

Materials

Complete Media (CM)	1x DMEM 4.5 mg/ml glucose 2 mM L-Glutamine 10% heat inactivated FCS 50 µg/ml penicillin 50 µg/ml streptomycin
Freezing Media	Complete media 10% DMSO
Phosphate Buffered Sodium (PBS)	140 mM NaCl 2.7 mM KCl 16 mM Na ₂ HPO ₄ 1.5 mM KH ₂ PO ₄ 0.8 mM EDTA
1x Trypsin in PBS	0.25% (w/v) Trypsin 0.03% (w/v) EDTA
CSK Buffer	10 mM Pipes pH 6.8 100 mM NaCl 300 mM Sucrose 3 mM MgCl ₂ 1 mM EGTA 1 mM DTT 50 mM sodium flouride 0.1 mM sodium vanadate 0.5% Triton 1 mM PMSF 1 µg/ml pepstatin 1 µg/ml chymostatin 1 µg/ml leupeptin 1 µg/ml aprotinin
4x Protein Sample Buffer	1M Tris pH 6.8 20% Glycerol 4% SDS 5% 2-Mercaptoethanol 1 mM PMSF
PMSF Solution	100 mM PMSF in Isopropanol

Polyacrylamide Stacking Gel	acrylamide mix to desired percentage 250mM Tris pH 6.8, 0.1% SDS 0.1% APS TEMED
Coommassie Stain	45% Methanol 10% Glacial Acetic Acid 0.2% Coommassie Brilliant Blue R250
10x SDS Running Buffer	250 mM Tris-HCl 192 mM Glycin 1% SDS
10X Transfer Buffer	250 mM Tris 192 mM Glycin
10x TBST Buffer	100 mM Tris-HCl, pH 8.0 1.54 M NaCl 1% (v/v) Tween 20
Block Milk	10 mM Tris pH 8.0 150 mM NaCl 0.1% Tween 20 0.5% NP40 0.5% BSA, Fraction V 2.5% non-fat dried milk
Coommassie Destain I	40% Methanol 10% Glacial Acetic Acid
Coommassie Destain II	7% Glacial Acetic Acid 5% Methanol
Cellophane wetting solution for Polyacrylamide gel storage	40% Methanol 2% Glycerol
10x Taqman Buffer A, pH 8.3	500 mM KCl 100 mM Tris-HCl 100 mM EDTA 600 nM ROX Passive Dye

Taqman reaction mix (50 μ l reaction) 10 μ l total RNA
 17.00 μ l RNase free ddH₂O
 11.00 μ l 25 mM MgCl₂
 5.0 μ l 10x Taqman Buffer A
 1.5 μ l 10 mM dATP
 1.5 μ l 10 mM dCTP
 1.5 μ l 10 mM dGTP
 1.5 μ l 20 mM dUTP
 0.25 μ l RNase inhibitor
 0.25 μ l TaqGold Polymerase
 0.05 μ l Reverse Transcriptase
 0.15 μ l 100 μ M target forward primer
 0.15 μ l 100 μ M target reverse primer
 0.05 μ l 100 μ M target taqman probe
 0.02 μ l 100 μ M β -actin forward primer
 0.02 μ l 100 μ M β -actin reverse primer
 0.05 μ l 100 μ M β -actin taqman probe

PI Solution 0.1% (v/v) Triton X-100
 2 mg DNase free-RNase
 200 μ g Propidium Iodide
 in 10 ml PBS

PVA/DABCO 4.8g Polyvinyl alcohol
 100mM Tris-HCl, pH 8
 1.25g DABCO
 in 50 ml ddH₂O

Enzymes

Taq polymerase AmpliTaq Gold 5U/ μ l (Applied Biosystems)
 M-MLV Reverse Transcriptase, RNase H Minus, 200U/ml (Promega)
 Rnasin Ribonuclease Inhibitor 20U/ μ l (Promega GmbH, Mannheim)
 Trypsin (Gibco, BRL)

Antibodies Primary-Polyclonal

Anti-hMCM2 (Becton Dickinson Catalog # 559542)
 Anti-hMCM3 (Becton Dickinson Catalog # 559543)
 Anti-hMCM4 (Becton Dickinson Catalog # 559544)

