

## 5. Materials

### 5.1 Laboratory equipment

adhesive sealing sheets	Advanced Biotechnologies, UK
autoclave	Getinge, Sweden
CAST™ slides	Schleicher & Schuell Bioscience, USA
cover slips	Engelbrecht, Germany
cryo tubes (Cellstar™)	Greiner bio-one, Germany
dessicator	Carl Roth, Germany
disposable cuvettes (UVette™)	Eppendorf, Germany
DNA microarray scanner (GenePix™ 4000B)	Axon Instruments, USA
DNA microarrayer (SDDC-3)	Virtek Vision, Canada
EMI shielding tape (# 1182)	3M, USA
FAST™ slides	Schleicher & Schuell Bioscience, USA
gel documentation system (EASY™ Win.32)	Herolab, Germany
gel electrophoresis equipment	Invitrogen, USA,
gel electrophoresis equipment	Amersham Pharmacia Bioscience, UK
gel loader tips	Eppendorf, Germany
gloves	Ansell Healthcare Products, USA
hybridization chambers	workshop MPIMG, Germany
incubator	Heraeus Instruments, Germany
incubator shaker	New Brunswick Scientific, Germany
light-optical microscope	Leica, Germany
magnetic stirrer and heater	Heidolph, Germany
MALDI target (AnchorChip™ 400/384)	Bruker Daltonik, Germany
MALDI target (Scout™ 384 MTP)	Bruker Daltonik, Germany
MALDI-TOF mass spectrometer (Autoflex™)	Bruker Daltonik, Germany
MALDI-TOF mass spectrometer (Biflex III™)	Bruker Daltonik, Germany
media lab bottles	Wheaton Science, USA
metal coated glass slides	MAT Dresden, Germany
microarray adapter, Bruker compatible	Rosemann & Sohn, Germany
microtiter plates	Genetix, UK
microwave	AEG, Germany
multi channel pipettes	Eppendorf, Germany
nylon membrane (Hybond N <sup>+</sup> ™, 0,45 µm)	Amersham Pharmacia Bioscience, UK
Parafilm™	American National Can, USA

PCR 96 well plate (Thermo-Fast™)	Advanced Biotechnologies, UK
PCR thermocycler (PTC-100)	MJ Research, USA
pH meter	Radiometer Copenhagen, Denmark
pipettes (Pipetman™)	Gilson, France
power supplies	workshop MPIMG, Germany
precision balance	Mettler, Germany
QMT™ Aldehyde slides	Quantifoil, Germany
quartz glas cuvettes	Hellma, Germany
reaction tubes and caps (MicroAmp™)	Applied Biosystems, USA
replicators, 384-/96-pin	Genetix, UK
shaker (Rocky™)	Fröbel Labortechnik, Germany
silicon wafers, thermically oxidized	Fraunhofer ISIT, Germany
speed vac system incl. vapor trap	Thermo Savant, USA
sterile filtering units (Nalgene™)	Nalge Nunc International, USA
table centrifuge (5415 C)	Eppendorf, Germany
table centrifuge (Biofuge 13)	Heraeus Instruments, Germany
test tubes PP (Cellstar™)	Greiner bio-one, Germany
thermomixer	Eppendorf, Germany
ultrasonic cleaner (Branson)	Branson Ultrasonics, USA
UV crosslinker (Stratalinker™ 2400)	Stratagene, USA
UV spectrophotometer (Biochrome 4060)	Amersham Pharmacia Bioscience, UK
UV spectrophotometer (UV-1602)	Shimadzu, Japan
vortex (Vortex Genie 2)	Scientific Industries, USA
water purification system (Milli-Q™)	Millipore, USA
Whatman™ paper	Whatman, USA
wrap (Saran™)	Dow Chemical, USA
XNA on Gold™ biochip	Thermohybrid, Germany

