DNA replication initiator proteins facilitate CENPA loading on early replicating compact chromatin

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Supplementary Information

All the strains and primers are listed in Supplemental tables S6 and S7, respectively.

Construction of URA3 integration strains

To construct the individual *URA3* integration cassettes, long primer pairs were designed (Supplementary table S7). Briefly, 70 bp regions both upstream and downstream to the site of integration were incorporated in the primers as overhangs. The 1.4 kb *URA3* gene was amplified from the plasmid *pUC19-URA3* (Mitra, Gomez-Raja et al. 2014) using the aforementioned primers. The PCR products were independently transformed in the *C. albicans* J200 (Thakur and Sanyal 2013). The transformants were selected on CM-Uri and confirmed by PCR (Supplemental Table S7). Three independent transformants of each integration type was taken ahead for the assays. All the distances of individual *URA3* insertions are indicated with respect to the mid-point of *CEN7* which has been taken as Ca21Chr7_427262.

Construction of MTW1-Protein A tagged strains

To tag an endogenous copy of *MTW1* with Protein-A, the MTW1-TAP fragment was amplified from CAKS13 (Roy, Burrack et al. 2011) using primers listed in Supplemental table S7. This fragment was then cloned as a *Notl/SpeI* fragment in pBS-NAT to obtain the plasmid *pMTW1-TAP(NAT)*. This plasmid was linearized by using *PacI* and the resulting cassette was transformed in strain RM1000AH to obtain LSK436 (*MTW1/MTW1-TAP(NAT)*). Subsequently, the *URA3* cassettes for the 4L and 4R insertions were transformed in LSK436. The neocentromere strains: LSK446, LSK459 (5'FOA sensitive) and LSK450, LSK465 (5'FOA resistant), were transformed with *pMTW1-TAP(NAT)* fragment to obtain the strains LSK469/ LSK470/ LSK473/ LSK474 (5'FOA sensitive) and LSK471/ LSK475/ LSK476 (5'FOA resistant). All strains were confirmed by western blot using anti-Protein A antibodies (Sigma cat. no P3775).

Construction of a conditional orc4 mutant

In order to create a conditional null mutant of *orc4* in *C. albicans*, a deletion cassette was constructed as follows: a 368 bp fragment (Ca21Chr5 480170-479721) upstream of *ORF19.4221* was amplified using the primers ORC413/ORC414 from the genomic DNA of SC5314 and cloned as a *KpnI/XhoI* fragment into pSFS2a (Reuss, Vik et al. 2004) to create pORC4US. A 490 bp fragment (Ca21Chr5 478025-477535) downstream to Orf19.4221 was amplified using ORC4 15/ORC416 and cloned as *SacII/SacI* fragment into pORC4US to generate pORC4DEL (Supplemental table S7 for primer list). The resulting plasmid was linearized using *KpnI* and *SacI*, and used to transform *C. albicans* 8675 (Joglekar, Bouck et al. 2008) and selected for nourseothricin resistance to obtain the strain LSK328. The marker was recycled to obtain the nourseothricin sensitive strain LSK329. To conditionally inactivate the remaining allele, a conditional mutant was constructed by cloning the N-terminus of

Orf19.4221(Ca21Chr5 479720-479221) as a *BamHI/Pst*I fragment in pCaDIS (Care, Trevethick et al. 1999). The resulting plasmid (pMET3ORC4) was linearized using *Bgl*II and transformed in LSK329 to obtain independent transformants of the conditional mutant LSK330, LSK331. Similar deletions were performed in SN148 background.

Construction of a conditional mcm2 mutant

In order to create a conditional null mutant of *mcm2* in *C. albicans*, a deletion cassette was constructed as follows: a 474 bp fragment (Ca21ChrR 857151-856675) upstream of *ORF19.4354* was amplified using the primers MCM213/MCM214 from the genomic DNA of SC5314 and cloned as a *KpnI/XhoI* fragment in pSFS2a to create pMCM2US. A 468 bp fragment (Ca21ChrR 853962-853494) downstream to Orf19.4354 was amplified using MCM215/MCM216 and cloned as *SacII/SacI* fragment in pMCM2US to generate pMCM2DEL (See Supplemental table S7 for primer list). The plasmid was digested using *KpnI* and *SacI*, used to transform *C. albicans* 8675 and selected for nourseothricin resistance to obtain the strain LSK309. The marker was recycled to obtain the nourseothricin sensitive strain LSK310. To inactivate the remaining allele, a conditional mutant was constructed by cloning the N-terminus of Orf19.4354 (Ca21ChrR 856674-856164) as a *Bam*HI/*PstI* fragment in pCaDIS (47). The resulting plasmid (pMET3MCM2) was linearized using *Bgl*II and used to transform LSK310 to obtain independent transformats of the conditional mutant LSK311, LSK312 and LSK313. Similar deletions were performed in SN148 background.

Construction of the CEN7 deletion strains (CaCEN7)

To delete one copy of *CEN7*, a cassette was constructed as follows. A 1.4 kb fragment containing a 66 bp upstream sequence (Ca21Chr7 424413-424472) and a 70 bp downstream sequence (Ca21Chr7 428994-429053) of *CEN7* and a marker gene (*CaHIS1*) were amplified from pBS-HIS using the primers mentioned in Supplementary Table S7. The PCR product was used to transform the 5'FOA resistant isolates from the strains LSK443 and LSK456 and their corresponding 5'FOA sensitive isolates. The transformants were selected on complete media lacking histidine (CM-His) and screened by PCR. Transformants in *cis*-orientation (for *URA3* and *HIS1*) were screened on the basis of Southern hybridisation (Southern 1975) (see Supplemental table S3 for Southern strategy).

Generation of Orc4 antibodies

The peptide sequence from *C. albicans* Orc4 (YLPKRKIDKEESSI) was chemically synthesized and conjugated with Keyhole Limpet Hemocyanin (KLH) (GeneMed Synthesis, USA). The conjugated peptide (1 mg/ml) was mixed with equal volumes of Freund's complete adjuvant (Sigma, Cat no. F5881) and used as an antigen to inject non-immunized rabbits as the priming dose. Three subsequent booster doses at an interval of two weeks (per immunization) were given using Freund's incomplete adjuvant (Sigma, Cat no. F5506). Following antibody detection using ELISA, major bleed was

performed. The anti-serum was collected, IgG fractionated and affinity purified against the free peptide (AbGenex, India). The specificity of the purified antibody preparation was confirmed by western blot and immunolocalization experiments.

