Distribio enhance quality by purifying all oligos and controlling by mass spectrometry all of them.

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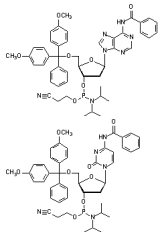
Technical informations

GeneCust provides oligonucleotides of the highest quality matching all research needs. Our Oligonucleotide Synthesis Service offers custom synthesis of high-quality oligonucleotides in a variety of scales and formats, including a broad range of modified oligos and purification levels.

How does it work?

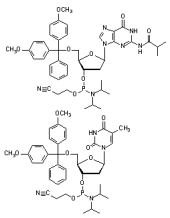
Oligonucleotides are chemically synthesized using phosphoramidites. A phosphoramidite is a normal nucleotide with protection groups added to its reactive amine, hydroxyl and phosphate groups.

Raw materials



dA(bz) Phosphoramidite

dC(bz) Phosphoramidite



dG(bz) Phosphoramidite

dT(bz) Phosphoramidite

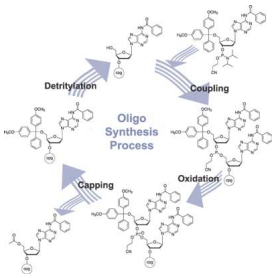
These protection groups prevent unwanted side reactions and force the formation of the desired product during synthesis. The 5' hydroxyl group is protected by DMT (dimethoxytrityl), the phosphate group by a diisopropylamino (iPr2N) group and a 2-cyanoethyl (OCH2CH2CN) group. The bases also have protecting groups on the exocyclic amine group (benzoyl or isobutyryl). With the completion of the synthesis process, all the protection groups are removed.

In solid-phase synthesis, the 3' end of the oligonucleotide is bound to a solid support column on which all reactions take place. The 3' group of the first base is immobilized via a linker to a solid support (polystyrene beads or similar). This allows for easy addition and removal of reactants. In each step, the solutions with the nucleotides for the next reaction are pumped through the column from an attached reagent delivery system and washed out before the next nucleotide is

added. At the end of the synthesis program, the oligonucleotide is cleaved off the solid support and eluted from the column.

The synthesis cycle

Oligonucleotide synthesis is done via a cycle of four chemical reactions that are repeated until all desired bases have been added:



- Step 1 - De-blocking (detritylation) : The DMT is removed with an acid, such as TCA (Tri Chloro Acetic Acid), and washed out, resulting in a free 5' hydroxyl group on the first base.

- Step 2 - Base condensation (coupling) : A phosphoramidite nucleotide (or a mix) is activated by tetrazole which removes the iPr2N group on the phosphate group. After addition, the deprotected 5' OH of the first base and the phosphate of the second base react to join the two bases together in a phosphite linkage. These reactions are not done in water but in tetrahydrofuran or in DMSO (Dimethylsulfoxid). Unbound bases and by-products are washed out.

- Step 3 - Capping : About 1% of the 5' OH groups do not react with the new base and need to be blocked from further reaction to prevent the synthesis of oligonucleotides with an internal base deletion. This is done by adding a protective group in the form of acetic anhydride and 1-methylimidazole which react with the free 5' OH groups via acetylation. Excess reagents are washed out.

- Step 4 - Oxidation: The phosphite linkage between the first and second base needs to be stabilized by making the phosphate group pentavalent. This is achieved by adding iodine and water which leads to the oxidation of the phosphite into phosphate. This step can be substituted with a sulphorylation step for thiophosphate nucleotides.

After accomplishing the synthesis, 2 post-processing steps are required. At first, the oligo must be cleaved from the solid support by treating the support-bound oligonucleotide with concentrated ammonia solution. Secondly, the protecting groups have to be removed from the adenine, guanine and cytidine bases and to release the exocyclic amino function, the ammonia oligonucleotide solution is incubated at higher temperatures (usually between 50°C and 80°C for 1 to 8 hrs. depending on the protocol and protecting groups used).

