TOSOH

核酸类药物的HPLC分析方法的评价（寡核苷酸、质粒DNA、MRNA）

2026年5月

TOSOH CORPORATION

寡核苷酸/核酸分离常用的HPLC分离模式

分离模式	分离机制	优点	缺点	流动相示例
反相色谱(RPC)	疏水性相互作用	• 高分辨率 • 异构体、不对称体的分离	• 异构体、不对称体峰较多 • 样品载量少	• 含离子对试剂的洗脱液 • 有机溶剂梯度洗脱
离子交换色谱(IEC)	离子相互作用	• 样品载量高 • 可根据磷酸基数量分离样品(N-1, N, N+1)	• 分离前需要脱盐、稀释等处理	• 中性至碱性溶液中，盐梯度洗脱 • 有时也添加有机溶剂
尺寸排阻色谱(SEC)	分子大小	• 基于碱基数分离 • 可在接近生理条件的洗脱液中分离	• 样品载量少 • 分离能力低于RPC、IEC	• 在中性附近的洗脱液中，等度洗脱 • 有时也添加有机溶剂
疏水色谱(HIC)	疏水相互作用	• 有机溶剂使用量少 • 分离DMT-on/off体 • 可部分分离N-1和P=O体	• 使用高浓度盐	• 使用硫酸铵的盐浓度梯度洗脱 • 有时也添加有机溶剂
亲水作用色谱(HILIC)	亲水相互作用	• 结构异构体、不对称体的分离 • 不使用离子对试剂	• 使用高浓度有机溶剂 • 易受样品溶剂组成的影响	• 使用含有高浓度有机溶剂的流动相 • 有机溶剂梯度洗脱

内容

01 寡核苷酸的分析

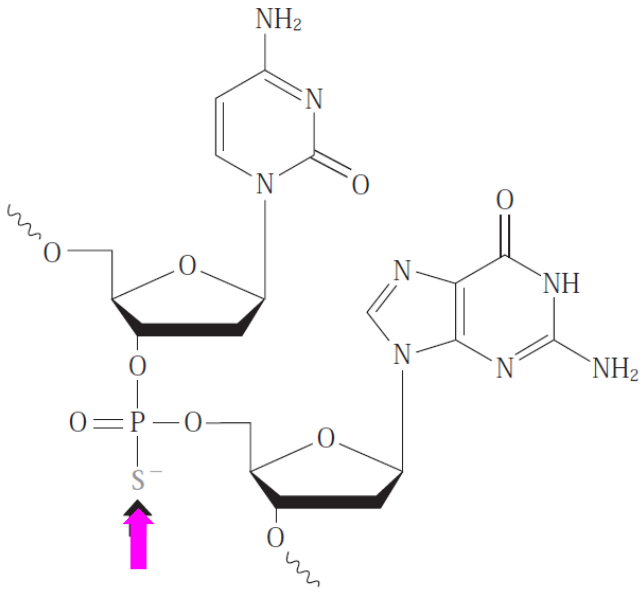
02 质粒DNA的分析

03 长链核酸(ssRNA、DNA)的分析

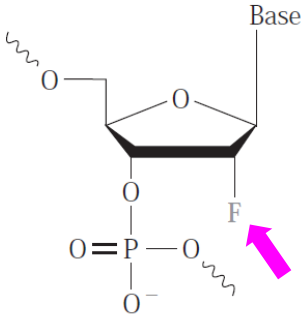
TOSOH

寡核苷酸的分析

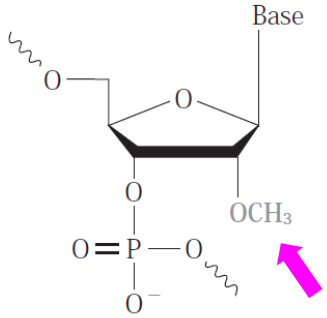
寡核苷酸的磷酸化及糖基修饰



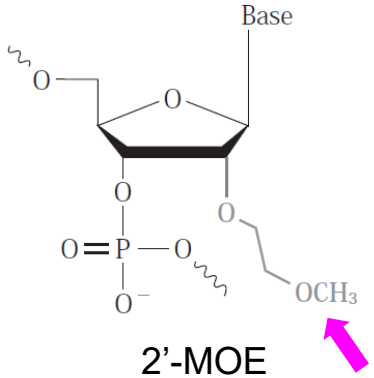
硫代磷酸酯



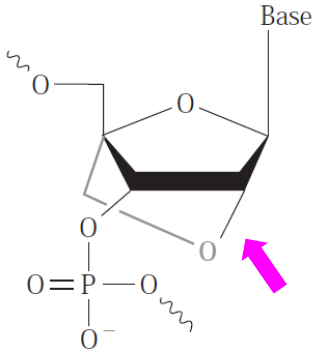
2’-F



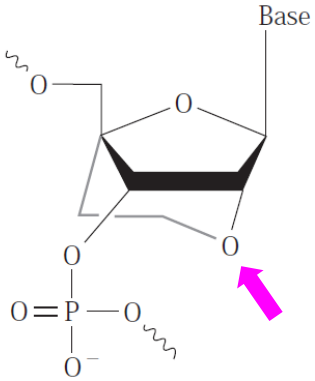
2’-O-Methyl



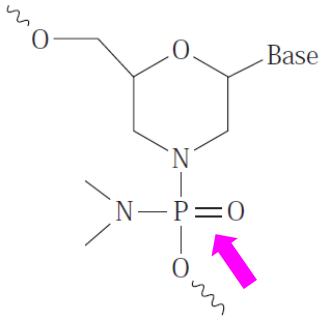
2’-MOE



LNA(2’-,4’-BNA)



ENA



Morpholino

- 磷酸化修饰：空间配置引起的异构体（但都是有效成分）
- 糖基修饰：通过架桥增强对核酸酶的耐受和降低毒性（如吗啉环形）
- 碱基修饰：适配体立体构造的多样性

存在于合成寡核苷酸的杂质

ICH Guideline Q3A(R2), Q3B(R2)

Non-genotoxic impurities; Organic, inorganic or residual solvents

Organic impurities; Process-related and Product-related

- **Class I**
  代谢物杂质；如 3’端、5’端缺失物等
- **Class II**
  仅含有天然核酸结构元素的杂质；如磷酸二酯类杂质等
- **Class III**
  母体寡核苷酸的序列变异杂质；如 n-1、n+1等
- **Class IV**
  由合成过程引起的各类杂质；如无碱基杂等

适用于寡核苷酸分析的色谱柱

离子对反相色谱(IP-RPLC)

商品名称	粒径 (μm)	孔径 (nm)	碳含量 (%)	尺寸 (mm I.D. x cm)
TSKgel ODS-120H	1.9、3、5	12	20 %	2.0 x 15

亲水相互作用色谱(HILIC)

商品名称	粒径 (μm)	孔径 (nm)	官能团	尺寸 (mm I.D. x cm)
TSKgel Amide-80	2、3、5	10	氨基甲酰	2.0 x 15

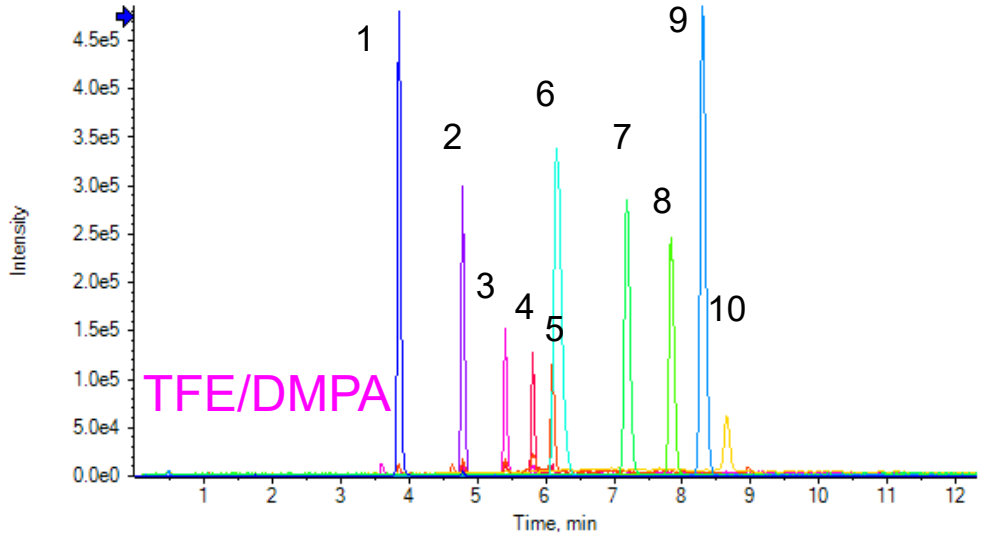
阴离子交换色谱(IEC)

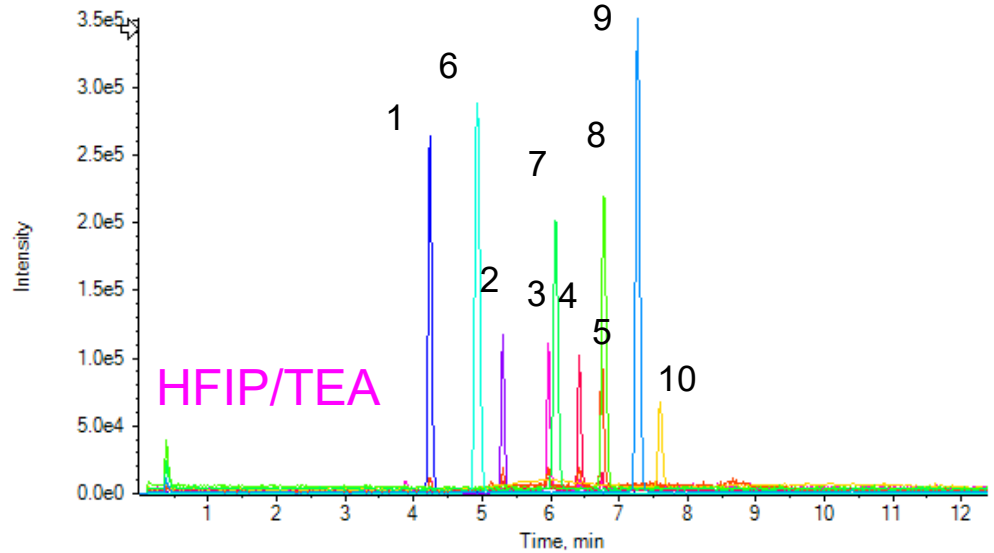
商品名称	粒径 (μm)	尺寸(mm I.D. x cm)	官能团
TSKgel DNA-STAT	5	4.6 X 10	季氨基

合成寡核苷酸的IP-RPLC分离

TOSOH

◘ 流动相添加剂对分离的影响





Identification of peaks :
1 ; $(dT)_{15}$, 2 ; $(dT)_{20}$, 3 ; $(dT)_{25}$, 4 ; $(dT)_{30}$, 5 ; $(dT)_{35}$,
6 ; $(dT)_{15}PS$, 7 ; $(dT)_{20}PS$, 8 ; $(dT)_{25}PS$, 9 ; $(dT)_{30}PS$,
10 ; $(dT)_{35}PS$

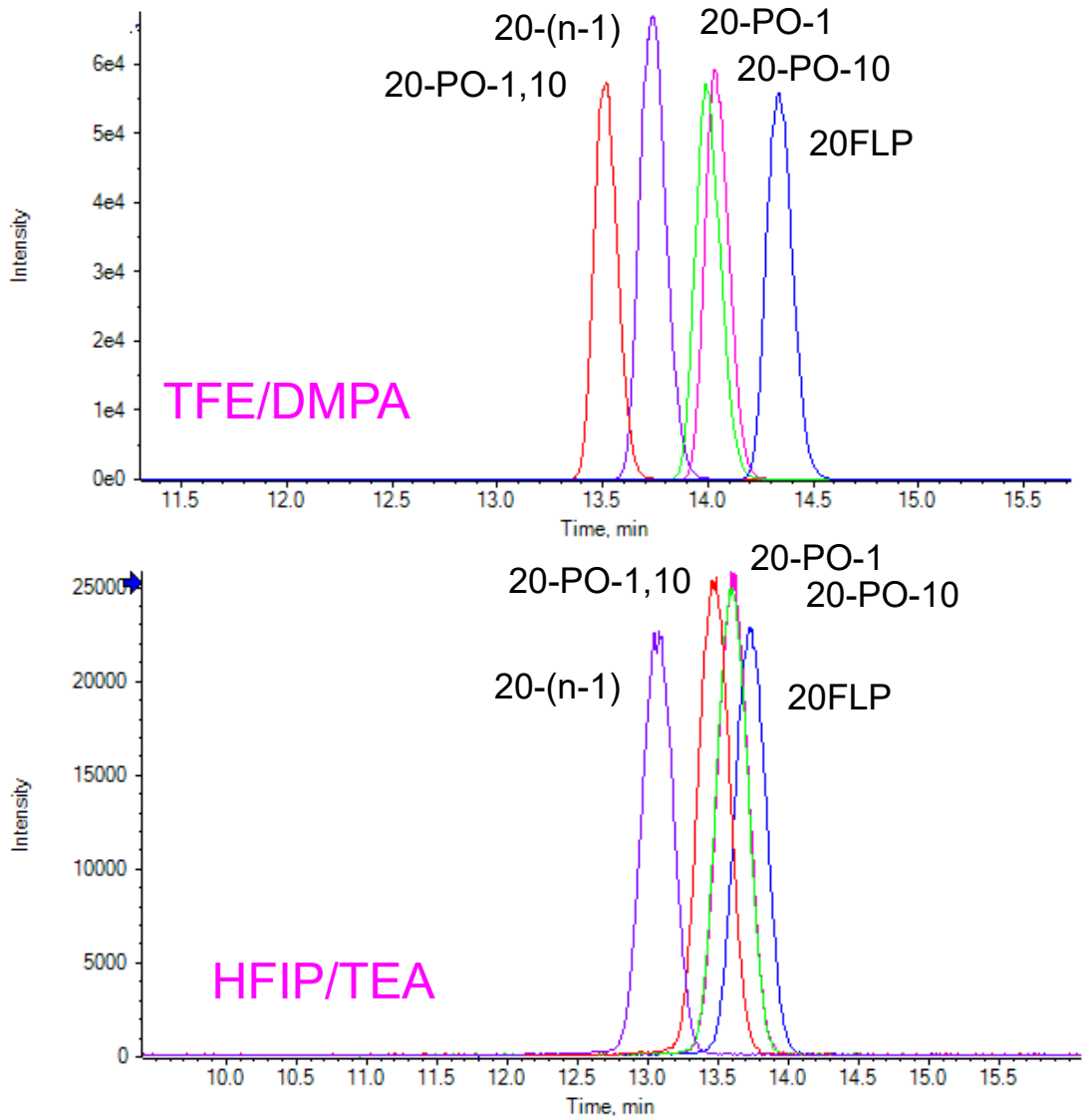
Conditions
Column : TSKgel ODS-120H (2.0 mm I.D. x 75 mm, 1.9 μm)
Eluent : A ; 10 mmol/L DMPA, 600 mmol/L TFE in water
B ; 10 mmol/L DMPA, 600 mmol/L TFE in methanol
Gradient : B conc.(0 min) 5 % →(10-12 min) 25 % →(12.1-20 min) 5 %
Flow rate : 0.4 mL/min Column temp. : 60 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