Media and growth conditions

All strains of *C. albicans* where *URA3* was integrated on Chr7 and Chr5 were propagated in YPD (1% yeast extract, 2% peptone, 2% dextrose) with uridine, unless otherwise specified. All transformations were done in YPDU. The auxotrophs were selected on appropriate selection media, as mentioned previously. For the 5'FOA plating assays, complete media with 2 % agar were supplemented with 1 mg/ml 5'FOA. ChIP experiments for the silenced colonies were done in a) complete media supplemented with 10 mg/ml uridine and 1mg/ml 5'FOA (CM+5'FOA) and b) CM-Uri. Strains with neocentromeres were grown in YPDU. *ORC4* and *MCM2* mutants were grown either in CM-methionine-cysteine or in CM + 5mM methionine +5mM cysteine for the indicated number of hours. CAKS3b (Sanyal and Carbon 2002) was grown in YP with succinate (2%) for expressing CENPA and YP with dextrose (2%) for depleting CENPA for 6 and 8 h for the ChIP experiments.

Silencing assay

Each of the *URA3* integrant was grown in YPDU overnight. Approximately, one million cells from three independent transformants of each kind of integration were plated on CM with 1 mg/ml 5'FOA. The plates were incubated at 30°C up to 72 h. One hundred colonies from each plate were patched on CM-Uri and YPDU. These were simultaneously patched on CM-His and CM-Arg plates to detect events such as loss of the marker gene *URA3* or gene conversion. The colonies showing growth in CM-Uri were counted and the percentage of reversible silencing was determined. These colonies were then taken from the corresponding YPD patch and streaked on CM+5'FOA plates to obtain single 5'FOA resistant colonies for the subsequent ChIP assays.

Chromatin Immunoprecipitation (ChIP)

ChIP experiments for the reversibly silenced colonies were performed as follows. Each colony that was isolated from CM+5'FOA media was inoculated simultaneously in liquid media of CM + 5'FOA and CM-Uri and grown till log phase. Crosslinking was done for 15 min (for CENPA) or 30 min (for Mtw1) using formaldehyde to a final concentration of 1% and cells were quenched using 0.135 mM glycine for 5 min at room temperature. For Orc4 ChIP, cultures were grown in YPDU and crosslinked for 1 h, and processed similarly. Quenched cells were incubated in a reducing environment in presence of 9.5 ml distilled water and 0.5 ml of beta mercapto-ethanol (HiMedia cat no. MB041). Rest of the protocol was followed from (Yadav, Sun et al. 2018). The DNA pellet was finally resuspended in 20 μ l of MilliQ water. All three samples (I, +, -) were subjected to PCR reactions.

Serial passaging of 5'FOA resistant strains in YPDU

5'FOA resistant colonies obtained from two independent transformants of LSK404 and LSK425 were inoculated from their respective glycerol stocks in CM+5'FOA. These cells were harvested, washed and reinoculated into YPDU. Cultures were monitored for their growth and samples were withdrawn after every 4 doublings, for ChIP, with F_0 being the initial culture grown in 5'FOA. Y₄, Y₈, Y₁₂, Y₂₀, Y₂₄ correspond to these reversibly silenced 5'FOA colonies grown on non-selective media for the indicated number of generations. Approximately 50 O.D. cells were harvested from each time point and ChIP was performed using anti-Protein A antibodies to examine the CENPA occupancy in these colonies. The cells from the last time point (Y₂₄) were washed and resuspended in fresh CM+ 5'FOA. Cells from the indicated time points (Y₁₂, Y₂₄) were washed and resuspend in sterile water. Serial dilutions of these along with the parental *URA3* insertion and 5'FOA resistant colony were made and spotted on CM-Uri and CM + 5'FOA. Plates were incubated for 72 h at 30°C and photographed.

Antibodies used

For western blot analysis, we used rabbit anti-Protein A in 1:5000 dilution, anti-Orc4 (1:1000) and anti-PSTAIRE (Abcam, cat No. 9866) in the dilution of 1: 5000. For ChIP, anti-Prot A (3ug/ml), rabbit anti-Orc4 antibodies ($10 \mu g/ml$) and mouse anti-GFP (Roche, cat no. 11814460001) (4 ug/ml) were used.

Western blotting

Approximately 3 O.D. equivalent cells were harvested and precipitated by 12.5% TCA overnight at - 20°C. The pellet was spun down at 13000 rpm and washed with 80% acetone. The pellet obtained was then dried and resuspended in lysis buffer (1% SDS, 1N NaOH) and SDS loading dye. Samples were boiled for 5 min and electrophoresed on a 10% polyacrylamide gel. Protein transfer was performed by semi-dry method for 40 min at 25V. Following protein transfer, the blot was blocked with 5% skimmed milk for an hour. The blot was incubated with primary antibodies (see *Antibodies used*). The blot was washed thrice in PBST (1X PBS + 0.05% Tween) and incubated with goat anti-rabbit IgG-HRP (1:10,000 Bangalore Genei cat No. 105499). Following three PBST washes, the blot was developed using chemi-luminescence method (Super Signal West Pico Chemiluminescent substrate, Thermo scientific, cat No. 34080)

Indirect immuno-fluorescence

Exponentially grown cultures of SC5314 was fixed with 37% formaldehyde. Spheroplasts were made using lysing enzyme and cells were fixed on poly-lysine coated slides using methanol and acetone. Cells were then incubated with 2% skimmed milk to block non-specific binding. Following ten PBS washes, cells were incubated with anti-Orc4 antibodies (1:100) for 1 h in a humid chamber. Post PBS washing, cells were incubated with the Alexa Fluor goat anti-rabbit IgG 568 (Invitrogen, cat. No. 11011) in the dilution of 1:500 for one hour. The slide was mounted on a coverslip using DAPI (10

ng/ul) Sigma cat no. 10236276001. Microscopic images were captured by a laser confocal microscope (Carl Zeiss, Germany) using LSM 510 META software with He/Ne laser (bandpass 565-615 nm) for Alexafluor 568 and a 2-photon laser near IR (bandpass~780 nm) for DAPI. Z-stacks were collected at 0.4-0.5 μ m intervals and stacked projection images were processed in Adobe Photoshop.

Microscopy

For conditional expression of genes under the *MET3* promoter, cells were grown in permissive media (CM -met-cys) overnight. They were then grown in presence of CM + 5 mM met+5mM cys for the indicated time point, corresponding to the repressive phenotype. In each case, the cells were washed twice with water and resuspended in distilled water which was placed on a 2% agarose bed on a glass slide. Images were captured in 100x using Zeiss Axio Observer 7 and processed using ImageJ and Adobe photoshop.