## 5.2 Chemicals

1-methyl-2-pyrrolidone	Aldrich, USA
6-trimethylammoniumhexyryl-	
N-hydroxy-succinimidylester	MPIMG self-synthesis
acetic acid (p.a.)	Merck, Germany
acetone (p.a.)	Merck, Germany
acetonitrile (p.a.)	Merck, Germany

acrylamide/bisacrylamide (0,8%)	Carl Roth, Germany
agarose - electrophoresis grade	Invitrogen, USA
$\alpha$ -cyano-4-hydroxy-cinnamic acid	Sigma, USA
$\alpha$ -cyano-4-hydroxy-cinnamic methyl ester	Bruker Daltonik, Germany
ammonium acetate	Fluka, Switzerland
ammonium chloride	Sigma, USA
ammonium sulfate	Merck, Germany
ampicillin	Sigma, USA
bacto tryptone	Difco Laboratories, USA
bacto yeast extract	Difco Laboratories, USA
betaine	Sigma, USA
boric acid	Merck, Germany
bromophenol blue	Sigma, USA
calcium chloride-dihydrate	Merck, Germany
chloramphenicol	Sigma, USA
coomassie blue	Merck, Germany
cresol red	Sigma, USA
dATP, dCTP, dGTP, dTTP	Amersham Pharmacia Bioscience, UK
dioxane (absolute)	Fluka, Switzerland
dithiothreithole (DTT)	Sigma, USA
ethanol (p.a.)	Merck, Germany
ethidium bromide (1%)	Merck, Germany
ethylenediamine tetraacetic acid (EDTA)	Merck, Germany
formamide	Fluka, Switzerland
glycerol	Merck, Germany
hydrochloric acid	Merck, Germany
isopropanol (p.a.)	Merck, Germany
magnesium chloride	Merck, Germany
magnesiumsulfate-heptahydrate	Merck, Germany
N,N,N',N'-tetramethyl-1,2-diaminomethane (TEMED)	BioRad Laboratories, Germany
perhydrole 30% (H <sub>2</sub> O <sub>2</sub> )	Merck, Germany
potassium chloride	Merck, Germany
potassium dihydrogenphosphate	Merck, Germany
potassium hydrogenphosphate	Merck, Germany
sodium borohydride	Merck, Germany
sodium chloride	Sigma, USA

sodium citrate	Sigma, USA
sodium dihydrogenphosphate	Merck, Germany
sodium dodecylsulfate (SDS)	Sigma, USA
sodium hydrogenphosphate	Merck, Germany
sodium hydroxide	Merck, Germany
sodium N-lauroyl sarcosine	Sigma, USA
succinic anhydride	Sigma, USA
tetraethylammonium chloride (TEACl)	Fluka, Switzerland
tetramethylammonium chloride (TMACl)	Fluka, Switzerland
triethylamine (TEA)	Merck, Germany
trifluoroacetic acid (TFA)	Fluka, Switzerland
tris-(hydroxymethyl)-aminomethane	Merck, Germany
Tween-20 (monolaurate)	Sigma, USA
xylencyanole FF	Sigma, USA

### 5.3 Kits

QIAquick™ PCR Purification Kit	Qiagen, Germany
QIAprep™ Spin Miniprep Kit	Qiagen, Germany
genopure™ oligo	Bruker, Germany

### 5.4 Enzymes

Pfu DNA polymerase	Roche, Germany
Taq DNA polymerase	Roche, Germany

### 5.5 Molecular weight markers

kb DNA ladder (fragments: 12; 10; 9; 8; 7; 6; 5; 4; 3; 2; 1,5; 1; 0,75; 0,5; 0,25 kb)	Stratagene, USA
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## 5.6 DNA oligonucleotides<sup>6</sup>

A071_1 REV	CTTCCTGGATGAGAACTGA
A071_2	GTAACCTTGGGCATTGGCAG
B219_1	TGGTAGACAGTTCATGCAGT
B219_2 REV	GGGGAATGGATGTTAGTTGT
H182_1 REV	AGGGGAATGGATGTTAGTTG
H182_2	TGTATTCAGCAGTTGGAAAG
K022_1 REV	GTGAGTGGCCCTGTGGCACT
K022_2 REV	GCAGGACAGAGATGCATGGG
M13 FWD	GTTTTCCCAGTCACGACG
M13 REV	AGGAAACAGCTATGACCAT
M13 REV spec	CAGGAAACAGCTATGAC
M13 UP	CGACGTTGTAAAACGACGGCCAGT
T <sub>15</sub> kk_G11 REV	(T) <sub>15</sub> TGGCACATATCACAACTCACTGCA
T <sub>15</sub> kk_M5 REV	(T) <sub>15</sub> GGAGTTGCTCTTCTCGAGGAG