Conditions
Column : TSKgel ODS-120H (2.0 mm I.D. × 75 mm, 1.9 μm)
Eluent : A ; 15 mmol/L TEA, 400 mmol/L HFIP in(water/methanol=80/20)
B ; 15 mmol/L TEA, 400 mmol/L HFIP in methanol
Gradient : B conc.(0 min) 0 % →(10-12 min) 20 % →(12.1-20 min) 0 %
Flow rate : 0.4 mL/min Column temp. : 60 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

合成寡核苷酸的IP-RPLC分离

TOSOH

◘ 流动相添加剂对分离的影响



Name	Sequence (5'→3')	Mw
20FLP	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^c	6349.30
20-PO-1	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^c	6333.23
20-PO-10	a^t^c^g^a^c^t^c^t^cg^a^g^c^g^t^t^c^t^c	6333.23
20-PO-1,10	at^c^g^a^c^t^c^t^cg^a^g^c^g^t^t^c^t^c	6317.16
20-(n-1)	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t	6044.05

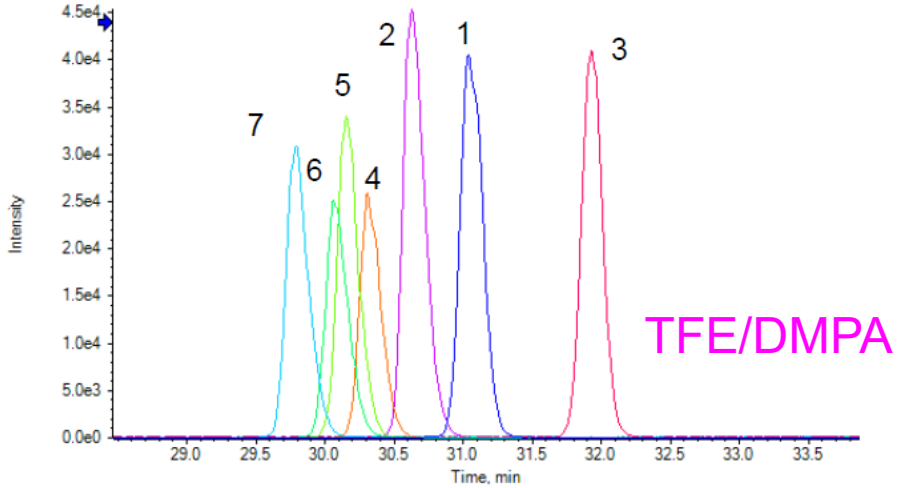
Conditions
Column : TSKgel ODS-120H (2.0 mm I.D. × 100 mm, 1.9 μm)
Eluent : A ; 10 mmol/L DMPA, 800 mmol/L TFE in water
B ; 10 mmol/L DMPA, 800 mmol/L TFE in acetonitrile
Gradient : B conc.(0 min) 0 % →(20-21 min) 10 % →(21.1-29 min) 0 %
Flow rate : 0.3 mL/min Column temp. : 50 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

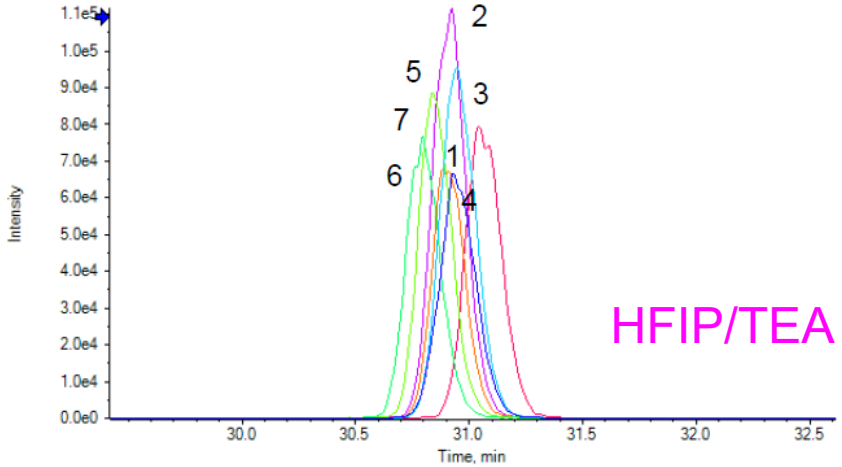
Conditions
Column : TSKgel ODS-120H (2.0 mm I.D. × 75 mm, 1.9 μm)
Eluent : A ; 15 mmol/L TEA, 400 mmol/L HFIP in (water/methanol=80/20)
B ; 15 mmol/L TEA, 400 mmol/L HFIP in methanol
Gradient : B conc.(0 min) 0 % →(20-21 min) 12 % →(21.1-30 min) 0 %
Flow rate : 0.4 mL/min Column temp. : 50 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

合成寡核苷酸的IP-RPLC分离

◘ 流动相添加剂对分离的影响

Name	Base	Sequence(5'→3')　　^ ; Phosphorothioated	M
50FLP	50	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c	15992.32
50-(n-1)	49	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t	15687.07
50-(n+1)	51	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g	16337.60
50-PO-49	50	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^tc	15976.25
50-PO-1	50	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c	15976.25
50-PO-1,49	50	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^tc	15960.18
50-PO-1,25,49	50	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^ac^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^tc	15944.11



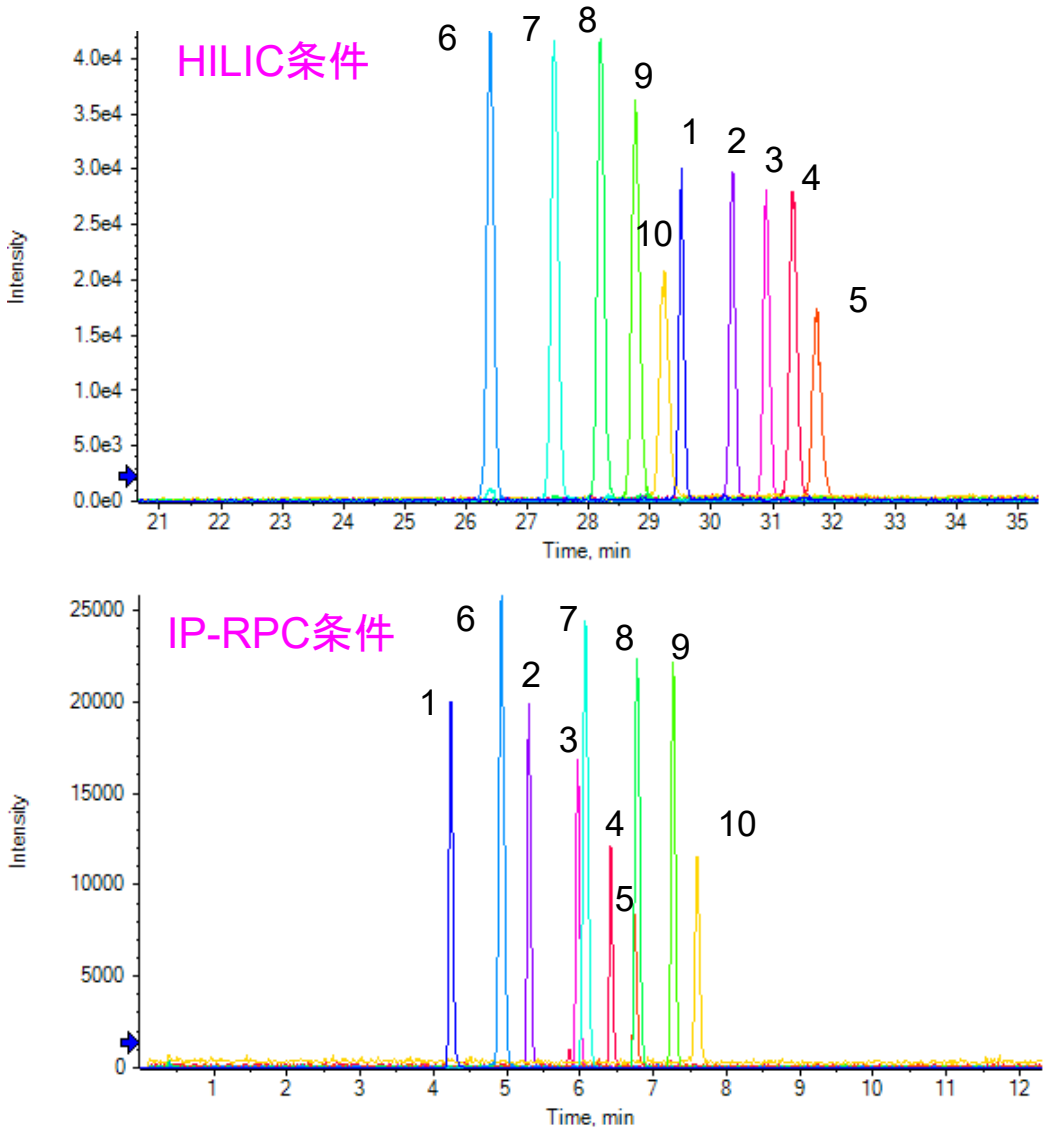


Conditions
Column : TSKgel ODS-120H (2.0 mm I.D.×100 mm, 1.9 μm)
Eluent : A ; 10 mmol/L DMPA, 600 mmol/L TFE in water
B ; 10 mmol/L DMPA, 600 mmol/L TFE in acetonitrile
Gradient : B conc.(0 min) 0 % →(40-41 min) 10 % →(41.1-50 min) 0 %
Flow rate : 0.4 mL/min　Column temp. : 60 ℃　Injection vol. : 5 μL
Ionization : ESI Negative

Conditions
Column : TSKgel ODS-120H (2.0 mm I.D.×75 mm, 1.9 μm)
Eluent : A ; 15 mmol/L TEA, 400 mmol/L HFIP in (water/methanol=80/20)
B ; 15 mmol/L TEA, 400 mmol/L HFIP in methanol
Gradient : B conc.(0 min) 0 % →(40-41 min) 12 % →(41.1-50 min) 0 %
Flow rate : 0.4 mL/min　Column temp. : 50 ℃　Injection vol. : 5 μL
Ionization : ESI Negative

合成寡核苷酸的HILIC分离

□ 分离模式对分离的影响



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6 ; $(dT)_{15}PS$, 7 ; $(dT)_{20}PS$, 8 ; $(dT)_{25}PS$, 9 ; $(dT)_{30}PS$,
10 ; $(dT)_{35}PS$

Conditions

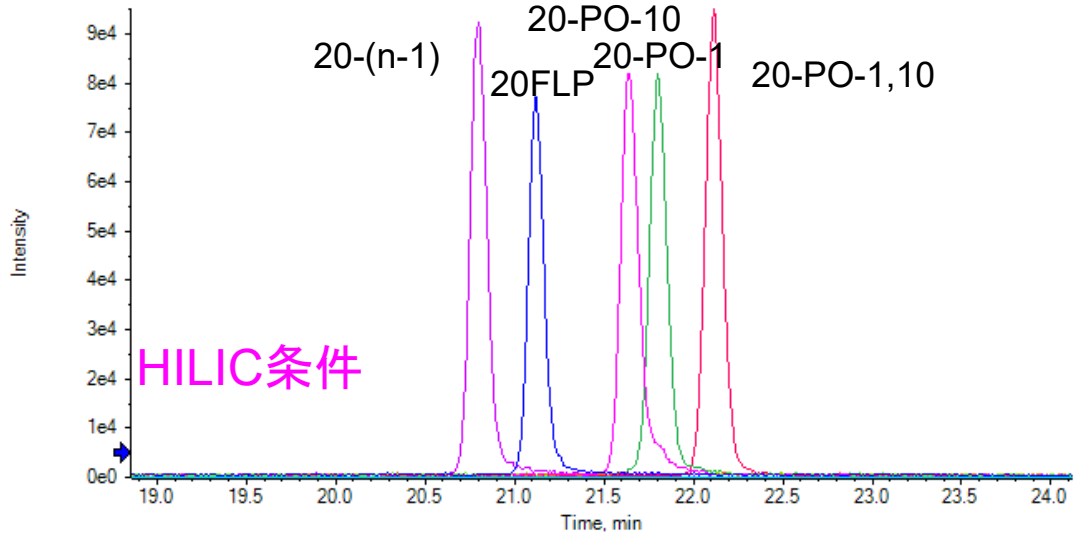
Column : TSKgel Amide-80 2μm (2.0 mm I.D. × 150 mm, 2 μm)
Eluent : A ; 50 mmol/L ammonium formate (pH 4.8)
B ; acetonitrile
Gradient : B conc.(0 min) 98 % →(40-42 min) 40 %→(42.5-55 min) 98 %
Flow rate : 0.2 mL/min Column temp. : 50 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