ChIP-qPCR analysis

The input and IP DNA were diluted appropriately and qPCR reactions were set up using primers listed in Supplemental table S7. The CENPA/ Mtw1/Orc4 enrichment was determined by the percentage input method. Two- way ANOVA and Bonferonni post tests were performed to determine statistical significance. All the percent IP values represented in the graphs comparing enrichment values in CM-Uri and CM+5'FOA are the ratio of percent IP of the regions indicated to the corresponding values of *CEN1*, which was used as an internal control to estimate the efficiency of the pulldown. For the ChIP experiments with the neocentromere strains, these values have not been normalised to *CEN1*.

ChIP-sequencing analysis

For the CENPA ChIP-seq, immunoprecipitated DNA and the corresponding DNA from whole cell extracts from strains LSK450 and LSK465 were quantified using Qubit before proceeding for library preparation. Around 5 ng ChIP and total DNA were used to prepare sequencing libraries using NEBNext Ultra DNA library preparation kit for Illumina (NEB, USA). The library quality and quantity were checked using Qubit HS DNA (Thermo Fisher Scientific, USA) and Bioanalyzer DNA high sensitivity kits (Agilent Technologies, USA) respectively. The QC passed libraries were sequenced on Illumina HiSeq 2500 (Illumina Inc., USA). HiSeq rapid cluster and SBS kits v2 were to generate 50 bp single end reads. The reads were independently aligned onto the *C. albicans* SC5314 reference genome (v. 21) and a genome with an altered version of Chr7 using bowtie2 (v. 2.3.2) aligner. For the Orc4 ChIP-seq, subtracted reads were aligned onto the *C. albicans* SC5314 reference genome (v. 21) using bowtie2 (v. 2.3.2) aligner (Langmead, Trapnell et al. 2009). More than 95% of the reads mapped onto the reference genome (Control:97.74%; IP:96.13%). All the alignment files (BAM) were processed to remove PCR duplicate reads using Mark Duplicates module of Picard tools.

These processed BAM files were further taken for identification of peaks by MACS2. These peaks were annotated with the *C. albicans* SC5314 reference and altered assembly annotation files. Visualisation of the aligned reads (BAM files) on the reference genome was performed using Integrative Genome Viewer (IGV) (<u>https://software.broadinstitute.org/software/igv/</u>).

Hi-C analysis

Wild-type C. albicans Hi-C data was downloaded from PRJNA308106 (Burrack, Hutton et al. 2016). FASTQ files containing 2 X 80bp paired-end (PE) reads were analyzed using hiclib package (http://mirnylab.bitbucket.org/hiclib/) (Imakaev, Fudenberg et al. 2012). First, each side of the PE reads was aligned separately to C. albicans reference genome (Ca21) using Bowtie 2 (Langmead and Salzberg 2012) with default parameters except for --very-sensitive option. This step was executed iteratively (iterative mapping) in which 3' truncated reads was aligned to reference genome, starting from first 20 bases with increment of 5 bases in subsequent iteration till it reached to the end of read length. The reads which were uniquely mapped with MAPQ score ≥ 1 were saved at each iteration and rest were subsequently analyzed in next iteration. The alignment results from both sides were paired, keeping those reads which had at least one side aligned and were assigned to restriction fragments. The output read pairs and their alignment information as well as the assigned restriction fragments were saved in HDF5 file format. The fragment filter then removed reads those have: 1) only one side aligned; 2) both sides aligned to same restriction fragment; 3) two sides which were too close to each other. PCR redundancies (duplicates) were also removed and all the unique valid pairs were binned into genomic intervals of 2 kb-5 kb (bin size). The resulted symmetric matrix was processed for bin filtering step, including removal of bins with <50% sequence information in reference genome and removal of 1% bins with low read coverage. Diagonal bins were excluded from further downstream analysis. The genome-wide interaction matrix was generated following bin bias correction as described (Imakaev, Fudenberg et al. 2012). The interaction matrix was then converted to a contact probability matrix where the sum of values in each row/column approached 1. The 3C profile anchored on a bin was generated using single row/column containing the anchor from the matrix. To plot distance-dependent contact probability curves, the mean cis contact probabilities (excluding the bins with 0 values) were calculated for each distance (bin size=2kb) for pericentric and non-pericentric regions. Mann-Whitney U test was performed for pericentric and all cis interactions, as well as for pericentric and non-pericentric interactions.

For the Orc4 binding regions, Hi-C interactions were analyzed according to the chromosome coordinates, different modes identified by DIVERSITY and also based on replication timing (early and late). The heatmap for the full genome was plotted using log-scaled values with a pseudocount of 0.000001 (10⁻⁶). The heatmap for the "ORC-only" was plotted using values for the 2 kb windows overlapping with the midpoints of the origins, using the same scaling and colour scale as the full-

genome heatmap. The violin plots were calculated for 1,000 randomizations of each dataset, where for each randomization, the chromosomal distribution and lengths of the regions were preserved.

Motif analysis

For motif analysis, the *de novo* motif discovery tool DIVERSITY (Mitra, Biswas et al. 2018) was used with default web-server options on the 417 Orc4 ChIP-seq peaks. DIVERSITY is specially developed for ChIP-seq experiments profiling proteins that may bind DNA in more than one way.

Replication timing analysis

To analyze the replication timing of the ORC binding regions, fully processed timing data available in GSE17963_final_data.txt (Koren, Tsai et al. 2010) was used. A larger replication time value implies earlier replication. All the 414 genomic origins were aligned according to their timing scores, and categorized as early (first 207) and late (last 207) origins.

Supplementary figures

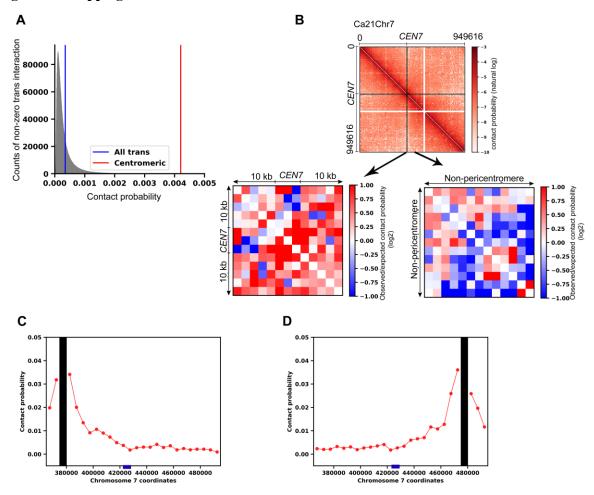


Figure S1. Mapping the inter and intra-chromosomal interactions in C. albicans.

