Supporting information

Chiral Resolution Capabilities of DNA Oligonucleotides

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Table of content

1. Chemicals and materials **3**

2. Capillary electrophoresis **3**

Figure S1 Representation of the modified cassette **4**

3. DNA preparation and injection vial configuration **4**

Figure S2 Representation of the modified injection vial **5**

Table S1 Resolution obtained for the 24 tested racemates using the ten screened DNA sequences **5**

Figure S3 Chiral resolution using **poly-dA** **6**

Figure S4 Chiral resolution using **poly-dC** **7**

Figure S5 Chiral resolution using **poly-dT** **8**

Figure S6 Chiral resolution using **dsDNA$_{-30}$** **9**

Figure S7 Chiral resolution using **hpDNA$_{-31}$** **10**

Figure S8 Chiral resolution using **$_3$WJDNA$_{-36}$** **11**

Figure S9 Chiral resolution using **hpDNA$_{-24}$** **12**

Figure S10 Chiral resolution using **hpDNA$_{-14}$** **13**

Figure S11 Chiral resolution using **mxDNA$_{-40}$** **14**

Figure S12 Chiral resolution using **tbDNA$_{-38}$** **15**

Figure S13 Mirror-image strategy **16**

Figure S14 Effect of the **$_3$WJDNA$_{-36}$** concentration on the resolution **17**

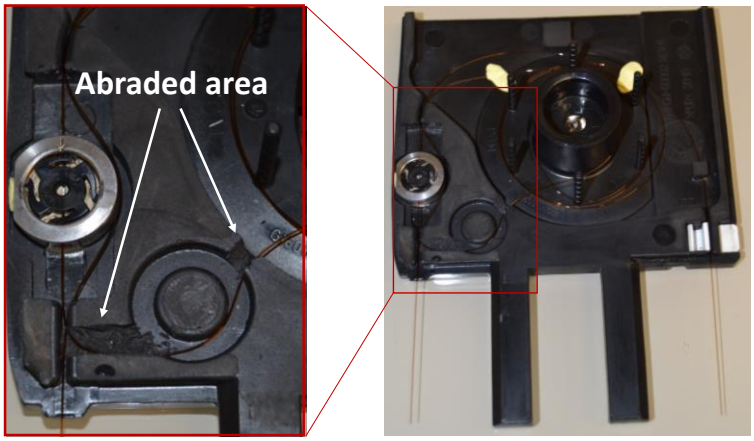
1. Chemicals and materials:

Threo-3-phenylserine was purchased from Acros Organic (Geel, Belgium). Tris(hydroxymethyl)aminomethane (Tris), 1-Methyl-Tryptophan, Atenolol, Ephedrine, Baclofen, Isoprenaline, Tramadol, Tyrosine methyl ester, Verapamil, Norepinephrine, Tryptophan, Phenylalanine, Tryptophan methyl ester and D-Amphetamine, were purchased from Sigma-Aldrich (Saint-Quentin, France). 1-Naphthylalanine, H-β-(3-Benzothienyl)-Ala-OH, H-β-(2-Quinolyl)-Ala-OH were purchased from Bachem (Budendorf, Switzerland). Methylenedioxymethamphetamine, Paramethoxyamphetamine and L-Amphetamine were purchased from Lipomed (Cambridge, USA). Propranolol was from AstraZeneca (Rueil-Malmaison, France). Chloroquine was from Sanofi (Paris, France). 2'-Deoxycytidine, 2'-Deoxyguanosine and 2'-Deoxyadenosine were purchased from ChemGenes Corp. (Wilmington, USA). Unless otherwise stated, racemic samples were used as analytes. NaCl was from Chimie-Plus laboratories (Bruyères de Pouilly, France). HCl was provided by Carlo Erba (Val de Reuil, France). Water was purified using a Purite Still Plus system (Thame, U.K.) fitted with a reverse osmosis cartridge. DNA oligonucleotides were synthesized and HPLC-purified by Eurogentec (Angers, France). The 0.2 mL PCR tubes were from VWR (Leuven, Belgium).

2. Capillary electrophoresis:

Capillary Electrophoresis (CE) experiments were carried out with a 3D-CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector. Bare-fused silica capillaries were purchased from Photon Lines (Saint-Germain-en-Laye, France). As described in Figure S1, the cassette was modified by abrading the plastic in order to create a channel for the capillary to re-enter in the cassette. The capillary was immobilized with removable adhesive gum. This format allows us to use a long capillary (105 cm) × 25 µm inner diameter while keeping short effective length (60 cm).

Figure S1 Representation of the modified cassette.



New fused silica capillaries were conditioned by performing the following washes: 1 M NaOH for 30 min and water for 10 min. The washing process between runs was performed with 1 M NaOH (5 min), water (1.5 min) and background electrolyte (5 min). An external pressure of 8 bar was used in both cases. The background electrolyte (BGE) was composed of 57.5 mM Tris + 46 mM HCl + 115 mM NaCl (pH 7.5, Ionic strength 160 mM) except when otherwise specified. The CE experiments were carried out using the capillary partial filling mode. After the washing process, a discrete plug of DNA oligonucleotide was introduced into the capillary by hydrodynamic injection (1 bar). Then, sequential injection of the racemates was performed and a voltage of 30 kV was next applied to achieve the separations.

The resolution was calculated with Agilent software by using the following equation:

$$Rs = \frac{1.18\left(t_2 - t_1\right)}{\delta_2 + \delta_1} \qquad (1)$$

where t is the apparent migration time of the analyte and δ the peak width at the half-height. The relative migration time of analytes was determined by the migration time ratio using the EOF peak as the reference point.

3. DNA preparation and injection vial configuration:

DNA stock solutions were prepared in pure water, aliquoted in small volumes (4 to 6 µL) into a PCR tube and stored at -20°C. The working DNA solutions were obtained by adequate dilution, of the aliquoted stock solution, in 2.2 times concentrated BGE. Prior to the first use, the working solutions were heated at 80°C for 5 min and left to stand at room temperature for 30 min. After a brief centrifugation to recover condensed water vapor, the top of the PCR tube was cut (Figure S2a). In order to use a very small amount of DNA sample, the injection vial configuration was also modified. As depicted in Figure S2a, Beckman vial cap was used and

was cut in two parts (part 2 and 3). The small part was introduced into the injection vial (Figure S2c) while the other was discarded. Then, the PCR tube was introduced in the injection vial, covered with parafilm and stored at 4°C until the time of the analysis, in order to limit the sample evaporation. Immediately after the DNA injection in the capillary, the vial is stored again at 4°C. The sample can be used throughout the day and at least for 16 runs.

Figure S2 Representation of the modified injection vial.