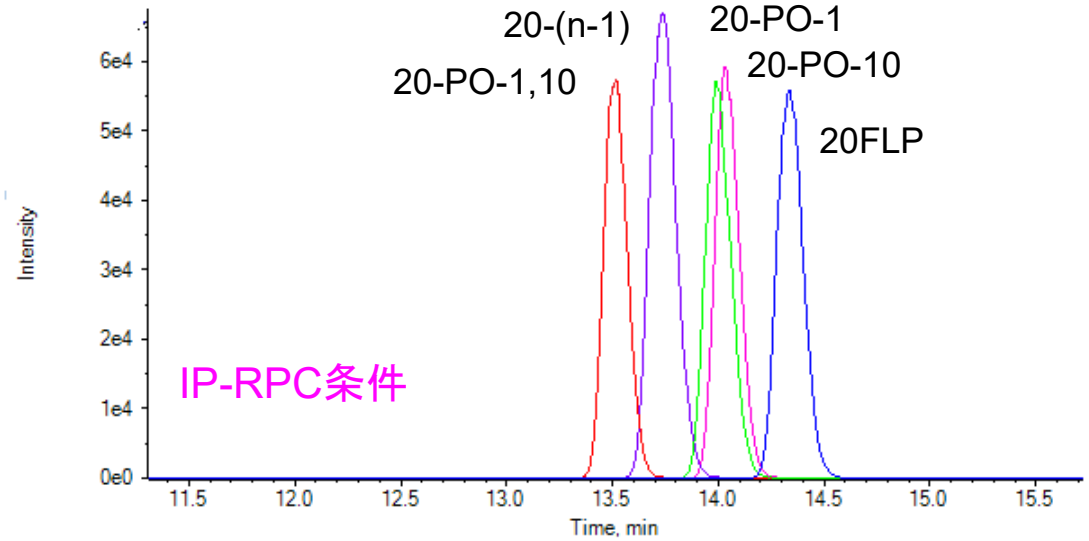
Conditions

Column : TSKgel ODS-120H (2.0 mm I.D. × 75 mm, 1.9 μm)
Eluent : A ; 15 mmol/L TEA, 400 mmol/L HFIP in water/methanol=80/20)
B ; 15 mmol/L TEA, 400 mmol/L HFIP in methanol
Gradient : B conc.(0 min) 0 % →(10-12 min) 20 % →(12.1-20 min) 0 %
Flow rate : 0.4 mL/min Column temp. : 60 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

合成寡核苷酸的HILIC分离

◘ 分离模式对分离的影响





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20-PO-1	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^c	6333.23
20-PO-10	a^t^c^g^a^c^t^c^t^cg^a^g^c^g^t^t^c^t^c	6333.23
20-PO-1,10	at^c^g^a^c^t^c^t^cg^a^g^c^g^t^t^c^t^c	6317.16
20-(n-1)	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t	6044.05

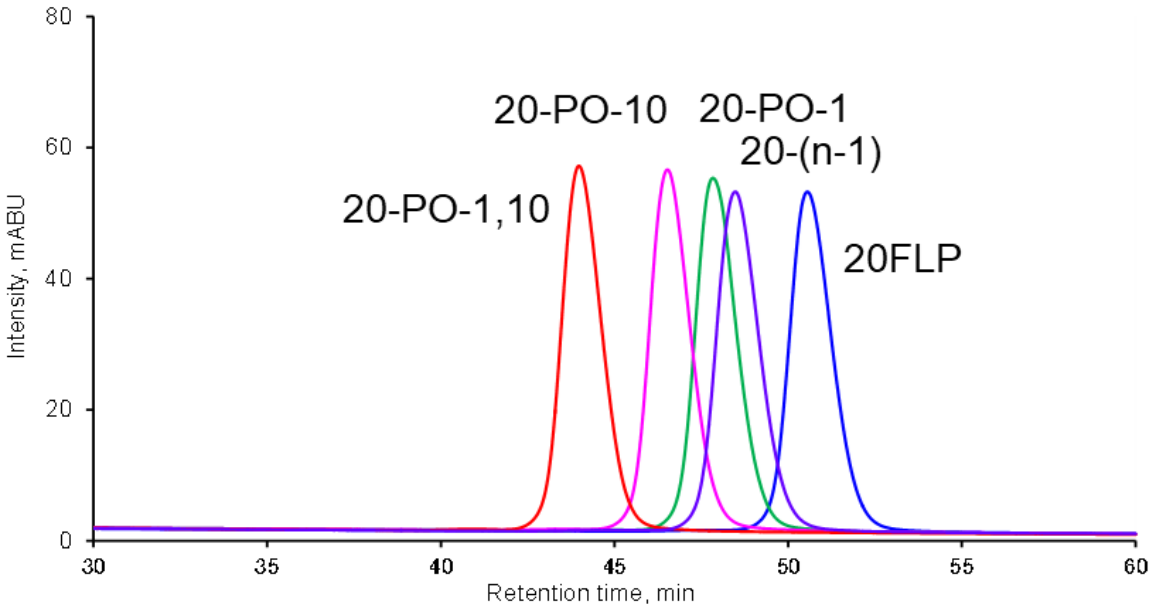
Conditions

Column : TSKgel Amide-80 2μm (2.0 mm I.D. × 150 mm, 2 μm)
Eluent : A ; 50 mmol/L ammonium formate (pH 4.8)
B ; acetonitrile
Gradient : B conc.(0 min) 98 % →(30-32 min) 20 % →(32.5-40 min) 98 %
Flow rate : 0.3 mL/min Column temp. : 50 ℃ Injection vol. : 2 μL
Ionization : ESI Negative

Conditions

Column : TSKgel ODS-120H (2.0 mm I.D. × 100 mm, 1.9 μm)
Eluent : A ; 10 mmol/L DMPA, 800 mmol/L TFE in water
B ; 10 mmol/L DMPA, 800 mmol/L TFE in acetonitrile
Gradient : B conc.(0 min) 0 % →(20-21 min) 10 % →(21.1-29 min) 0 %
Flow rate : 0.3 mL/min Column temp. : 50 ℃ Injection vol. : 5 μL
Ionization : ESI Negative

合成寡核苷酸的IEC分离



Name	Sequence (5'→3')	Mw
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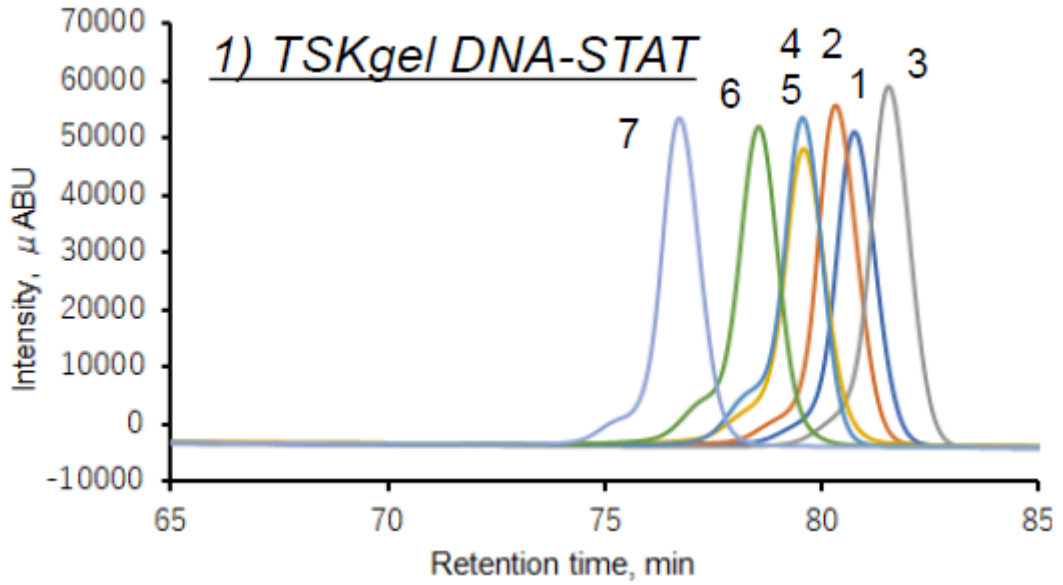
20-PO-10	a^t^c^g^a^c^t^c^t^cg^a^g^c^g^t^t^c^t^c	6333.23
20-PO-1,10	at^c^g^a^c^t^c^t^cg^a^g^c^g^t^t^c^t^c	6317.16
20-(n-1)	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t	6044.05

Conditions
Column : TSKgel DNA-STAT (4.6 mm I.D. × 100 mm, 5 μm)
Eluent :
A ; acetonitrile/40 mmol/L Tris-HCl+2 mmol/L EDTA・2Na (pH8.5)/water=30/50/20
B ; acetonitrile/40 mmol/L Tris-HCl+2 mmol/L EDTA・2Na+ 2 mol/L $NaClO_4$(pH8.5)/water=30/50/20
Gradient : B conc.(0 min) 20 % →(60 min)40 %
→(60.1-65 min) 100 % →(65.1-75 min) 20 %
Flow rate : 0.5 mL/min
Column temp. : 60 ℃
Injection vol. : 10 μL
Detector : UV 260 nm

合成寡核苷酸的IEC分离

Name	Base	Sequence(5'→3')　^ ; Phosphorothioated	M
50FLP	50	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c	15992.32
50-(n-1)	49	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t	15687.07
50-(n+1)	51	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g	16337.60
50-PO-49	50	a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^tc	15976.25
50-PO-1	50	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c	15976.25
50-PO-1,49	50	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^tc	15960.18
50-PO-1,25,49	50	at^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^gac^t^c^t^c^g^a^g^c^g^t^t^c^t^a^t^c^g^a^c^t^c^tc	15944.11



Conditions

Column : TSKgel DNA-STAT (4.6 mm I.D. × 100 mm, 5 μm)

Eluent :

A ; acetonitrile/40 mmol/L Tris-HCl+2 mmol/L EDTA・2Na (pH8.5)/water=30/50/20

B ; acetonitrile/40 mmol/L Tris-HCl+2 mmol/L EDTA・2Na+ 2 mol/L $NaClO_4$(pH8.5)/water=30/50/20

Gradient : B conc.(0 min) 20 % →(90-105 min)60 % →(105.1-135 min) 20 %

Flow rate : 0.5 mL/min

Column temp. : 60 ℃

Injection vol. : 2 μL

Detector : UV 260 nm

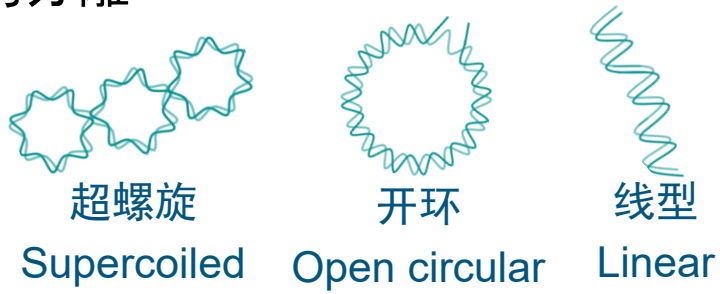
TOSOH

质粒DNA的分析

适用于质粒DNA分析的色谱柱

TOSOH

◘ 质粒DNA拓扑异构体的分離



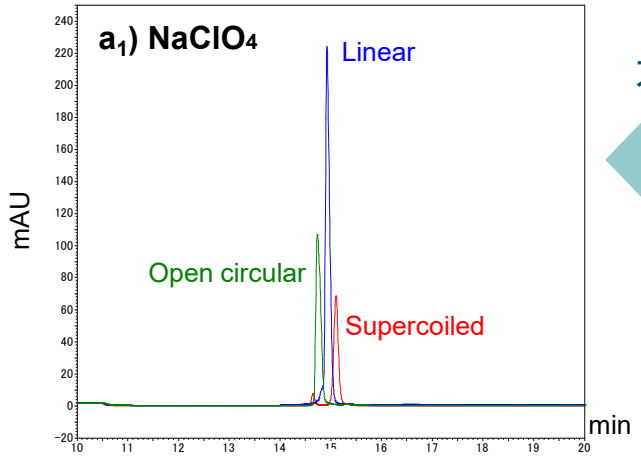
- 多使用无孔微粒聚合物基材(-NPR, -STAT)
  - 无孔聚合物基材 >>> 减少样品扩散，实现高分离和高回收率
  - 微粒填料 >>> 高塔板数

商品名称	粒径 (μm)	尺寸 (mm I.D. x cm)	官能团	适用样品
TSKgel® DNA-NPR	2.5	4.6 X 7.5	二乙胺乙基（DEAE）	链长5 kbp以下
TSKgel DNA-STAT	5	4.6 X 10	季氨基	
TSKgel Q-STAT	7	4.6 X 10	季氨基	链长5 kbp以上
TSKgel Q-STAT	10	3.0 X 3.5	季氨基	

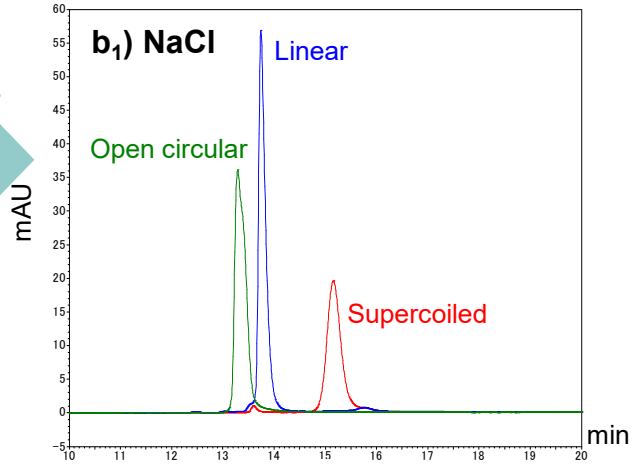
质粒DNA拓扑异构体的IEC分离

□ 样品: Plasmid DNA pBR322 (4.3 kbp)

