

## 4. MATERIALS

### 4.1 Laboratory apparatus

<b>NAME</b>	<b>MANUFACTURER OR GERMAN DISTRIBUTOR</b>
Biofuge A	Heraeus Sepatech, Kendro Laboratory Products GmbH, Berlin
Centrifuge 5804R	Eppendorf, Hamburg
FACScan	Becton Dickinson, Heidelberg
Gel electrophoresis chamber	Gibco, Karlsruhe/Heidelberg
GPR Centrifuge	Beckmann Instruments GmbH, München
Heraeus CO-AUTO-ZERO	Heraeus Sepatech, Kendro Laboratory Products GmbH, Berlin
Light Cycler	Roche Diagnostics, Mannheim
Microscope	Zeiss, Jena
Motopipet	Hirschmann Labor Geräte,
Neubauer Cell Chamber	Zeiss, Jena
PCR cycler	Biozym, Hessisch Oldendorf
Photometer	Pharmazia, Freiburg
Pipets	Eppendorf, Hamburg
Polaroid film camera (Reprostar II)	CAMAG, Berlin
Template Tamer	Oncor Appligene, Heidelberg
Thermobloc TB1	Biometra, Göttingen
Vortex	IKA Labortechnik; without address
Waterbath	Merck Eurolab, Berlin

### 4.2 Kits

<b>NAME</b>	<b>MANUFACTURER OR GERMAN DISTRIBUTOR</b>
Cy3 staining Kit	Ambion, Cambridgeshire, UK
DNA isolation Kit	Gentra Systems, Minneapolis, USA
LightCycler-DNA Master Hybridization Probes	Roche Diagnostics, Mannheim
LightCycler-DNA Master SYBR Green I	Roche Diagnostics, Mannheim
Omniscript Reverse Transcriptase Kit	Qiagen, Hilden
QIAprep Miniprep Kit	Qiagen, Hilden

RNeasy Mini Kit	Qiagen, Hilden
Silencer siRNA Construction Kit	Ambion, Cambridgeshire, UK
TOPO TA Cloning Kit	Invitrogen, Karlsruhe/Heidelberg

### 4.3 Enzymes

<b>NAME</b>	<b>MANUFACTURER OR GERMAN DISTRIBUTOR</b>
DNase	Qiagen, Hilden
DNA methylase M.Sss I	New England Biolabs, Beverly, USA
Hha II restrictase	New England Biolabs, Beverly, USA
Hpa II restrictase	New England Biolabs, Beverly, USA
Hga I restrictase	New England Biolabs, Beverly, USA
RNase A	Roche Diagnostics, Mannheim
RNase-Inhibitor	Roche Diagnostics, Mannheim
Taq-Polymerase	Roche Diagnostics, Mannheim
Trypsin	Invitrogen, Karlsruhe/Heidelberg

### 4.4 Reagents

<b>NAME</b>	<b>MANUFACTURER OR GERMAN DISTRIBUTOR</b>
Agarose SEeKEM LE	Biozym, Hessisch Oldendorf
dNTP	Roche Diagnostics, Mannheim
Ethanol	Baker
Ethidium Bromide	Sigma, Steinheim
DEPC water	Invitrogen, Karlsruhe/Heidelberg
GTC	Sigma, Steinheim
DNA marker	Life Technologies, Paisley, Scotland
Hybridization Probes	Metabion, München
Isopropanol	Sigma, Steinheim
Low Melting Agarose XI	Sigma, Steinheim
Oligo-dT-Primer	Roche Diagnostics, Mannheim
Oligofecamine	Gibco - Invitrogen, Karlsruhe/Heidelberg
PCR buffer	Roche Diagnostics, Mannheim

PCR grade water	Sigma, Steinheim
Polyadenylic Acid	Pharmacia Biotech
Primers	Metabion, München
siPORT Lipid	Ambion, Cambridgeshire, UK
TBE	Life Technologies, Paisley, Scotland

#### 4.5 Other materials

<b>Product</b>	<b>MANUFACTURER OR GERMAN DISTRIBUTOR</b>
24 well cell culture dish	NUNC, Wiesbaden
Culture flasks (small, med, large)	NUNC, Wiesbaden
Falcons 50ml	Becton Dickinson, Heidelberg
Pipettes (1ml, 5ml, 10ml, 25ml)	Becton Dickinson, Heidelberg
Tips (10µl,100µl,1000µl)	Biozym, Hamburg

#### 4.6 Cell culture mediums

<b>NAME</b>	<b>MANUFACTURER OR GERMAN DISTRIBUTOR</b>
Opti-MEM I	Invitrogen, Karlsruhe/Heidelberg
RPMI 1640	Invitrogen, Karlsruhe/Heidelberg
FBS	Invitrogen, Karlsruhe/Heidelberg
PenStrep	Invitrogen, Karlsruhe/Heidelberg
Glutamax	Invitrogen, Karlsruhe/Heidelberg
Hepes	Invitrogen, Karlsruhe/Heidelberg
Sodium Bicarbonate	Invitrogen, Karlsruhe/Heidelberg
PBS	Biochrom KG, Berlin
Phenolred Solution	SIGMA, Deisenhofen
Trypanblue Solution	SIGMA, Deisenhofen