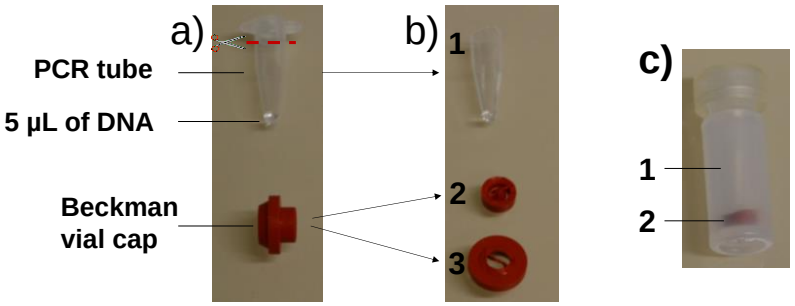
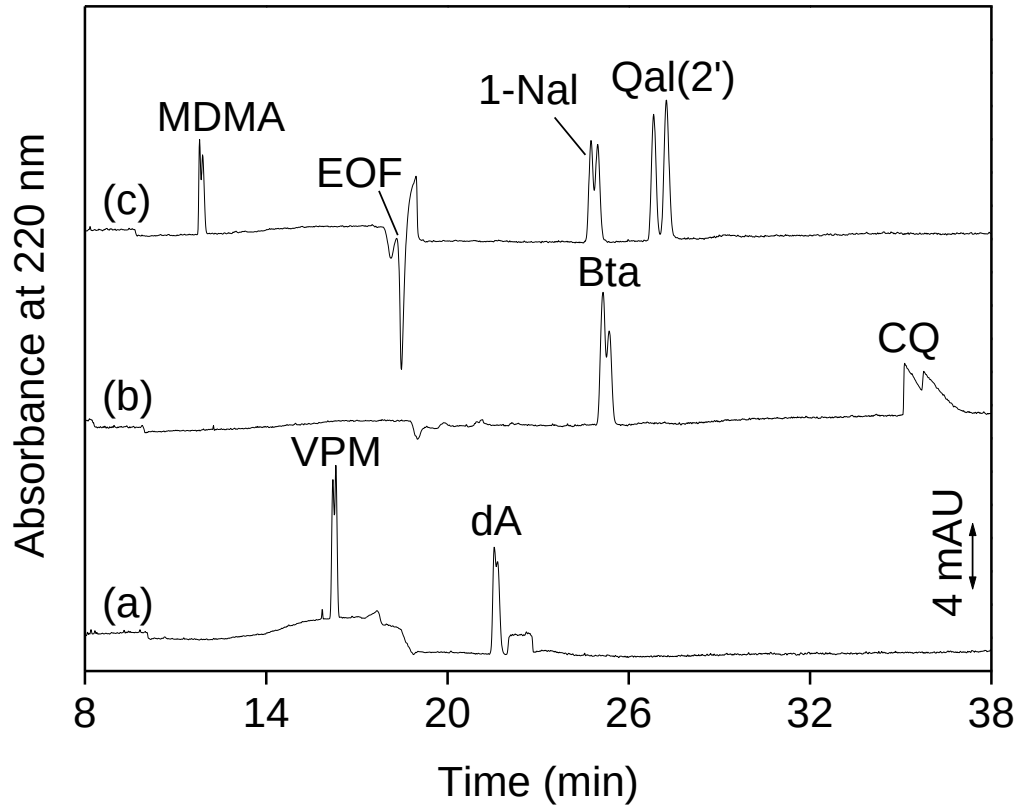


Table S1 Resolution obtained for the 24 tested racemates using the ten screened DNA sequences.

	DNA Oligonucleotides										
Analytes	**Poly-dA**	**Poly-dC**	**poly-dT**	**dsDNA$_{-30}$**	**hpDNA$_{-31}$**	**$_{3}$WJDNA$_{-36}$**	**hpDNA$_{-24}$**	**hpDNA$_{-14}$**	**mxDNA$_{-40}$**	**tbDNA$_{-38}$**	**Best Rs**
1-MT	–	–	1.45	1.50	13.25	4.05	7.59	2.18	8.59	4.98	13.25
dA	0.62	–	–	–	2.59	1.64	–	0.55	–	–	2.59
Trp	–	–	1.48	0.47	4.17	1.43	5.61	1.17	3.58	2.87	5.61
dG	–	–	–	–	3.51	2.31	–	1.34	3.36	7.69	7.69
1-Nal	0.68	–	0.70	2.17	5.10	2.63	3.89	3.99	–	–	5.10
CQ	0.62	2.66	4.24	–	–	–	1.50	–	–	–	4.24
PL	–	–	0.91	–	–	–	0.91	–	0.58	–	0.91
PMA	–	–	–	–	1.72	2.33	0.63	1.12	1.78	0.45	2.33
AMP	–	–	–	–	–	1.40	0.38	–	–	–	1.40
dC	–	–	–	–	–	0.68	–	–	–	–	0.68
TRA	–	–	–	–	2.00	0.73	0.71	–	2.14	1.38	2.14
TME	–	–	–	–	–	0.86	–	–	0.76	–	0.86
ISP	–	–	–	–	1.76	–	–	–	–	–	1.76
NE	–	–	–	–	2.23	–	–	–	–	–	2.23
VPM	0.60	1.40	1.61	1.38	0.62	–	1.19	4.67	1.21	1.93	4.67
WME	–	–	–	–	2.51	–	–	–	1.33	1.34	2.51
Phe	–	–	–	–	–	–	–	–	–	–	–
ATE	–	–	–	–	1.61	–	–	–	–	–	1.61
Bta	0.70	0.58	0.70	2.65	5.62	2.47	5.56	1.66	5.49	5.96	5.96
BCL	–	–	–	–	–	–	–	–	–	–	–
EPH	–	–	–	–	0.88	–	–	–	–	–	0.88
TPS	–	–	–	–	–	–	–	–	–	–	–
Qal(2')	1.39	–	0.70	1.11	–	0.89	1.08	1.60	–	1.95	1.95
MDMA	0.61	–	–	0.91	1.69	1.10	2.35	1.47	–	0.67	2.35