TSKgel DNA-NPR

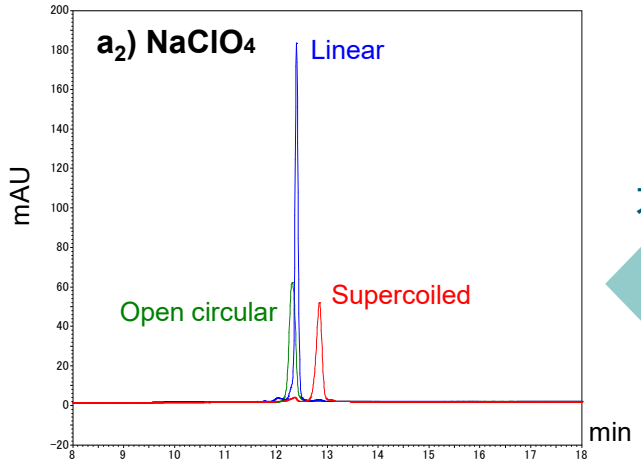


不同盐

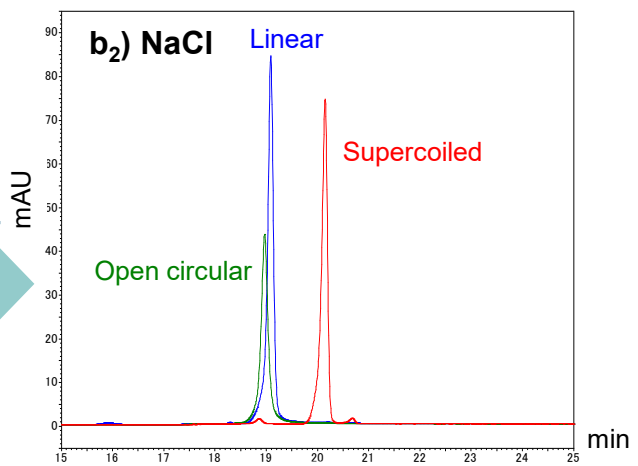


不同色谱柱

TSKgel DNA-STAT



不同盐

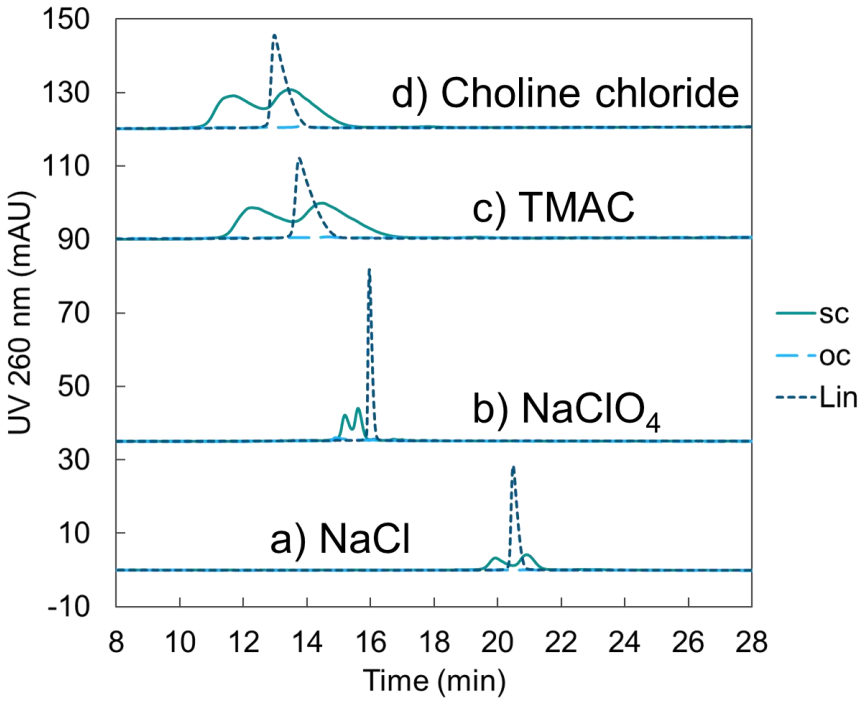


Conditions
Column : TSKgel DNA-NPR (4.6 mm I.D. x 7.5 cm)
: TSKgel DNA-STAT (4.6 mm I.D. x 10 cm)
Eluent : a)A; 20 mM Tris-HCl (pH 8.5)
B; 20 mM Tris-HCl +1 M NaClO4 (pH 8.5)
b)A; 20 mM Tris-HCl (pH 8.5)
B; 20 mM Tris-HCl +1 M NaCl (pH 8.5)
Gradient : a_1) B; 15-35 % (30 min)
b_1) B; 50-70 % (30 min):
a_2) B: 30 -50 % (30 min)
b_2) B: 75-95 % (30 min)
Flow rate : 0.5 mL/min
Detection : UV (260 nm)
Temperature: 25 ℃
Inj.vol. : 2 µL
Samples : 1) Linear (0.2 g/L)
2) Open circular (0.2 g/L)
3) Supercoiled (0.1 g/L)

质粒DNA拓扑异构体的IEC分离

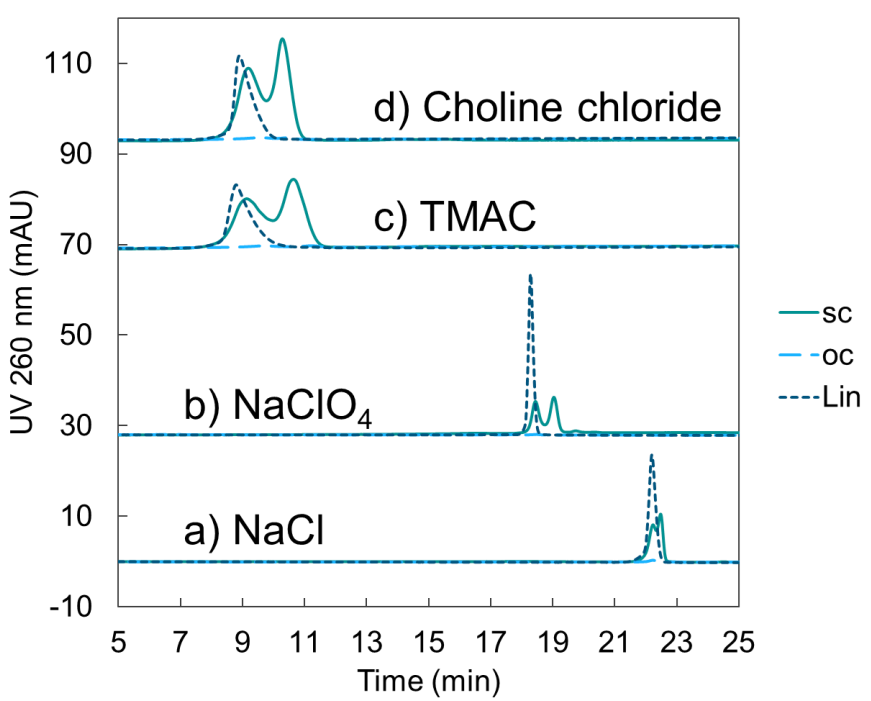
□ 样品: Plasmid DNA pHelper (11.6 kbp)

TSKgel DNA-NPR (2.5 µm)



a) 36%~51% (45 min); b) 13%~28% (45 min)
c) 54%~64% (40 min); d) 49%~59% (40 min)

TSKgel DNA-STAT (5 µm)



a) 50%~65% (45 min); b) 20%~35% (45 min)
c) 84%~94% (40 min); d) 72%~82% (40 min)

Conditions;
Column;TSKgel DNA-NPR (4.6 mm I.D. x 7.5 cm)
TSKgel DNA-STAT (4.6 mm I.D. x 10 cm)
Eluent A; 20 mM Tris-HCl (pH 8.5)
Eluent B;a) A + 1.5 mol/L NaCl pH 8.5
b) A + 1.5 mol/L $NaClO_4$ pH 8.5
c) A + 2 mol/L TMAC* pH 8.5
d) A + 2 mol/L choline chloride pH 8.5
e) A + 2 mol/L Guanidinium chloride pH 8.5
Gradient; shown below each chromatogram
Flow rate; 0.5 mL/min
Detection; UV (260 nm)
Temperature; 25 ℃
Inj.vol.; 10 µL
Samples; 1) Linear (0.02 mg/ml)
2) Open circular (0.02 mg/ml)
3) Supercoiled (0.02 mg/ml)

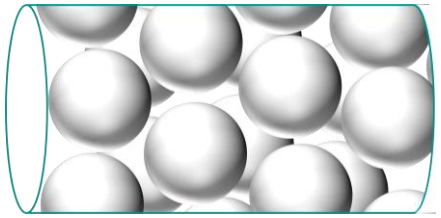
*TMAC: Tetramethylammonium Chloride

- 使用TMAC与Choline chloride时分离程度相似,可使用Choline chloride替代毒性较强的TMAC
- 但以上条件均未确认到OC的洗脱

质粒DNA拓扑异构体的IEC分离

□ 样品: Plasmid DNA pHelper (11.6 kbp)

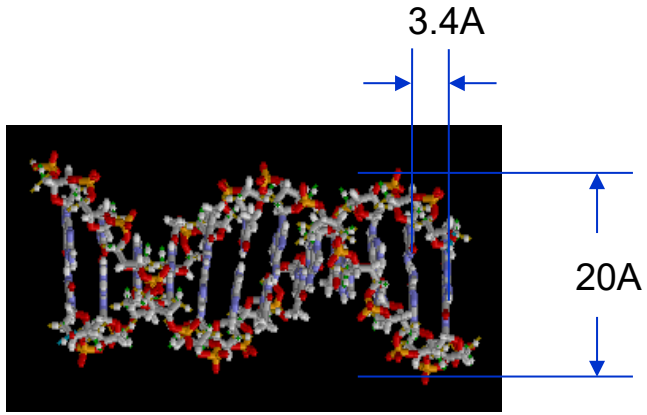
色谱柱示意图



填料粒子间的空隙尺寸 ⇒ 粒径的15 % – 35 %

TSKgel DNA-NPR (2.5 µm) ⇒ 375 nm – 875 nm

TSKgel DNA-STAT(5 µm) ⇒ 750 nm – 1750 nm



Plasmid DNA pHelper : 11.6 kbp

超螺旋型 ⇒ 全碱基长度的41 %⇒ 1617 nm

线型 ⇒ 3944 nm

1617 nm < 开环型 < 3944 nm

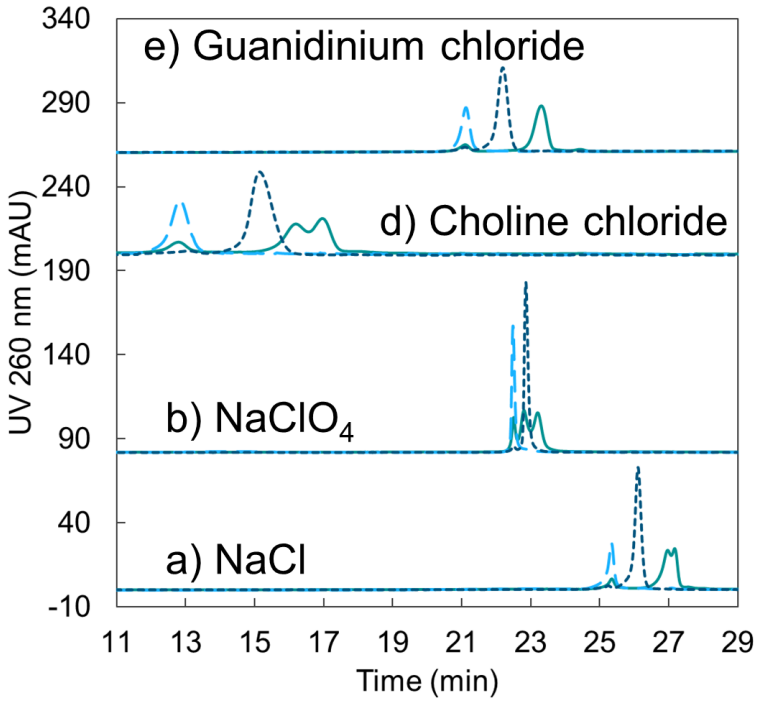
SC和Linear易被拉伸程线状，可以穿过色谱柱间隙
OC保持松弛型环状结构，尺寸较大，因此容易滞留在色谱柱间隙中

质粒DNA拓扑异构体的IEC分离

TOSOH

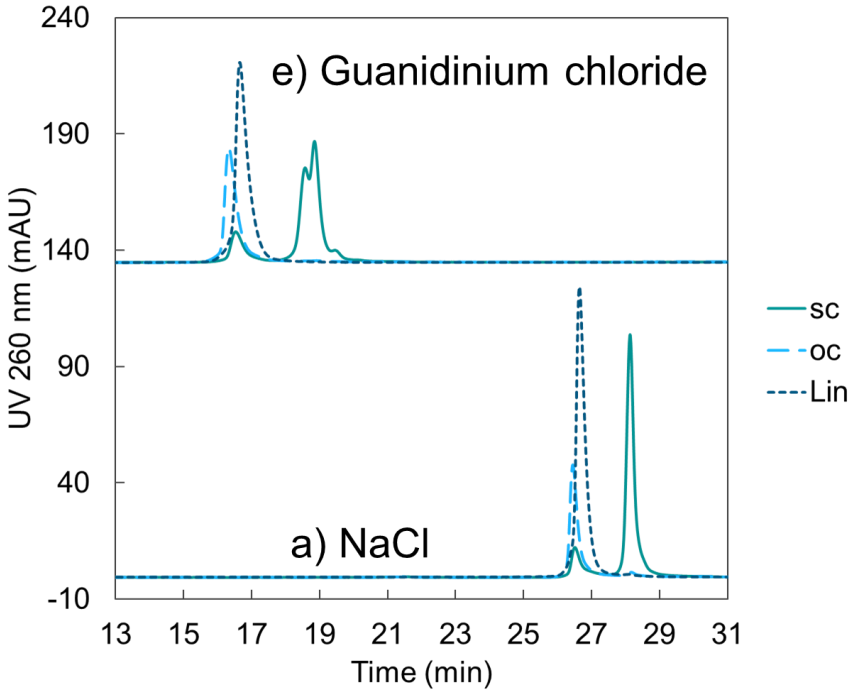
□ 样品: Plasmid DNA pHelper (11.6 kbp)

