Material

Cell lines

HEK 293	ATCC
HEK 293T	(Lebkowski et al., 1985)
HeLa	ATCC
Hela M2	(Urlinger et al., 2000)
HtTA	(Gossen and Bujard, 1992)
Hi5	Invitrogen
Sf9	Invitrogen

Bacterial strains

E.Coli Xl1-blue	lab stock
E.Coli DH5α	lab stock
E.Coli DH10BAC	lab stock
E.Coli GM 48	lab stock

Cell culture reagents

Foetal calf serum	Biochrom
Minimum essential medium	Sigma
DMEM	Sigma
Grace's media	Applichem
TMN-FH media	Sigma
Pluronic F-68	Invitrogen
Yeastolate	Invitrogen
Blasticidin S	InvivoGen
G418®	Invitrogen
Penicillin / Streptomycin	Invitrogen

Other Lab reagents

All other regular lab chemicals were procured from Sigma, Invitrogen, Roth, Biochrom, Serva and Applichem. The chemicals were normally of p.a. grade.

Radiochemical

[α-^{32}P]dCTP and [γ-^{32}P]dATP (3000 Ci/mmol, 10 mCi/ml) NEG

Enzymes

Restriction enzymes	NEB / Amersham
Taq DNA polymerase	Eppendorf
Pwo DNA polmerase	Hybaid-AGS
T4 DNA ligase	USB
TEV protease	Invitrogen
Shrimp alkaline phosphatase	USB
T4 DNA polymerase	Amersham

Antibodies

Mouse anti-TetR	Mobitec, Germany
Rabbit anti-HsOrc2	Pharmingen
Rat anti-HsOrc3	Gift from Dr. Schepers, Munich
Mouse anti-HsOrc4	BD Biosciences
Rabbit anti-HsOrc1	(Anand Ranjan, PhD work)
Mouse anti Flag M2	Sigma
Human IgG	Pierce
Goat anti-mouse IgG	Pierce
Goat anti-rat IgG	Pierce
Goat anti-rabbit IgG	Pierce

Kits*

Plasmid Midi and Maxi prep.	Qiagen
Quick Spin coumns (G-25)	Roche
SilverQuest silver staining kit	Roth
NEBlot random DNA labeling	New England Biolabs
Quick ligation kit	New England Biolabs
Galacto-Light Kit	Applied biosystems

* All kits were used according to manufacturer's protocol

Plasmids

Name	Important Features	Source
scTet	CMV, monomerized TtA, SV40PolyA	Wolfgang Hillen
4F	CMV TetR fused at N' Orc4, F-linker, Flag Tag SV40PolyA	Vishal
4G	CMV TetR fused at N' Orc4, G-linker, Flag Tag, SV40PolyA	Vishal
2F	CMV TetR fused at N' Orc2, F-linker, Flag Tag,SV40PolyA	Vishal
2G	CMV TetR fused at N' Orc2, G-linker, Flag Tag, SV40PolyA	Vishal
PFastbac1	Expression vector for insect cells	Gibco
Fastbac-4F	4F ORF in fastbac	Vishal
Fastbac-4G	4G ORF in fastbac	Vishal
Fastbac-2F	2F ORF in fastbac	Vishal
Fastbac-2G	2G ORF in fastbac	Vishal

tetO7-4G	Heptamerized tetO inserted in 4G	Vishal
tetO7-2G	Heptamerized tetO inserted in 2G	Vishal
pUHG102-3 Orc1C	Tet promoter, Orc1, C' TAP tag	Anand Ranjan
Orc1GFP	Tet-Promoter, GFP fused with C' of Orc1,	Vishal
Orc1Tet	Tet-Promoter, scTet fused to C' of Orc1	Vishal
Fastbac Orc1-GFP	Orc1-GFP ORF in fastbac	Vishal
Fastbac Orc1Tet	Orc1-Tet ORF in fastbac	Vishal
phygro Orc1Tet	CMV, scTet fused to C' of Orc1, hygromycin resistance	Vishal
pCDNA/to4/Lacz	2XtetO in CMV, Lacz	Invitrogen
pTRE/LacZ	minimal CMV with tet regulatory element(TRE), LacZ	Clontech
Pub/bsd	Blasticidin resistance	Invitrogen
Pub/bsdt7	Heptamerized tetO in Pub/bsd	Manfred Gossen
Replication green	Heptamerized tetO, EF, GFP	Vishal
Replication red	Heptamerized tetO, CMV, RFP	Vishal
Loxp-EGFP	EF, loxP sites flanking GFP	Mathias Hampf
EGFP-C1	CMV, EGFP	Clontech
pCDNA/TR6	CMV, Tet Repressor	Invitrogen
pUHC131-1	CMV, Firefly luciferase	Manfred Gossen