-: no chiral resolution

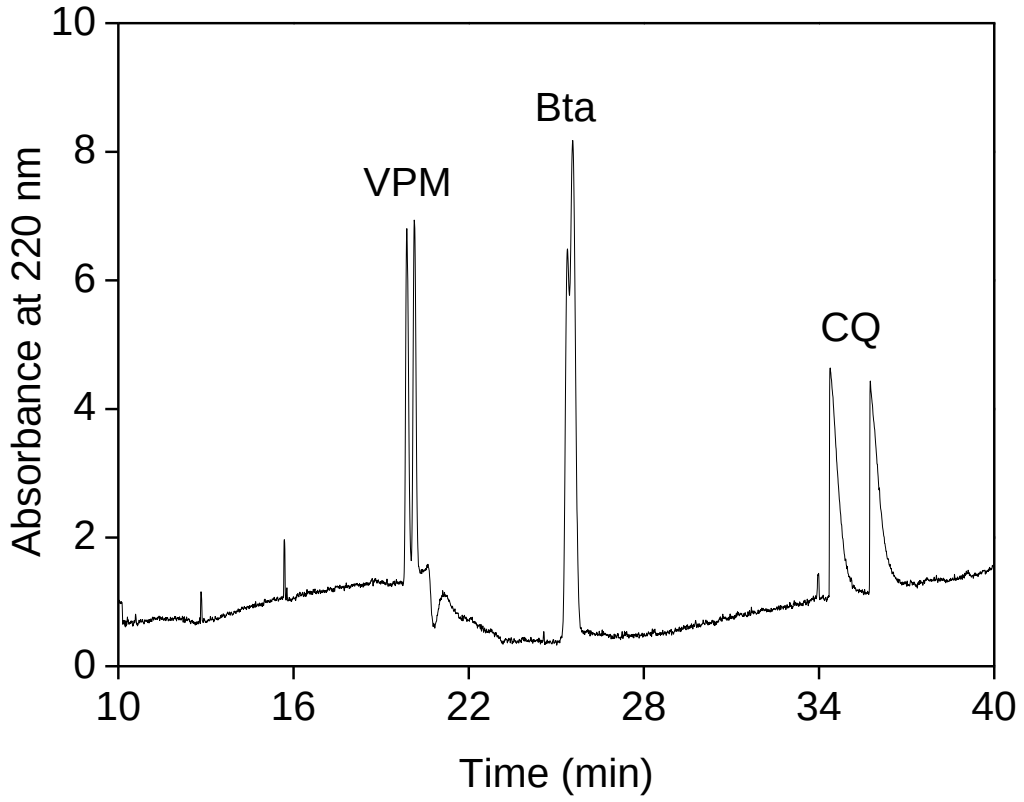
Figure S3 Chiral resolution using **poly-dA**



Experimental conditions:

Trace	[**poly-dA**]	**poly-dA** injection time	T	Racemate sequential injection
(a)	1.60 mM in 3 time diluted BGE	4.5 min	10°C	**1/** VPM 0.96 g/L (5 kV, 8 s) **2/** dA 0.88 g/L (10 kV, 5 s)
(b)	1.60 mM in 3 time diluted BGE	4.5 min	10°C	**1/** Bta 0.20 g/L + ACN 5% v/v (10 kV, 20 s) **2/** CQ 0.98 g/L (10 kV, 10 s)
(c)	1.60 mM in 3 time diluted BGE	4.5 min	10°C	**1/** MDMA 0.28 g/L (10 kV, 10 s) **2/** 1-Nal 0.14 g/L + ACN 5% v/v (15 kV, 10 s) **3/** Qal(2') 0.17 g/L (10 kV, 15 s)

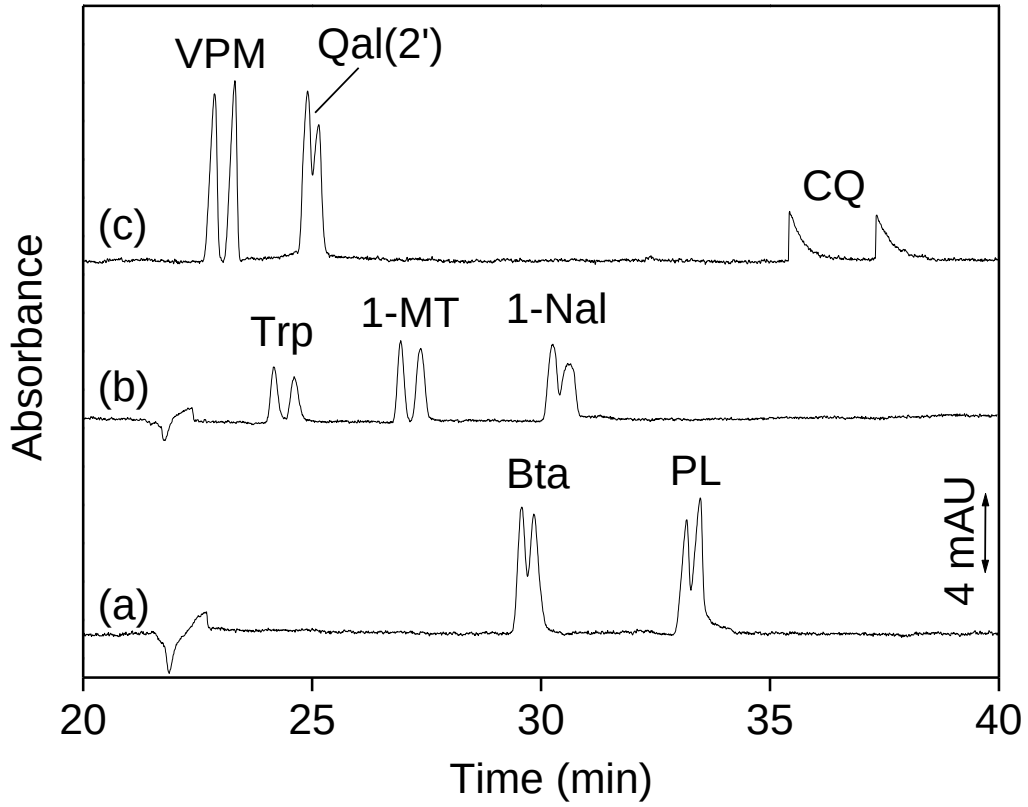
Figure S4 Chiral resolution using **poly-dC**



Experimental conditions:

[**poly-dC**]	**poly-dC** injection time	T	Racemate sequential injection
1.60 mM in 3 time diluted BGE	4.5 min	10°C	**1/** VPM 0.87 g/L (5 kV, 8 s) **2/** Bta 0.21 g/L + ACN 5% v/v (10 kV, 20 s) **3/** CQ 0.96 g/L (10 kV, 10 s)