Figure S1. The inter and intra-chromosomal interactions in *C. albicans.* (**A**) A histogram of nonzero *trans* contact probabilities (grey) from the genome-wide interaction matrix depicts that the mean contact probability of all *trans* (black line) is much lower than interactions among centromeric bins (red) (bin size=2kb). (**B**) Heatmaps of observed/expected contact probabilities (bin size=2kb) at Chr7 zoomed into a pericentromeric region (left) and a non-pericentric region (Chr7:440000-466000) with same size (right). The expected matrix was obtained from mean contact probabilities of all *cis* interactions at each distance. (**C**) and (**D**) The 3C profile (bin size=5kb) anchored on a bin 50 kb upstream (Chr7:375000-380000) (black) (B) and another bin 50kb downstream (Chr7:475000-480000) (black) (C) of *CEN7* (blue box). The red dots represent contact probabilities.



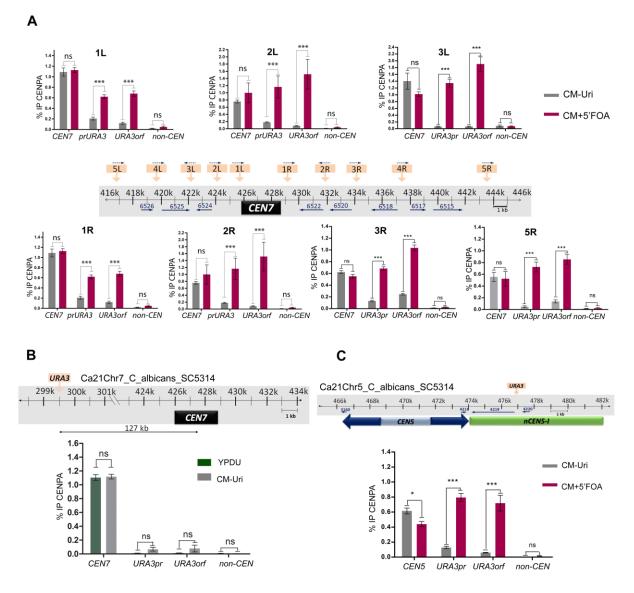


Figure S2. Ectopic centromeres are formed at pericentromeric regions of *C. albicans*. (A) Schematic of *URA3* (orange boxes) integrated at pericentromeres of Chr7 is shown (middle panel). Arrowheads and numbers indicate positions and identities of the ORFs. Corresponding sites (1L,2L...5R) are mentioned as graph titles (see Supplemental table S1 for integration coordinates). Standard ChIP-qPCR analysis (using anti-protein A antibodies) of the 5'FOA resistant colonies obtained from these strains was used to compare CENPA enrichment on the indicated loci (*CEN7*, *URA3pr, URA3orf*, non-*CEN* region) in CM-Uri (grey) and CM+5'FOA (red). (**B**) Schematic of *URA3* integration at a far-CEN locus, 127 kb away from *CEN7*. ChIP q-PCR results of this strain in YPDU (green bar) and CM-Uri (grey bar) show no significant enrichment of CENPA at the *URA3* locus. (**C**) *CEN5* of *C. albicans*, contains a mid-core (light blue) flanked by inverted repeats (dark blue arrows). *URA3* was integrated at the indicated location, at one of the neocentromere hotspots (*nCEN5-II*). ChIP qPCR results for the 5'FOA resistant colonies obtained from the integrant was grown in CM + 5'FOA (red bar) and CM- Uri (grey bar). Percent input values were normalised to corresponding values on *CEN1*. Statistical significance was determined by two-way ANOVA followed by Bonferroni post-tests (*** p<0.001, ** p<0.01, ns: p>0.05).

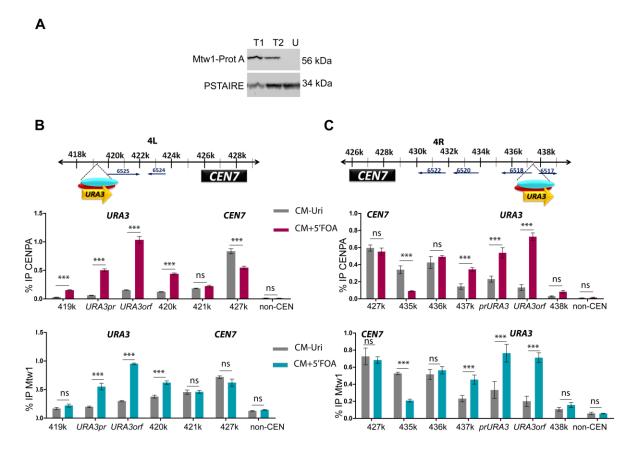


Figure S3. Kinetochore binding to ectopic centromeres is restricted to the silent *URA3* **locus.** (A) Western blot analysis determines the expression level of the endogenous copy of *MTW1* tagged with Protein A (Prot A) in the strain RM1000AH. Mtw1-Prot A could be detected as a 56 kDa band (T₁, T₂) which was absent in the untagged control (U). PSTAIRE was used as the loading control. (B) Both CENPA and Mtw1 bind to the ectopic centromere at *URA3* when the 5'FOA resistant colonies from LSK404 (*4L/4L::URA3 CSE4/CSE4-TAP*) (top panels) and LSK437 (*4L/4L::URA3 CEN7 MTW1/MTW1-TAP*) (bottom panels), are grown in CM +5'FOA (red/ blue bar) or CM-Uri (grey bar). Primers flanking the *URA3* locus (Supplementary table S7) were used to check for the extended binding of CENPA and Mtw1 beyond *URA3*. (C) Similar ChIP-qPCR assays were done for 5'FOA resistant colonies from LSK425 (*4R/4R::URA3 CEN7 CSE4/CSE4-TAP*) and LSK440 (*4R/4R::URA3 MTW1/MTW1-TAP*). Percent input values were normalised to *CEN1*. ChIP q-PCR was performed in three independent transformants and technical triplicates for each transformant. Statistical significance was determined by two-way ANOVA followed by Bonferroni post-tests (*** p<0.001, ** p<0.01, ns: p>0.05).