TSKgel Q-STAT (7 µm)



Flow rate: 0.5 mL/min
a) 49%~64% (45 min); b) 18%~33% (45 min)
d) 72%~82% (40 min); e) 38%~48% (40 min)

TSKgel Q-STAT (10 µm)



Flow rate: 0.1 mL/min
a) 49%~64% (45 min);e) 38%~48% (40 min)

Conditions;
Column;TSKgel Q-STAT (4.6 mm I.D. x 10 cm)
TSKgel Q-STAT (3.0 mm I.D. x 3.5 cm)
Eluent A; 20 mM Tris-HCl (pH 8.5)
Eluent B;a) A + 1.5 mol/L NaCl pH 8.5
b) A + 1.5 mol/L $NaClO_4$ pH 8.5
d) A + 2 mol/L choline chloride pH 8.5
e) A + 2 mol/L Guanidinium chloride pH 8.5
Gradient; shown below each chromatogram
Flow rate; shown below each chromatogram
Detection; UV (260 nm)
Temperature; 25 ℃
Inj.vol.; 10 µL
Samples; 1) Linear (0.02 mg/ml)
2) Open circular (0.02 mg/ml)
3) Supercoiled (0.02 mg/ml)

*TMAC: Tetramethylammonium Chloride

TOSOH

长链核酸（MRNA和DNA）的分析

mRNA疫苗的制造与质量评价

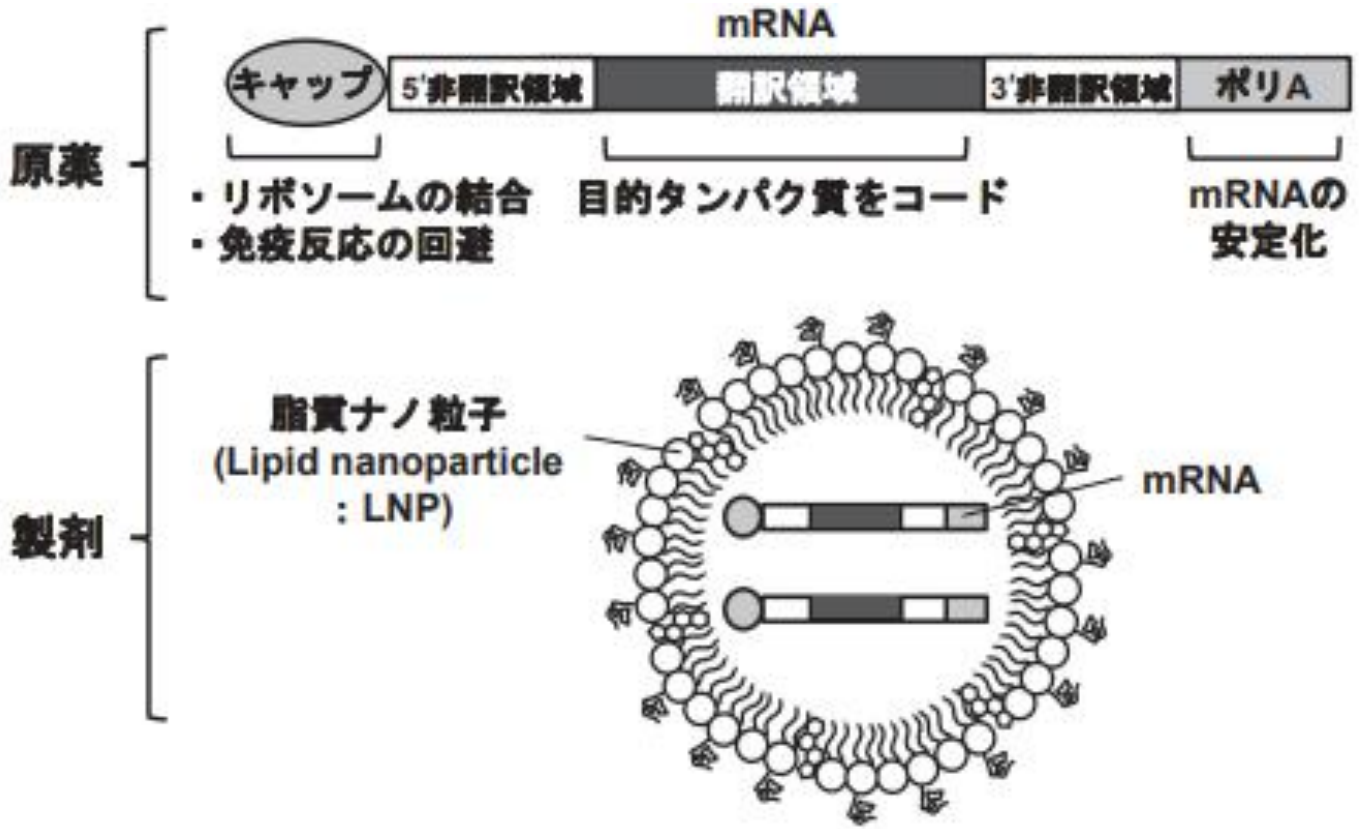


Fig. 1　mRNA医薬の構造

产品相关杂质；双链RNA、片段化RNA、加帽不完全mRNA等

工艺相关杂质；帽子相关杂质、残留蛋白酶、有机溶剂

［医薬品医療機器レギュラトリーサイエンス，PMDRS, 54（4），300～311（2023）］

mRNA疫苗和治疗产品的质量分析方法指南<USP1040>

Purity	mRNA purity	Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC)
	5′ capping efficiency	Reverse-phase liquid chromatography mass spectroscopy (RP–LC-MS/MS) [D]
		Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC)
		Liquid chromatography mass spectroscopy (LC-MS/MS) [D]
	3′ poly(A) tail length	Liquid chromatography mass spectroscopy (LC-MS/MS) [D]
		Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC)
	Product related impurities - dsRNA	Immunoblot
		Enzyme-linked immunosorbent assay (ELISA)
	Product related impurities - aggregate quantitation	Size exclusion-high-performance liquid chromatography (SEC-HPLC) [D]
	Product related impurities - percentage of fragment mRNA	Reversed-phase HPLC (RP-HPLC) [D]
	Process related impurities - residual DNA template	quantitative PCR (qPCR)
	Process related impurities - quantitation of free/non-incorporated nucleosides	Reverse-phase liquid chromatography mass spectroscopy (RP–LC-MS/MS) [D]
	Process related impurities - residual NTP and capping agent	Anion exchange high-performance liquid chromatography (AEX-HPLC) [D]
	Process related impurities - residual T7 RNA polymerase content	Enzyme-linked immunosorbent assay (ELISA)

适用于ssRNA分析的色谱柱

尺寸排阻色谱（SEC）

- 可在接近生理条件的洗脱液系中分离

商品名称	基材	粒径 (μm)	孔径 (nm)	尺寸 (mm I.D. x cm)
TSKgel G4000PW$_{XL}$	亲水性乙烯基聚合物	10	50以下	7.8 x 30
TSKgel G4000SW$_{XL}$	高纯度二氧化硅	8	45	7.8 x 30

离子对反相色谱法（IP-RPLC）

- 高分离

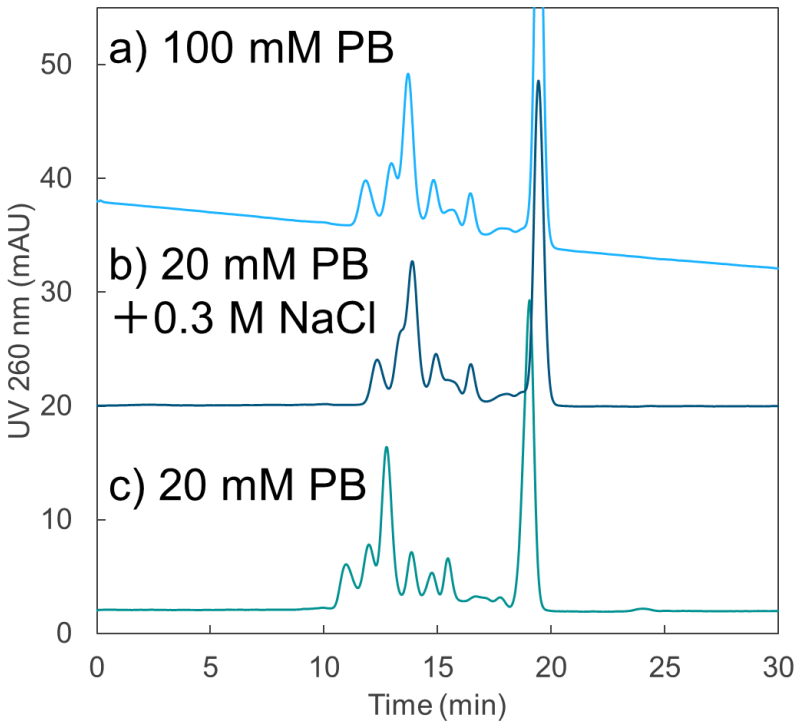
商品名称	粒径 (μm)	孔径 (nm)	碳含量 (%)	尺寸 (mm I.D. x cm)
TSKgel ODS-120H	1.9、3、5	12	20 %	2.0 x 15
TSKgel Oligo DNA RP	5	25	12 %	4.6 x 15

不同链长ssRNA的SEC分离

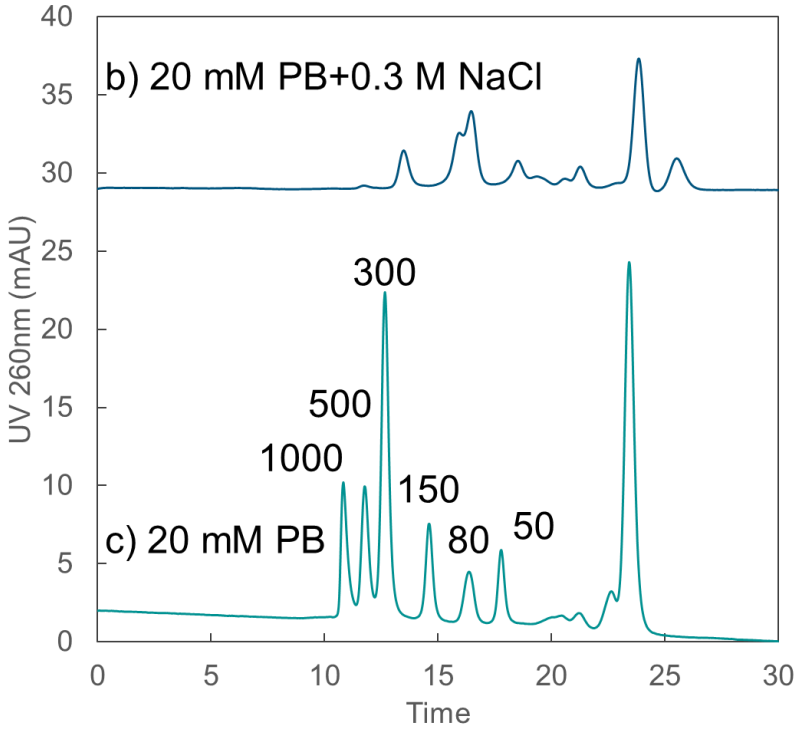
◘ 洗脱液种类对分离的影响

➢ 样品:ssRNA Ladder (50~1000 bases ssRNA) (New England BioLabs)

TSKgel G4000PW$_{XL}$



TSKgel G4000SW$_{XL}$



Conditions;
Columns; 1) TSKgel G4000PW$_{XL}$ (7.8 mm I.D. x 30 cm)
2) TSKgel G4000SW$_{XL}$ (7.8 mm I.D. x 30 cm)
Eluent; a) 100 mM PB (pH 7.0)
b) 20 mM PB + 0.3 M NaCl (pH 7.0)
c) 20 mM PB (pH 7.0)
Flow rate; 0.5 mL/min
Detection; UV (260 nm)
Temperature; 1) 80 ℃
2) 65 ℃
Inj.vol.; 2 µL
Samples; 50,80,150,300,500,1000 base ssRNA mixture (500 µg/mL)

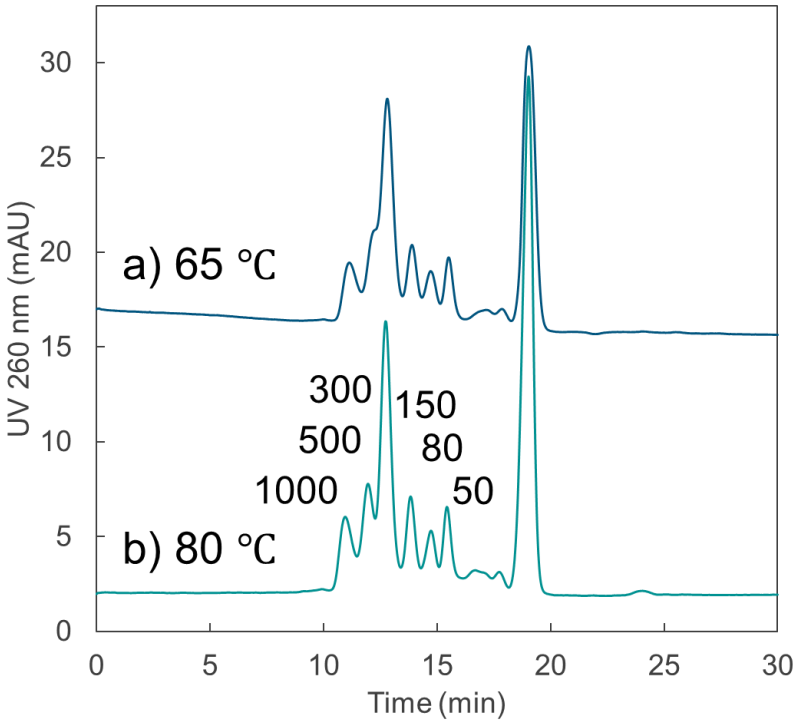
注:与标准温度相比，在65℃使用TSKgel G4000SW$_{XL}$时，色谱柱寿命可能会缩短