## 4.7 Cell lines

Cell line	Type of neoplasia	Source	Provider
BT-474	Ductal Breast Cancer	Human	DSMZ
CaCo2	Colon Adenocarcinoma	Human	DSMZ
Colo 205	Colon Adenocarcinoma	Human	ATCC
Colo 206F	Colon Carcinoma	Human	DSMZ
Colo 320,	Colon Adenocarcinoma	Human	DSMZ
CX 2	Colon carcinoma	Human	ATCC
CX F94	Colon carcinoma	Human	ATCC
DLD 1	Colon Adenocarcinoma	Human	DSMZ
HBL 100	Breast Cancer	Human	ATCC
HCT 116	Colon Carcinoma	Human	ATCC
HEL	Erythroleukemia	Human	DSMZ
HL60	AML	Human	DSMZ
HT 29	Colon Adenocarcinoma	Human	DSMZ
K562	CML in blast crisis	Human	DSMZ
LS 174T	Colon Adenocarcinoma	Human	ATCC
MCF-7	Breast Cancer	Human	DSMZ
MDA MB 435S	Breast Cancer	Human	CLS
MDA MB 436	Breast Cancer	Human	CLS
MDA MB 468	Breast Cancer	Human	CLS
MX-1	Breast Cancer	Human	CLS
SW 403	Colon Adenocarcinoma	Human	DSMZ
SW 480	Colon Adenocarcinoma	Human	DSMZ
SW 620	Colon Adenocarcinoma	Human	ATCC
SW 948	Colon Adenocarcinoma	Human	DSMZ
SW1116	Colon Adenocarcinoma	Human	ATCC
T47-D	Breast Cancer	Human	ATCC

Cells were grown in proper media supplied with 20% heat inactivated FBS, 1% penicillin-streptomycin, 1% Glutamax and 20mM Hepes at 37°C, 5% CO<sub>2</sub> in 95% relative humidity. Medium was changed in 2 to 3 day intervals.

## 4.8 Blood and tissue samples

Blood and tissue samples were collected during routine medical procedures after the informed consent was given. The analysis was done in a framework of studies approved by local ethics committee.

## 4.9 qRT-PCR Primers and Hybridization Probes

Primers	Forward	Reverse
WT1*	5'- GGGTTACAGCACGGTCACCT -3'	5'- GCGTCCTCAGCAGCAAAG -3'
GATA1*	5'- ACTACCTATGCAACGCC -3'	5'- TTGCGGTTTCGAGTCT -3'
PAX2*	5'- GGTGAGAAGAGGAAACG -3'	5'- GCAGATAGACTCGACTTG -3'
PAX8*	5'- CCAGAACCCTACCATGT -3'	5'- GTCAATGCTTAGTCGGC -3'
PBGD*	5'- TGCAGGCTACCATCCATGTCCCTGC -3'	5'- AGCTGCCGTGCAACATCCAGGATGG -3'
GAPDH**	5'- AACAGCGACACCCACTCCTC -3'	5'- GGAGGGGAGATTCAGTGTGGT -3'

  

Hybridization Probes	Fluorescein	LC RED
WT1*	5'- CAGCAGTACTCGGTGCC GCCCCC X -3'	5'- Y TCTATGGCTGCCACACCCCCACCG P -3'
GATA1*	5'- AACTGCCAGACGACCACC X -3'	5'- Y GACACTGTGGCGGAGAAATGC P -3'
PAX2*	5'- CTCAGTCCCCAATGGAGATTCC X -3'	5'- Y GAGTGGTGTGGACAGTTTGGC P -3'
PAX8*	5'- GGACCAAAGTGCAGCAACCA X -3'	5'- Y CAACCTCCCTATGGACAGCT P -3'
PBGD*	5'- CGTGGAAATGTTACGAGCAGTGATGCCTACC X -3'	5'- Y TGTGGGTCATCCTCAGGGCCATCTTC P -3'
GAPDH**	5'- CATGGCCTCCAAGGAGTAAGACCCCT X -3'	5'- ACCACCAGCCCCAGCAAGAGCA P -3'

\* Taken from Siehl et al., 2003.

\*\*Taken from Hubert Schullein, Gastroenterology Charité, personal communication.

## 4.10 PCR Primers

Name	Forward primer	Reverse primer
WT1 promoter proximal*	5'- CAA CCC CAT CTC TAC TCC CA -3'	5'- CGC TGC CTT GAA CTC CTT AC -3'
WT1 promoter distal*	5'- TAT AAC TGG TGC AAC TCC CGG C -3'	5'- AAA GAG TGG TTT GGA GGG AGG G -3'
WT1 enhancer	5'- CTT TCC AGG GCA ACT GAG -3'	5'- ACC TGG CTC TTT CCG ACT -3'
PAX8 promoter	5'- GTC ACC CAG GGG TTA GCT -3'	5'- GGCTTC CTC CCG TAG GTC C -3'
PAX2 promoter	5'- CAG CGC TGG CGA ATC ACA -3'	5'- GGG CGG GGG AGG CGA CTC -3'

GATA1  
promoter

5'- GGC AAG CTC AGT GTG ATC CC -3'

5'- GCA GCC AGG AAG ACG CAC AT -3'

\*Taken from Mares et al., 2001.