Purification

The selection of purification method depends on the type of oligonucleotide and on your purity and yield requirements. There is a trade-off between purity and yield. The higher the purity, the smaller the yield and the lower the purity, the higher the yield.

Reverse-phase cartridge purification

Purification with reverse-phase cartridge offers the lowest level of purity (typically 80%). The basis of the separation is the difference in hydrophobicity between full length product with DMT protecting groups and truncated sequences (without DMT groups).

Because the differences in hydophobicity between the full length- DMT product and non-DMT truncated sequences are reduced as the oligo length is increased, cartridge purification is not recommended for oligos < 50 bases.

HPLC Reverse-phase purification

Reverse-phase HPLC operates on the same principle as the reverse-phase cartridges, but typical yields a product of 90% purity. The capacity and resolving properties of HPLC columns are also much greater than cartridge devices, so HPLC is the method of choice for purifying

larger quantities of oligos (> 1 µmol). As with cartridges, reverse-phase HPLC is usually not recommended for purifying oligos longer than 50 bases.

Polyacrylamide Gel Purification (PAGE)

Purification by this method is considered as the Gold Standard for oligonucleotide purification and yields 95-99% purity. Gel purification can be used for any length of oligonucleotide. Gel purification is strongly advised for all applications involving cloning of the product, such as mutagenesis and gene construction applications. Yields from PAGE are lower than from other methods due to the relative inefficient extraction of oligos from the gel.

Calculations : how to estimate the required scale ?

Oligonucleotides are ordered by the scale instead of the quantity. In fact, every oligonucleotide is a unique molecule, with unique synthetic properties based on sequence and modifications. That's why the yields of synthesis can be different from one order to another and it can be reported in different units of mass such as milligrams or grams, in mole or in OD. So, what scale should you request in order to obtain sufficient quantities ?

How is yield measured ?

All oligonucleotides are measured for yield using absorbance. UV spectroscopy is a much more accurate way to measure the yield of oligonucleotides since it ignores salt, water, and other residuals of the synthesis that would affect the weight. The oligonucleotide is dissolved in water or buffer and the absorbance at 260 nm determined.

Conversions

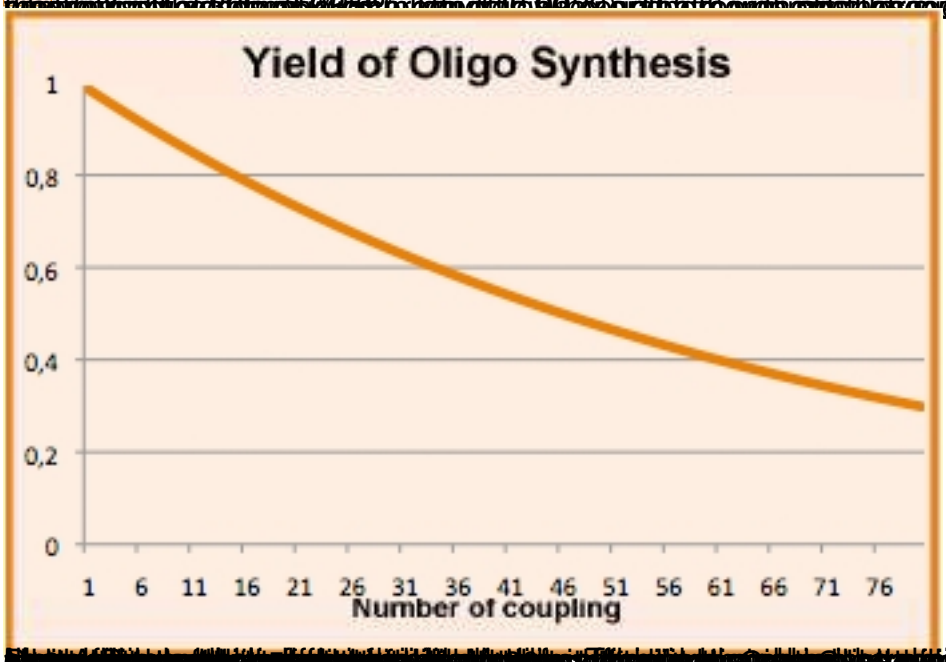
OD_{260} units can be converted to mmoles using Beer's Law that relates absorbance to concentration using the extinction coefficient (ε), which is a constant unique to every substance :

which can be derived to:

$$C[\mu mole/mL] = \frac{OD_{260nm}}{(15{,}4 \times A + 11{,}8 \times G + 7{,}4 \times C + 9{,}6 \times T)}$$