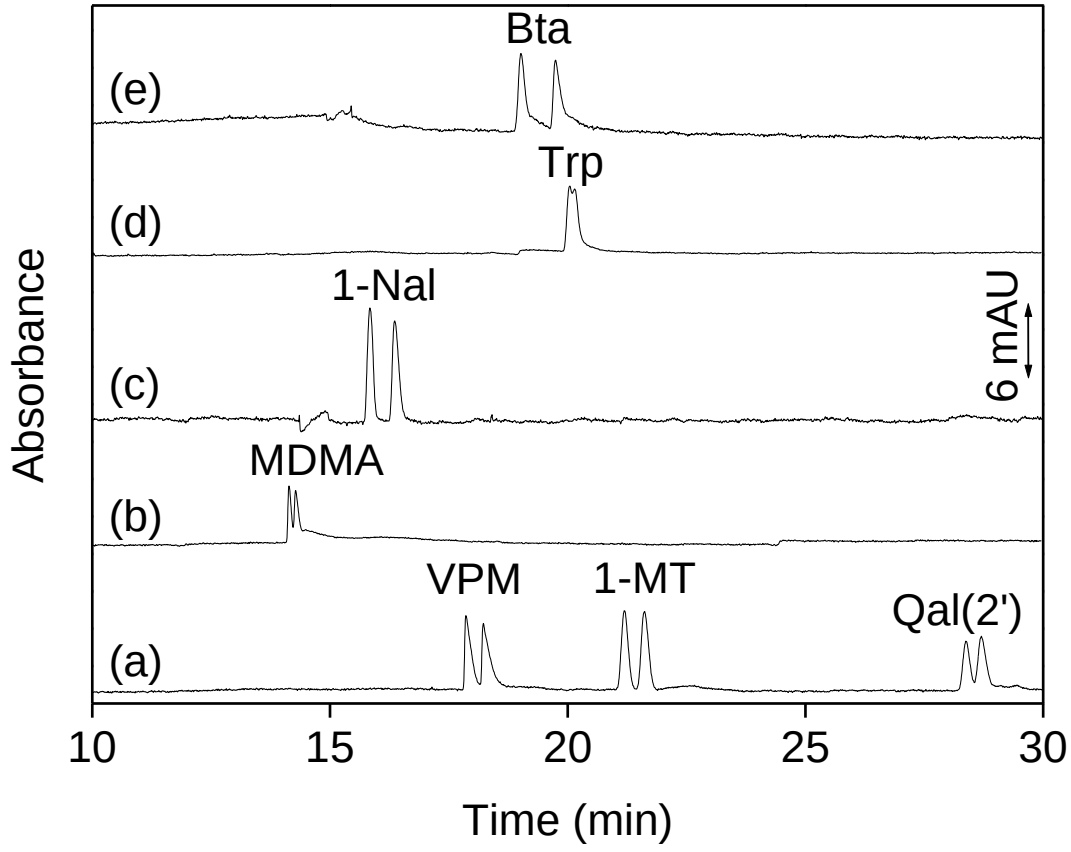
Figure S5 Chiral resolution using **poly-dT**



Experimental conditions:

Trace	[**poly-dT**]	**poly-dT** injection time	T	Racemate sequential injection	UV detection
(a)	1.60 mM in 3.0 time diluted BGE	4.5 min	10°C	**1/** Bta 0.20 g/L + ACN 5% v/v (10 kV, 20 s) **2/** Propranolol 0.50 g/L (10 kV, 5 s)	220 nm
(b)	1.60 mM in 2.9 time diluted BGE	4.5 min	10°C	**1/** Trp 0.19 g/L (10 kV, 12 s) **2/** BGE (30 kV, 120 s) **3/** 1-MT 0.16 g/L (10 kV, 15 s) **4/** BGE (30 kV, 48 s) **5/** 1-Nal 0.14 g/L + ACN 5% v/v (10 kV, 20 s)	230 nm
(c)	1.60 mM in 2.9 time diluted BGE	4.5 min	10°C	**1/** VPM 0.95 g/L (10 kV, 8 s) **2/** Qal(2') 0.17 g/L (10 kV, 15 s) **3/** CQ 0.99 g/L (10 kV, 5 s)	220 nm

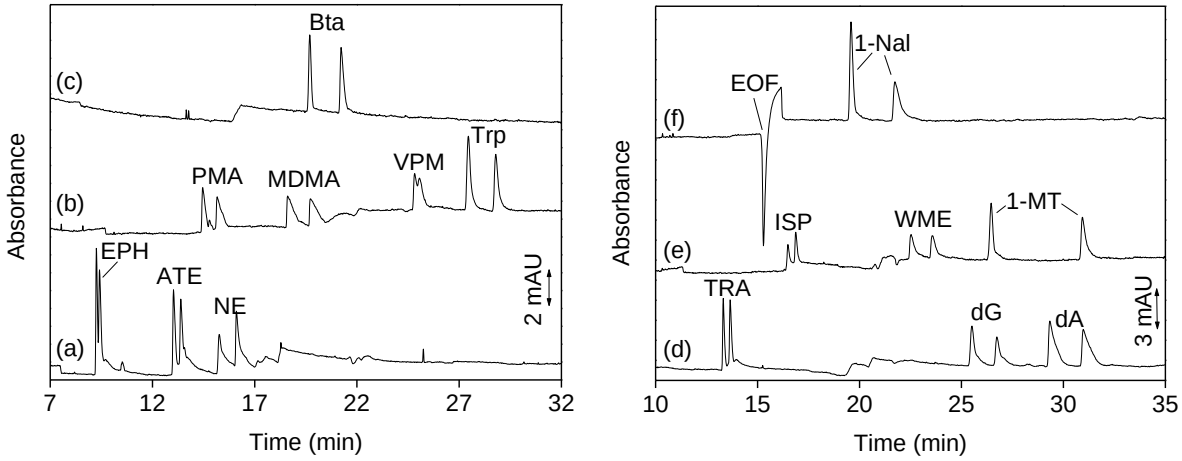
Figure S6 Chiral resolution using **dsDNA$_{-30}$**



Experimental conditions:

Trace	[**dsDNA$_{-30}$**]	**dsDNA$_{-30}$** injection time	T	Racemate sequential injection	UV detection
(a)	0.85 mM in 2.9 time diluted BGE	4.5 min	15°C	**1/** 1-MT 0.16 g/L (15 kV, 15 s) **2/** BGE (30 kV, 180 s) **3/** VPM 0.87 g/L (10 kV, 10 s) **4/** Qal(2') 0.18 g/L (10 kV, 10 s)	230 nm
(b)	0.85 mM in 2.9 time diluted BGE	5.5 min	10°C	MDMA 0.28 g/L (10 kV, 10 s)	220 nm
(c)	0.21 mM in 1.2 time diluted BGE	3.5 min	30°C	1-Nal 0.08 g/L + ACN 5% v/v (10 kV, 20 s)	220 nm
(d)	0.85 mM in 3.0 time diluted BGE	3.5 min	30°C	Trp 0.19 g/L (50 mbar, 20 s)	220 nm
(e)	0.85 mM in 3.0 time diluted BGE	4.0 min	20°C	Bta 0.20 g/L + ACN 5% v/v (10 kV, 20 s)	220 nm