Antibodies Primary-Monoclonal

Anti-hMCM5 (Becton Dickinson Catalog # 611750)
Anti-hMCM6 (Becton Dickinson Catalog # 611622)
Anti-hMCM7 (Santa Cruz Catalog # (141.2): sc-9966)
Anti-hPCNA (Santa Cruz Catalog # (PC10): sc-56)
Anti- β -ACTIN (Sigma Catalog # A5441, clone AC-15)
Human Anti-phospho-Ser/Thr-Pro, MPM2 (Upstate, Catalog # 05-368)
FITC conjugated anti-BrdU (BD)

Secondary peroxidase conjugated antibodies

Goat Anti-Rabbit IgG, Fc Fragment Specific (Pierce Catalog # 31463)
Goat Anti-Mouse IgG, Fcg Fragment Specific (Pierce Catalog # 31439)
FITC conjugated anti-mouse (SIGMA)

Chemiluminescence

SuperSignal West Pico Chemiluminescent Substrate (Pierce #34080)
Immobilon-P Transfer Membrane Polyvinylidene fluoride, PVDF, 0.45 μ m
(Millipore Catalog # IPVH 000 10)

Chemicals and Compounds

all chemicals were purchased through Carl Roth GmbH + Co. KG Karlsruhe, Germany, unless otherwise noted.

Ammonium Persulfate
 β -Mercaptoethanol
Mimosine, Sigma
Nocodazole, Sigma
TEMED-N,N,N',N'-tetramethylethylenediamine
EDTA
EGTA
NaF
 Na_3VO_4
Sucrose
SDS
NaCl
HCl
Bromphenol Blue
Coomassie Blue R25
Tween
NP40, Noniodit
 MgCl_2

Chemicals and Compounds continued

NA₂B₄O₇·10 H₂O (Borax)
Penicillin
Streptomycin
DimethylSulfoxide
Sodium Phosphate
Potassium Phosphate
Dithiothreitol
TRIS
Glycerol
Sodium Laurel sulfate
Sodium Chloride
Potassium Chloride
Triton x-100
30% acrylamide
Methanol
Ethanol
Glacial Acetic Acid
Glycin
Bovine Serum Albumin, fraction V
Magnesium chloride
Propidium Iodide
PVA, Sigma
DABCO, Sigma

Computers

All computer work was performed on an Apple G4 Macintosh Computer.

Programs

Primer Express, version 1.0 to design Taqman amplicons
Sequence Detection Systems, version 1.6.3 for Taqman analysis
appliedbiosystems.com

CellQuest™ Pro for Flow cytometry analysis
bdbiosciences.com

Microsoft office 2003 for OSX and OS9 for word processing
microsoft.com

Machines, laboratory equipment and chemicals

ABI Sequence Detector 7700, PCR machine
appliedbiosystems.com

Becton Dickinson FACS Calibur, Flow cytometry cell scanner
bdbiosciences.com

SmartSpec 3000 Spectrophotometer
PowerPac 200, 200,3000 power supplies for electrophoresis
biorad.com

Mini Protean II multi screen for immunocytochemistry
Hofer mini VE Basic for PAGE
Hofer Blotter for transfer of proteins from PAGE to nitrocellulose
amershambiosciences.com

5414D Ambient temperature table top Centrifuge
5417R Refrigerated table top centrifuge
5804R Refrigerated clinical centrifuge
eppendorf.com

