ALTA BIOSCIENCE LTD

DNA Oligo Synthesis

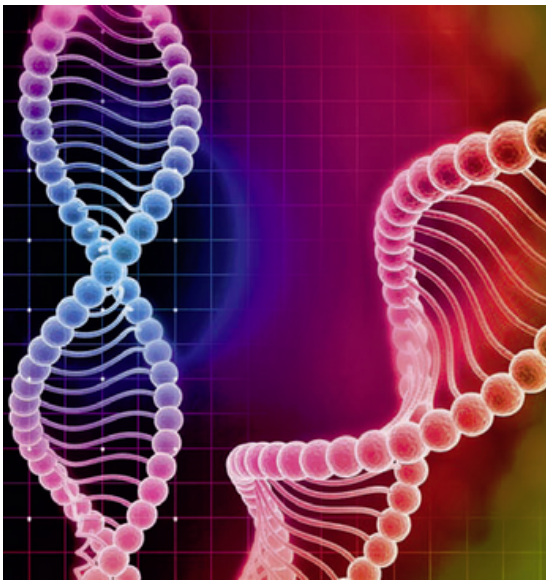
Technical Document

Alta Bioscience offers custom DNA Oligo Synthesis with a wide range of scale and purification options.

Oligonucleotides are short, single-stranded molecules of DNA which can be made to specific lengths and sequence requirements. They are used in a large range of applications and play a pivotal role in aiding genetic testing, medical research and forensic investigation.

AltaBioscience has more than 30 years experience of offering high-quality, ready to use custom oligonucleotides and primers which we supply at exceptional speed. Synthesis of ssDNA (single stranded DNA) is typically 15-25 bases long, although we can make oligos up to 100 bases. We offer a range of modifications, including fluorescent tags and purification options to suit requirements.

In conjunction with our peptide synthesis service, we can also synthesis peptide-DNA oligo conjugation.



Why Use AltaBioscience?

AltaBioscience is certified to ISO 9001 quality management system for the laboratory, which guarantees that our products meet both customer and regulatory requirements. Recognised as specialists in modified oligo syntheses, both range of scale and purity options are available and many modified bases are held in stock.

Typical Applications

- Probes for sequence detection – usually containing a dye to give a signal.
- Primers for amplification and sequencing.
- Mutagenesis for insertion into genes or proteins.
- Hybrids – using peptides to chaperone DNA into cells.
- Artificial gene synthesis.

Methodology

AltaBioscience have been making oligos for over 30 years and employ a robust and rapid phosphoramidite method of oligonucleotide production, as devised by Beaucage and Carruthers in 1981. Using solid phase synthesis chemistry, the initial nucleotide base is covalently bound via its 3'-terminal hydroxyl group to controlled pore glass (CPG). Chain extension of each additional base is then undertaken in a stepwise monitored process involving 4 clearly defined operations towards the 5' end of the oligo, remaining on the CPG until complete, when it can be cleaved from the support. The method of building the oligo comprises of four steps: deblocking; coupling; capping; and oxidation.

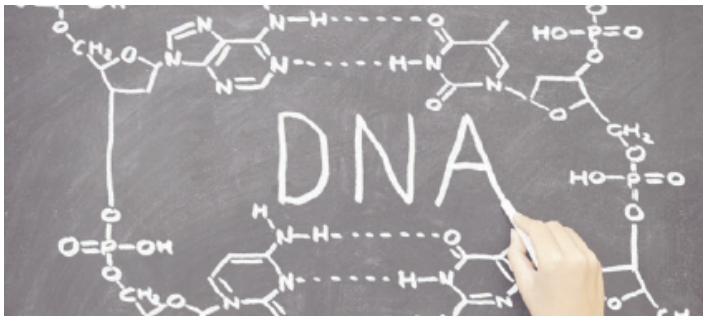
- **Deblocking:** The first step removes the protective DMT (4,4'-dimethoxytrityl) group that is attached to the reactive 5'-hydroxyl group of the nucleotide base, using 3% trichloroacetic acid. This forms a distinctive orange colour in the acid, which is used as a measure of reaction efficiency.
- **Coupling:** The reactive 5'-OH of the nucleotide is now free to couple with amidite with the aid of an activating solution (tetrazole). This step is fast, taking only 60-90 seconds for the reaction to take place.
- **Capping:** Any unreacted 5'-OH is acetylated to block further extension of truncated molecules and prevent internal deletions.
- **Oxidation:** This final step converts the unstable phosphite-triester into the stable phospho-triester achieved by iodine oxidation in the presence of water and pyridine. Oligonucleotide phosphorothioates are produced by substituting the oxidation step with sulphurisation.

