

## 2. Materialien

### 2.1 Hersteller und Lieferfirmen

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- |  |                                   |
|--|-----------------------------------|
| (1) Adobe                                | (21) MWG, Ebersberg               |
| (2) Affymetrix, Santa Clara, USA         | (22) Perkin Elmer, Langen         |
| (3) Amersham Pharmacia Biotech, Freiburg | (23) Pharmacia, Freiburg          |
| (4) Amersham, Braunschweig               | (24) Promega, Heidelberg          |
| (5) Beecher Instruments,                 | (25) Quiagen, Hilden              |
| (6) Bio-Rad, München                     | (26) Roche-Boehringer, Mannheim   |
| (7) Biozym Diagnostik, Hameln            | (27) Roth, Karlsruhe              |
| (8) Clontech, Heidelberg                 | (28) Schleicher & Schuell, Dassel |
| (9) Eppendorf, Hamburg                   | (29) Serva, Heidelberg            |
| (10) Fluka, Neu-Ulm                      | (30) Sigma-Aldrich, Deisenhofen   |
| (11) Genetic Microsystems (GMS)/USA      | (31) Stratagene, Heidelberg       |
| (12) Gibco BRL, Karlsruhe                | (32) TiBMolbiol, München          |
| (13) Greiner, Frickenhausen              | (33) USB, Bad Homburg             |
| (14) Heraeus, Hanau                      | (34) Memmert, Schwabach           |
| (15) Invitrogen, Heidelberg              | (35) Biometra, Göttingen          |
| (16) Life Technologies, Berlin           | (36) Millipore, Eschborn          |
| (17) MBI Fermentas, Vilnius, Litauen     | (37) CLF, Emmesacker              |
| (18) Menzel-Gläser                       |                                   |
| (19) Merck, Darmstadt                    |                                   |
| (20) Microsoft                           |                                   |
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## 2.2 Chemikalien

<u>1,4 Phenylendiisothiocyanat</u>	(30)
<u>3-Aminopropyltriethoxysilan</u>	(30)
<u>3-Aminopropyltrimethoxysilan</u>	(30)
<u>6-Amino-1-Hexanol</u>	(30)
<u>Ammoniumhydroxid</u>	(10)
<u>Aceton</u>	(19)
<u>Acrylamid/Bisacrylamid</u>	(27)
<u>Agarose</u>	(12)
<u>Ammoniumperoxodisulfat</u>	(27)
<u>Ampicillin</u>	(26)
<u>Aqua ad iniectabilia (Ampullen-H<sub>2</sub>O)</u>	(23)
<u>Betaine</u>	(30)
<u>Bromphenolblau</u>	(12)
<u>Cot1 DNS</u>	(12)
<u>Cy3-dUTP</u>	(4)
<u>Cy5-dUTP</u>	(4)
<u>Dimethylsulfoxid (DMSO)</u>	(24)
<u>Dinatriumhydrogenphosphat</u>	(19)
<u>Dithiothreitol (DTT)</u>	(19)
<u>dNTPs</u>	(26)
<u>EDTA</u>	(27)
<u>Ethanol</u>	(19)
<u>Ethidiumbromid (EtBr)</u>	(27)
<u>Glyzerol</u>	(19)
<u>Glykogen</u>	(19)
<u>Isopropyl-<math>\beta</math>-thiogalactopyranosid (IPTG)</u>	(29)
<u><math>\beta</math>-Mercaptoethanol</u>	(19)
<u>Methanol</u>	(19)

<u>Millipore-Wasser (deionisiertes Wasser)</u>	(36)
<u>Mineralöl</u>	(27)
<u>NTPs</u>	(24)
<u>N`N Diisopropylethylamin</u>	(30)
<u>N`N Dimethylformamid</u>	(10)
<u>Natriumchlorid (NaCl)</u>	(19)
<u>Natriumcarbonat (Na<sub>2</sub>CO<sub>3</sub>)</u>	(19)
<u>Natriumdodecylsulfat (SDS)</u>	(19)
<u>Natriumhydroxid (NaOH)</u>	(19)
<u>Phenol</u>	(27)
<u>Pyridin</u>	(10)
<u>Rinderserumalbumin (BSA)</u>	(26)
<u>Salzsäure (HCl)</u>	(10)
<u>Tetramethylammoniumchlorid</u>	(19)
<u>Tris</u>	(30)
<u>Trypton</u>	(26)
<u>5-Brom-4-chloro-3-indolyl-β-D-galactopyranosid (X-Gal)</u>	(26)


## 2.3 Enzyme

<u>T7-Gene-6-Exonuclease</u>	(33)
<u>T4-DNS-Ligase</u>	(16)
<u>SP6/T7 Transkription Kit</u>	(26)
<u>Red-Taq-Polymerase</u>	(30)
<u>AmpliTaq-Polymerase</u>	(22)
<u>Tth-DNS-Polymerase</u>	(26)
<u>Thermosequenase</u>	(33)
<u>Expand-PCR-System</u>	(26)

