5. Materials

5.1 Laboratory equipment and disposables

Adhesive PCR film, Cat.#: AB-0558	ABgene, UK
Agaros-electrophoresis Grade, ultra pure, Cat. No. 15510-019	Invitrogen, USA
Agilent 2100 Bioanalyser	Agilent Technologies, USA
Bio-Photometer	Eppendorf, Germany
CCD camera of type CH350	Photometrix Ltd., UK
cuvette, centre Height 8.5mm, cat. No. 95201 005_1	Eppendorf, Germany
Disposable cuvettes (UVette™)	Eppendorf, Germany
Filters	Andover Corporation, USA
Gel documentation system (EASY™ Win.32)	Herolab, Germany
Gel electrophoresis equipment	Amersham Pharmacia Bioscience, UK
Gel electrophoresis equipment	Invitrogen, USA
Gel loader tips	Eppendorf, Germany
Gloves	Ansell Healthcare Products, USA
Hettich Zentrifugen, Universal 30RF	Germany
Microscope slide (76*26mm)	Menzel, Germany
Microtiter plates	Genetix, UK
Microwave, AEG	Germany
PCR thermocycler (PTC-100)	MJ Research, USA
PCR 384 well plate (Thermo-Fast™)	Advanced Biotechnologies, UK
pH meter Radiometer	Copenhagen, Denmark
Photostrip, double face, 10m/15mm, teas	Beiersdorf, Hamburg, Germany
Pipettes (Pipetman™)	Gilson, France
Precision balance	Mettler, Germany
Quartz cuvettes, Qs 10 mm	Hellma, Germany
Reaction tubes and caps (MicroAmp™)	Applied Biosystems, USA
sciFLEXARRAYER	Scienion, Germany
Table centrifuge (5415 C)	Eppendorf, Germany
Table centrifuge (Biofuge 13)	Heraeus Instruments, Germany
Thermal cycler	Eppendorf, Germany

Thermo-controllable spectrophotometer	Avantes Fiberoptic, The Netherlands
TopSpot/E Micro-Arrayer module	IMTEK, Germany
Tube-strip Picofuge	Stratagene, USA
UTHSCASA Image Tool v3.00	University of Texas Health Science Center, TX, USA)
UV crosslinker (Stratalinker™ 2400)	Stratagene, USA
Vortex (Vortex Genie 2)	Scientific Industries, USA
Varian UV-Vis-NIR Spectrometers	Varian, USA
Vivid™ Gene Array Slides (PN511)	Pall, USA
Water purification system (Milli-Q™)	Millipore, USA

5.2 Chemicals

Acetic acid (p.a.)	Merck, Germany
Casein Hammerten ultrapure	USB Corporation, USA
DEPC-treated water, 9915G	Ambion, USA
Ethanol (p.a.)	Merck, Germany
Ethanol (100%)	Menzel, Germany
Ethidium bromide (1%)	Merck, Germany
Ethylendiamine tetraacetic acid (EDTA)	Merck, Germany
Mineral oil	Sigma, Germany
PlusOne repel-Silane ES	Amersham Biosciences, Sweden
Polyoxyethylenesorbitan monolaurate (Tween 20)	Sigma, USA
Sodium chloride	Merck, Germany
Sodium hydratide	Merck, Germany
Sodium hydrogen phosphate dihydrate	Merck, Germany
Sybr Green I	Molecular Probes, OR, USA

5.3 Kits

MEGAScript T7	Ambion, USA
RNeasy Mini Kit (50)	Qiagen, Germany
QIAquick™ PCR Purification Kit	Qiagen, Germany
Agilent RNA 6000 Nano Kit	Agilent Technologies, USA

5.4 Enzymes

Taq DNA polymerase	Roche, Germany
T7 Gene 6 Exonuclease	USB Corporation, USA
DNase I	Ambion, USA

5.5 Molecular weight markers

GeneRuler^{TM} DNA ladder Mix	Fermentas, USA

5.6 Buffers and solutions

10% SDS (v/w)	10 g SDS 100 ml H_2O
Taq DNA polymerase Reaction buffer (2x1 ml)	Invitrogen, USA
Gel loading buffer (6x)	Bromophenol blue 0,25% (w/v) Xylenecyanol FF 0,25% (w/v) Glycerol 30% (v/v)
SSC (1x) (pH 7,0)	NaCl 150 mM Na-citrate 15 mM
TAE (1x) (pH 8,0)	Tris-Acetate 40 mM EDTA 1 mM
PCR buffer (10x) (pH 8,3)	Tris-HCl 500 mM KCl 500 mM MgCl2 15 mM Tween-20 1% (v/v)
Blocking buffer	10 mM Tris pH8.0 150 mM NaCl 1% SDS 3% (w/v) Casein Hammersten