Mettler MP200 pH meter
mt.com

Curix 60 film developer
AGFA.COM

CK40 Inverted microscope
olympus.com

CB series CO2 incubator
binder-world.com

Gelair cell culture hood
icnpharm.com

Mcm4 cDNA

atg tgg toc ccg ggg tgg acc ccg agc cgc ggc agc ccg cgt gga agc gcc acc ccg gcc cag acy oct cgg agt gag gat gcc agy tca tct ccc tct cag aga cgt aga ggc gag gat toc acc tcc acg ggg gag ttg cag ccg atg cca acc tgg oct gga gtg gac ctg
 M S S P A S T P S R R G S R R G R A T P A Q T P R S E D A R S S P S Q R R R G E D S T S T G E L Q P M P T S P G V D L
 cag agc act gct ccg cag gac gtg ctg ttt toc agc oct ccc caa atg cat tct toa gct atc cct ctt gac ttt gat gtt agt toa cca ctg aca tac gcc act ccc agc tot ccg gta gag gga acc cca aga agt ggt gtt agg ggc aca cct gtg aga cag agg oct gac ctg
 Q S T A A Q D V L F S S P P Q M H S S A I P L D F D V S S P L T Y G T P S S R V E G T P R S G V R G T P V R Q R P D L

Genbloc 22174

ggc tct gca cag aag ggc ctg caa gtg gat ctg cag tct gac ggg gca gca gca gaa gat ata **CTG GCA AGT GAG CAG TCT CTA GG** cca aaa ctt gtg atc tgg gga aca gat gta aat gtg gca gca tgc aaa gaa aac ttt cag aga ttt ctt cag
 G S A Q K G L Q V D L Q S D G A A A E D I V A S E Q S L G Q K L V I W G T D V N V A A C K E N F Q R F L Q
 cgt ttt att gac oct ctg gct aaa gaa gaa aat gtt gca ata gat att act gaa oct cta tac atg caa cga ctt ggg gag att aat gtt att ggt gag caa ttt tta aat gtg aac tgt gaa cac atc aaa toa ttt gac aaa aat ttg tac aga caa ctc atc tot tac cca
 R F I D P L A K E E E N V G I D I T E P L Y M Q R L G E I N V I G E Q F L N V N C E H I K S F D K N L Y R Q L I S Y P
 cag gaa gtt att oca act ttt gac atg gct gtc aat gaa atc ttc ttt gac cgt tac oct gac toa atc tta gaa cat cag att caa gta aga oca ttc aac gca ttg aag act aag aat atg aga aac ctg aat oca gaa gac att gac cag ctc atc acc atc agc agt gtg
 Q E V I P T F D M A V N E I F F D R Y P D S I L E H Q I Q V R P F N A L K T K N M R N L N P E D I D Q L I T I S G M V
 atc agy ana toc cag ctg att ccc gag atg cag gag gcc ttc ttc cag tgc caa gtg tgt gcc cac acy acc ccg gtg gag atg gac cgc cgc att gca gag ccc agt gtg tgc ggg cgc tgc cac acc cac agc atg gca ctc atc cac aac cgc toc ctc ttc tot gac
 I R T S Q L I P E M Q E A F F Q C Q V C A H T T R V E M D R G R I A E P S V C G R C H T T H S M A L I H N R S L F S D
 aag cag atg atc aag ctt cag gag tct ccg gaa gac atg oct gca ggg cag aca oca cac aca gtt atc ctg ttt gct cac aat gat cct gtt gac aag gtc cag oct ggg gac aga gtg aat gtt aca ggc atc tat oga gct gtg oct att oga gtc aat oca aga gtg agt aat
 K Q M I K L Q E S P E D M P A G Q T P H T V I L F A H N D L V D K V Q P G D R V N V T G I Y R A V P I R V N P R V S N
 gtg aag tot gtc tac aaa acc cac att gat gtc att cat tat ccg aaa acy gat gca aaa cgt ctg cat ggc ctt gat gaa gaa gca gaa gaa aaa ctt ttt toa gag aaa cgt gtg gaa ttg ctt aag gaa ctt toc agy aaa cca gac att tat gag agy ctt gct toa gcc ttg
 V K S V Y K T H I D V I H Y R K T D A K R L H G L D E E A E Q K L F S E K R V E L L K E L S R K P D I Y E R L A S A L
 gct oca agc att tat gaa cat gaa gat ata aag gga att ttg oct cag ctc ttt ggc ggg aca agy aag gat ttt agt cac act gga agy ggc aaa ttt ccg gct gag atc aac atc ttg ctg tgt ggc gac oct ggt acc agc aag toc cag ctg ctg cag tac gtg tac aac
 A P S I Y E H E D I K K G I L L Q L F G G T R K D F S H T G R G K F R A E I N I L L C G D P G T S K S Q L L Q Y V Y N
 ctc gtc ccc agy ggc cag tac acy tct ggg aag gcc toc agt gca gtt gcc ctc acc ccg tac gta atg aaa gac oca agy cag ctg gtc ctg cag aca ggt gct ctt gtc ctg agt gac aac gcc atc tgc tgt atc gat gag ttc gac aag atg aat gaa agt aca aga
 L V P R G Q Y T S G K G S S A V G L T A Y V M K D P E T R Q L V L Q T G A L V L S D N G I C C I D E F D K M N E S T R