## 2.4 Synthetische Oligonukleotide (Primer)

HPLC gereinigte Oligonukleotide wurden von Metabion (Martinsried), und MWG Biotech AG (Ebersberg) bezogen. Konventionelle Oligonukleotide wurden für die Herstellung von PCR-Produkten verwendet. Allel-spezifische Oligonukleotide zum Aufbringen auf Glas-Objektträger waren am 5`-Ende aminomodifiziert. Für die Erzeugung von Einzelstrang-DNS-Targets durch Exonuklease-Verdau von PCR Produkten, wurden Phosphothioat-modifizierte Oligonukleotide verwendet, welche am 5`-Ende drei PTO-Gruppen tragen. Alle Oligonukleotide wurden im lyophilisiertem Zustand geliefert. Gelöst wurden die Oligonukleotide je nach Bedarf in Wasser oder in 400mM Na<sub>2</sub>CO<sub>3</sub> pH 9,0 , in einer Endkonzentration von 100µM. Bis zur Verwendung wurden diese bei -20°C gelagert.

**Tab. 2.1:** 5`-aminomodifizierte Oligonukleotide mit (T)<sub>15</sub>-Distanzsequenz zum Aufbringen auf Objektträger

Name	5`  3`
3196 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTatgatatcatctcaacttag
3196 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTatgatatcatctcaacttaa
3243 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTgggtttgtaagatggcaga
3243 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTgggtttgtaagatggcagg
3252 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTaagatggcagagcccggtaa
3252 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTaagatggcagagcccggtag
3290 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtttacagtcagaggttcaat
3290 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtttacagtcagaggttcaac
3302 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTggttcaattcctcttcttaa
3302 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTggttcaattcctcttcttag
3394 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcgaacgaaaattctaggct
3394 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcgaacgaaaattctaggcc

3397	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>acgaaaaattctaggctata</b>
3397	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>acgaaaaattctaggctatg</b>
3447	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>gccctacgggctactacaa</b>
3447	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>gccctacgggctactacag</b>
3460	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>tactacaacccttcgctgacg</b>
3460	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>tactacaacccttcgctgaca</b>
4136	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>ttatgaattcgaacagcata</b>
4136	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>ttatgaattcgaacagcatg</b>
4160	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>cgattccgctacgaccaact</b>
4160	c	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>cgattccgctacgaccaacc</b>
4216	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>cctagcattactatgat</b>
4216	c	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>cctagcattactatgac</b>
4317	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>gtaaataataggagctaaa</b>
4317	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>gtaaataataggagctaaag</b>
4336	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>acccttattctaggact</b>
4336	c	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>acccttattctaggacc</b>
4529	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>ttgcaggcacactcatcaca</b>
4529	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>ttgcaggcacactcatcact</b>
4646	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>cagaagctgcatcaagtat</b>
4646	c	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>cagaagctgcatcaagtac</b>
4917	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>ccaaactctccctcactaa</b>
4917	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>ccaaactctccctcactag</b>
7028	c	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>acacgtactacgttagc</b>
7028	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>acacgtactacgttagct</b>
8344	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>taagttaaagattaagagaa</b>
8344	g	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>taagttaaagattaagagag</b>
8851	t	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>agccatggccatccccttat</b>
8851	c	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>agccatggccatccccttac</b>
8993	a	NH <sub>2</sub> - TTTTTTTTTTTTTTTT <b>tctattcaaccaatagccca</b>

8993 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctcattcaaccaatagccce
8993 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctcattcaaccaatagccc <b>g</b>
8993 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctcattcaaccaatagcc <b>ct</b>
10398 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTacaaaaaggattagactgaa
10398 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTacaaaaaggattagactgag <b>g</b>
10463 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtaaattatgataatcatatt
10463 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtaaattatgataatcatat <b>e</b>
11251 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtcccctactcatcgca <b>cta</b>
11251 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtcccctactcatcgca <b>ctg</b>
11332 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTaaactatcaaactcctgag <b>cc</b>
11332 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTaaactatcaaactcctgag <b>ct</b>
11467 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtacttgccgcagtactct <b>ta</b>
11467 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtacttgccgcagtactct <b>tg</b>
11778 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtacgaacgcactcacag <b>tcg</b>
11778 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtacgaacgcactcacag <b>tca</b>
12246 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctaactcatgccccatg <b>tc</b>
12246 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctaactcatgccccatg <b>tg</b>
12308 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcattggtcttaggccc <b>caaa</b>
12308 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcattggtcttaggccc <b>caag</b>
12311 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtggcttaggcccc <b>aaaat</b>
12311 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtggcttaggcccc <b>aaaac</b>
12372 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctataaccaccctaacc <b>ctg</b>
12372 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctataaccaccctaacc <b>cta</b>
12612 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTccataatattcatcc <b>ctgta</b>
12612 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTccataatattcatcc <b>ctgtg</b>
12634 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTattgttcggtacatggtc <b>ca</b>
12634 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTattgttcggtacatggtc <b>cct</b>
12705 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtcttcaaatatctactc <b>ate</b>
12705 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtcttcaaatatctactc <b>att</b>