20X SSC (pH7.5)	3 M NaCl 300 mM Na-Citrate
buffer-2	20 mM Tris pH7.0 50 mM NaCl 0,1% Tween-20
Hybridisation buffer	50 mM Na_2PO4 pH7.0 10% BSA 0,2% Tween-20
Stripping buffer	1X SSC pH7.5 0.1% SDS dissolved in buffer-2
5X RNA fragmentation buffer	200 mM Tris acetatem pH8,1 (Trizma base, pH adjusted with glacial acetic acid) 150 mM MgOAc 500 mM KOAc

5.7 Primers

5.7.1 Primer pair for normal PCR amplification

M13-Forward	5'-CGC CAG GGT TTT CCC AGT CAC GAC-3'
M13-Reverse	5'-TTT CAC ACA GGA AAC AGC TAT GAC-3'

5.7.2 Primer pair for in vitro RNA transcription

M13-Forward-T7	5'-GTA ATA CGA CTC ACT ATA GGG TTT TCC CAG TCA CGA-3'
M13-Reverse-T7	5'-GTA ATA CGA CTC ACT ATA GGG CAC AGG AAA CAG CTA TGA-3'

5.7.3 Primer pair for single-stranded DNA preparation

M13-Forward-S	5'-C*G*C* C*A*G GGT TTT CCC AGT CAC GAC-3'
M13-Reverse-S	5'-T*T*T* C*A*C ACA GGA AAC AGC TAT GAC-3'

(The bases indicated with * are phosphorothioated. And the letter S represents phosphorothioation)

5.8 Non-modified and LNA-modified oligoprobes

For Table 1 and Table 2, the capital letters in the sequences denote the LNA-modified bases. Letter A- E and L indicate LNA.

Table 1 Characteristics of 7-mer LNA-modified oligoprobes used in this study

Oligo ID	Sequence	GC-content (%)	Matching rate (%)	Tm (^{o}C)
OP-01L	5'-Cy5-tcAgaAg-3'	43	37.9	6
OP-02A	5'-Cy5-cTgaagc-3'	57	18.2	7
OP-02L/02B	5'-Cy5-CtgaaGc-3'	57	18.2	7
OP-02C	5'-Cy5-cTGaaGc-3'	57	18.2	19
OP-02D	5'-Cy5-cTGaAGc-3'	57	18.2	23
OP-02E	5'-Cy5-cTGAAGc-3'	57	18.2	28
OP-03L	5'-Cy5-aTgAgGa-3'	43	19.7	13
OP-04L	5'-Cy5-CtgaaGg-3'	57	37.9	9
OP-05L	5'-Cy5-TgcTgGg-3'	71	45.5	25
OP-06L	5'-Cy5-tTcctcc-3'	57	60.6	1
OP-07L	5'-Cy5-caggaCa-3'	57	16.7	8
OP-08L	5'-Cy5-TccTgct-3'	57	16.7	6
OP-09L	5'-Cy5-aAgTgct-3'	43	18.2	10
OP-10L	5'-Cy5-tCacTgt-3'	43	18.2	8
OP-11L	5'-Cy5-cacTgca-3'	57	28.8	10
OP-12L	5'-Cy5-tcCtgGa-3'	57	37.9	9
OP-13L	5'-Cy5-cctccTg-3'	71	42.4	9
OP-14L	5'-Cy5-agcTgag-3'	57	10.6	7
OP-15L	5'-Cy5-cTcctCc-3'	57	15.2	12
OP-16L	5'-Cy5-cagccTc-3'	71	9.1	10
OP-17L	5'-Cy5-tGCtgGt-3'	57	18.2	16
OP-18L	5'-Cy5-cTgCcct-3'	86	30.3	19
OP-19L	5'-Cy5-ttGccAa-3'	43	28.8	9
OP-20L	5'-Cy5-ttcTctg-3'	43	37.9	-1
OP-21L	5'-Cy5-agTccTc-3'	57	4.5	7
OP-22L	5'-Cy5-aaTgaGg-3'	43	15.2	11
OP-23L	5'-Cy5-aGcTcag-3'	57	18.2	10
OP-24L	5'-Cy5-gTcTgga-3'	57	12.1	10
OP-25L	5'-Cy5-gTcTggc-3'	71	13.6	17
OP-26L	5'-Cy5-TgagcTg-3'	57	28.8	9

Table 2. The 6-mer and 5-mer LNA-modified oligoprobes used in this study

Name	Sequence	GC-content (%)	Tm (^{o}C)
OP-01L-6nt	5'-Cy5-cAgaAg-3'	50	-2
OP-04L-6nt	5'-Cy5-ctGAag-3'	50	-2
OP-06L-6nt	5'-Cy5-Tcctcc-3'	67	-5
OP-17L-6nt	5'-Cy5-tgCTgg-3'	67	9
OP-17L-5nt	5'-Cy5-tgCTg-3'	60	unpredictable
OP-19L-6nt	5'-Cy5-tGccAa-3'	50	0