Our oligos are made on Expedite instruments where trityl data collection and monitoring are incorporated as part of our QC process. Once the oligo is made, it is stable on the glass until it is cleaved using an alkaline solution of methylamine and ammonia. Following cleavage, the oligo is quantified using a spectrophotometer to provide an accurate measure of yield then lyophilised, ready for use or for further purification.

Scale

AltaBioscience provides custom DNA synthesis ranging from 0.2 umole to 15umole in a single synthesis run. There is no minimum order requirement for any scale.

The scales specify the amount of starting material used for each oligo synthesis. The final yield will be reduced due to inefficiencies in the chemical process and can also be affected by the incorporation of modifications, purification and oligo length. Expected synthesis yield is generally about half of the starting scale, although this is sequence specific and length dependant.



Purification

Various purification options are available depending on the application of the oligo:-

- **Unpurified:** If the oligo is to be used for simple PCR work or gene sequencing, then purification is not necessary.
- **Desalted:** Essential for mutagenesis. Removes excess salts and the base protection groups from the final product.
- **HPLC:** Useful for cloning, insertion work and double dye work and molecular beacons, removing all truncated oligo, salts and side-chain protecting groups from the target oligo.

Modifications

Modified oligos have many applications, for example, in crystallographic studies, DNA damage/repair investigations, for conjugation to other compounds or for phosphorothiote backbones and end-caps, to make them more resistant to nuclease attack. We offer an extensive range of modification options and hold many modifiers in stock to ensure a faster turnaround of your specialised oligo. Many modifications are available at both 3' and 5' ends of the oligo.

Modification options available include :

- Degenerate oligos (incorporation of mixed bases at single positions) provided at no extra cost. AltaBioscience uses the IUPAC code for mixed bases.
- Labelled compounds (e.g. FAM, BHQ, Dabcyl, DylightTM dyes),
- non-fluorescent modifications (biotin, thiol, amino groups, phosphate groups)
- sugar, base and backbone modifications (Bromo dU, inosine, phosphorothioate, oxo-dG).
- Spacers (PEG, available with a range of spacer atoms)

We are happy to quote for novel compounds and work with our customers so we can produce the product they need. Please contact us for more details should you require other compounds not listed.

DNA-peptide hybrids

AltaBioscience offers custom synthesis of oligo-peptide hybrids. By synthesising cell penetrating peptides, the cargo DNA can be delivered into the cell. Both the peptide and oligo can be modified to incorporate dyes and tags to assist in identification within the cell.

The peptide-oligo bond can be either stable using specific bifunctional linkers or cleavable, linked via a disulphide bridge which will be reduced within the cell to yield the unbound DNA.

You will receive

You will receive a Certificate of Analysis detailing Tm, MW, OD values and dilution information along with your oligo product which will be supplied quantified and lyophilised ready for use. We archive all of our oligo sequences to make re-ordering a quick and easy process for our customers.

Storage and resuspension

All oligos are supplied dried in individual glass vials and are considered stable until re-suspended. These should be stored at -20°C until used. For immediate use, reconstitution using sterile water is suitable. When aliquoting in to smaller amounts for storage at -20°C, TE buffer (Tris-EDTA, pH 7.5-8.0) provides some resistance to nuclease degradation. We recommend that each aliquot is thawed only once as repeated freeze-thaw cycles can cause degradation.

Contact

To discuss any aspect of this service please contact us by phone on:
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Alternatively you can email us at:
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