Oligonucleotide Purification

Automated chemical synthesis of DNA has improved rapidly, with substantial gains made in the chemistry enabling routine coupling yields in excess of 99% and very reliable automation with each synthesis cycle of less than 3 min. The final oligonucleotide product obtained in the 20-30mer range is substantially pure with very low truncated sequences, thus requiring no further purification for most routine applications involving Polymerase Chain Reaction (PCR*) and DNA sequencing. Purification may be required for other applications, and is recommended for cloning, site directed mutagenesis, ligation etc.

Purification of oligonucleotides can be accomplished by various methods, the selection based on the particular requirement. The common techniques available and used frequently are polyacrylamide gel purification (PAGE), HPLC, and Reverse Phase Cartridge (RPC). The table below summarizes the purification range of each of the above techniques.

	PAGE	**HPLC**	**RPC**
8-40mer	Yes	Yes	Yes
41-200mer	Yes	No	No

Polyacrylamide Gel Purification (PAGE)

Purification by this method is considered the Gold Standard for oligonucleotide purification. PAGE purification can be used for any length of oligonucleotide (as compared to HPLC and RPC cartridges, which are limited to oligonucleotides preferably below 35mer). This technique is also the most labor-intensive method. Appropriate percentage of polyacrylamide gel (10-20%) is prepared and the oligonucleotide electrophoresed. The major product is the slowest migrating band, which is identified by UV shadowing and excised out. The gel slice is then processed for oligo elution commonly by crush and soak method.

HPLC

HPLC purification is usually based on reverse phase utilizing hydrophobic matrices. The oligonucleotide is synthesized with Trityl ON (the triphenylmethyl group at the 5' OH of the last base of the synthetic oligonucleotide) and the elution profile first elutes all non-tritylated truncated sequences followed by elution of the hydrophobically bound full length oligonucleotide. This method yields greater than 95% purity, depending upon the sequence and length of the oligonucleotide. Reverse phase based HPLC fails above 35-40mer oligonucleotide, as longer oligos are inherently hydrophobic and bind non-specifically.

Reverse Phase Cartridge (RPC)

This is an inexpensive alternate to HPLC reverse phase purification. The cartridge for reverse phase purification usually contains a hydrophobic matrix e.g. C18 silica, the principle of purification being the same as HPLC, RPC achieves purification of ~95% purity depending upon the sequence and length of the oligonucleotide. Reverse phase cartridge based purification also fails above 35-40mer oligonucleotide, as longer oligos are inherently hydrophobic and bind non-specifically to the column matrix.

	Synthesis Scale, $/base				
	50 nmol	**200 nmol**	**1 µmol**	**10 µmol**	**15 µmol**
Unmodified Oligomers *	**$0.90**	**$2.00**	**$3.25**	**$25.00**	**$28.00**
*minimum charges for 15mer applies					

Purification					
	50 nmol	**200 nmol**	**1 µmol**	**10 µmol**	**15 µmol**
Gel Purification	$75.00	$75.00	$150.00	$600.00	$600.00
Reverse Phase Cartridge (RPC)	$30.00	$30.00	$90.00	$240.00	$240.00

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Prices subject to change without notice

All Gene Link products are for research use only.