不同链长ssRNA的SEC分离

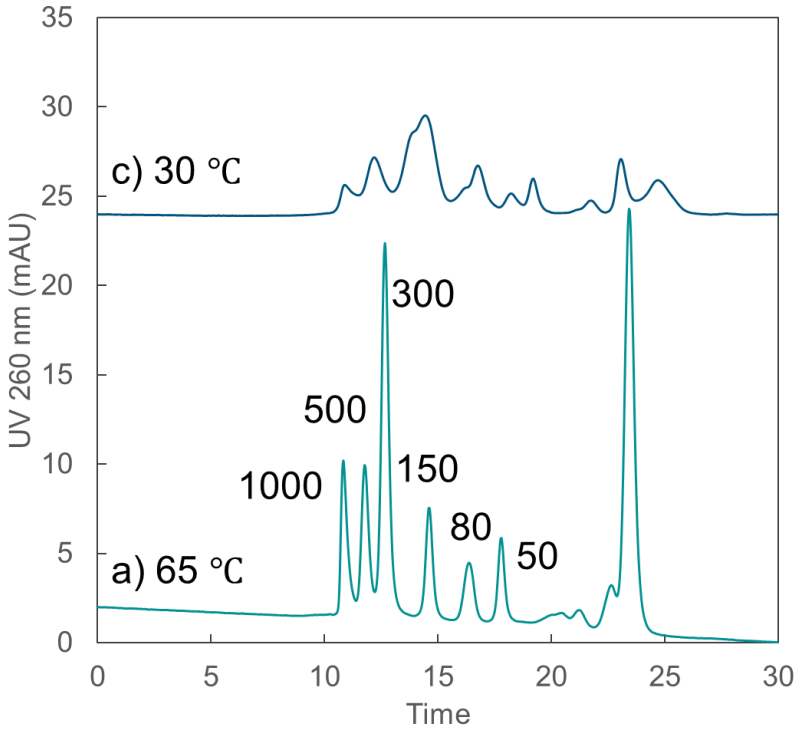
◘ 温度对分离的影响

➢ 样品:ssRNA Ladder (50~1000 bases ssRNA) (New England BioLabs)

TSKgel G4000PW$_{XL}$



TSKgel G4000SW$_{XL}$



Conditions;
Columns; 1) TSKgel G4000PW$_{XL}$ (7.8 mm I.D. x 30 cm)
2) TSKgel G4000SW$_{XL}$ (7.8 mm I.D. x 30 cm)
Eluent; 20 mM PB (pH 7.0)
Flow rate; 0.5 mL/min
Detection; UV (260 nm)
Temperature; a) 65 ℃
b) 80 ℃
c) 30 ℃
Inj.vol.; 2 µL
Samples; 50,80,150,300,500,1000 base ssRNA mixture (500 µg/mL)

注:与标准温度相比，在65℃使用TSKgel G4000SW$_{XL}$时，色谱柱寿命可能会缩短

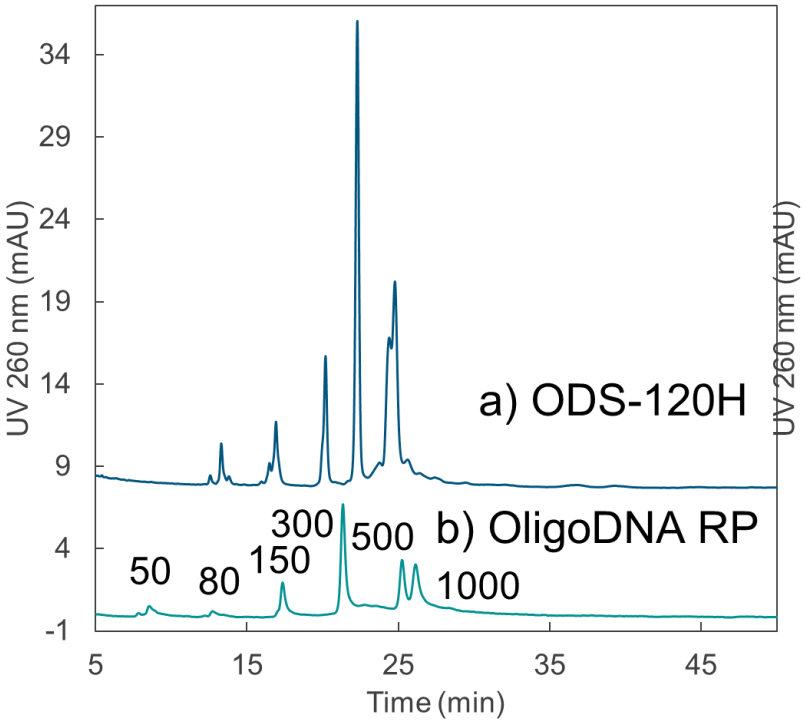
不同链长ssRNA的IP-RPLC分离

TOSOH

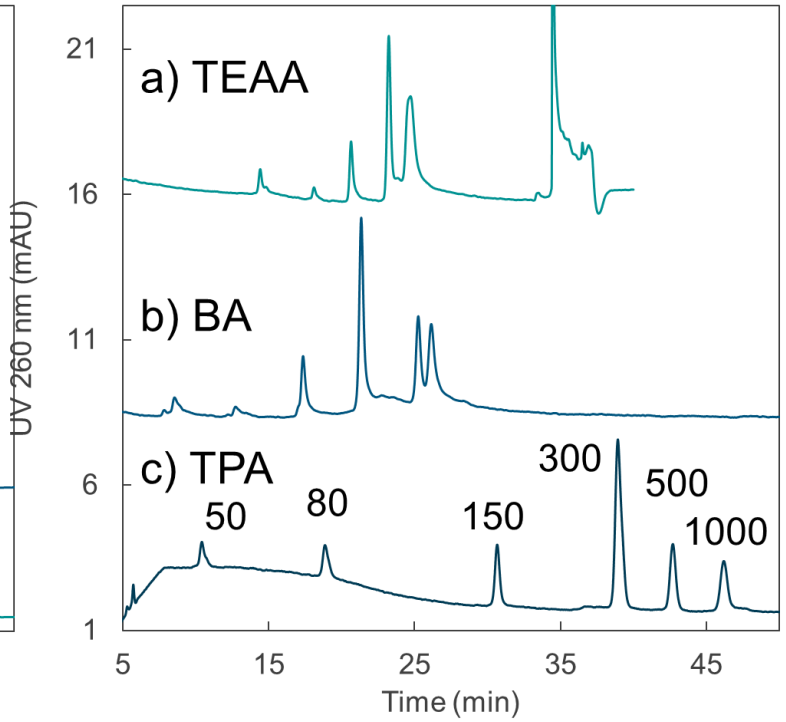
◘ 色谱柱及离子对试剂对分离的影响

➢ 样品:ssRNA Ladder (50~1000 bases ssRNA) (New England BioLabs)

色谱柱的影响



离子对试剂的影响



Conditions1
Column;a) TSKgel ODS-120H (2.0 mm I.D. x 15 cm; 3 μm)
b) TSKgel OligoDNA RP (4.6 mm I.D. x 15 cm; 5 μm)
Eluent; A_1: 100 mmol/L BA pH 7.0
B_1: 100 mmol/L BA pH 7.0＋80 % acetonitrile
A_2: 100 mmol/L BA pH 7.0
B_2: 100 mmol/L BA pH 7.0＋50 % acetonitrile
Gradient; a) 20 %-35 % B, 48 min
b) 30 %-60 % B, 60 min
Flow rate;a) 0.2 mL/min
b) 1.0 mL/min
Detection; UV (260 nm)
Temperature; 50 ℃
Inj.vol.; 2 μL
Samples; 50,80,150,300,500,1000 base ssRNA mixture(500 μg/mL)

Conditions 2
Column; TSKgel OligoDNA RP (4.6 mm I.D. x 15 cm)
Eluent; A_1: 100 mmol/L *TEAA pH 7.0
B_1: 100 mmol/L TEAA pH 7.0＋50 % acetonitrile
A_2: 100 mmol/L *BA pH 7.0
B_2: 100 mmol/L BA pH 7.0＋50 % acetonitrile
A_3: 100 mmol/L *TPA pH 7.0
B_3: 100 mmol/L TPA pH 7.0＋50 % acetonitrile
Gradient; a) 15 %-30 % B, 30 min
b) 30 %-60 % B, 60 min
c) 60 %-90 % B, 60 min
Flow rate; 1.0 mL/min
Other conditions are the same as in Condition 1

TEAA: Triethylammonium Acetate
BA: Butylamine
TPA: Tripropylamine

适用于DNA分析的色谱柱

TOSOH

尺寸排阻色谱(SEC)

商品名称	基材	粒径 (μm)	孔径 (nm)	尺寸 (mm I.D. x cm)
TSKgel G3000SW$_{XL}$	高纯度二氧化硅	5	25	7.8 x 30
TSKgel G4000SW$_{XL}$	高纯度二氧化硅	8	45	7.8 x 30
TSKgel G-DNA-PW	亲水性乙烯基聚合物	10	100以上	7.8 x 30

阴离子交换色谱(IEC)

商品名称	粒径 (μm)	尺寸(mm I.D. x cm)	官能团
TSKgel DNA-STAT	5	4.6 X 10	季氨基

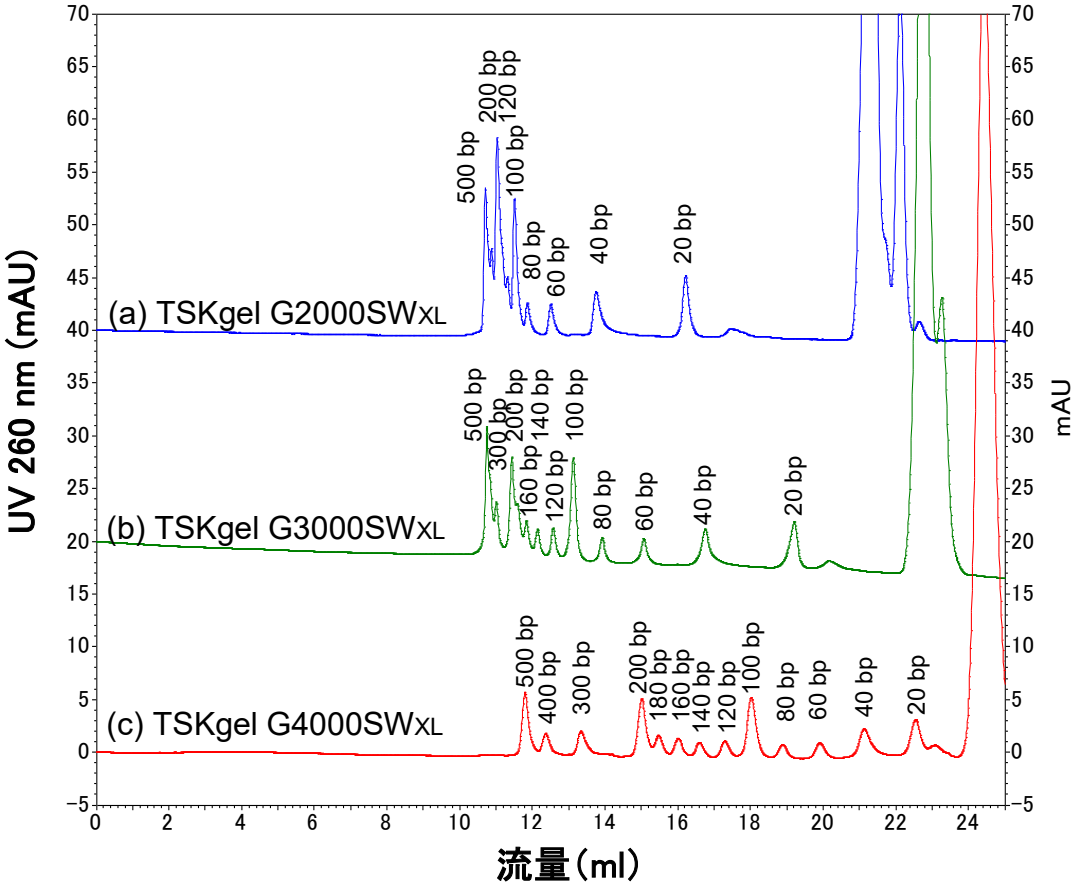
障碍（Slalom）色谱(SC)

商品名称	基材	粒径 (μm)	孔径 (nm)	尺寸 (mm I.D. x cm)
TSKgel UP-SW3000	高纯度二氧化硅	2	12.5	7.8 x 30

不同链长DNA的SEC分离

◘ 色谱柱对分离的影响

➢ 样品:DNA Ladder (20~500 bp DNA) (Takala Bio)



Conditions

Columns : (a) TSKgel G2000SWXL x 2 (7.8 mm I.D. ×30 cm)
(b) TSKgel G3000SWXL x 2(7.8 mm I.D. ×30 cm)
(c) TSKgel G4000SWXL x 2 (7.8 mm I.D. ×30 cm)

Eluent : 20 mmol/L PB + 0.3 mol/L NaCl + 1 mmol/L EDTA (pH7.0)