Oligos

Label	Sequence
GFP-C	AGACCCCAACGAGAAGCG
GFP-N	TGTGGCCGTTTACGTCGC
3’ Orc1-tet	CCGACTAGTGCATGCTTAGGCCGGCCCTCCACTTTCACA
5’ orc-tet	GAGTACGAGACGTCTAGACTGGACAAGAGCAA
UBBhind-sd2	CTGCAAGGCGATTAAGTT
SV40	CACTGCATTCTAGTTGTGG
Rev bsd	CTGCAATAAACAAGTTTCG
Fwd bsd	CTCATTGAAAGAGCAACGGC
PFB3	CTACAAATGTGGTATGGCTG
PFB5	TATTCCGGATTATTCATACC
5’mid-orc4	CCTTATTATCGGACCCCGAG
Intern sctetR	GCGATGGAGCAAAAGTACAT
CHIP/CMV-1R	GGGCGTACTTGGCATATG
Bla-xho	CGAAAAGTGCCACCTGACG
5’tetR-sc	TATGCCGCCATTATTACGAC
5’-orc1gfp	CGAGTACGAGACGGTGAGCAAGGGCGAGGAG
3’-orc1gfp	CAGTCTAGATTACTTGTACAGCTCGTCCA
5’ TetO (34bp)	GGTGGTGGTCTCTATCACTGATAGGGAGGTGGTGG
5’ T-Orc2	AGGGCCGGCCCTAGGGAGTAAACCAGAATTAAAGGAAGAC
3’ T-Orc2	GTCCCGGGAGCCTCCTCTTCCTTTTCCAAGAAATC
M13rev	AGCGGATAACAATTTCACACAGG
Orc1 for seq2	TGCAGAAGCTAACAGGCC
Horc4-F	CCGACGCACCATGAGCAGTCGTAAATCAAAG
Horc4orf-B	GGCGTCTCTCATAACCAGCTTAGTGAGGA

Media and Buffers

Lysis buffer	1X PBS 2 mM $MgCl_2$ 0.1% NP40 10% Glycerol Protease inhibitors
Bactrial lysis buffer	50 mM NaH_2PO_4 300 mM NaCl 1 % Trition X 100 2.5 mM $MgCl_2$ 20mM Imidazole pH 8 Protease inhibitors
1X DMEM (Complete media)	2 mM L-Glutamine 10% heat inactivated FCS 50 µg/ml penicillin 50 µg/ml streptomycin
Luciferase buffer	15mM MgSO4 25mM glycol glycine, pH 7.8 2mM ATP 50uM Luciferin
Freezing Media	Complete media 10% DMSO
Phosphate Buffered Sodium (PBS)	140 mM NaCl 2.7 mM KCl

	16 mM Na_2HPO_4 1.5 mM KH_2PO_4 0.8 mM EDTA
4x Protein Sample Buffer	1M Tris pH 6.8 20% Glycerol 4% SDS 5% 2 Mercaptoethanol
1x Trypsin in PBS	0.25% (w/v) Trypsin 0.03% (w/v) EDTA
4x SDS loading Buffer	62.5 mM Tris pH 6.8 20% Glycerol 12% SDS 20% 2-Mercaptoethanol 0.1% Bromophenol blue
PMSF Solution	100 mM PMSF in Isopropanol
Coomassie Stain	45% Methanol 10% Glacial Acetic Acid 0.2% Coomassie 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
20x SSC	175 g NaCl 88 g Sodium citrate Add H_2O to 1 litre,pH 7.0
Denaturation solution	1.50 M NaCl 0.50 M NaOH
Neutralization solution	1.50 M NaCl 0.50 M Tris.HCl, pH 7.2 0.001 M EDTA
Blot wash I	2x SSC, 0.10 % SDS
Blot wash II	1x SSC, 0.10 % SDS
Blot wash III	0.1x SSC, 0.10 % SDS
10X TBE	108 g Tris Base 55 g Boric acid 40 ml 0.5 M EDTA, pH 8.0 Add H_2O to 1 litre
50X TAE	242 g Trsis Base 57.1 ml glacial acetic acid 37.2 g $Na_2EDTA.2H_2O$

Super Blotto	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
Coomassie Destain I	40% Methanol 10% Glacial Acetic Acid
Coomassie Destain II	5% Methanol 7% Glacial Acetic Acid
Cellophane wetting solution for Polyacrylamide gel storage	40% Methanol 2% Glycerol

Material for in vitro DNA Binding assay

Streptavidin beads	NEB
Poly (dA). Poly (dT)	Pharmacia
ssDNA	Lab stock
Biotin labeled tetO	Biotez
5x binding buffer	100 mM $MgCl_2$, 100 mM Tris, pH 7.5, 50% glycerol
Talon beads	Clontech
High salt binding buffer	500mM NaCl, 20mM Tris-HCl (pH 7.5) 1mM EDTA

Consumables

Immobilon-P PVDF membrane	Millipore
Hybond N + Nylon membrane	Millipore
X-MR films	KODAK
Cell culture dishes	Techno Plastic Products (TPP)

Special software

DNA strider for analyzing vector maps.
BAS reader & TINA program for analyzing southern blots.
Image quantification was done with NIH Image J programme.
DNA sequence analysis software from Applied Biosystems was used for analyzing DNA sequencing data.