Forward Taqman oligo

Taqman Probe

Reverse Taqman oligo

tgg gta ttg cat gaa gtc atg gaa cag **CAG ACT CTG TCC ATT GCA AAG G-T GGG ATC ATC TGT CAG CTC AAT GCG** c **C ACC TCT GTC CTG GCA GCA** gca aat ccc att gag tot cag tgg
 S V L H E V M E Q Q T L S I A K A G I I C Q L N A R T S V L A A A N P I E S Q W
 aat cct aaa aaa aca acc att gaa aac atc cag ctg oct cat act tta tta toa agy ttt gat ttg atc ttc ctc atg ctg gac oct cag gac gaa gcc tat gac agy cgt ctg gct cac cac ctg gtc gca ctg tac tac cag agc gag cag gaa gag gag ctc ctg gac
 N P K K T T I E N I Q L P H T L L S R F D L I F L M L D P Q D E A Y D R R L A H H L V A L Y Y Q S E E Q A E E E L L D
 atg gcy gtg cta aag gac tac att gcc tac ccg cac agc acc atc atg ccg cgt cta agt gag gaa gcc agc cag gct ctc atc gag gct tat gta gac atg agy aag att gcc agt agc ccg gga atg gtt tot gca tac cct cga cag cta gag toa tta atc cgc tta gca gaa
 M A V L K D Y I A Y A H S T I M P R L S E E A S Q A L I E A Y V D M R K I G S S R G M V S A Y P R Q L E S L I R L A E
 gcc cat gct aaa gta aga ttg tot aac aaa gtt gaa gcc att gat gtg gaa gag gcc aaa cgc ctc cat ccg gaa gct ctg aag cag tot gca act gat ccc ccg act gcc atc gtg gac ata tot att ctt act acy ggg atg agt gcc acc tot cgt aaa ccg aaa gaa gaa tta
 A H A K V R L S N K V E A I D V E E A K R L H R E A L K Q S A T D P R T G I V D I S I L T T G M S A T S R K R K E E L
 gct gaa gca ttg aaa aag ctt att tta tot aag ggc aaa oca cca gct cta aaa tac cag caa ctt ttt gaa gat att ccg gga caa tot gac ata gca att act aaa gat atg ttt gaa gaa gca ctg cgt gcc ctg gca gat gat ttc ctg aca gtg act ggg aag acc gtg
 A E A L K K L I L S K G K T P A L K Y Q Q L F E D I R G Q S D I A I T K D M F E E A L R A L A D D D F L T V T G K T V
 cgc ttg ctc
 R L L

Figure 2. Open reading frame of Mcm4 cDNA showing the positions Genbloc 22174 and the Taqman primers.