13368 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTactatttatgtgctccggg
13368 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTactatttatgtgctccgga
13708 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTaaacccattaaacgcctgg
13708 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTaaacccattaaacgcctga
14233 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctactaatcaacgccataa
14233 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctactaatcaacgccatag
14484 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTgtatatccaaagacaacat
14484 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTgtatatccaaagacaaccac
14709 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTacaaccacgaccaaagata
14709 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTacaaccacgaccaaagata
14905 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcaggactattcctagccatg
14905 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcaggactattcctagccata
15043 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTgcctcttctacacatcggg
15043 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTgcctcttctacacatcggga
15205 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcaaactactatccgccatc
15205 t	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcaaactactatccgccatt
15257 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctgaggaggctactcagtag
15257 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTctgaggaggctactcagtaa
15326 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcattattgcagccctagcaa
15326 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcattattgcagccctagcag
15452 c	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcctcggettactctcttcc
15452 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTcctcggettactctcttca
15607 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtccgatccgctcctaaca
15607 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtccgatccgctcctaaca
15924 a	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtaatacaccagtcttga
15924 g	NH <sub>2</sub> - TTTTTTTTTTTTTTTTtaatacaccagtcttga

**Tab. 2.2:** 5`-aminomodifizierte Oligonukleotide (50mere) zum Aufbringen auf Objektträger

Name		5` $\longrightarrow$ 3`
Amino-3196	g 50mer	NH <sub>2</sub> -gcctacttcacaaagcgcttccccgtaaatgatatcatctcaacttag
Amino-3196	a 50mer	NH <sub>2</sub> - gcctacttcacaaagcgcttccccgtaaatgatatcatctcaacttaa
Amino-3243	a 50mer	NH <sub>2</sub> - tagtattataccacaccaccaagaacagggttgtaagatggcaga
Amino-3243	g 50mer	NH <sub>2</sub> - tagtattataccacaccaccaagaacagggttgtaagatggcagg
Amino-3252	a 50mer	NH <sub>2</sub> - acccacaccaccaagaacagggttgtaagatggcagagcccggtaa
Amino-3252	g 50mer	NH <sub>2</sub> - acccacaccaccaagaacagggttgtaagatggcagagcccggtag
Amino-3290	t 50mer	NH <sub>2</sub> - agagcccggtaatgcataaaaacttaaaactttacagtcagaggttcaat
Amino-3290	c 50mer	NH <sub>2</sub> - agagcccggtaatgcataaaaacttaaaactttacagtcagaggttcaac
Amino-3302	a 50mer	NH <sub>2</sub> - tcgcataaaaacttaaaactttacagtcagaggttcaattcctcttcttaa
Amino-3302	g 50mer	NH <sub>2</sub> - tcgcataaaaacttaaaactttacagtcagaggttcaattcctcttcttag
Amino-3394	t 50mer	NH <sub>2</sub> - tctaategcaatggcattcctaatactaccgaacgaaaattctaggct
Amino-3394	c 50mer	NH <sub>2</sub> - tctaategcaatggcattcctaatactaccgaacgaaaattctaggcc
Amino-3397	a 50mer	NH <sub>2</sub> - aatcgcaatggcattcctaatactaccgaacgaaaattctaggctata
Amino-3397	g 50mer	NH <sub>2</sub> - aatcgcaatggcattcctaatactaccgaacgaaaattctaggctatg
Amino-3447	a 50mer	NH <sub>2</sub> - tacaactacgcaaaggcccaacgttgtaggccctacgggctactacaa
Amino-3447	g 50mer	NH <sub>2</sub> - tacaactacgcaaaggcccaacgttgtaggccctacgggctactacag
Amino-3460	g 50mer	NH <sub>2</sub> - aggcccaacgttgtaggccctacgggctactacaacccttcgctgacg
Amino-3460	a 50mer	NH <sub>2</sub> - aggcccaacgttgtaggccctacgggctactacaacccttcgctgaca
Amino-4136	a 50mer	NH <sub>2</sub> - accaagaccctacttctaacctccctgttcttatgaattcgaacagcata
Amino-4136	g 50mer	NH <sub>2</sub> - accaagaccctacttctaacctccctgttcttatgaattcgaacagcatg
Amino-4160	t 50mer	NH <sub>2</sub> - ctgttcttatgaattcgaacagcataccccgattccgctacgaccaact
Amino-4160	c 50mer	NH <sub>2</sub> - ctgttcttatgaattcgaacagcataccccgattccgctacgaccaace
Amino-4216	t 50mer	NH <sub>2</sub> - cctcctatgaaaaaacttctaccactcacctagcattacttatatgat
Amino-4216	c 50mer	NH <sub>2</sub> - cctcctatgaaaaaacttctaccactcacctagcattacttatatgac
Amino-4317	a 50mer	NH <sub>2</sub> - tatgtctgataaaagagttactttgatagagtaataataggagcttaa