Flow rate : 0.15 mL/min

Temperature : 25 ℃

Detection : 260 nm

Inj.vol : 5 µL

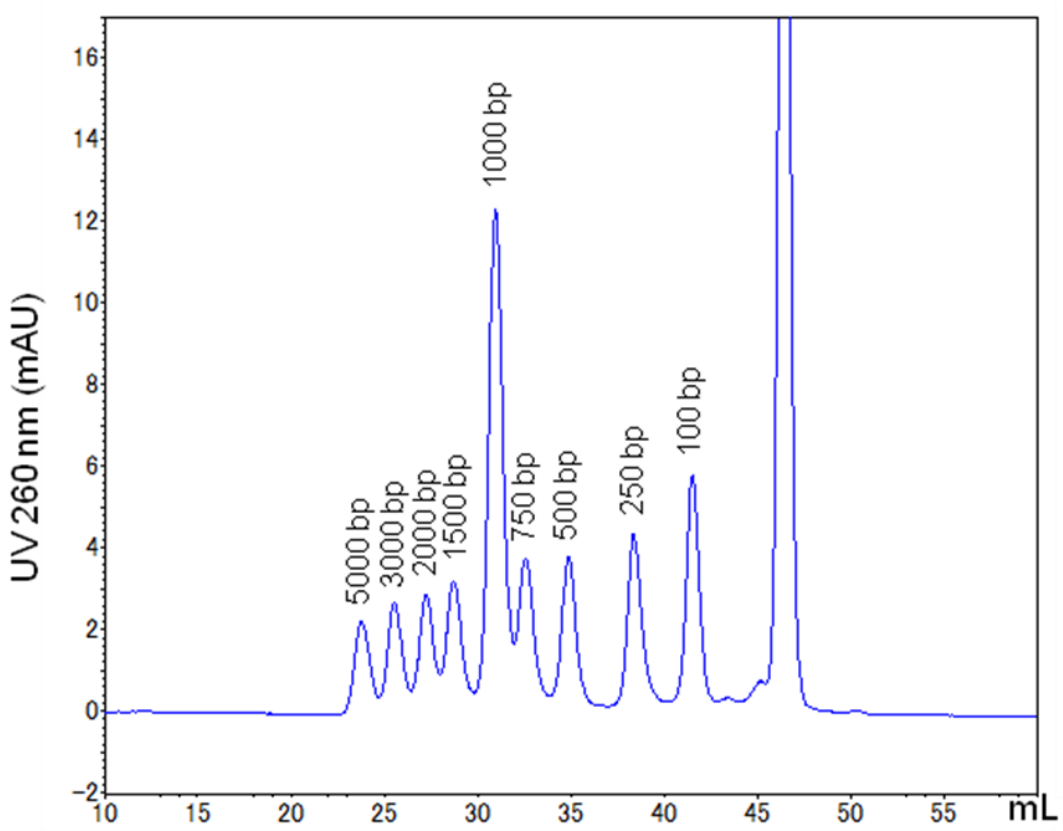
Sample : DNA Laddar, 20, 40, 60, 80,100, 120, 140, 160, 180, 200, 300, 400, 500 bp DNA mixture

不同链长DNA的SEC分离

TOSOH

◘ 长链DNA的分离

➢ 样品:DNA Ladder (100~5000 bp DNA)

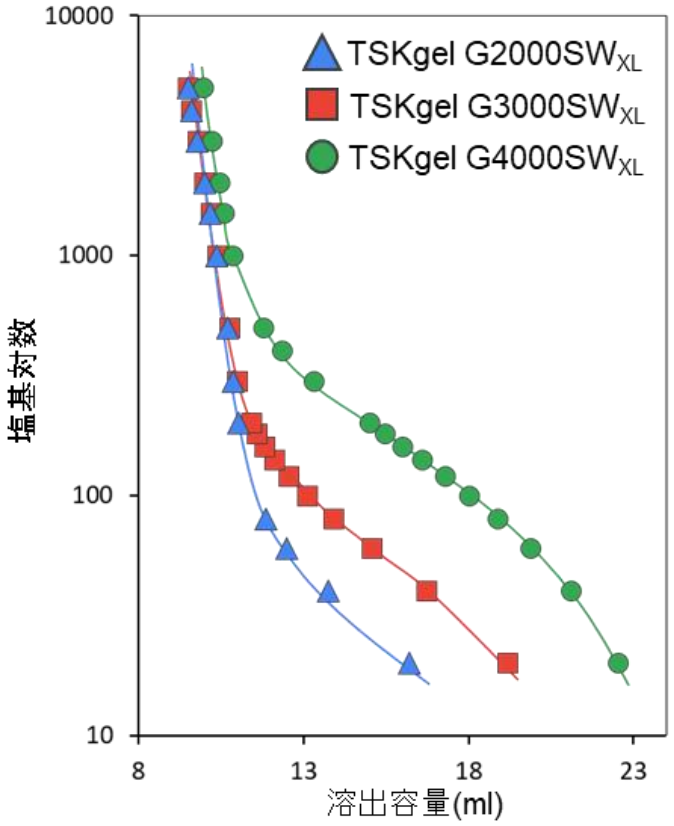


Conditions

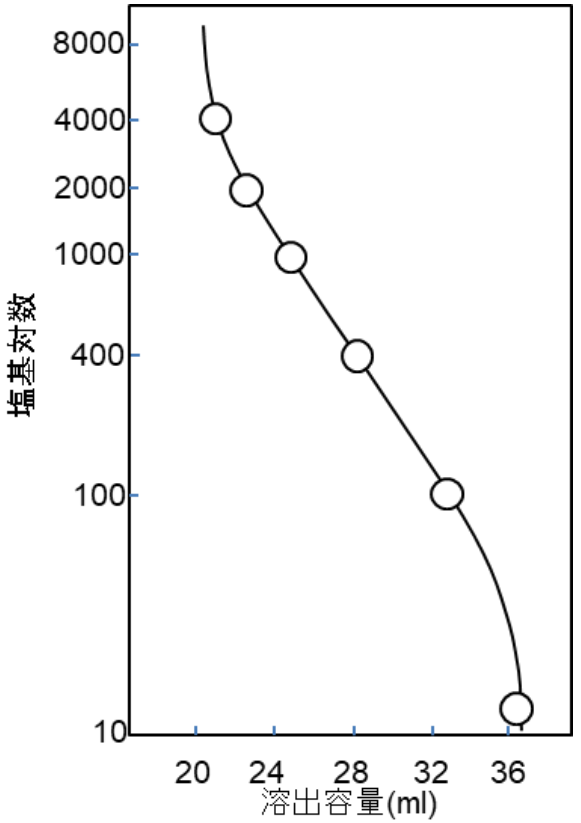
Columns	: TSKgel G-DNA-PW x 4 (7.8 mm I.D. x 30 cm)
Eluent	: 20 mmol/L PB + 0.3 mol/L NaCl + 1 mmol/L EDTA (pH7.0)
Flow rate	: 0.15 mL/min
Temperature	: 25 ℃
Detection	: 260 nm
Inj.vol	: 5 µL
Sample	: DNA Laddar, 100, 250, 500, 750,1000, 1500, 2000, 5000 bp DNA mixture

不同链长DNA的SEC分离

□ 校正曲线



TSKgel SWXL系列的校正曲线
(7.8 mm I.D. × 30 cm × 2)

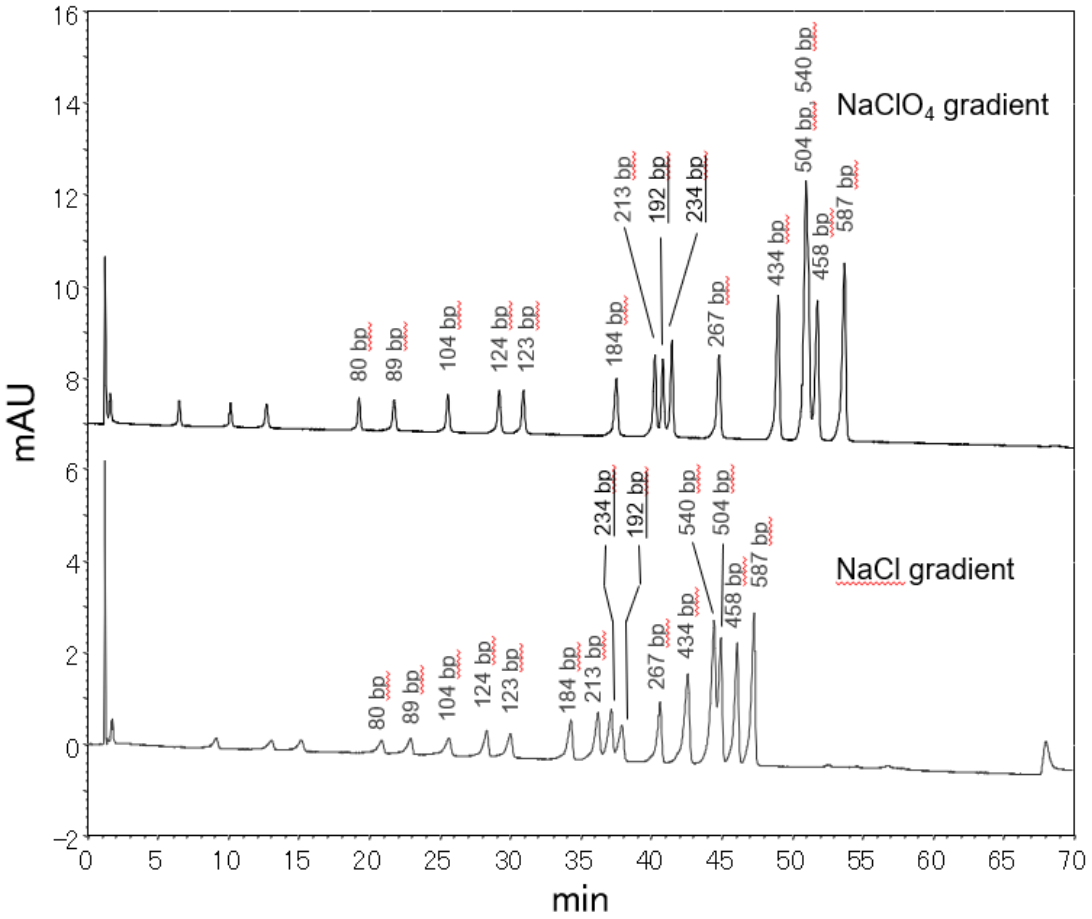


TSKgel G-DNA-PW的校正曲线
(7.8 mm I.D. × 30 cm × 4)

不同链长DNA的IEC分离

◘ 盐种类对分离的影响

➢ 样品:pBR322-HaeⅢdigest



Column :TSKgel DNA-STAT (4.6 mmI.D. x 100 mm)
Eluent :A; 20 mmol/L Tris-HCl (pH8.5)
B; 20 mmol/L Tris-HCl + 0.5 mol/L $NaClO_4$ (pH8.5)
Gradient : B 55 %→70 % (60 min)
Flow rate : 0.5 mL/min
Detection : UV (260 nm)
Temp. : 25 C
Inj. volume: 5 µL
Conc. : 25 mg/L
Sample : pBR322-*Hae*Ⅲdigest

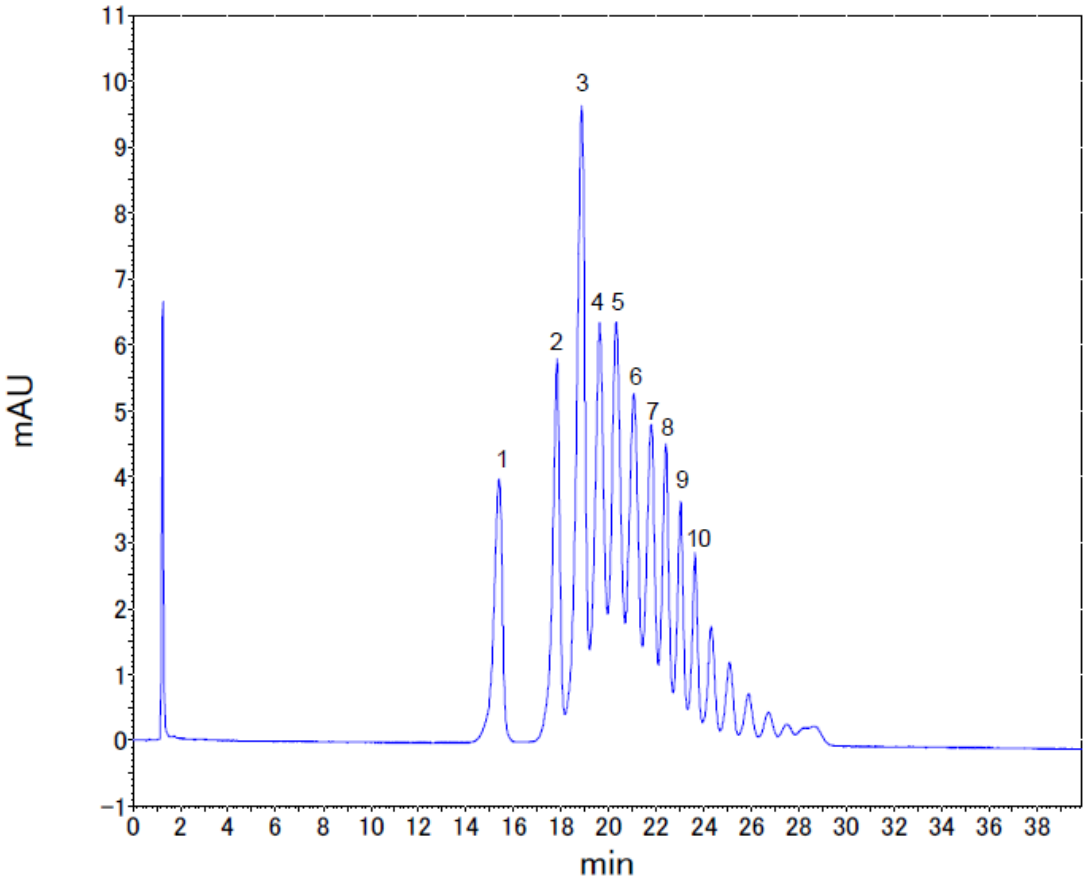
Column :TSKgel DNA-STAT (4.6 mmI.D. x 100 mm)
Eluent :A; 20 mmol/L Tris-HCl (pH8.5)
B; 20 mmol/L Tris-HCl + 1.0 mol/L NaCl (pH8.5)
Gradient : B 70 %→90 % (60 min)
Flow rate : 0.5 mL/min
Detection : UV (260 nm)
Temp. : 25 C
Inj. volume: 5 µL
Conc. : 25 mg/L
Sample : pBR322-*Hae*Ⅲdigest

不同链长DNA的IEC分离

TOSOH

◘ 长链DNA的分离

➢ 样品:DNA Ladder (1k~10 kbp DNA) (Takala Bio)