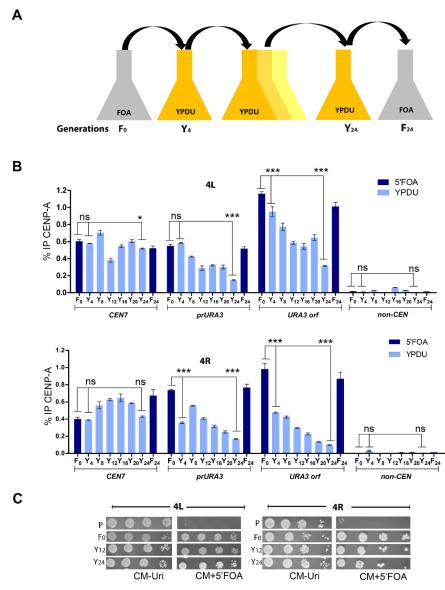


Figure S4. Ectopic kinetochore formed at *URA3* **is transient and unstable.** (A) Schematic of the experiment showing serial passaging of 5'FOA resistant colonies obtained from the strains LSK404 (*4L/4L::URA3 CSE4/CSE4-TAP*) and LSK425 (*4R/4R::URA3 CSE4/CSE4-TAP*) in non-selective media (YPDU). The primary 5'FOA resistant colony was grown in YPDU for the indicated number of generations and then regrown in CM+5'FOA. (B) ChIP using anti-Protein A antibodies followed by qPCR analysis reveals a steady decline in enrichment at *URA3* when cells were passaged in the non-selective media (light blue) Percent input values were normalised to *CEN1*. ChIP-qPCR was performed in two independent transformants of 4L (top) and 4R (bottom) with technical triplicates for each transformant. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-tests (*** p<0.001, ** p<0.01, ns: p>0.05). (C) A spotting assay showing the frequency of reversible silencing of the 5'FOA resistant colonies from strains 4L (left) and 4R (right) after they were grown in non-selective media for the indicated number of generations. Individual panels show serially diluted cultures of the 5'FOA sensitive strain (P), primary 5'FOA resistant colony (F₀) and 5'FOA resistant colony grown in YPD for 12 (Y₁₂) and 24 (Y₂₄) generations, spotted on CM+5'FOA and CM-Uri plates. Plates were incubated for 48 h at 30°C and then photographed.

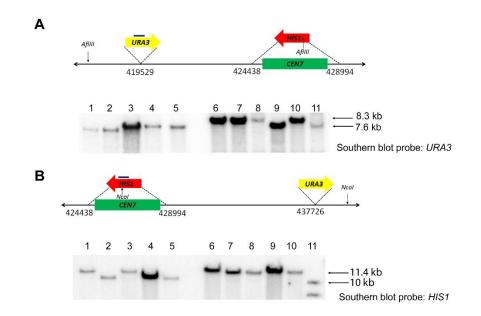


Figure S5. Southern analysis of *CEN7* **deletion strains.** (**A**) A line diagram showing restriction sites of pericentromeres of Chr7 when *URA3* (yellow arrow) is located at 4L (7.7 kb left of *CEN7*). *CEN7* (CaChr7 424475-428993) (green) has been replaced with *HIS1* (red). Genomic DNA from strains LSK445, LSK446, LSK 447, LSK 448, LSK449, LSK450, LSK451, LSK452, LSK453, LSK454 and LSK455 (lanes 1-11) were digested with *AfI*III, Southern hybridized and probed with a *URA3* fragment. The desired band of 8.3 kb suggests the presence of *URA3* and *HIS1* on the same homolog of Chr7. (**B**) A line diagram showing restriction digestion of pericentromeres of Chr7 when *URA3* is located at 4R (10.4 kb right of *CEN7*). *CEN7* (CaChr7 424475-428993) has been replaced with *HIS1*. Genomic DNA from strains LSK459, LSK460, LSK461, LSK462, LSK463, LSK464, LSK465, LSK466, LSK467 and LSK468 (lanes 1-10) were digested with *Nco*I, Southern hybridized and probed with a *HIS1* fragment. The desired band of 11.4 kb suggests the presence of *URA3* and *HIS1* on the same homolog of Chr7.

Figure S6

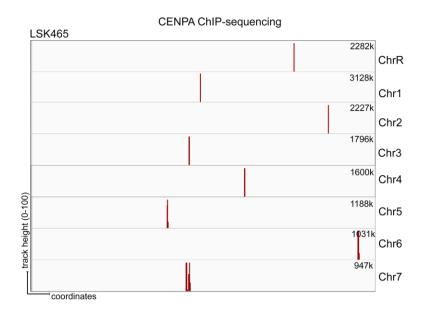


Figure S6. CENPA ChIP sequencing of a *CEN7* **deletion strain.** ChIP-sequencing using anti-GFP antibodies in the strain LSK465 (*CSE4/CSE4-GFP-CSE4 4R/4R::URA3 CEN7/CEN7::HIS1*) reveals a single peak on all chromosomes, except Chr7 that shows two closely spaced CENPA peaks, centromere (*CEN7*) and neocentromere (*URA3nCEN7-II*).

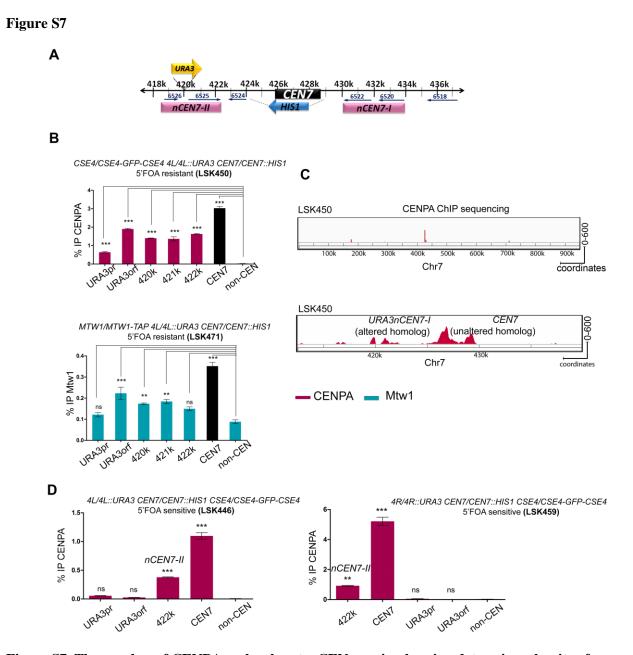
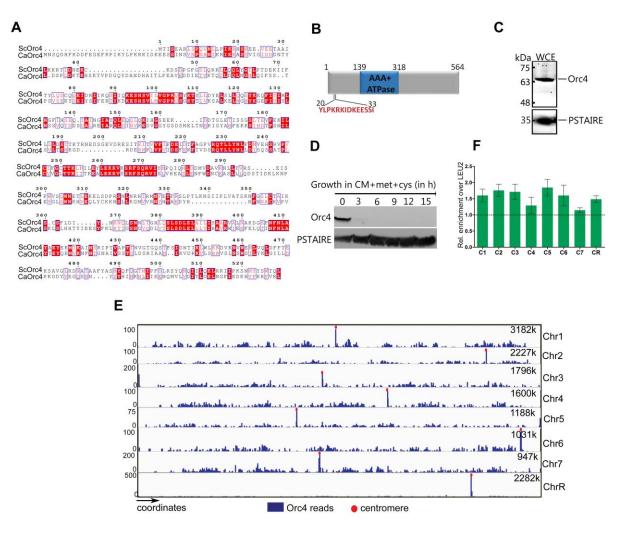
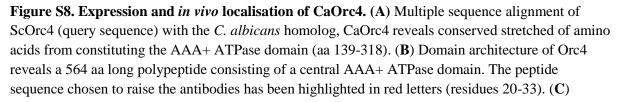