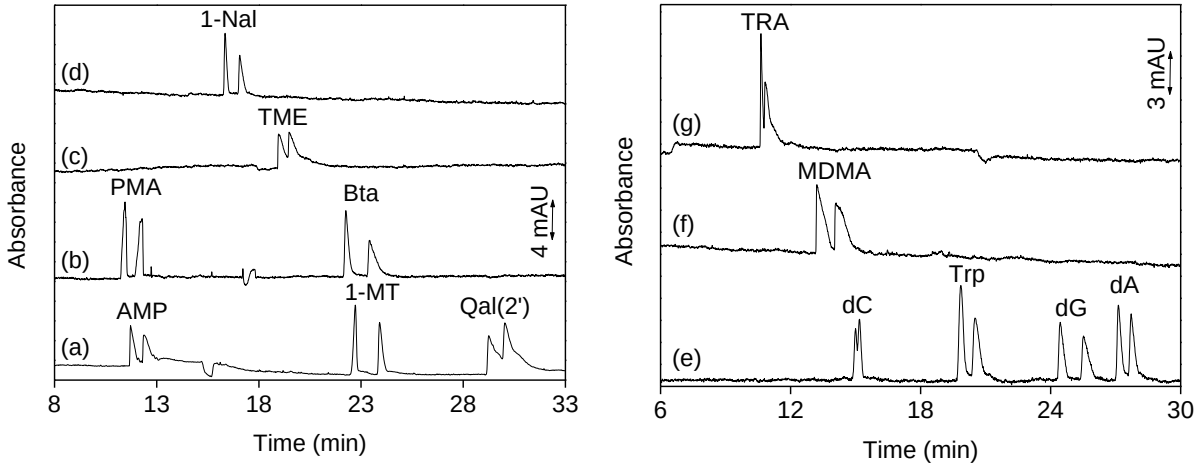
Figure S7 Chiral resolution using **hpDNA$_{-31}$**



Experimental conditions:

Trace	[**hpDNA$_{-31}$**]	**hpDNA$_{-31}$** injection time	T	Racemate sequential injection	UV detection
(a)	1.80 mM in 2.9 time diluted BGE	5.0 min	10°C	**1**/ EPH 0.69 g/L (15 kV, 10 s) **2**/ ATE 0.51 g/L (15 kV, 10 s) **3**/ BGE (30 kV, 240 s) **4**/ NE 0.55 g/L (10 kV, 10 s)	210 nm
(b)	1.80 mM in 2.9 time diluted BGE	5.0 min	10°C	**1**/ PMA 0.25 g/L (10 kV, 15 s) **2**/ MDMA 0.28 g/L (20 kV, 15 s) **3**/ VPM 0.22 g/L (20 kV, 15 s) **4**/ BGE (30 kV, 180 s) **5**/ Trp 0.32 g/L (10 kV, 10 s)	220 nm
(c)	0.90 mM in 1.5 time diluted BGE	4.0 min	25°C	Bta 0.20 g/L + ACN 5% v/v (20 kV, 10 s)	220 nm
(d)	1.80 mM in 2.9 time diluted BGE	5.0 min	10°C	**1**/ TRA 0.54 g/L (10 kV, 10 s) **2**/ dG 1.00 g/L (10 kV, 10 s) **3**/ BGE (30 kV, 120 s) **4**/ dA 1.00 g/L (10 kV, 10 s)	220 nm
(e)	1.80 mM in 2.9 time diluted BGE	5.0 min	10°C	**1**/ ISP 0.74 g/L (10 kV, 10 s) **2**/ WME 0.43 g/L (10 kV, 10 s) **3**/ 1-MT 0.20 g/L (10 kV, 15 s)	230 nm
(f)	0.90 mM in 1.5 time diluted BGE	3.0 min	35°C	1-Nal 0.13 g/L + ACN 5% v/v (15 kV, 10 s)	220 nm

Figure S8 Chiral resolution using **$_3$WJDNA$_{-36}$**

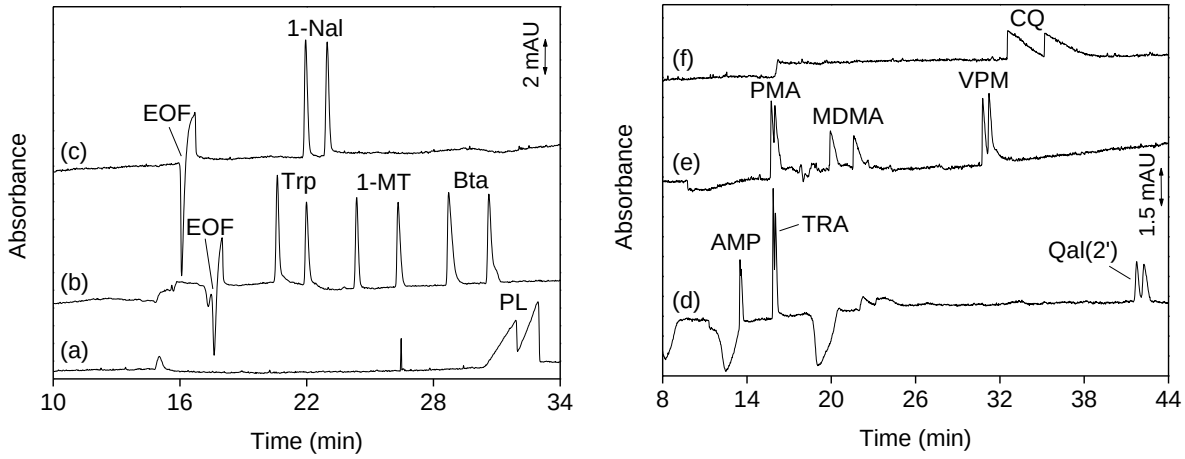


Experimental conditions:

Trace	[**$_3$WJDNA$_{-36}$**]	**$_3$WJDNA$_{-36}$** injection time	T	Racemate sequential injection	UV detection
(a)	0.95 mM in 2.9 time diluted BGE	4.5 min	15°C	**1/** AMP 0.36 g/L (20 kV, 15 s) **2/** 1-MT 0.16 g/L (20 kV, 10 s) **3/** Qal(2') 0.17 g/L (20 kV, 10 s)	210 nm
(b)	0.97 mM in 2.9 time diluted BGE	4.5 min	15°C	**1/** BGE (30 kV, 480 s) **2/** PMA 0.25 g/L (20 kV, 20 s) **3/** BGE (30 kV, 120 s) **4/** Bta 0.20 g/L + ACN 5% v/v (20 kV, 20 s)	210 nm
(c)	0.85 mM in 2.0 time diluted BGE1[a]	5.0 min	10°C	TME 0.48 g/L (10 kV, 18 s)	210 nm
(d)	0.15 mM in 1.1 time diluted BGE	4.0 min	25°C	1-Nal 0.07 g/L + ACN 5% v/v (20 kV, 10 s)	210 nm
(e)	0.97 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** dC 0.30 g/L (10 kV, 15 s) **2/** BGE (30 kV, 120 s) **3/** Trp 0.64 g/L (20 kV, 15 s) **4/** BGE (30 kV, 180 s) **5/** dG 1.00 g/L (20 kV, 10 s) **6/** BGE (30 kV, 180 s) **7/** dA 0.97 g/L (10 kV, 8 s)	254 nm
(f)	0.97 mM in 2.9 time diluted BGE	4.0 min	30°C	**1/** BGE (30 kV, 600 s) **2/** MDMA 0.28 g/L (20 kV, 20 s)	210 nm
(g)	0.15 mM in 2.9 time diluted BGE[b]	3.5 min	45°C	TRA 0.47 g/L (10 kV, 10 s)	210 nm

[a]BGE1 is constituted by NaCl/Tris/HCl 80/40/32 mM (pH 7.5). [b]10% (v/v) of EtOH was added to the BGE and to the DNA sample.