Amino-4317	g 50mer	NH <sub>2</sub> - tatgtctgataaaagagttactttgatagagtaaataataggagcttaag
Amino-4336	t 50mer	NH <sub>2</sub> - actttgatagagtaaataataggagcttaaacccccctatttctaggact
Amino-4336	c 50mer	NH <sub>2</sub> - actttgatagagtaaataataggagcttaaacccccctatttctaggacc
Amino-4529	a 50mer	NH <sub>2</sub> - tggcccaaccgctcatctactctaccatctttgcaggcacactcatcaca
Amino-4529	t 50mer	NH <sub>2</sub> - tggcccaaccgctcatctactctaccatctttgcaggcacactcatcact
Amino-4646	t 50mer	NH <sub>2</sub> - ttctaacaaaaaataaacctcgttccacagaagctgcatcaagtat
Amino-4646	c 50mer	NH <sub>2</sub> - ttctaacaaaaaataaacctcgttccacagaagctgcatcaagtac
Amino-4917	a 50mer	NH <sub>2</sub> - acaaaaactagccccatctcaatcatataccaaatctctccctcactaa
Amino-4917	g 50mer	NH <sub>2</sub> - acaaaaactagccccatctcaatcatataccaaatctctccctcactag
Amino-7028	c 50mer	NH <sub>2</sub> - caaactcatcactagacatcgctactacacgacacgtactacgttgtagcc
Amino-7028	t 50mer	NH <sub>2</sub> - caaactcatcactagacatcgctactacacgacacgtactacgttgtagct
Amino-8344	a 50mer	NH <sub>2</sub> - cactgtaaagctaacttagcattaacctttaagttaagattaagagaa
Amino-8344	g 50mer	NH <sub>2</sub> - cactgtaaagctaacttagcattaacctttaagttaagattaagagag
Amino-8851	t 50mer	NH <sub>2</sub> - tacaccaaccaccaactatctataaacctagccatggccatccccttat
Amino-8851	c 50mer	NH <sub>2</sub> - tacaccaaccaccaactatctataaacctagccatggccatccccttac
Amino-8993	a 50mer	NH <sub>2</sub> - atactagttattatcgaaaccatcagcctactcattcaaccaatagccca
Amino-8993	c 50mer	NH <sub>2</sub> - atactagttattatcgaaaccatcagcctactcattcaaccaatagcccc
Amino-8993	g 50mer	NH <sub>2</sub> - atactagttattatcgaaaccatcagcctactcattcaaccaatagccccg
Amino-8993	t 50mer	NH <sub>2</sub> - atactagttattatcgaaaccatcagcctactcattcaaccaatagccct
Amino-10398	a 50mer	NH <sub>2</sub> - cctagccctaagtctggcctatgagtgactacaaaaaggattagactgaa
Amino-10398	g 50mer	NH <sub>2</sub> - cctagccctaagtctggcctatgagtgactacaaaaaggattagactgag
Amino-10463	t 50mer	NH <sub>2</sub> - gtttaacaaaacgaatgatttcgactcattaaattatgataatcatatt
Amino-10463	c 50mer	NH <sub>2</sub> - gtttaacaaaacgaatgatttcgactcattaaattatgataatcatatc
Amino-11251	a 50mer	NH <sub>2</sub> - acttctattctacaccctagtaggctcccttcccctactcactcgcacta
Amino-11251	g 50mer	NH <sub>2</sub> - acttctattctacaccctagtaggctcccttcccctactcactcgcactg
Amino-11332	c 50mer	NH <sub>2</sub> - acattctactactcactctcactgcccagaactatcaaactcctgagcc
Amino-11332	t 50mer	NH <sub>2</sub> - acattctactactcactctcactgcccagaactatcaaactcctgagct
Amino-11467	a 50mer	NH <sub>2</sub> - atgtcgaagccccatcgctgggtcaatagtacttgccgcagtactctta
Amino-11467	g 50mer	NH <sub>2</sub> - atgtcgaagccccatcgctgggtcaatagtacttgccgcagtactcttg

