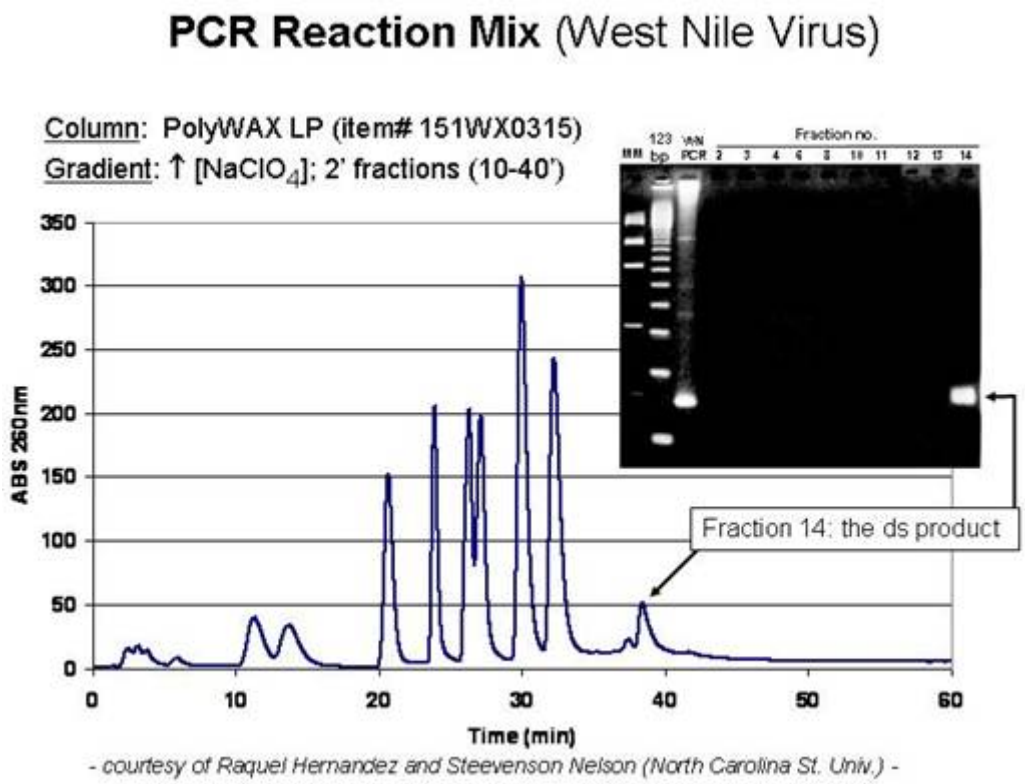
Oligonucleotides and PCR Products

Larger oligonucleotides, their analogs, and dsDNA fragments are analyzed and purified by anion-exchange chromatography. Our **PolyWAX LP™** material affords excellent results in such applications, especially the 3-µm, 1500-Å version. G-rich nucleic acids cause problems with some separation methods but not with PolyWAX LP™ columns eluted with a $NaClO_4$ gradient.

PCR Reaction Products: Results from Raquel Hernandez' group (North Carolina St. Univ.) demonstrate that PCR reaction products can be purified on a column of this material much more conveniently than with a PAGE gel and with higher recovery [BELOW]. This is true even of GC-rich products.



- courtesy of Raquel Hernandez and Steevenson Nelson (North Carolina St. Univ.) -

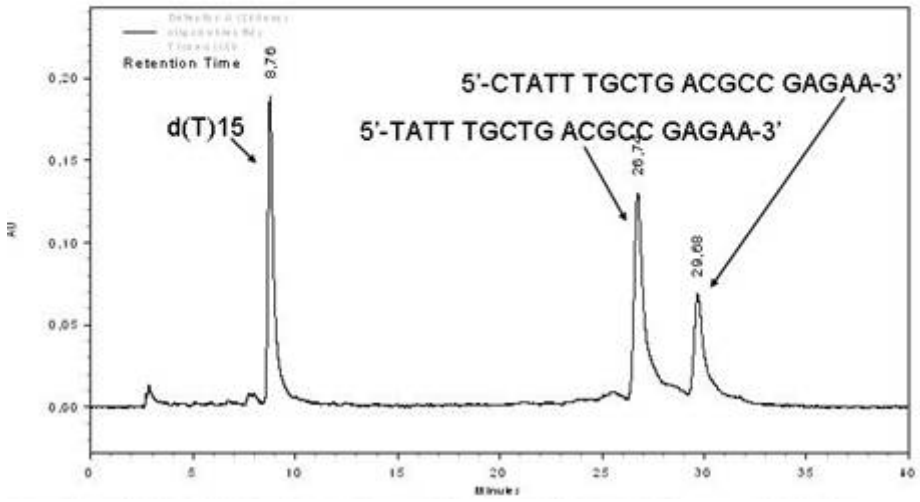
Oligonucleotides: The 3-µm version of **PolyWAX LP™** affords unusually good resolution of oligonucleotides [BELOW].

北京崇文区左安门内大街 8 号伟图大厦 301 室 PC：100061
Tel：010-67136152/67100708 Fax：010-67114016/67113925
http://www.chromatogr.com E-mail：china.hplc@163.com

Anion-Exchange of Oligonucleotides

COLUMN: PolyWAX LP (item# 104WX0315)

Excellent separation of oligos differing by one base!