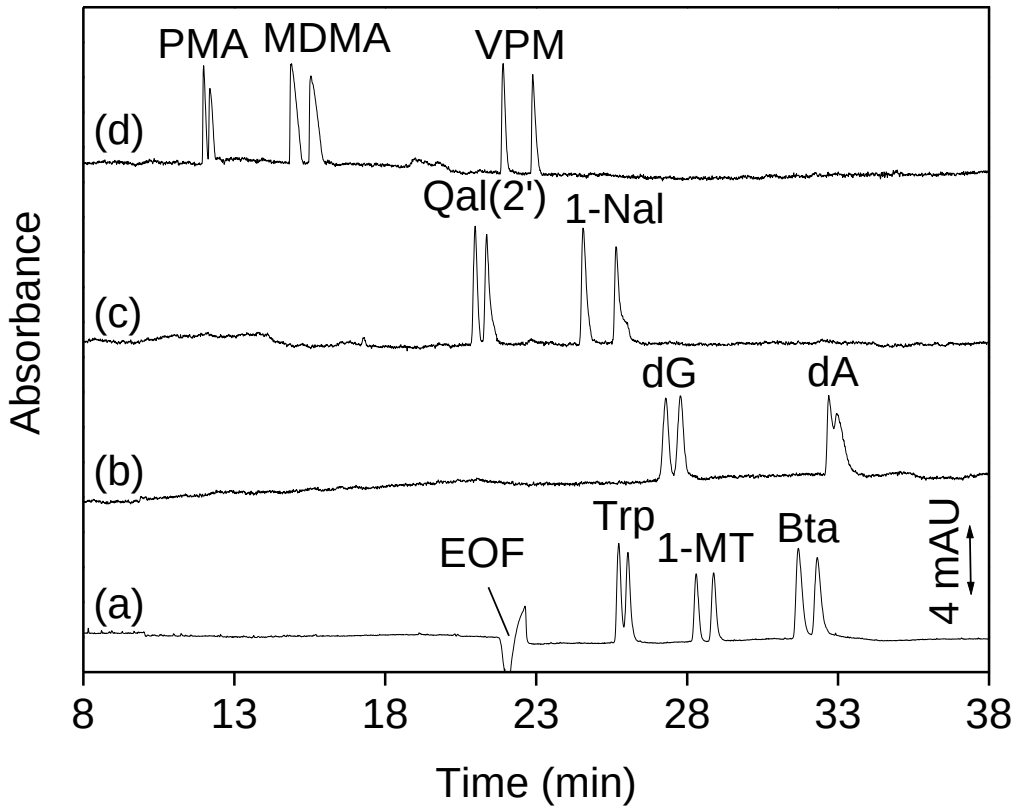
Figure S9 Chiral resolution using **hpDNA**$_{-24}$



Experimental conditions:

Trace	[**hpDNA**$_{-24}$]	**hpDNA**$_{-24}$ injection time	T	Racemate sequential injection	UV detection
(a)	1.23 mM in 1.5 time diluted BGE	4.0 min	25°C	PL 0.6 g/L (10 kV, 10 s)	220 nm
(b)	2.46 mM in 2.9 time diluted BGE	4.5 min	15°C	**1/** Trp 0.32 g/L (10 kV, 8 s) **2/** BGE (30 kV, 120 s) **3/** 1-MT 0.20 g/L (10 kV, 15 s) **4/** Bta 0.20 g/L + ACN 5% v/v (20 kV, 10 s)	220 nm
(c)	1.23 mM in 1.5 time diluted BGE	4.0 min	25°C	1-Nal 0.13 g/L + ACN 5% v/v (15 kV, 10 s)	220 nm
(d)	2.46 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** AMP 0.34 g/L (10 kV, 15 s) **2/** TRA 0.58 g/L (10 kV, 10 s) **3/** Qal(2') 0.18 g/L (10 kV, 10 s)	220 nm
(e)	2.46 mM in 3.0 time diluted BGE	5.0 min	10°C	**1/** PMA 0.25 g/L (10 kV, 15 s) **2/** MDMA 0.28 g/L (20 kV, 15 s) **3/** VPM 0.22 g/L (20 kV, 15 s)	230 nm
(f)	0.61 mM in 1.2 time diluted BGE	4.0 min	20°C	CQ 0.86 g/L (10 kV, 10 s)	230 nm

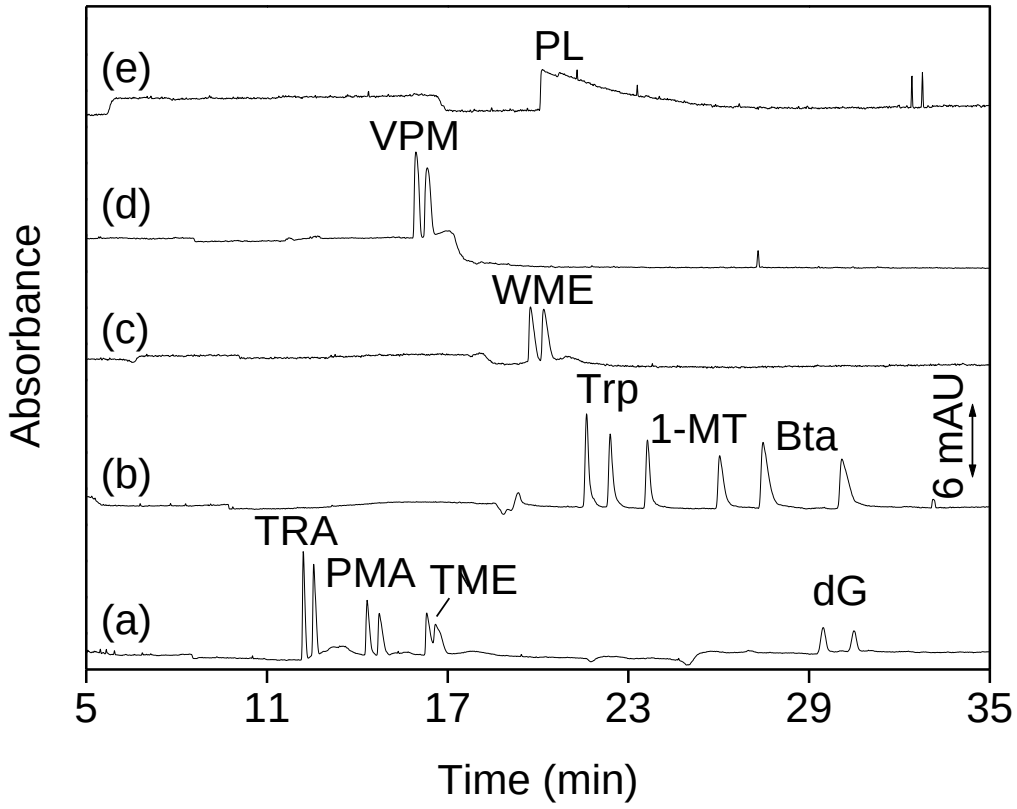
Figure S10 Chiral resolution using **hpDNA**$_{-14}$