Table 3. The non-modified DNA oligoprobes used in this study

Name	Sequence	GC-content (%)	Tm (^{o}C)
OP-01	5'-Cy5-tcagaag-3'	43	-5
OP-02	5'-Cy5-ctgaagc-3'	57	3
OP-03	5'-Cy5-atgagga-3'	43	-7
OP-06-6nt	5'-Cy5-tcctcc-3'	67	-13
OP-13	5'-Cy5-cctcctg-3'	71	4
OP-13-8nt	5'-Cy5-tcctcctg-3'	63	10
OP-15	5'-Cy5-ctcctcc-3'	71	3
OP-17	5'-Cy5-tgctggt-3'	57	3
OP-19	5'-Cy5-ttgccaa-3'	43	0
OP-27	5'-Cy5-tcctcct-3'	57	-3
OP-28	5'-Cy5-cctgccc-3'	86	12
OP-29	5'-Cy5-gctcgtt-3'	57	6
OP-30	5'-Cy5-gctcctg-3'	71	7
OP-31	5'-Cy5-ttctctg-3'	43	-5
OP-32	5'-Rox-ctccaag-3'	57	0
OP-33	5'-Bodipy-agccgattg-3'	56	21

5.9 Synthetic single-stranded target DNAs and dsDNA clones

Table 4. The sequences of the synthetic single-stranded DNA targets

Name	Sequence
DNA-1	5'- TTTGCAGGAAGAGTCCCAATCGGCTTT –3'
DNA-2	5'- TTTGCAGGAGGAGTCCCAATCGGCTTT –3'
DNA-3	5'-TAGTTCTTGGAGAAGG–3'
DNA-4	5'- TCCTCATTCCAGGAACACAGCAGC–3'
DNA-5	5'- TTCACCAGCAAGGCTCCTCATTTGC–3'
DNA-6	5'- TTTGGCTGCGGCACAGTGACAGGAGCA–3'
DNA-7	5'-CCCAAATAACATTCACCAGCATTGGAGGAGGTT–3'
DNA-8	5'-TGCTTTGTCACACCAGCCACTCACACTGGGGAAG–3'
DNA-9	5'-CACCAGCATCCTCATTGGGAGAGACAGAGAAA–3'
DNA-10	5'-CAGGGCAGGTTTGCTTCAGTTT–3'

Table 5. Characteristics of dsDNA sequences matching the human chromosome Xq28 cosmid clone (GeneBank accession number: AL034384) (Continued on the next page)

Name of the clone	Length	Matching range
A06	1405	106930-108334
A11	1275	107016-108290
C03	1050	106948-107997
C14	1425	119158-120528
D11	1604	133440-135043
D18	976	108393-109368
D20	1094	100248-101341
E04	1287	110703-111989
E08	1628	136037-137664
E11	1828	130619-132046
E14	1630	133384-135013
E24	1151	129244-130394
F05	1243	127628-128870
F12	1507	95346-96852

Table 5. Characteristics of dsDNA sequences matching the human chromosome Xq28 cosmid clone (GeneBank accession number: AL034384) (Continued on the next page)

Name of the clone	Length	Matching range
F13	1431	133133-134563
F17	1116	95418-96533
F18	1142	95073-96214
G09	1644	115299-116942
G11	1001	131072-132072
G20	1476	101508-102983
G24	1448	108495-109942
H02	640	95575-96214
H04	755	104914-105668
H07	1507	119664-121170
H08	1181	95375-96555
H20	1263	100143-101405
H21	1541	130527-132067
H24	1218	101473-102690
I02	1665	130617-132281
I09	1344	130867-132210
I10	1642	108563-110204
I17	1719	130331-132049
I21	1221	101504-102725
J06	1098	98987-100084
J10	1146	95522-96667
J19	1377	119514-120890
J20	1202	99112-100313
J22	1338	108542-109879
K03	1237	106967-108203
K04	1630	107029-108658
K09	1661	130350-132010
K18	1438	107048-108485
L03	1399	99818-101216
L09	1394	121785-123178
L11	1353	132887-134239

Table 5. Characteristics of dsDNA sequences matching the human chromosome Xq28 cosmid clone (GeneBank accession number: AL034384) (Continued)

Name of the clone	Length	Matching range
L23	1269	101213-102481
L24	288	101070-101357
M04	1348	100958-102305
M05	861	135020-135880
M09	1415	95882-97296
M10	1359	119436-120794
M19	1244	98687-99930
M23	1303	103096-104398
N02	881	89182-90062
N05	1259	101189-102447
N06	1216	100005-101220
N07	1196	119102-120297
N21	1648	133843-135490
O12	1323	133510-134832
O18	1446	125337-126782
O19	1320	122143-123462
O20	1237	130879-132115
O23	1068	95768-96835
P06	1088	100850-101936
P07	1477	122011-123487
P18	1126	106967-108092