Amino-11778 g 50mer	NH <sub>2</sub> - tcattactattctgcctagcaaactcaaactacgaacgcactcacagt <b>cg</b>
Amino-11778 a 50mer	NH <sub>2</sub> - tcattactattctgcctagcaaactcaaactacgaacgcactcacagt <b>ca</b>
Amino-12246 c 50mer	NH <sub>2</sub> - cttatttaccgagaaagctcacaagaactgctaactcatgccccat <b>gtc</b>
Amino-12246 g 50mer	NH <sub>2</sub> - cttatttaccgagaaagctcacaagaactgctaactcatgccccat <b>gtg</b>
Amino-12308 a 50mer	NH <sub>2</sub> - tttctcaacttttaaggataacagctatccattggcttaggcccc <b>aaa</b>
Amino-12308 g 50mer	NH <sub>2</sub> - tttctcaacttttaaggataacagctatccattggcttaggcccc <b>aag</b>
Amino-12311 t 50mer	NH <sub>2</sub> - ctcaacttttaaggataacagctatccattggcttaggcccc <b>aaaat</b>
Amino-12311 c 50mer	NH <sub>2</sub> - ctcaacttttaaggataacagctatccattggcttaggcccc <b>aaaac</b>
Amino-12372 g 50mer	NH <sub>2</sub> - tccaaataaaaagtaataaccatgcacactactataaccacc <b>taaacctg</b>
Amino-12372 a 50mer	NH <sub>2</sub> - tccaaataaaaagtaataaccatgcacactactataaccacc <b>taaaccta</b>
Amino-12612 a 50mer	NH <sub>2</sub> - tctccctaagcttcaaactagactacttctccataatattcatcc <b>ctgta</b>
Amino-12612 g 50mer	NH <sub>2</sub> - tctccctaagcttcaaactagactacttctccataatattcatcc <b>ctgtg</b>
Amino-12634 a 50mer	NH <sub>2</sub> - ctacttctccataatattcatccctgtagcattgttcg <b>ttacatggtcca</b>
Amino-12634 t 50mer	NH <sub>2</sub> - ctacttctccataatattcatccctgtagcattgttcg <b>ttacatggtcct</b>
Amino-12705 c 50mer	NH <sub>2</sub> - atataaactcagacccaaacattaatcagttcttcaa <b>aatctactcacc</b>
Amino-12705 t 50mer	NH <sub>2</sub> - atataaactcagacccaaacattaatcagttcttcaa <b>aatctactcatt</b>
Amino-13368 g 50mer	NH <sub>2</sub> - acatctgtaccacgccttcttcaaagccatactat <b>ttatgtgctccggg</b>
Amino-13368 a 50mer	NH <sub>2</sub> - acatctgtaccacgccttcttcaaagccatactat <b>ttatgtgctccgga</b>
Amino-13708 g 50mer	NH <sub>2</sub> - taacattaacgaaaataaccccaccctactaa <b>acccattaaacgcctgg</b>
Amino-13708 a 50mer	NH <sub>2</sub> - taacattaacgaaaataaccccaccctactaa <b>acccattaaacgcctga</b>
Amino-14233 a 50mer	NH <sub>2</sub> - caccaacaacaatggtcaaccagtaactactacta <b>atcaacgcccataa</b>
Amino-14233 g 50mer	NH <sub>2</sub> - caccaacaacaatggtcaaccagtaactactacta <b>atcaacgcccatag</b>
Amino-14484 t 50mer	NH <sub>2</sub> - tcaggatactcctcaatagccatcgctgtagtata <b>tccaaagacaacat</b>
Amino-14484 c 50mer	NH <sub>2</sub> - tcaggatactcctcaatagccatcgctgtagtata <b>tccaaagacaaccac</b>
Amino-14709 t 50mer	NH <sub>2</sub> - aaagcatacatcattattctcgcacggactaca <b>accacgaccaatgataf</b>
Amino-14709 g 50mer	NH <sub>2</sub> - aaagcatacatcattattctcgcacggactaca <b>accacgaccaatgatag</b>
Amino-14905 g 50mer	NH <sub>2</sub> - ttggcgctgctgctgaccccaatcaccacagg <b>actattcctagccatg</b>
Amino-14905 a 50mer	NH <sub>2</sub> - ttggcgctgctgctgaccccaatcaccacagg <b>actattcctagccata</b>
Amino-15043 g 50mer	NH <sub>2</sub> - acgccaatggcgctcaatattctttatctgcct <b>ttcctacacatcggg</b>

Amino-15043 a 50mer	NH <sub>2</sub> - acgccaatggcgcctcaatattctttatctgcctcttcctacacatcgga
Amino-15205 c 50mer	NH <sub>2</sub> - aaatatcattctgaggggccacagtaattacaaacttactatccgccatc
Amino-15205 t 50mer	NH <sub>2</sub> - aaatatcattctgaggggccacagtaattacaaacttactatccgccatt
Amino-15257 g 50mer	NH <sub>2</sub> - atacattgggacagacctagttcaatgaatctgaggaggctactcagtag
Amino-15257 a 50mer	NH <sub>2</sub> - atacattgggacagacctagttcaatgaatctgaggaggctactcagtaa
Amino-15326 a 50mer	NH <sub>2</sub> - attctttaccttcacttcatcttgccttcattattgcagccctagcaa
Amino-15326 g 50mer	NH <sub>2</sub> - attctttaccttcacttcatcttgccttcattattgcagccctagcag
Amino-15452 c 50mer	NH <sub>2</sub> - ctccacccttactacacaatcaagacgccctcggcttacttctcttcc
Amino-15452 a 50mer	NH <sub>2</sub> - ctccacccttactacacaatcaagacgccctcggcttacttctcttca
Amino-15607 a 50mer	NH <sub>2</sub> - aatgatatttctattcgcctacacaattctccgatccgtccctaacaaa
Amino-15607 g 50mer	NH <sub>2</sub> - aatgatatttctattcgcctacacaattctccgatccgtccctaacaag
Amino-15924 a 50mer	NH <sub>2</sub> - ctcaaattgggctgtccttgtagtataaactaatacaccagtcttgtaa
Amino-15924 g 50mer	NH <sub>2</sub> - ctcaaattgggctgtccttgtagtataaactaatacaccagtcttgtaag

