

Oligonucleotide synthesis

Exiqon uses the phosphoramidite synthetic method for oligonucleotide synthesis. The principle of this method was developed by McBride and Caruthers in 1983. In short, the oligonucleotides are synthesized on solid supports from the 3'-end and the first monomer at this end is normally attached to a CPG or polystyrene. Attached monomers are protected at the 5'-end with an acid labile and lipophilic trityl group and the A, G, C and mC monomers are protected with base labile protection groups at the nucleobase positions. Each monomer is attached through a synthetic cycle depicted in Figure 1.

The cycle consists of four steps: de-protection, coupling, oxidation and capping. In the classic de-protection step the trityl group attached to the 5' carbon of the pentose sugar of the recipient nucleotide is removed by trichloroacetic acid (TCA) leaving a reactive hydroxyl group.

In the coupling step, the phosphoramidite monomer is added in the presence of an activator such as a tetrazole, a weak acid that attacks the coupling phosphoramidite nucleoside forming a tetrazolyl phosphoramidite intermediate. This structure then reacts with the hydroxyl group of the recipient and the 5' to 3' linkage is formed (Figure 1). The tetrazole is reconstituted and the process continues.

The oxidation step stabilizes the phosphate linkage in the growing oligonucleotide. The traditional method of achieving this is by treatment with iodine in water.

The final step of the synthesis cycle is the capping reaction. Any remaining free 5'-hydroxyl groups are blocked at the capping step in an irreversible process. This step prevents the synthesis of oligonucleotides with missing bases. Following this step, the oligonucleotide is ready for the next monomer.

Figure 1

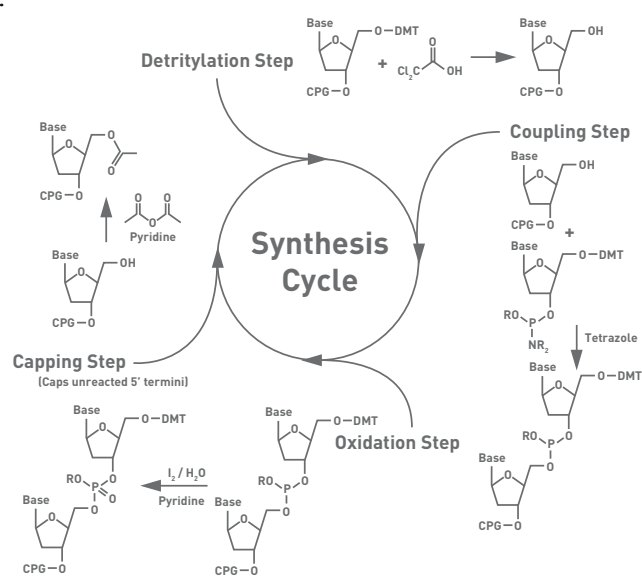


Figure 1. The synthetic cycle of oligonucleotide synthesis

After having synthesized the full length sequence, the oligonucleotide is then released from the solid support using a base, such as aqueous ammonia or a mixture of ammonia and methylamine. This will also remove protection groups from the nucleobases. The oligonucleotide is now ready for either desalting or purification. For dual HPLC purification, the final trityl group is left on the oligonucleotide prior to treatment with ammonia. First, the oligonucleotide is purified with RP-HPLC where the retention time is to a large extent determined by the lipophilic trityl group. Following this step, the trityl group is removed and the oligonucleotide is again HPLC purified.