Primers were designed with Oligo 6.0 software.

#### 4.11 Matrix ssDNA Oligos

Name	Position	Sense sequence oligo	Antisense sequence oligo
WT1 seq 1	338	5'- AATCCTCCGGCCCTGGAGACGCCTGTCTC -3'	5'- AACGTCTCCAGGGCCGGAGGACCTGTCTC-3'
WT1 seq 2	385	5'- AACCCGCACGTCGGAGCCCATCCTGTCTC -3'	5'- AAATGGGCTCCGACGTGCGGGCCTGTCTC -3'
WT1 seq 3	607	5'- AAGCCCCAGCTCGGCTCCTGTCTC -3'	5'- AAACAGGAGCCGAGCTGGGGCCTGTCTC -3'
WT1 seq 4	967	5'- AACTCATGCTTGAATGAGTGGCCTGTCTC -3'	5'- AACCACTCATTCAAGCATGAGCCTGTCTC -3'
WT1 seq 5	2832	5'- AAAGTACTTATACAGTCCTAACCTGTCTC -3'	5'- AATTAGGACTGTATAAGTACTCCTGTCTC -3'
PAX2 seq 1	742	5'- AAGTAGTACCTGCCAGGATTCCTGTCTC -3'	5'- AAAATCCTGGGCAGGTACTACCCTGTCTC -3'
PAX2 seq 2	482	5'- AAGTCGCCTCTCAAACCTCAACCCTGTCTC -3'	5'- AAGTTGAGTTTGAGAGGGCACCCTGTCTC -3'
PAX2 seq 3	808	5'- AACACTTTGGGCGTCGCCACTCCTGTCTC -3'	5'- AAAGTGGCGACGCCCAAAGTGCCTGTCTC -3'
PAX2 seq 4	1183	5'- AAACCACTCTGGGAATCTCCACCTGTCTC -3'	5'- AATGGAGATCCCAGAGTGGTCCTGTCTC -3'
PAX2 seq 5	1636	5'- AAGGAACTTAGTAAGGCGGGCCTGTCTC -3'	5'- ACCCCCGCCTTACTAAGTCCCCTGTCTC -3'
PAX8 seq 1	153	5'- AAGTAGTACCTGCCAAGGATCCCTGTCTC -3'	5'-AAGATCCTTGGCAGGTACTACCCTGTCTC -3'
PAX8 seq 2	234	5'- AACCCAATCTTCTCCACCACCCTGTCTC -3'	5'- AAGGTGGTGGAGAAGATTGGGCCTGTCTC -3'
PAX8 seq 3	378	5'- AAGTTGAATGGTTGCTGCACTCCTGTCTC -3'	5'- AAAGTGCAGCAACCATTCAACCCTGTCTC -3'
PAX8 seq 4	631	5'- AAGCATCCGTGCGAAGGTGCTCCTGTCTC -3'	5'- AAAGCACCTTCGCACGGATGCCCTGTCTC -3'
GATA1 seq 1	1145	5'- AATGAAGCCACCTCCCCACAACCTGTCTC -3'	5'- AATTGTGGGGAGGTGGCTTCACCTGTCTC -3'
GATA1 seq 2	1411	5'- AAAGACTTGGGTTGTCCAGAACCTGTCTC -3'	5'- AATTCTGGACAACCCAAGTCTCCTGTCTC -3'
GATA1 seq 3	890	5'- AACGTGGTGGTCGTCTGGCAGCCTGTCTC -3'	5'- AACTGCCAGACGACCACCAGCCTGTCTC -3'
GATA1 seq 4	653	5'- AAAGAGGAATAGGCTGCTGAACCTGTCTC -3'	5'- AATTCAGCAGCCTATTCTCCTGTCTC -3'
PBGD seq 1	147	5'- AAGGGTACGAGGCTTCAATGCCTGTCTC -3'	5'- ACCATTGAAAGCCTCGTACCCCTGTCTC -3'
PBGD seq 2	293	5'- AAAGTGAACAACCAGGTCCACCCTGTCTC -3'	5'- AAGTGGACCTGGTTGTTCACTCCTGTCTC -3'
PBGD seq 3	531	5'- AACGAAGCCGGGTGTTGAGGTCTGTCTC -3'	5'- AACCTCAACACCCGGCTTCGCCTGTCTC -3'
PBGD seq 4	1319	5'- AACAAAGGCTGTTGCTTGGACCCTGTCTC -3'	5'- AAGTCCAAGCAACAGCCTTTGCCTGTCTC -3'

siRNA DNA oligos designed with Ambion siRNA design tool available at [www.ambion.com](http://www.ambion.com)