Figure S7. The number of CENPA molecules at a CEN proximal region determines the site of neocentromere formation. (**A**) In the diploid *C. albicans*, only one homolog of Chr7 where *CEN7* (CaChr7 424475-428994) has been replaced with *HIS1* in a *URA3* integrant (CaChr7 419529-419530) is shown. (**B**) Top panel indicates relative enrichment of CENPA at native *CEN7* from the unaltered homolog (black) and the neocentromere locus *URA3nCEN7-I* (red) in the 5'FOA resistant strain LSK450 (*CSE4/CSE4-GFP-CSE4 4L/4L::URA3 CEN7/CEN7::HIS1*). Bottom panel indicates relative enrichment of Mtw1 at *CEN7* (black bar) and *URA3nCEN7-I* (blue) at the native centromere (427k) in LSK471 (*CSE4/CSE4-GFP-CSE4 4L/4L::URA3 CEN7/CEN7::HIS1 MTW1/MTW1-TAP*). Relative enrichment of CENPA and Mtw1 indicate that neocentromere formed on the altered homolog

(*URA3nCEN7-II*) was mapped to a region surrounding the integration locus (CaChr7 435078-440387) (***p<0.001, ** p<0.01, ns p>0.05). (C) CENPA ChIP-sequencing confirmed the presence of neocentromere in the strain LSK450, where the profile is a combination of two peaks, the one at *CEN7* is on the unaltered homolog the one at *URA3nCEN7-I* is on the altered homolog. A 30 kb region harbouring *CEN7* depicts the track height (as on IGV) on *y*-axis and coordinates on the *x*-axis. (D) CENPA ChIP followed by qPCR in the 5'FOA sensitive strains LSK446 (*CSE4/CSE4-GFP-CSE4 4L/4L::URA3 CEN7/CEN7::HIS1*) (left panel) and LSK459 (*CSE4/CSE4-GFP-CSE4 4R/4R::URA3 CEN7/CEN7::HIS1*) (right panel) indicates that neocentromeres are activated at the previously identified hotspot *nCEN7-II*. There was no CENPA enrichment seen on *URA3*. The experiment was performed in two independent transformants for each type of neocentric strain. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-test (*** p<0.001, ** p<0.01, ns: p>0.05).

Figure S8





Expression of Orc4 was verified by western blot with anti-Orc4 antibodies using whole cell extract (WCE) from C. albicans SC5314. Orc4 yielded a band at the expected molecular weight at ~64 kDa in a denaturing SDS PAGE. PSTAIRE was used as the loading control. (D) Western blot analysis using anti-Orc4 antibodies indicates time course depletion of Orc4 in the conditional mutant LSK330 when the strain was grown for the indicated time (0, 3h, 6h, 9h, 12h, 15h) in presence of 5mM met +5mM cys. PSTAIRE was used as the loading control. (E) ChIP-sequencing analysis revealed that Orc4 binds to discrete genomic sites in C. albicans. The total Orc4 reads (blue histogram) were obtained by subtracting the relative number of sequencing reads from the whole cell lysate from the Orc4 ChIP sequence reads and aligning them to the reference genome C. albicans SC5314 Assembly 21. The x-axis represents chromosome coordinates while the yaxis represents track height as visualised in IGV. (F) Orc4 ChIP followed by standard qPCR assays were used to validate the binding of Orc4 on one region in each of the eight C. albicans chromosomes. Primers corresponding to an Orc4-enriched region on each chromosome (C1- C7, CR) were used to amplify the Orc4 ChIP DNA. qPCR analysis was performed to calculate the relative enrichment of Orc4 at each of these chromosomal regions over a control region (LEU2). Relative enrichment values were plotted as mean of three technical replicates \pm SD.



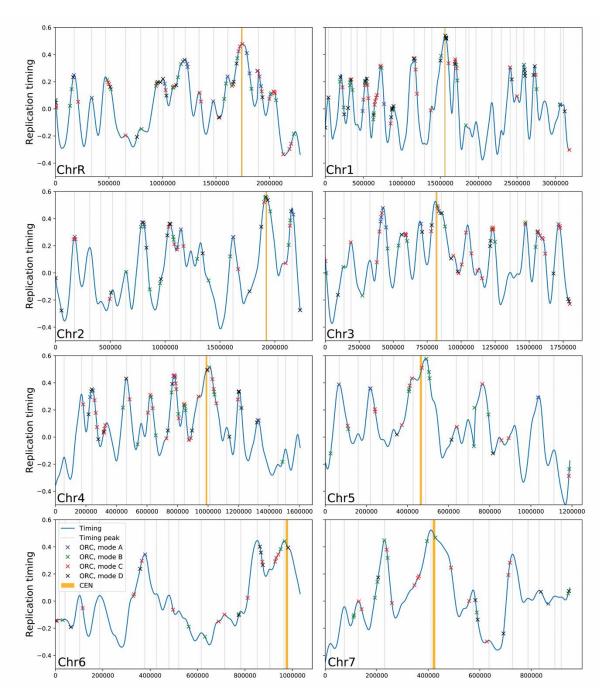


Figure S9. Replication timing profile of various modes associated with Orc4 binding. Orc4 ChIP-seq peaks were aligned to the replication timing profile obtained from *C. albicans* from a previous report (Koren, Tsai et al. 2010). Color-coded stars indicate each of the four motifs identified by DIVERSITY which covers all the 414 chromosomal origins. Peaks represent early replicating regions, including the centromere (yellow lines). A significant fraction of the modes cluster towards the local maxima of the peaks. The *x*-axis represents chromosomal coordinates and *y*-axis shows replication timing scores.

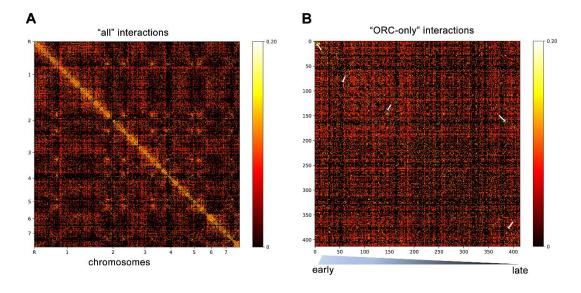


Figure S9. Early replicating regions interact among themselves to form clusters/ replication factories. (**A**) The Hi-C heatmap shows a whole-genome "all" heatmap representation of the Hi-C data (Burrack, Hutton et al. 2016) as a 7145x7145 matrix. The maximum value in the data was 0.2015 and the minimum was zero. For plotting, the values were log-transformed with a pseudocount of 0.0001. (**B**) The Hi-C "ORC-only" heatmap shows interactions between the 414 chromosomal ORC binding regions, ordered by timing (early to late), to the same colour scale as in (A). White arrows directing towards the yellow pixels indicate clustered/ strongly interacting origins. The analysis was performed at a resolution of 2 kb.