Tab. 2.3: 50mer Oligonukleotide zum Einsatz als Target für die Primer-Elongationsreaktion


Name	5'  3'
Oligo 50mer A Hin	5'-tgtattagcaaactcatcactagaa <u>a</u> atcgactacacgacacgtactacg-3'
Oligo 50mer C Hin	5'-tgtattagcaaactcatcactagac <u>a</u> atcgactacacgacacgtactacg-3'
Oligo 50mer G Hin	5'-tgtattagcaaactcatcactagag <u>a</u> atcgactacacgacacgtactacg-3'
Oligo 50mer T Hin	5'-tgtattagcaaactcatcactagat <u>a</u> atcgactacacgacacgtactacg-3'
Oligo 50mer A Rück	5'-cgtagtacgtgctgtgtagtacgat <u>a</u> tctagtgatgagtttgctaataca-3'
Oligo 50mer C Rück	5'-cgtagtacgtgctgtgtagtacgat <u>t</u> ctagtgatgagtttgctaataca-3'
Oligo 50mer G Rück	5'-cgtagtacgtgctgtgtagtacgat <u>g</u> tctagtgatgagtttgctaataca-3'
Oligo 50mer T Rück	5'-cgtagtacgtgctgtgtagtacgat <u>t</u> tctagtgatgagtttgctaataca-3'
Amino-mt-6995 A	NH <sub>2</sub> -TTTTTTTTTTTTTTTTcgtagtacgtgctgtgtagtacgata
Amino-mt-6995 C	NH <sub>2</sub> -TTTTTTTTTTTTTTTTcgtagtacgtgctgtgtagtacgac
Amino-mt-6995 G	NH <sub>2</sub> -TTTTTTTTTTTTTTTTcgtagtacgtgctgtgtagtacgatg
Amino-mt-6995 T	NH <sub>2</sub> -TTTTTTTTTTTTTTTTcgtagtacgtgctgtgtagtacgatt

**Tab. 2.4:** Oligonukleotide als Startermoleküle zur Herstellung von PCR-Produkten aus mtDNS

Name	5' → 3'
A 3153 L	5`-ttcaciaaagcgcttccccc-3`
A 4678 H	5`-aggattatggtagcggttgcttg-3`
B 5424 L	5`-catacaaaaccacccattcctc-3`
B 5920 H	5`-caacggtcggcgaacatcagt-3`
C 9964 L	5`-atgtctccatctattgatgagggtcttactct-3`
C 10628 H	5`-ggagtgggtgttgagggttatgagagt-3`
D 11158 L	5`-cacccgatgaggcaaccagc-3`
D 12017 H	5`-tgagtgagccccattgtgttg-3`
E 12210 L	5`-aaagctcacaagaactgctaactcatgc-3`
E 12788 H	5`-gatataattcctacgccctctcagcc-3`
F 13324 L	5`-tgtaccacgccttcttcaaagc-3`
F 14347 H	5`-tgatgggggtggtggttg-3`
G 14815 L	5`-cccaccaacatctccgcatgatgaaac-3`
G 15522 H	5`-gctagggtataattgtctgggtgcct-3`
H 15149 L	5`-tgaggccaaatattctgaggggc-3`
H 16052 H	5`-atgggtgagtcaatacttgggtg-3`
I 3153 L	5`-ttcaciaaagcgcttccccc -3`
I 3579 H	5`-tatgggggaggggggttcatagtagaa-3`
J 4094 L	5`-ccctacttctaacctcctgttcttat-3`
J 4976 H	5`-tccacctcaactgctgctatgatgg-3`
K 5424 L	5`-catacaaaaccacccattcctc-3`
K 5920 H	5`-caacggtcggcgaacatcagt-3`
L 6325 L	5`-cctccgtagacctaacattcttctc-3`
L 7117 H	5`-agggtgtagcctgagaataggggaaa-3`
M 8316 L	5`-ttaacctttaagttaaga-3`

