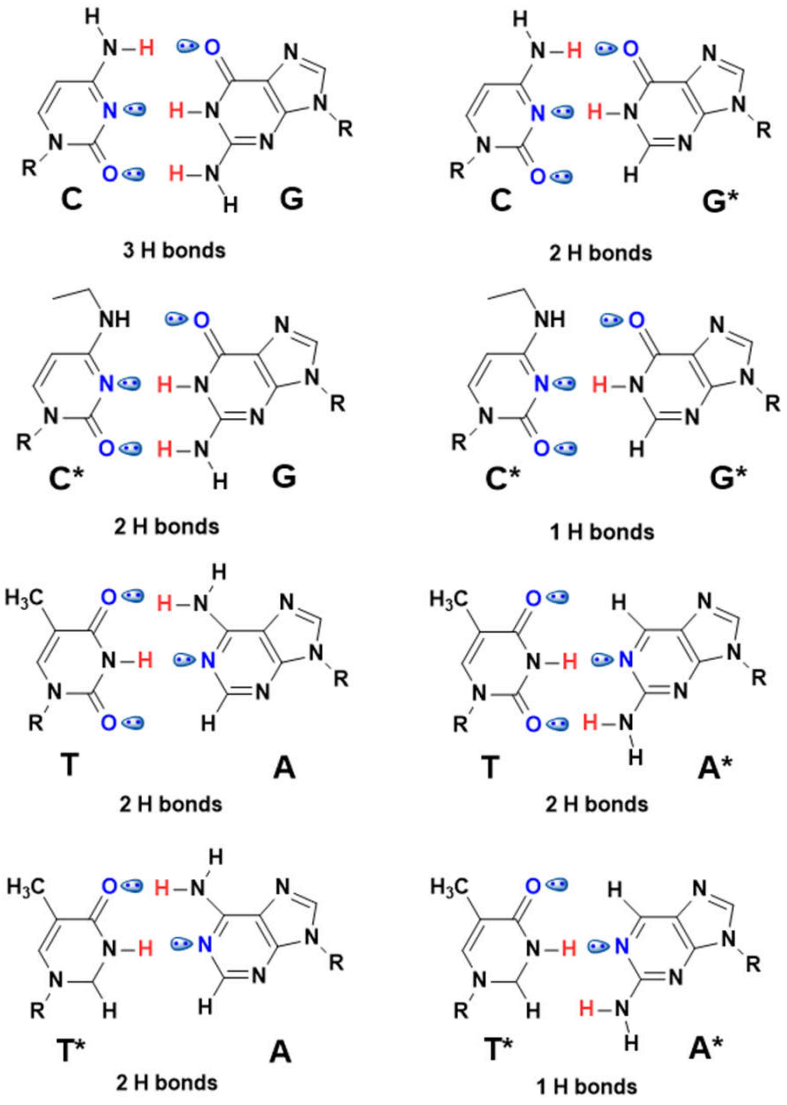
Please inquire for availability of ribonucleoside derivatives.

SAMRS™

Self Avoiding Molecular Recognition System

Nucleic acid amplification reactions suffer from the formation of off-target amplification products. This problem increases with the number of primers in the reaction. It mainly occurs because of primer-primer interactions. Thus, selectively removing hydrogen bonding units from the primer bases results in *self-avoiding* DNA primers that can be added to enable and improve multiplexed PCR (Hoshika et al., 2010).

As shown below, SAMRS bases, indicated with a *, can base pair with standard bases (in the target or amplicon) but not with other SAMRS bases.



SAMRS:
design rules

General rules for placement of SAMRS bases into primers used in PCR and other amplification techniques. Upper case letters indicate the standard base, lower case letters indicate the SAMRS base.

1. Recommended lengths are 20-35 nts. SAMRS bases should be utilized in the first 4, (up to 8), positions at the 3' end of the oligonucleotide but not in the very first 3' base.

2. Between two and four SAMRS bases should be used per oligonucleotide, with two or three SAMRS modifications being preferred.

3. The SAMRS t base has not demonstrated as much reduction in primer dimer formation so it is preferable to substitute a, g or c rather than t when given a choice.

4. Avoid using SAMRS bases in a string of three and four consecutive identical bases. For example: ggg, ccc, aaa, ttt, gggg, cccc, aaaa, and tttt are to be avoided.

5. Separate SAMRS bases with standard bases, for example: gGg, cCc, aAa, and tTt.

6. If the 3'-end of an oligo is standard A or T, the second base should also be a standard base, followed by two or three SAMRS modifications.