A, G, C, T: number of bases in the oligonucleotide

To determine the mass of the oligonucleotide, multiply the number of moles by the



Quality Control

A stringent quality control system ensures that you can expect the quality of our oligonucleotides to be of the highest standard. Before the synthesis, all chemicals are checked to meet our quality standard. During the fully automatic oligo synthesis, all steps of the synthesis are monitored by multiple control functions on the DNA synthesizers. This way we can assure the coupling efficiency of the synthesis to meet our demands.

The oligonucleotides are subsequently deprotected, desalted, and the optical density is measured at 260 nm. In addition, oligos are randomly analyzed by gel electrophoresis and each oligonucleotide will be controlled by the measurement of the molecular mass. All the oligos showing a bad / poor MS profile will be re-synthesized at the expense of GeneCust.

Standard oligonucleotide synthesis

Synthesis Scales

GeneCust offers four different synthesis scales: 10 nmol, 40 nmol, 200 nmol and 1000 nmol. For non-labeled, standard oligos, up to 30 bases, we guarantee a minimum yield:

* 10 nmol scale: 4,5 nmoles
* 40 nmol scale: 20 nmoles
* 200 nmol scale: 95 nmoles
* 1000 nmol scale: 400 nmoles

Maximum Lenghts : 10 nmol : 40 bases
40 nmol : 70 bases
200 nmol : 90 bases

For longer oligos, GeneCust uses a special 1000 nmol synthesis protocol.

Modified Oligonucleotides

GeneCust offers a wide variety of high quality modifications synthesized with High-Purity synthesis protocol. On the menu "Price List", you will find a list of some of the dyes we offer for oligonucleotide labeling. These can be placed either on the 5' end, the 3' end, or internally. You will also find others modifications such as phosphorylations, thiol modifications, biotin labeling or synthesis of RNA oligonucuotides, molecular beacons...

If there is a label or a modification you would like to use but you do not see here, please contact us, we may have it or we may be able to locate it for you.

Shipment :

The oligos are shipped dry. Under normal conditions standard oligos are shipped within one week. Shipment of larger orders, purified oligos and labeled oligos will take a few days more. Please contact us for further information about delivery times.

Shipment costs when using express mail services :

For Europe :	18,00 €
Rest of the world :	65,00 €

Quotations and Ordering

For quotations, please contact us at info@genecust.com. However, you may also contact us by phone (+352 27620411)
or fax (+352 27620412).

For ordering, please download and complete our Order Form and email it to info@genecust.com.

Notice : Chemistry Reagents by ChemGenes

Genecust can propose you a wide range of oligonucleotide synthesis reagents. All these products are ChemGenes made, stricktly identical to the products you use and this with very competitive prices worldwide. GeneCust is the European retailer for the ChemGenes product range.

ChemGenes' product lines include phosphoramidites for RNA and DNA synthesis, modified bases for DNA, as well as RNA modification. In addition, a variety of modified phosphoramidites for the introduction of chromophores and ligands are produced. A large variety of Reagents for the synthesis of DNA/RNA are also available, which include many natural and modified nucleoside bases, tetrazole, 5-thioethyltetrazole, DMT-chloride, DMT protected deoxy and ribo nucleosides, 2'-O-methyl and 2'-O-propargyl nucleosides, silyl protected ribonucleosides, several types of phosphorylating reagents, and many more.

Please, contact us at info@genecust.com for a free quote.