Experimental conditions:

Trace	[**hpDNA**$_{-14}$]	**hpDNA**$_{-14}$ injection time	T	Racemate sequential injection	UV detection
(a)	2.51 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** Trp 0.32 g/L (10 kV, 8 s) **2/** 1-MT 0.16 g/L (10 kV, 15 s) **3/** Bta 0.20 g/L + ACN 5% v/v (20 kV, 10 s)	220 nm
(b)	4.99 mM in 4.0 time diluted BGE	5.0 min	10°C	**1/** dG 0.97 g/L (10 kV, 10 s) **2/** BGE (30 kV, 120 s) **3/** dA 0.97 g/L (10 kV, 10 s)	254 nm
(c)	2.51 mM in 2.9 time diluted BGE	4.0 min	20°C	**1/** Qal(2') 0.17 g/L (10 kV, 10 s) **2/** BGE (30 kV, 120 s) **3/** 1-Nal 0.12 g/L + ACN 5% v/v (15 kV, 10 s)	220 nm
(d)	2.51 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** PMA 0.25 g/L (10 kV, 15 s) **2/** MDMA 0.28 g/L (20 kV, 10 s) **3/** VPM 0.19 g/L (20 kV, 15 s)	220 nm

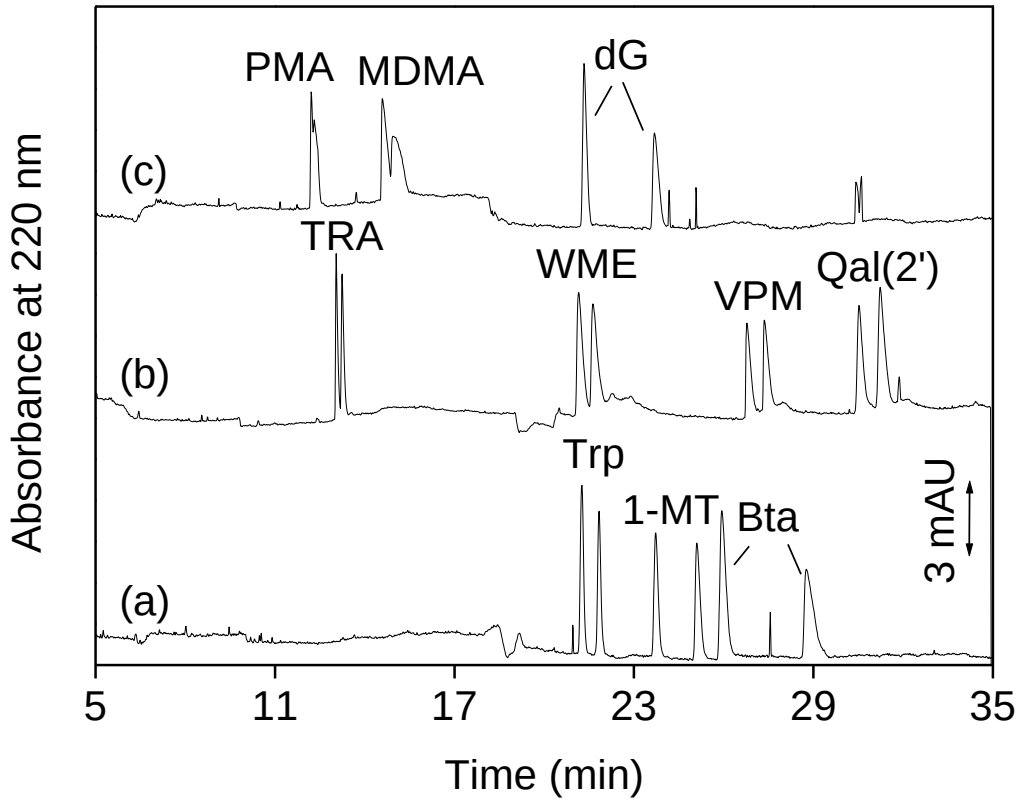
Figure S11 Chiral resolution using **mxDNA$_{-40}$**



Experimental conditions:

Trace	[**mxDNA$_{-40}$**]	**mxDNA$_{-40}$** injection time	T	Racemate sequential injection	UV detection
(a)	1.08 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** TRA 0.58 g/L (10 kV, 10 s) **2/** BGE (30 kV, 150 s) **3/** PMA 0.25 g/L (10 kV, 15 s) **4/** TME 0.50 g/L (10 kV, 20 s) **5/** dG 1.06 g/L (10 kV, 10 s)	220 nm
(b)	1.08 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** Trp 0.32 g/L (10 kV, 8 s) **2/** 1-MT 0.20 g/L (10 kV, 15 s) **3/** Bta 0.20 g/L + ACN 5% v/v (20 kV, 10 s)	220 nm
(c)	1.08 mM in 2.9 time diluted BGE	5.0 min	10°C	WME 0.51 g/L (10 kV, 8 s)	210 nm
(d)	0.54 mM in 1.5 time diluted BGE	5.0 min	10°C	VPM 0.22 g/L (20 kV, 15 s)	220 nm
(e)	0.27 mM in 1.2 time diluted BGE	4.0 min	20°C	PL 0.60 g/L (20 kV, 10 s)	210 nm

Figure S12 Chiral resolution using **tbDNA**$_{-38}$



Experimental conditions:

Trace	[**tbDNA**$_{-38}$]	**tbDNA**$_{-38}$ injection time	T	Racemate sequential injection
(a)	1.09 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** Trp 0.32 g/L (10 kV, 8 s) **2/** 1-MT 0.20 g/L (10 kV, 15 s) **3/** Bta 0.20 g/L + ACN 5% v/v (20 kV, 10 s)
(b)	1.10 mM in 2.8 time diluted BGE	5.0 min	10°C	**1/** TRA 0.54 g/L (10 kV, 10 s) **2/** WME 0.45 g/L (10 kV, 10 s) **3/** VPM 0.22 g/L (20 kV, 15 s) **4/** Qal(2') 0.18 g/L (15 kV, 10 s)
(c)	1.09 mM in 2.9 time diluted BGE	5.0 min	10°C	**1/** PMA 0.25 g/L (10 kV, 15 s) **2/** MDMA 0.28 g/L (20 kV, 15 s) **3/** dG 0.99 g/L (10 kV, 10 s)

Figure S13 Mirror-image strategy. A non-racemic mixture containing an excess of L-enantiomer (L/D molar ratio: 2/1) was injected to clearly show the reversed migration order of enantiomers. Experimental conditions: [**hpDNA**$_{-14}$] and [**L-hpDNA**$_{-14}$] at ~2.1 mM. T = 10°C. DNA injection volume 230 nL. UV detection at 230 nm. Sample: (L) 1-MT 0.1 g/L + (D) 1-MT 0.05 g/L. To note, the lower migration time and resolution observed with **L-hpDNA-14** was attributed to its lower purity.