(Benner et al., 2015; Glushakova et al., 2015a, 2015b; Hoshika et al., 2010; Sharma et al., 2014; Yang et al., 2015 and unpublished data)

As with all primer designs, Firebird makes no guarantee as to the performance of oligonucleotides made by following these rules.

SAMRS:
Phosphoramidites

t* phosphoramidite

1 g	$ 1050
10 g	$ 8220

a* phosphoramidite

1 g	$ 655
10 g	$ 5340

c* phosphoramidite

1 g	$ 394
10 g	$3150

g* phosphoramidite

1 g	$ 110
10 g	$ 880

Oligonucleotides are available that incorporate the SAMRS bases.

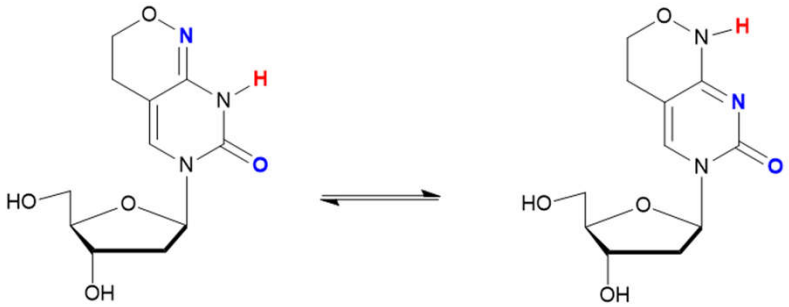
SAMRS can be combined with AEGIS in oligos to get highly multiplexed and highly clean PCR, as well as isothermal amplification. Please inquire.

Biversal™ nucleotides

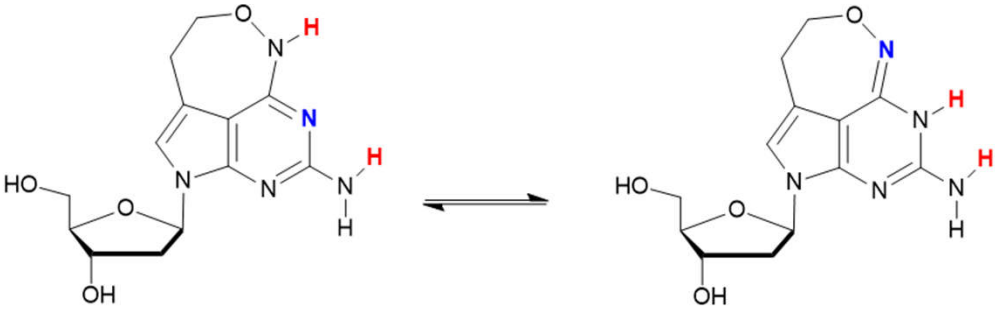
The divergence present in pathogen genomes makes it difficult or impossible to design a single probe or primer that is the exact complement of all possible target sequences. In practice this is handled using degenerate or "universal bases" (e.g. inosine).

Firebird scientists have created two "biversals"™. Each exists in two tautomeric forms that provide alternate hydrogen bonding patterns. This allows the pyrimidine biversal to pair with either guanine or adenine and the purine biversal to pair with either thymine or cytosine. These bases provide a new alternative to the existing methods of handling divergent sequences for both PCR amplification and SNP detection (Yang et al., 2018).

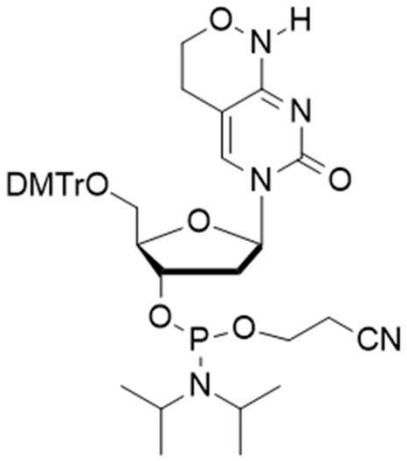
Tautomeric forms of the Pyrimidine biversal:



Tautomeric forms of the Purine biversal:



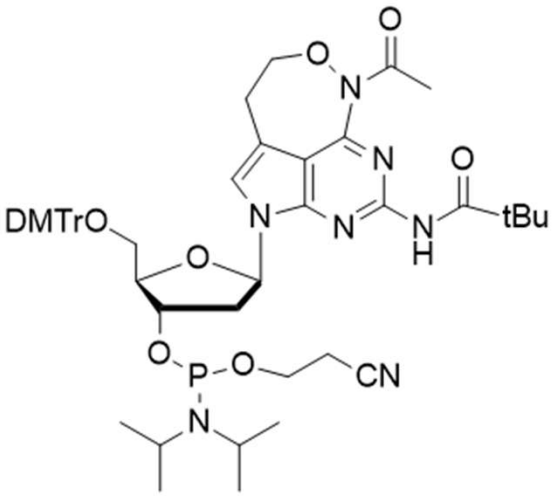
Biversal:
Phosphoramidites



Pyrimidine biversal
phosphoramidite
Linear Formula $C_{41}H_{50}N_5O_8P$
Mol Weight 771.84

Cat No. Pyr-BiVer

100 mg	$ 500
1 gram	$ 4000



Purine biversal
phosphoramidite
Linear Formula $C_{50}H_{62}N_7O_9P$
Mol Weight 936.04

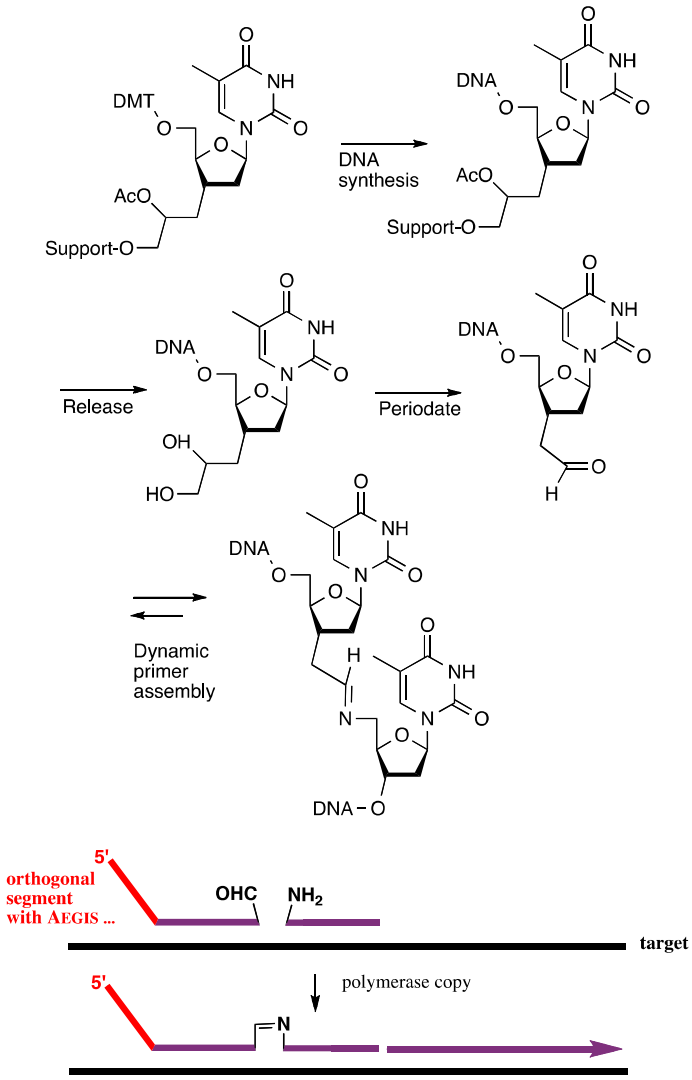
Cat No. Pur-BiVer

100 mg	$ 2850
1 gram	Inquire

Firebird sells oligonucleotides that contain the purine and pyrimidine biversals. Please inquire.

SNAP2™ Oligonucleotides: Priming with high specificity

Specific DNA priming requires oligonucleotides with complementarity of ≥16 nucleotides but the ability to discriminate against single mismatches. Dynamic assembly of a primer on a template can provide this (Leal et al., 2006).



Controlled pore glass with aldehyde precursor
SNAP2T_HJK001
100 mg $600.00

Firebird sells phosphoramidites, oligos and libraries with SNAP2 ends. Please inquire.

SNAP2 primers are not to be confused with Snap-Tag® and other registered trademarks of New England BioLabs.

Purchasing, shipping and handling

Contact

Orders
By email: orders@firebirdbio.com
By telephone: (386) 418-0347
9:00 - 5:00 Eastern Time

Technical inquiries
support@firebirdbio.com

Reagents and enzymes are shipped at the customer's expense, on dry ice as appropriate.

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