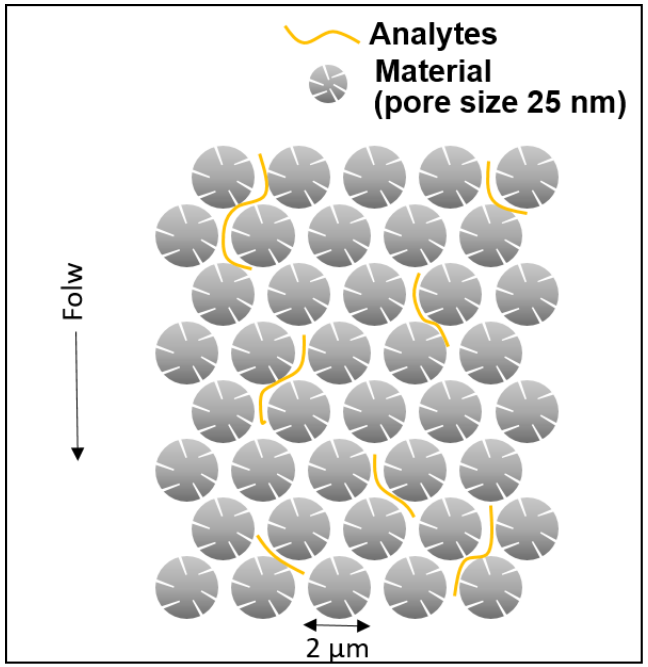


Column :TSKgel DNA-STAT (4.6 mmI.D. x 100 mm)
Eluent :A; 20 mmol/L Tris-HCl (pH8.5)
B; 20 mmol/L Tris-HCl + 1.0 mol/L NaCl (pH8.5)
Gradient : B 80 %→95 % (60 min)
Flow rate : 0.5 mL/min
Detection : UV (260 nm)
Temp. : 25 C
Inj. volume: 3 µL
Conc. : 200 mg/L
Sample : 1 k, 2 k, 3 k, 4 k, 5 k, 6 k, 7 k, 8 k, 9 k, 10 kbp DNA mixture

不同链长DNA的SC分离

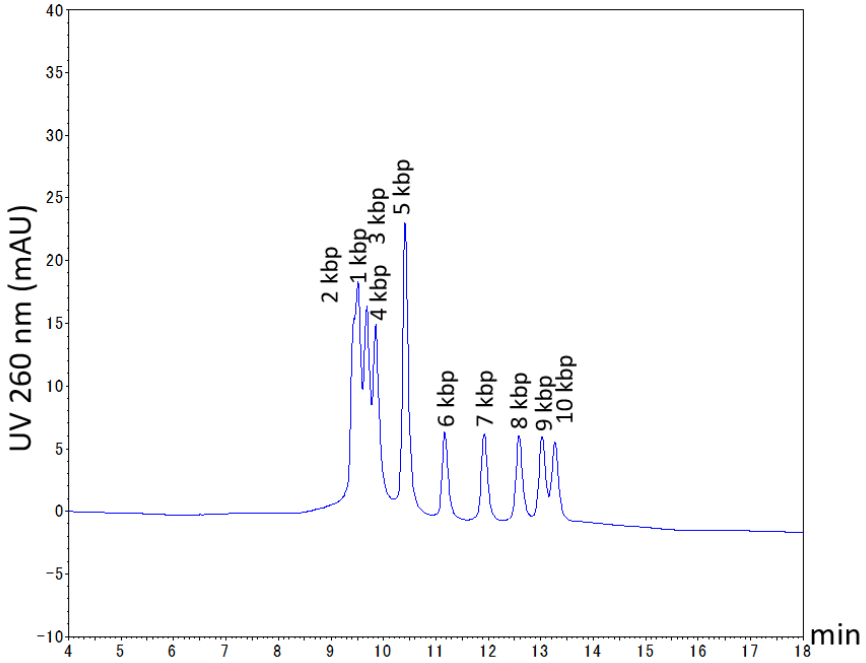
TOSOH

Slalom chromatography 示意图



对于长链核酸，链长越长，洗脱越晚

➢ 样品:DNA Ladder (1k~10 kbp DNA) (Takala Bio)

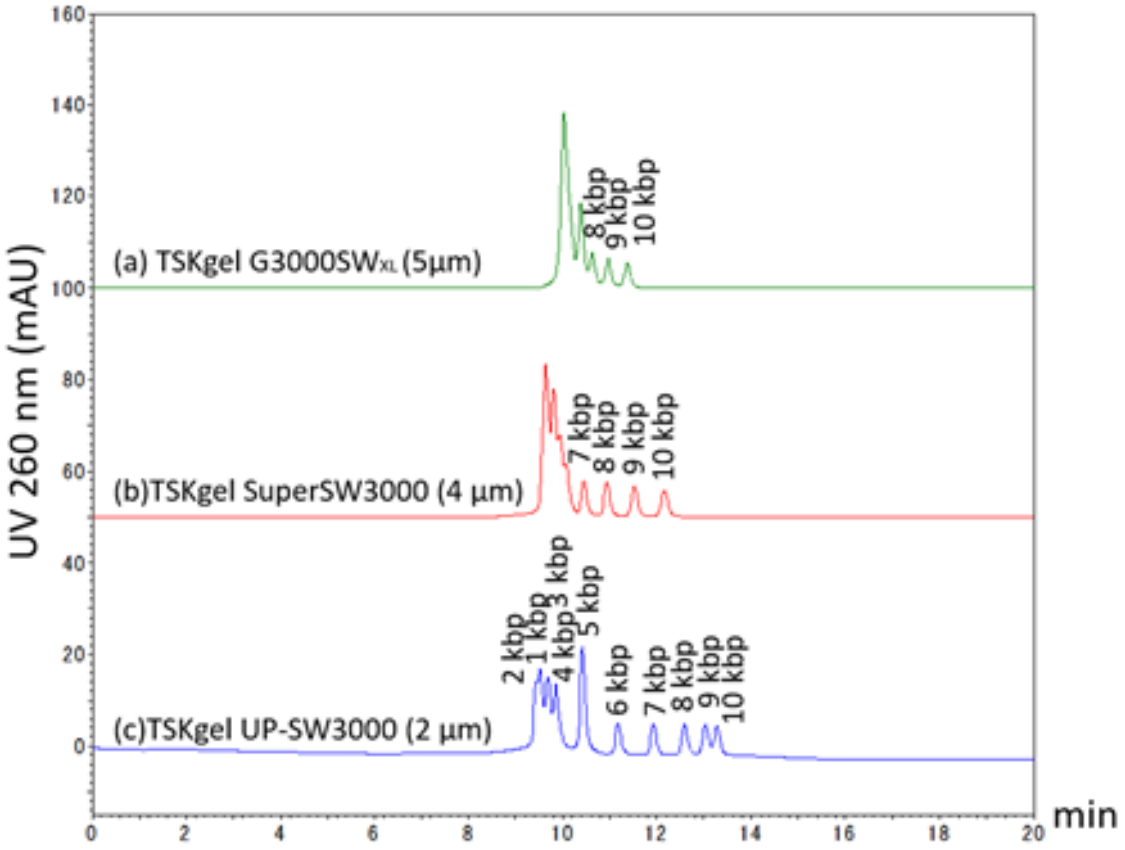


Column	:TSKgel UP-SW3000 x 2 (4.6 mmI.D. x 30 cm)
Eluent	: 20 mM PB + 0.3 M NaCl + 1 mmol/L EDTA pH 7.0
Flow rate	: 0.35 mL/min
Detection	: UV (260 nm)
Temp.	: 25 C
Inj. Volume	: 2 µL
Sample	: 1 k, 2 k, 3 k, 4 k, 5 k, 6 k, 7 k, 8 k, 9 k, 10 kbp DNA mixture

不同链长DNA的SC分离

◘ 粒径对分离的影响

➢ 样品:DNA Ladder (1k~10 kbp DNA) (Takala Bio)



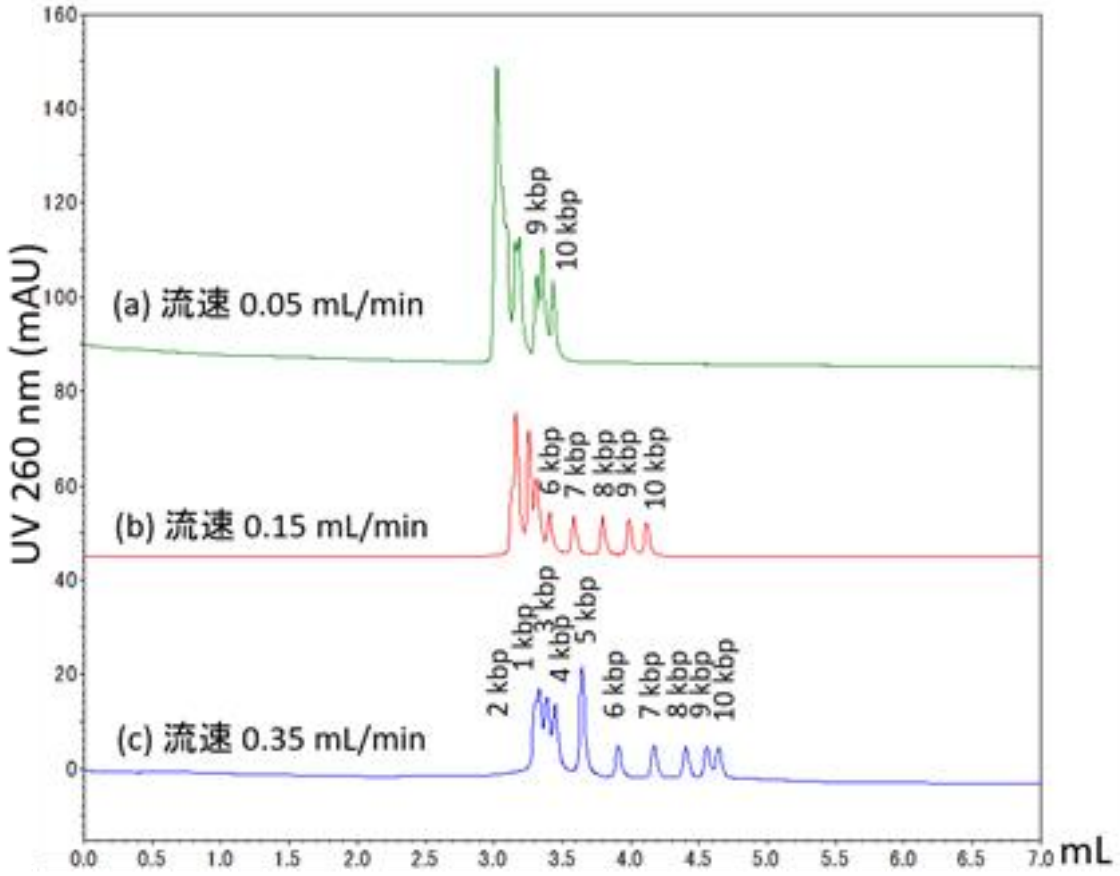
Column :(a) TSKgel G3000SW$_{XL}$ x 2 (7.8 mm I.D.×30 cm×2)
(b) TSKgel SuperSW3000 x 2 (4.6 mm I.D.×30 cm×2)
(c) TSKgel UP-SW3000 x 2 (4.6 mm I.D.×30 cm×2)
Eluent: 20 mmol/L PB+ 0.3 mol/L NaCl + 1 mmol/L EDTA (pH 7.0)
Flow rate: (a) 1.00 mL/min（線速:2.1 cm/min）
(b) 0.35 mL/min（線速:2.1 cm/min）
(c) 0.35 mL/min（線速:2.1 cm/min）
Temperature: 25 ℃
Detection: 260 nm
Inj. volume: (a) 5 µL
(b) 2 µL
(c) 2 µL
Sample :1 k, 2 k, 3 k, 4 k, 5 k, 6 k, 7 k, 8 k, 9 k, 10 kbp DNA mixture

不同链长DNA的SC分离

TOSOH

◘ 流速对分离的影响

➢ 样品:DNA Ladder (1k~10 kbp DNA) (Takala Bio)



Column :TSKgel UP-SW3000 x 2 (4.6 mmI.D. x 30 cm x 2)
Eluent : 20 mM PB + 0.3 M NaCl + 1 mmol/L EDTA pH 7.0
Flow rate : (a) 0.05 mL/min
(b) 0.15 mL/min
(c) 0.35 mL/min
Detection : UV (260 nm)
Temp. : 25 C
Inj. Volume: 2 µL
Sample : 1 k, 2 k, 3 k, 4 k, 5 k, 6 k, 7 k, 8 k, 9 k, 10 kbp DNA mixture

总结

分离模式	产品名称	适用样品	影响因子
反相色谱 (RPC)	• TSKgel ODS-120H	• 寡核苷酸、500 bases ssRNA	• 添加剂种类、离子对试剂对分离影响大
	• TSKgel Oligo DNA RP	• 1000 bases ssRNA	
亲水作用色谱(HILIC)	• TSKgel Amide-80	• 寡核苷酸	• 使用高浓度有机溶剂，样品洗脱顺序与反相不同
离子交换色谱 (IEC)	• TSKgel DNA-NPR	• 5 kbp以下质粒DNA拓扑异构体	• 粒径、盐种类对分离影响大
	• TSKgel DNA-STAT	• 寡核苷酸、5 kbp以下质粒DNA拓扑异构体、DNA	
	• TSKgel Q-STAT	• 5 kbp以上质粒DNA拓扑异构体	
尺寸排阻色谱 (SEC)	• TSKgel G4000SW$_{XL}$	• 1000 bases ssRNA、1000 bp DNA	• 温度以及盐浓度对分离影响大
	• TSKgel G4000PW$_{XL}$	• 1000 bases ssRNA、1000 bp DNA	
	• TSKgel G-DNA-PW	• 5000 bp DNA	
障碍Slalom色谱(SC)	• TSKgel UP-SW3000	• 5000 bp以上DNA	• 粒径与流速对分离影响大

TOSOH

感谢聆听