## 5.7 PNA oligonucleotides

**Table 5.1** charge-tagged PNA set "6mer global"

PNA	sequence <sup>7</sup>	mass (calc.) [Da]
6P005	CCTCCC	1695.8
6P013	CTCTCC	1710.8
6P054_1/2	CACTCC	1719.8
6P108_1/2	ACACCC	1728.8
6P006	CTGCCC	1735.8
6P100_1/2	CAATCC	1743.8
6P087	TCCTGC	1750.8
6P012	CTCAGC	1759.8
6P114_1/2	CAGACC	1768.8
6P166_1/2	CTGTAC	1774.8
6P046_1/2	CCTGAA	1783.8
6P009	CCAAAG	1792.8
6P038_1/2	TCTGAA	1798.8
6P018	AAACTG	1807.8
6P015	AGCAAA	1816.8
6P016	CTGAAG	1823.8
6P028_1/2	ACAGAG	1832.8
6P124_1/2	AGATTG	1838.8
6P008	AGGCAG	1848.8
6P007	GAGAAA	1856.8
6P021L	O-CTCCCA	1865

<sup>6</sup> sequence: 5'-end → 3'-end

<sup>7</sup> sequence: N-terminus → C-terminus

**Table 5.1 continued**

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
6P020L	O-CACCAC	1874
6P143	GGATGG	1879.8
6P323L	O-CTTTTC	1885.9
6P236L	O-CTCCTG	1895.9
6P014L	O-CTGCCA	1905
6P262L	O-TCTCTG	1910.9
6P119L	O-CTCCGG	1921
6P042L	O-CTCAGA	1929
6P098L	O-TCCTGG	1936
6P022L	O-CCTGAG	1945
6P023L	O-CCAGAG	1954
6P017L	O-CAAGAA	1962
6P034L	O-GAGGCC	1970
6P120L	O-GCTGGT	1976
6P095L	O-ATATGG	1984
6P010L	O-AAATGG	1993
6P156L	O-ATGGTG	2000
6P234L	O-TGAGGA	2009
6P011L	O-GAAGGA	2018

**Table 5.2 charge-tagged PNA set "6mer sub1"**

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
6P009	CCAAAG	1792.8
6P018	AAACTG	1807.8
6P015	AGCAAA	1816.8
6P016	CTGAAG	1823.8
6P008	AGGCAG	1848.8
6P007	GAGAAA	1856.8
6P143	GGATGG	1879.8
6P323L	O-CTTTTC	1885.9
6P236L	O-CTCCTG	1895.9
6P262L	O-TCTCTG	1910.9
6P042L	O-CTCAGA	1929
6P098L	O-TCCTGG	1936
6P022L	O-CCTGAG	1945
6P023L	O-CCAGAG	1954
6P017L	O-CAAGAA	1962
6P034L	O-GAGGCC	1970
6P120L	O-GCTGGT	1976
6P095L	O-ATATGG	1984
6P010L	O-AAATGG	1993
6P156L	O-ATGGTG	2000
6P234L	O-TGAGGA	2009

**Table 5.3 charge-tagged PNA set "6mer sub2"**

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
6P032 2	CAGACA	1792.8
6P038 1/2	TCTGAA	1798.8
6P028 1/2	ACAGAG	1832.8

**Table 5.3 continued**

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
6P124 1/2	AGATTG	1838.8
6P014L	O-CTGCCA	1905
6P052L_2	O-ACTGCT	1920
6P048L_2	O-AGCCAT	1929
6P056L_2	O-ACATTG	1944
6P024L_2	O-AAATGC	1953
6P055L_2	O-CAAAGA	1962
6P037L_2	O-TGGACA	1969
6P029L_2	O-ATTTGG	1975
6P158L_2	O-AGTTAG	1984
6P026L_2	O-ATGAAG	1993
6P118L_2	O-AGGTTG	2000
6P073L_2	O-AATGGG	2009
6P011L	O-GAAGGA	2018

