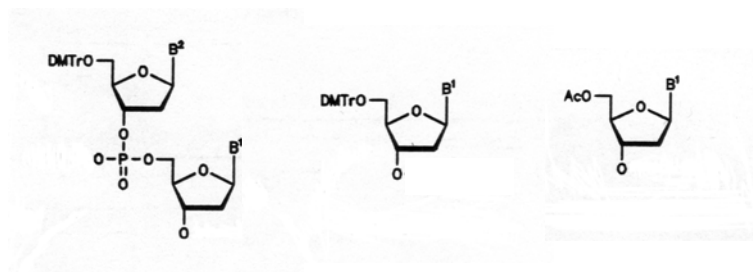


Why are we performing Reversed Phase HPLC as standard purification?

In order to guarantee a consistently high product quality and reproducibility of your experiments, Thermo Electron was the first company to systematically purify all synthesised oligonucleotides by RP-HPLC.

Looking at the synthesis efficiencies achieved with solid support chemistry, we believe that mere desalting is not the way to go.

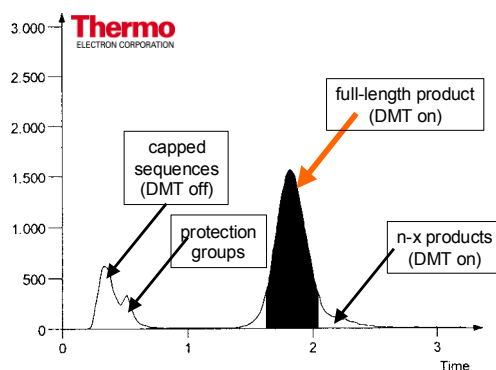
The unpreventable formation of by-products during synthesis increases with the oligo length:



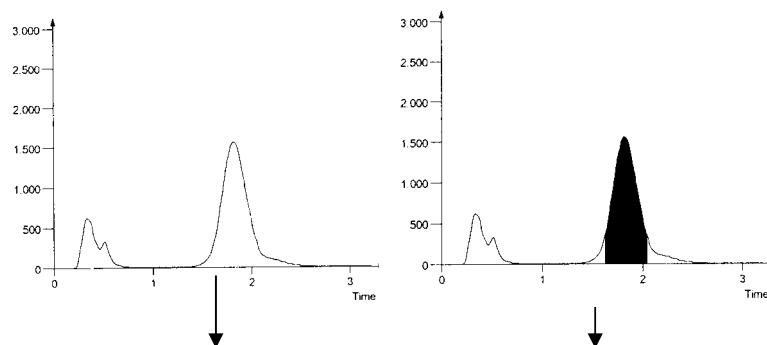
	desired product	shorter products	capped sequences
1 st cycle	98%	0,4%	1,6%
20 mer	67%	6,6%	26,4%
30 mer	54%	9,2%	36,8%
40 mer	45%	13%	52%

A **crude** 30 mer oligonucleotide contains nearly 50% of by-products – so this would mean, that half of the „product“ you get is not the oligo you wanted to get!

The HPLC chromatograph of a **crude oligonucleotide** looks like this:



Comparison of Desalting and RP-HPLC purification:



	Desalting	RP-HPLC
removes	trityl and other reagents	trityl, other reagents and capped sequences
30 mer contains	54% desired product 9,2% shorter products 36,8% capped sequences	54% desired product 9,2% shorter products
ratio of full-length oligo in product	54%	85%

PAGE (Poly-Acrylamide-Gel-Electrophoresis) purification of oligonucleotides

The efficiency of chemical oligonucleotide synthesis depends on sequence length, the kind of bases and the kind of modification. The amount of chemical by-products may vary a lot, due to the highly sophisticated chemistry. Most of the by-products are separated by the standard method of HPLC (High-Performance-Liquid-Chromatography). A more enhanced purification is offered by PAGE.

PAGE-purification is recommended particularly to distinguish molecules with small size differences. The matrix of the vertically arranged poly-acrylamide-gel combined with denaturing buffer enables separation of oligonucleotides which only a one base difference in length.

PAGE-Purification offers a purity of oligonucleotides of >95%, depending on the length and the loading-amount. The standard HPLC-purification results in an approximate purity of 80%-90% for standard oligonucleotides.

Thermo Electron strongly recommend an additional PAGE-Purification for **oligonucleotides longer than 50 bases** and for oligonucleotides used in **sequencing, cloning, mutagenesis or gene synthesis**.