M 8435 H	5`-tgatgaggaatagtgtgaagg-3`
N 8821 L	5`-tctataaacctagccatggc-3`
N 8940 H	5`-gataaggggtgtaggtgtgc-3`
O 8961 L	5`-aaccatcagcctactcattc-3`
O 9080 H	5`-ttaatggttgatattgctag-3`
P 14456 L	5`-atcgctgtagtatatccaaa-3`
P 14576 H	5`-tgtagcgggtgtggacgggt-3`
Q 14681 L	5`-gcacggactacaaccacgac-3`
Q 14800 H	5`-gaatgagtggtaattaatt-3`
R 3211 L	5`-ccaccaagaacagggttg-3`
R 3330 H	5`-gagtaggaggttgccatgg-3`
S 8301 L	5`-aaagctaacttagcattaac-3`
S 8420 H	5`-taaggagtatgggggtaatt-3`
T 11741 L	5`-tgctagcaaaactcaaacta-3`
T 11860 H	5`-gaggttagcaggcttgcta-3`
U 15221 L	5`-gcactagttcaatgaatctg-3`
U 15340 H	5`-taggaggtggagtgttgcta-3`
V 15421 L	5`-aatcaaagacgcctcggct-3`
V 15540 H	5`-gggggtttaaaggggttggc-3`

**Tab. 2.5:** Oligonukleotide als Startermoleküle zur Herstellung von asymmetrischen PCR-Produkten aus mtDNS

Name	5`  3`
R-7080	5`-agcctcctatgatggcaaat-3`
R-8400	5`-atggtaggccatacggtagt-3`
R-8900	5`-agggcatttttaactctaga-3`
R-8980	5`-gaatgagttaggctgatggtt-3`
R-10450	5`-taatttaatgagtcgaaatc-3`
R-10520	5`-tagaagtgagatggtaaatg-3`

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R-11310	5`-tgggcagtgagagtgagtag-3`
R-11390	5`-ctgaaagaggtatctttact-3`
R-11520	5`-gtcaggggggttgagaatgag-3`
R-11830	5`-aaaagctattagtgggagta-3`
R-12300	5`-ctaagaccaatggattgctg-3`
R-12360	5`-ggttatagtagtggcatgg-3`
R-12430	5`-atgagttttttgtaggg-3`
R-12670	5`-ggctgagtttatatcac-3`
R-14290	5`-aggagaggggtcagggtga-3`
R-14760	5`-cgtattggggtcattggtg-3`
R-14960	5`-ctcgagtgatgggcgatt-3`
R-15100	5`-gataatgccgatgttcagg-3`
R-15260	5`-tgttactgagtagcctct-3`
R-15510	5`-ttgtctgggtcgcctaggag-3`
R-15980	5`-gtggagttaagacttttc-3`
R-4392	5`-gggtgataggtggcacggaga-3`
R-4678	5`-aggattatggatgcggttgctg-3`
R-10289	5`-tcatggtaggggtaaaaggaggcaa-3`
R-12549	5`-ggttgtggctcagtgctcagtcg-3`
R-11502	5`-agtgtgagcgtattataccatagc-3`
R-5680	5`-tgggtttaagtccttggcttagt-3`
R-15161	5`-atattggcctcacggaggacat-3`
R-15396	5`-tatcggaatgggaggtgattcctag-3`
R-5882	5`-gctgagtgaagcattggactg-3`
R-13415	5`-cctattttcgaatatctgttcat-3`
R-13755	5`-ggaaatgtttagtaatgagaaat-3`
R-14533	5`-gggaggttatatgggttaatag-3`
R-15650	5`-ctaggatgaggatggatag-3`
R-12115	5`-gtcgggggttgaggataggaggaga-3`
L-7925	5`-ggcggactaatcttcaactcctacat-3`

Tab. 2.6: Phosphothioat-modifizierte Oligonukleotide als Startermoleküle zur Herstellung von PCR-Produkten aus mtDNS

Name	5' → 3'
PTO mtDNS R-3579	5`- <i>gtat</i> ggggaggggggttcata-3`
PTO mtDNS R-3495	5`- <i>ggg</i> tttaggggctctttgg-3`
PTO mtDNS R-3343	5`- <i>tgg</i> tacaatgaggagtaggag-3`
MtDNS L-3170	5`-aggegccttccccgtaaat-3`
PTO mtDNS R-5920	5`- <i>caac</i> ggtcggcgaacatcagt-3`
MtDNS L-9970	5`-catctattgatgagggtcttactct-3`
PTO mtDNS R-10628	5`- <i>gga</i> gtgggtgttgagggttat-3`
PTO mtDNS R-12017	5`- <i>tga</i> tgagccccattgtgttg-3`
PTO mtDNS R-12788	5`- <i>gat</i> ataattcctacgcctctcagcc-3`
PTO mtDNS R-14347	5`- <i>tga</i> tgggggtggtggttg-3`
MtDNS L-14816	5`-ccatccaacatctccgat-3`
PTO mtDNS R-15523	5`- <i>ggc</i> taggtataattgtctgggt-3`
PTO mtDNS R-16052	5`- <i>atg</i> ggtgagtcaatactgggtgg-3`
PTO mtDNS R-3579	5`- <i>tat</i> ggggaggggggttcata-3`
PTO mtDNS R-4976	5`- <i>tcc</i> acctcaactgcctgctatgatgg-3`
MtDNS L-6325	5`-cctccgtagacctaacatt-3`
PTO mtDNS R-7117	5`- <i>gtg</i> tagcctgagaataggggaaa-3`
PTO mtDNS R-7117-1	5`- <i>agg</i> gttagcctgagaataggggaaa-3`
PTO mtDNS R-8810	5`- <i>gtt</i> ggtgtaaatgagtggca-3`
MtDNS F-6639	5`-ttatcctaccaggcttcggaataa-3`
PTO mtDNS R-7060	5`- <i>aca</i> gctcctattgataggacatagt-3`
PTO(5x) mt R-3343	5`- <i>tgg</i> tacaatgaggagtaggag-3`
PTO(5x) mt R-7117	5`- <i>gtg</i> tagcctgagaataggggaaa-3`
PTO(5x) mt R-12017	5`- <i>tga</i> tgagccccattgtgttg-3`
PTO(5x) mt R-16052	5`- <i>atg</i> ggtgagtcaatactgggtgg-3`