**Table 5.4 charge-tagged PNA set "6mer sub3"**

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
6P005	CCTCCC	1695.8
6P013	CTCTCC	1710.8
6P054 1/2	CACTCC	1719.8
6P108 1/2	ACACCC	1728.8
6P107_2	ACCTTC	1734.8
6P100 1/2	CAATCC	1743.8
6P047_2	CCAAAC	1752.8
6P051_2	ACATTC	1758.8
6P114 1/2	CAGACC	1768.8
6P166 1/2	CTGTAC	1774.8
6P046 1/2	CCTGAA	1783.8
6P021L	O-CTCCCA	1865
6P020L	O-CACCAC	1874
6P061L_2	O-ATCTCC	1880
6P119L	O-CTCCGG	1921

**Table 5.5 charge-tagged PNA set "7mer global"**

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
7P031	CCTTCCC	1961.9
7P021	CCTCCCA	1970.9
7P227	TCTCTCC	1976.9
7P325	TTCCCCA	1985.9
7P051	CTTCTTC	1991.9
7P027	CCCAGCC	1995.9
7P023	CCCCAAA	2003.9
7P029	CAGCCTC	2010.9
7P047	TCCCAAA	2018.9
7P065	GCTGCCC	2026.9
7P262	TTCTCTG	2031.9
7P059	CAGGCCC	2035.9
7P215	TCACTGT	2040.9
7P024	CAGCAGC	2059.9

Table 5.5 continued

PNA	sequence	mass (calc.) [Da]
7P383	ACAAAGC	2067.9
7P030	CTGGGCA	2090.9
7P102	GAGGCCA	2099.9
7P291L	O-CTCCTCC	2107
7P020	AGGCTGG	2130.9
7P323L	O-CTTTTCC	2137
7P101L	O-CCTCCTG	2147
7P207L	O-TTTCTTC	2152
7P174L	O-GCCCCGC	2157.1
7P087L	O-TCCTGCT	2162
7P119L	O-CTCCGGC	2172.1
7P236L	O-GCTCCTG	2187.1
7P337L	O-TTCAGCA	2195.1
7P133L	O-TTCCTGG	2202.1
7P098L	O-TCCTGGA	2211.1
7P417L	O-TGCACAG	2220.1
7P120L	O-TGCTGGT	2242.1
7P088L	O-AAAGGCA	2253.1
7P070L	O-AAATGTG	2259.1
7P204L	O-GTGGCTG	2267.1
7P022L	O-AAAGGAA	2277.1
7P234L	O-ATGAGGA	2284.1
7P156L	O-ATGGTGG	2291.1
7P143L	O-AGGATGG	2300.1
7P060L	O-GGAGAGA	2309.1
7P080L	O-GGAGGAG	2325.1

Table 5.6 charge-tagged PNA set "7mer sub1"

PNA	sequence	mass (calc.) [Da]
7P051	CTTCTTC	1991.9
7P047	TCCAAA	2018.9
7P065	GCTGCC	2026.9
7P262	TTCTCTG	2031.9
7P215	TCACTGT	2040.9
7P383	ACAAAGC	2067.9
7P102	GAGGCCA	2099.9
7P020	AGGCTGG	2130.9
7P323L	O-CTTTTCC	2137
7P207L	O-TTTCTTC	2152
7P337L	O-TTCAGCA	2195.1
7P133L	O-TTCCTGG	2202.1
7P098L	O-TCCTGGA	2211.1
7P120L	O-TGCTGGT	2242.1
7P088L	O-AAAGGCA	2253.1
7P070L	O-AAATGTG	2259.1
7P204L	O-GTGGCTG	2267.1
7P022L	O-AAAGGAA	2277.1
7P156L	O-ATGGTGG	2291.1
7P143L	O-AGGATGG	2300.1