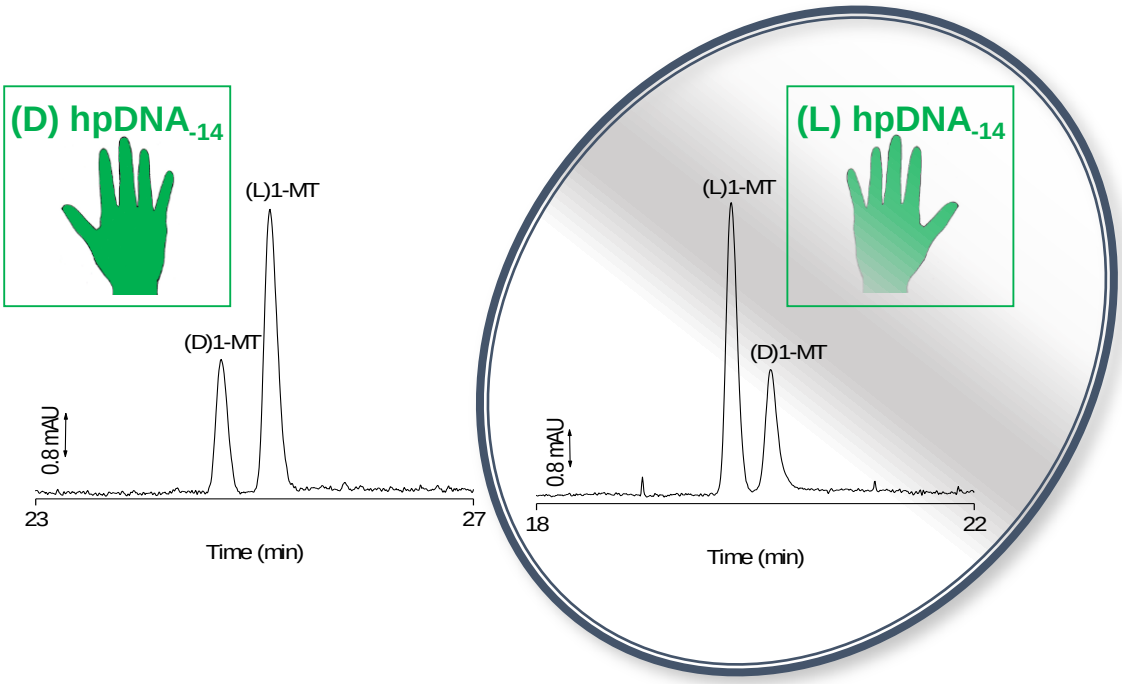
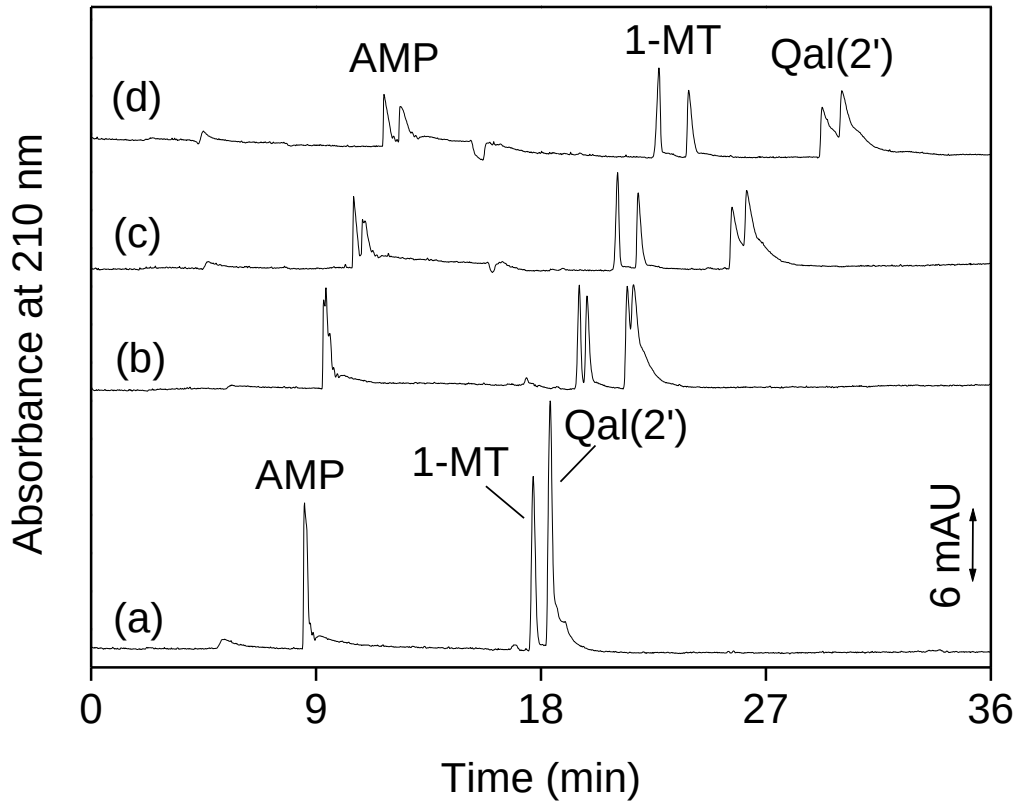


Figure S14 Effect of the **$_3$WJDNA$_{-36}$** concentration on the resolution



Experimental conditions:

Trace	[$_3$WJDNA$_{-36}$]	$_3$WJDNA$_{-36}$ injection time	T	Racemate sequential injection
(a)	0.06 mM in BGE	4.5 min	15°C	**1/** AMP 0.36 g/L (20 kV, 15 s) **2/** 1-MT 0.16 g/L (20 kV, 10 s) **3/** Qal(2') 0.17 g/L (20 kV, 10 s)
(b)	0.24 mM in 1.2 time diluted BGE			
(c)	0.48 mM in 1.5 time diluted BGE			
(d)	0.95 mM in 2.9 time diluted BGE			