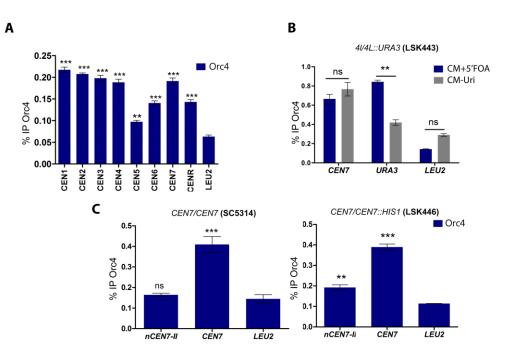


Figure S11

Figure S11. Orc4 binds to ectopic and neocentromeres in C. albicans. (A) Orc4 ChIP followed by standard qPCR assays was used to validate the enrichment of Orc4 at all C. albicans centromeres. Primers corresponding to the central core of each CEN was used to amplify IP DNA (Supplementary table S7). LEU2 was used as a control region. The experiment was performed in two replicates of SC5314. Statistical significance was determined by one-way ANOVA followed by Bonferroni posttests (*** p<0.001, ** p<0.01, ns: p>0.05). (B) Orc4 shows significant enrichment in the conditional ectopic centromere formed on URA3. Orc4 ChIP followed by qPCR analysis in the strain LSK443 (4L/4L::URA3) revealed the significant enrichment of Orc4 on URA3orf in CM+5'FOA over CM-Uri. ChIP was performed in two independent transformants and q-PCR was performed with three technical replicates for each transformant. Statistical significance was determined by two-way ANOVA followed by Bonferroni post-tests (*** p<0.001, ** p<0.01, ns: p>0.05). (C) Orc4 binds to neocentromeres in C. albicans. Orc4 ChIP qPCR in the wild type (CEN7/CEN7) (left side) and CEN7 deletion strain LSK446 (CEN7/CEN7::HIS1) (right panel) indicates significant enrichment of Orc4 at nCEN7-II, the neocentromere hotspot over the control region (LEU2). ChIP qPCR was performed in three independent transformants with three technical replicates for each transformant. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-tests (*** p<0.001, ** p<0.01, ns: p>0.05).

Figure S12

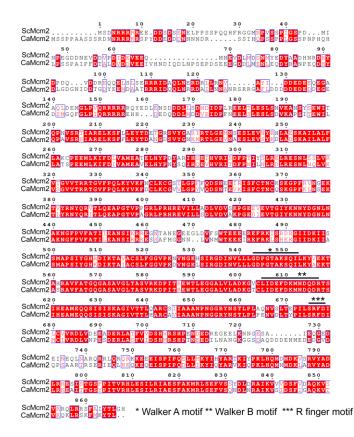


Figure S12. Mcm2 is a highly conserved protein in *C. albicans*. A multiple sequence alignment showing the protein sequences of *S. cerevisiae* Mcm2, ScMcm2 (query sequence) and *C. albicans* Orf19.4354 (CaMcm2) displays the conserved MCM box containing the Walker A, Walker B and the R finger motifs, indicated as stars.



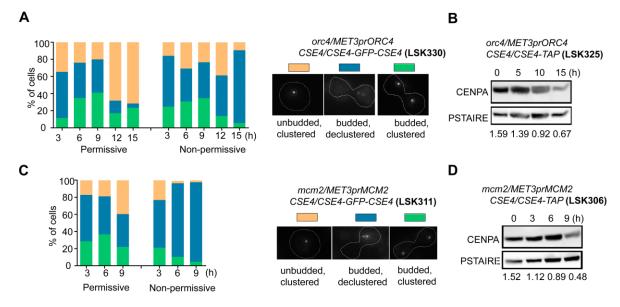


Figure S13. Orc4 and Mcm2 affect chromosome segregation and CENPA stability. (A) CENPA (clustered kinetochore) segregation pattern was examined in an orc4 conditional mutant LSK330. The strain was grown either in CM-met-cys or CM+5mM met +5mM cys for 3,6,9,12 and 15h to shut down the expression of ORC4 and the percentage of cells showing a specific segregation phenotype of clustered kinetochores in small budded (yellow), unsegregated budded (blue) and segregated budded (green) was counted. Approximately 100 cells from three independent transformants of orc4 mutant were analyzed for this assay, where 80% of the orc4 mutants displayed abrogated kinetochore segregation. (B) Western blot using anti-Protein A antibodies shows time dependant decrease in CENPA levels when Orc4 is depleted for 0,5,10,15 h, when normalized with the loading control, PSTAIRE. (C) CENPA (clustered kinetochore) segregation pattern was examined in an mcm2 conditional mutant LSK311. The strain was grown either in CM-met-cys or CM+ 5mM met+ 5mM cys for 3,6,9 h to shut down the expression of MCM2. Approximately 100 cells from three independent transformants of orc4 mutant were analyzed for this assay, where 80% of the mcm2 mutants displayed unsegregated kinetochore. (D) Western blot showing protein levels of CENPA upon depletion of Mcm2 for 3,6,9 h showed dramatic reduction in CENPA after 6 h of Mcm2 depletion. Normalization was performed using PSTAIRE.

Supplemental tables:

Type of insertion	Coordinate of insertion	Distance from mid-CEN
5L	Ca21Chr7 417202-417203	10 kb (left of CEN7)
4L	Ca21Chr7 419529-419530	7.7 kb (left of CEN7)
3L	Ca21Chr7 422037-422038	5.2 kb (left of CEN7)
2L	Ca21Chr7 423682-423683	3.5 kb (left of CEN7)
1L	Ca21Chr7 425563-425564	1.7 kb (left of CEN7)

Supplemental table S1. Coordinates for URA3 insertion in C. albicans

1R	Ca21Chr7 429198-429199	1.9 kb (right of CEN7)
2R	Ca21Chr7 432145-432146	4.9 kb (right of CEN7)
3R	Ca21Chr7 434069-434070	6.8 kb (right of CEN7)
4R	Ca21Chr7 437729-437730	10.4 kb (right of CEN7)
5R	Ca21Chr7 443546-443547	16.2 kb (right of CEN7)
Far-CEN	Ca21Chr7 299510-299511	127 kb (left of CEN7)
CEN5int	Ca21Chr5 477918-477919	7.5 kb (right of CEN5)

Supplemental table S2. Frequency of reversible silencing of URA3 integration strains