**Table 5.7** charge-tagged PNA set "7mer sub2"

<b>PNA</b>	<b>sequence</b>	<b>mass (calc.) [Da]</b>
7P031	CCTTCCC	1961.9
7P021	CCTCCCA	1970.9
7P227	TCTCTCC	1976.9
7P325	TTCCCCA	1985.9
7P027	CCCAGCC	1995.9
7P023	CCCCAAA	2003.9
7P029	CAGCCTC	2010.9
7P059	CAGGCCC	2035.9
7P024	CAGCAGC	2059.9
7P030	CTGGGCA	2090.9
7P291L	O-CTCCTCC	2107
7P101L	O-CCTCCTG	2147
7P174L	O-GCCCCGC	2157.1
7P087L	O-TCCTGCT	2162
7P119L	O-CTCCGGC	2172.1
7P236L	O-GCTCCTG	2187.1
7P417L	O-TGCACAG	2220.1
7P234L	O-ATGAGGA	2284.1
7P060L	O-GGAGAGA	2309.1
7P080L	O-GGAGGAG	2325.1

## 5.8 Buffers, solutions, and media

### Denaturing solution

SSC	3x
NaOH	400 mM
EDTA	10 mM

### LB agarose

Agarose	2% (w/v)
LB Broth	

### LB broth

<b>Gel loading buffer (6x)</b>		Bacto tryptone	1% (w/v)
Bromophenol blue	0,25% (w/v)	Bacto yeast extract	0,5% (w/v)
Xylenecyanol FF	0,25% (w/v)	NaCl	170 mM
Glycerol	30% (v/v)		

### MALDI matrix solution I

<b>HMFM (10x)</b>		Acetone	99% (v/v)
K <sub>2</sub> HPO <sub>4</sub>	360 mM	TFA	0,002% (v/v)
KH <sub>2</sub> PO <sub>4</sub>	132 mM	$\alpha$ -cyano-4-hydroxy-	
Na-citrate	17 mM	cinnamic acid	saturated
MgSO <sub>4</sub>	4 mM		
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	68 mM		
Glycerol	44% (v/v)		

**MALDI matrix solution II**

Acetone	90% (v/v)
TFA	0,05% (v/v)
$\alpha$ -cyano-4-hydroxy-cinnamic acid	saturated

**MALDI matrix solution III**

Dioxane	20% (v/v)
Formamide	20% (v/v)
TFA	0,6% (v/v)
$\alpha$ -cyano-4-hydroxy-cinnamic acid	saturated

**Na-borohydride solution**

NaBH <sub>4</sub>	40 mM
PBS (1x)	77% (v/v)
Ethanol	23% (v/v)

**Neutralization Solution (pH 7,0)**

Tris-HCl	0,5 M
NaCl	1,5 M

**PBS (1x) (pH 7,5)**

Na <sub>2</sub> HPO <sub>4</sub>	8 mM
NaH <sub>2</sub> PO <sub>4</sub>	2 mM
NaCl	140 mM
KCl	2,6 mM

**PCR buffer (10x) (pH 8,3)**

Tris-HCl	500 mM
KCl	500 mM
MgCl <sub>2</sub>	15 mM
Tween-20	1% (v/v)

**SSARC**

NaCl	600 mM
Na-citrate	60 mM
Na N-lauroyl sarcosine	7,2% (v/v)

**SSC (1x) (pH 7,0)**

NaCl	150 mM
Na-citrate	15 mM

**SuperAmine blocking solution (pH 8,0)**

Succinic anhydride	55 mM
Na-borate	42,9 mM
1-methyl-2-pyrrolidone	96% (v/v)

**TAE (1x) (pH 8,0)**

Tris-Acetate	40 mM
EDTA	1 mM

**TBS (1x) (pH 8,0)**

Tris-HCl	10 mM
NaCl	150 mM

**TBS-T (1x) (pH 8,0)**

TBS	1x
Tween-20	0,1% (v/v)

**TE (low, 1x) (pH 8,0)**

Tris-HCl	5 mM
EDTA	0,1 mM

**TEA/CO<sub>2</sub> (~pH 8,0)**

Triethylamine	2 M
CO <sub>2</sub>	saturated

**YT broth (2x)**

Bacto tryptone	1,6% (w/v)
Bacto yeast extract	1% (w/v)
NaCl	85 mM