Real-time rtPCR amplicons

Amplicon	Sequence	TM	GC%
Mcm2 f	CGCATCACCAACCACATCC	59	57
Mcm2 r	CGGATCAGCTGGTTCAGAT	58	55
Mcm2 probe	CGCATCTCCCACCTGCCTCTGGT	69	65
Mcm3 f	GGGCTACAGGACTCACTGCTGT	59	59
Mcm3 r	TGGTCTGAGATCTCCCGATCCT	58	57
Mcm3 probe	TGCTCTTCATCATGCTGGATCAGATGGAT	69	44
Mcm4 f	CAGACTCTGTCCATTGCAAAGG	58	50
Mcm4 r	TGCTGCCAGGACAGAGGTTG	60	63
Mcm4 probe	TGGGATCATCTGTCTCAGCTCAATGCG	68	52
Mcm5 f	CCATGGAGCAGCAGACCAT	59	58
Mcm5 r	CAGCGGGAGTTCAGGGTG	60	67
Mcm5 probe	CTATCGCCAAGGCTGGGATCACCA	66	58
Mcm6 f	ACAGCAGTTCAAATACACACAGCC	59	46
Mcm6 r	GAATACGAACCTTTTGAAAATCAA	59	31
Mcm6 probe	TCTGCCGAAATCCAGTTTGTGCCAAC	69	50
Mcm7 f	GACCGCACAGCCATCCAC	59	67
Mcm7 r	GGGCATTGAGTGTGGTGAGAA	60	52
Mcm7 probe	AGCAGCAGACCATCTCCATTGCCAAG	69	54
Cdc6 f	CCGTAACCTGTTCTCCTCGTG	62	57
Cdc6 r	TGTCATCGCCCAGACGTTT	57	53
Cdc6 probe	AAAAGCCCTGCCTCTCAGCCCCA	62	57
Cdc45 f	ACAGTGATGGGTCAGAGCCTTC	59	55
Cdc45 r	ATACTGCTCGTAGTCAAAGAGGATGTC	59	44
Cdc45 probe	AGCGCACACGGTTAGAAGAGGAGATAGTGG	69	53
Orc1 f	CCCCACCAAGTCTATGTGCA	58	60
Orc1 r	TGCTTTGCCAGCAGTTCTG	57	53
Orc1 probe	ACAGGCCAAAAGCAACAGCCAA	66	48
Orc2 f	CACAATTTGGATAGCCAGATGTTG	59	42
Orc2 r	AAAGACTCTGCTTTGCATGATCC	59	44
Orc2 probe	CATTGACCACCTCAATGCTCCTCATG	68	50
Orc3 f	TACTTCAGTGCTGCCCATGC	58	55
Orc3 r	AAGGATTGTTGAGTGCAGTATGGA	59	42
Orc3 probe	TTAAATGCTGCTCCGCGAATTGCC	68	50
Orc4 f	GAAGCTTTGCTGAAAACCTTTCA	59	39
Orc4 r	AGTGTGTTGGTTTTATGATGAGCAA	59	31
Orc4 probe	TGACCGAACTAGCAGTTGCCAGTGATCT	70	52

Real-time rtPCR amplicons continued

Amplicon	Sequence	TM	GC%
Orc5 f	AGCCGTTTGTCTTATATTTCCCTG	59	42
Orc5 r	AGAAATCAGCTGAATACTCTGGAGG	58	44
Orc5 probe	GCAACCTTCAAAGATCCTGTCCCATGATC	69	47
Orc6 f	TCATGTGCCTGGACCTTGCAGC	59	58
Orc6 r	ACACTCAAAAGATTTAAGACAGCTCTGA	59	36
Orc6 probe	TCCTGGATGAAGTGCCCCTTGGAC	68	58
β -Actin f	GTTGAGACCTTCAACACCCCA	60	50
β -Actin r	GACCAGAGGCATACAGGGACA	59	57
β -Actin probe	CCATGTACGTAGCCATCCAGGCTGTG	68	58

N.B.: all amplicon sequences are written written 5' to 3'. All probes are labelled at the 5' end with FAM and at the 3' end with TAMRA. The β -Actin probe is labelled at the 5' end with VIC and at the 3' end with TAMRA. f-forward primer, r-reverse primer, probe-Taqman probe

Phosphorothioated antisense oligonucleotides

mcm 4 Genebloc (GB) sequences designed from Genbank NM182746

GB# 22173 guugcagACTGCTTCAgagcuuc
 GB# 22174 ccuagagACTGCTCACuugccac
 GB# 22175 acucgaaTAGGCACAGcucgaua
 GB# 22176 auggugaTGAGCTGGTcaauguc
 GB# 22177 caucuguTCCCAGATcacaagu
 GB# 22178 ucuuccaCATCAATGGcuucaac
 GB# 22179 caaagagCTGAAGCAAauuccc
 GB# 22180 uggucuuGAACTCCTGaccucau