Tab. 2.7: Weitere verwendete Oligonukleotide

Name	5' → 3'
5`A-12308G-Cy3	NH <sub>2</sub> - cttggggcctaagaccaatg-Cy3-3`
T7-R-3495	5`-aaacgacggccagtgaattgtaatacgactcactatagggcg gggttttaggggctctttgg-3`
T7-R-3339	5`-aaacgacggccagtgaattgtaatacgactcactatagggcg tacaatgaggagtaggaggt-3`
IA-XhoI-CL2	5`-aactcgagctgcactccagcctggg
T7-IA-XhoI-CL2	5`-aaacgacggccagtgaattgtaatacgactcactatagggcg aactcgagctgcactccagcctggg
T7-L3153	5`-aaacgacggccagtgaattgtaatacgactcactatagggcg ttcacaagegccttcccc
T7-R3549	5`-aaacgacggccagtgaattgtaatacgactcactatagggcg tatggggaggggggttcatagtagaa

## 2.5 Lösungen und Puffer

### LB-Amp-Medium pH 7,5

Trypton	1%	(w/v)
Hefeextrakt	0,5%	(w/v)
NaCl	1%	(w/v)
Ampicillin	50µg/ml	

### SOC-Medium

Trypton	20	g/l
Hefeextrakt	5	g/l
NaCl	0,5	g/l



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<b>TE-Puffer pH 7,5</b>	KCl	2,5 mM
	EDTA	1 mM
	Tris	10 mM
<b>10 x TBE-Puffer</b>	Tris	1,34 M
	Borsäure	45 mM
	EDTA	25 mM
<b>20 x SSC pH 7,0</b>	NaCl	3 M
	Na-Citrat	0,3 M
<b>Silanisierungslösung</b>	95 %	Aceton
	1%	3-Aminopropyltriethoxysilan
<b>Aktivierungslösung</b>	0,2 %	1,4 Phenylendiisothiocyanat
	15 %	Pyridin
		in N`N Dimethylformamid

## 2.6 Kits

<u>QIAamp DNS Blood, Mini Extraction Kit</u>	(25)
<u>QIAquick PCR Purification Kit</u>	(25)
<u>Topo TA Cloning Kit</u>	(24)

## 2.7 Glasobjektträger

<u>Aminosilan Objektträger</u>	(30)
<u>In situ PCR Objektträger</u>	(22)
<u>Super Frost Plus</u>	(18)

## 2.8 Geräte

<u>Microarray Scanner 428</u>	(2)
<u>Arrayer (A4800)</u>	(5)
<u>Brutschrank</u>	(34)
<u>Concentrator 5301</u>	(9)
<u>Gelkammern</u>	(35)
<u>GeneAmp PCR-System 2400/9700</u>	(9)
<u>Millipore-Anlage</u>	(36)
<u>Scanner</u>	(5)
<u>Spektralphotometer</u>	(9)
<u>Trockenschrank</u>	(34)
<u>UV-Transilluminator</u>	(4)
<u>Wasserbad</u>	(37)
<u>Zentrifuge (Biofuge Stratos), Rotor # 3048 für 96-well MTP</u>	(14)
<u>Zentrifuge 5810R, Rotor A-4-62 für 96-well MTP</u>	(9)
<u>Zentrifuge 5417R, 5415C</u>	(9)
<u>Zykler MJ-Research</u>	(7)

## 2.9 Sonstige Materialien

<u>Färbegestell</u>	(27)
<u>Frame Seal Chambers</u>	(7)
<u>Glaskästen</u>	(27)
<u>Mikrotiterplatten</u>	(27)
<u>Objektträgerkästen</u>	(27)
<u>Secure-Seal Hybridisation chambers</u>	(30)
<u>UV-Küvetten</u>	(9)

## 2.10 Computerprogramme

Exel 97	(20)
IP Lab / Microarray Suite	(5)
Photoshop 5.2	(1)
Powerpoint 97	(20)
Word 97	(20)