Mobile Phase: A) 25mM Tris-Cl, pH 8.0, with 30% ACN, B) Same +1 M NaCl

Gradient: 60-100% B in 50' **Flow Rate:** 0.5ml/min **Temp:** 60° C

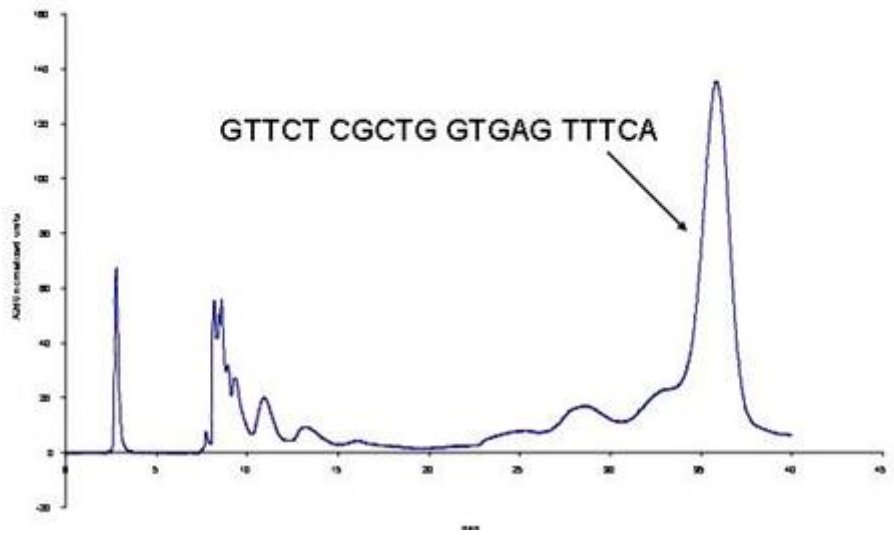
Detection: A_{250}

Phosphorothioates: These elute in broader peaks than do regular oligonucleotides, since the phosphorus atoms are optically active centers. Thus, phosphorothioates consist of 2^n diastereomers (n = # of bases). An example is shown below.

Anion-Exchange of Crude Phosphorothioate

COLUMN: PolyWAX LP (item# 104WX0315)

Good selectivity for failure sequences



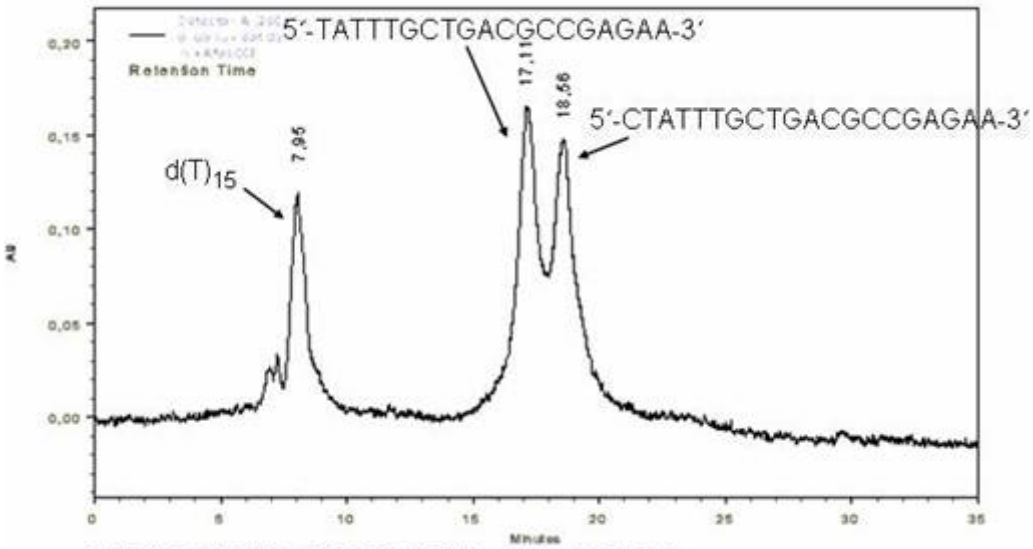
北京崇文区左安门内大街 8 号伟图大厦 301 室 PC：100061
Tel：010-67136152/67100708 Fax：010-67114016/67113925
http://www.chromatogr.com E-mail：china.hplc@163.com

Mobile Phase: A) 25mM Tris, pH 8.0, with 30% ACN, B) Same +1 M $NaClO_4$
Gradient: 0-3': 0% B; 3-4': 0-60% B; 4-34': 60-100% B; 34-38': 100% B
Flow Rate: 0.5ml/min **Temp:** Ambient **Detection:** A_{260} **Sample**: 1.7µg

Hydrophilic Interaction Chromatography: This is an alternative to anion-exchange that can be used with volatile solvents. The following example consists of phosphorothioates with the same base sequence as the conventional oligonucleotide above. Peaks are broader, although it is unclear how much of this is due to:

a) The mode used;
b) The diastereomeric composition of phosphorothioates; and
c) The use of a 5-µm column for HILIC instead of 3-µm.

COLUMN: PolyHYDROXYETHYL A (item# 204HY0503)



MOBILE PHASE: 75-60% ACN in 100 mM TEAA.

- Courtesy of Martin Buncek (Charles Univ., Czech Republic) -

北京崇文区左安门内大街 8 号伟图大厦 301 室 PC：100061
Tel：010-67136152/67100708 Fax：010-67114016/67113925
http://www.chromatogr.com E-mail：china.hplc@163.com