GB# 22174 mismatch control (MM) ccuacagAGTCCACTCuugcgac

Orc1 Geneblocs designed from Genbank NM_004153

GB# 22165 gaaucucGGTGAACAaccuucu
 GB# 22166 uguucagGTGAAGCCTucuuguc
 GB# 22167 uucagagCTGGAGACAagguuug
 GB# 22168 guucugaGAAAGTGGccugaau
 GB# 22169 aucaucaTCCCCTTCAucaaca
 GB# 22170 gguuaccTAAGTTGCCaagcucc
 GB# 22171 guggaagCCTCTTCTTcgucacu
 GB# 22172 agugcugCTACCTTCCuggcuac
 GB# 24526 aggugaaGCCTTCTTGucauccu
 GB# 24527 uuacaggCAGGGACTTcacagaa
 GB# 24528 ucaaggaGTTTGCTTccacaaa
 GB# 24529 agacucuTCTTTGGCAccuucgu
 GB# 24530 gugaagcCTTCTTGTCauccuca
 GB# 24531 uccuuugGGGCTAAAGguaucac
 GB# 24532 ugaagccCCTTTACTCgucuuuc
 GB# 24533 aaucggaCAAACCACTguacucg

Cdc6 Geneblocs designed from Genbank NMU9550

GB# 21062 gaggagaACAGGTTACgguuugg
 GB# 21063 agugcagGATCCTTCTcacgucu
 GB# 21064 aaccaguGTCTCCAAaucucca
 GB# 21065 uagcucuCTAAATGCCagcugaa
 GB# 21066 caacaugGTAAAACCCugucucu
 GB# 21067 gcaacacAATCACTTcaaccugg
 GB# 21068 ggcaccuGTAATCCCAgcuacuc
 GB# 21069 uugucagCTGATTGTCaaauacc

Phosphorothioated antisense oligonucleotides continued*Cdc45 Geneblobs designed from Genbank AF062495*

GB# 21054 gaacuucTGCTTCACCugcuuca
GB# 21055 acggagaGTGTGTTCTccucauuc
GB# 21056 ucauucuGGAAGAATCaaauucc
GB# 21057 ugacgacATTGACTGGccuauug
GB# 21058 cugagagGTCAAATGguugugc
GB# 21059 uaggacaGGAGGGAAuaagugc
GB# 21060 aaaaugGCTTCTACAucucaaa
GB# 21061 ccaauagGTCTACATTagcucca
GB# 22157 ugcaggaCACCAACATcagucac

GB# 22158 gucacacTGAACAAGgccugaa
GB# 22159 uucaucaGGTTGAAGAauaucca
GB# 22160 gucacugTGCCATGCTccaugcu
GB# 22161 aggacctCTGGCTCTggaccac
GB# 22162 auguccaTGGCCTGGAacuucug
GB# 22163 gucgugGCCAGAAACuugugcu
GB# 22164 gaggauGCTCTTCTCggggccu

All sequences are written 5' to 3'.

Small cap sequences are 2' O-methyl modified ribonucleotides.

Large cap sequences are DNA.

Bold letters represent mismatched bases.

Potential targets for GB22174 allowing more than 5 mismatch base pairings

hNuclear indicates targeting of heteronuclear species. numbers indicate location of matching sequences

MCM2 hNuclear		CAC CGT TCA CTC GTC AGA GAT CC
		:: :: ::: :
	14050	TTG GCG AGT GCT CAG TAT CTG GC

MCM3 mRNA		CAC CGT TCA CTC GTC AGA GAT CC
		:: : : ::: :: ::: :
	1710	ATG GGA AGT CAG CAG AGA ACA TT

MCM6 hNuclear		CAC CG- TTC ACT CGT CAG AGA TCC
		::: :: ::: : :: ::: :::
	22770	GTG GCT AAG AGC ACA GTC TCT GAA

MCM10 hNuclear		CAC CGT TCA CTC GTC AGA GAT CC
		::: :: :: ::: ::: :: :::
	30000	CAA GCA AGC AAG CAG TCT TTA GG