Integration type	Transformant no.	No. of 5'FOA resistant colonies analyzed	% reversible silencing (% 5'FOA ^r URI ⁺ HIS ⁺ ARG ⁺)
5L	1	107	ND
	2	73	ND
	3	95	ND
4L	1	116	0.862
	2	103	ND
	3	117	0.854
3L	1	86	1.162
	2	96	2.083
	3	98	2.04
2L	1	97	1.03
	2	117	2.564
	3	100	ND
1L	1	110	10
	2	158	14.556
	3	160	3.125
CEN7::URA3/CEN7	J151	74	97.297
	J153	61	78.688
	J154	79	94.936
1R	1	101	98.019
	2	58	100
	3	78	91.025
2R	1	118	1.694
	2	111	0.9
	3	100	1
3R	1	111	0.9
	2	108	0.925
	3	88	1.136
4R	1	97	3.09
	2	96	1.04
	3	101	1.98

5R	1	138	0.724
	2	157	1.273
	3	100	ND
far-CEN	1	200	ND
	2	200	ND
	3	205	ND
CEN5 int	1	98	4.08
	2	114	1.75
	3	89	ND

ND= Not determined

Supplemental table S3. Southern blot strategy for *CEN7* deletion strains

Strain	Restriction	Primers used to amplify	Size of the expected
	enzyme	probe (length of probe)	band/wild type
4.5 kb CEN7 deletion	AflIII	URA3RT1, URA3 RT4	8.3/7.6 kb
in URA3 at 4L locus		(870 bp)	
4.5 kb CEN7 deletion	NcoI	HIS ORF_2, HIS ORF_1	11.4/10 kb
in URA3 at 4R locus		(480 bp)	

Supplemental table S4. Neocentromere coordinates of *CEN7* deletion strains (from CENPA ChIP-sequencing analysis)

Strain	Description	Coordinates for neocentromere
LSK450	4.5 kb CEN7 deleted in 5'FOA resistant URA3	Ca21Chr7 419629-422084
	integrant (4R)	
LSK465	4.5 kb CEN7 deleted in 5'FOA resistant URA3	Ca21Chr7 435078-440387
	integrant (4L)	

Supplemental table S5. Chromosomal coordinates for Orc4 binding at centromeres based on *C*. *albicans* Assembly 21

Centromere	CENPA binding region	Orc4 binding region
	Coordinates	Coordinates
	(length)	(length)
1	Ca21Chr1 15662315-1566930	Ca21Chr1 1562748- 1566244
	(4616 bp)	(3497 bp)
2	Ca21Chr2 1925206- 1929688	Ca21Chr2 1926183- 1929443
	(4483 bp)	(3261 bp)

3	Ca21Chr3 822762-827727	Ca21Chr3 823057- 826863
	(4966 bp)	(3807 bp)
4	Ca21Chr4 991382-996030	Ca21Chr4 992010- 995522
	(4649 bp)	(3513 bp)
5	Ca21Chr5 4673814-472497	Ca21Chr5 468552- 471618
	(5114 bp)	(3067 bp)
6	Ca21Chr6 979686-984007	Ca21Chr6 980541- 983910
	(4322 bp)	(3370 bp)
7	Ca21Chr7 425129- 431652	Ca21Chr7 425910- 429297
	(6524 bp)	(3388 bp)
R	Ca21ChrR 1742833- 1748598	Ca21ChrR 1743951- 1747274
	(5766 bp)	(3324 bp)

Supplementary table S6. Strains used in the study. (FOA^r, 5'FOA resistant; FOA^s, 5'FOA

sensitive)

Name	Genotype	Description	Referenc
			e
J200	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	CSE4-TAP(NAT) in	(Thakur
	∆his1::hisG arg4::HIS1/ARG4 CSE4/CSE4-	RM1000AH	and
	TAP(NAT)		Sanyal
			2013)
8675	<u> Лига3::imm434/Лига3::imm434,</u>	CSE4-GFP-	(Joglekar,
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	CSE4/CSE4	Bouck et
	CSE4-GFP-CSE4/CSE4		al. 2008)
LSK401	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	5L_T1	This
	Δhis1::hisG arg4::HIS1/ARG4 5L/5L::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK402	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	5L_T2	This
	Δhis1::hisG arg4::HIS1/ARG4 5L/5L::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK403	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	5L_T3	This
	Δhis1::hisG arg4::HIS1/ARG4 5L/5L::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK404	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	4L_T1	This
	Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK405	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	4L_T2	This
	Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3		study
	CSE4/CSE4-TAP(NAT)		

LSK406	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	4L_T3	This
LSK400	Δhis1::hisG arg4::HISI/ARG4 4L/4L::URA3	4L_13	study
	<i>CSE4/CSE4 -TAP(NAT)</i>		study
LSK407	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	3L T1	This
LJIX407	Δhis1::hisG arg4::HISI/ARG4 3L/3L::URA3	56_11	study
	CSE4/CSE4-TAP(NAT)		study
LSK408	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	3L T2	This
LSK400	Δhis1::hisG arg4::HISI/ARG4 3L/3L::URA3	512_12	study
	CSE4/CSE4-TAP(NAT)		study
LSK409	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	3L_T3	This
LOIGHU	Δhis1::hisG arg4::HISI/ARG4 3L/3L::URA3	51_15	study
	CSE4/CSE4-TAP(NAT)		study
LSK410	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	2L_T1	This
LOIGHIO	Δhis1::hisG arg4::HISI/ARG4 2L/2L::URA3	20_11	study
	CSE4/CSE4-TAP(NAT)		study
LSK411	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	2L_T2	This
LOILIII	Δhis1::hisG arg4::HIS1/ARG4 2L/2L::URA3	20_12	study
	CSE4/CSE4-TAP(NAT)		study
LSK412	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	2L T3	This
LOILIIL	Δhis1::hisG arg4::HISI/ARG4 2L/2L::URA3	22_10	study
	CSE4/CSE4-TAP(NAT)		
LSK413	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	1L_T1	This
	Ahis1::hisG arg4::HIS1/ARG4 1L/1L::URA3	_	study
	CSE4/CSE4-TAP(NAT)		5
LSK414	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	1L_T2	This
	Δhis1::hisG arg4::HIS1/ARG4 1L/1L::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK415	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	1L_T3	This
	Δhis1::hisG arg4::HIS1/ARG4 1L/1L::URA3		study
	CSE4/CSE4TAP(NAT)		
J151	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	CEN7::URA3_T1	(Thakur
	Δhis1::hisG arg4::HIS1/ARG4 CEN7/CEN7::URA3		and
	CSE4/CSE4-TAP(NAT)		Sanyal
			2013)
J153	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	CEN7::URA3_T2	(Thakur
	∆his1::hisG arg4::HIS1/ARG4 CEN7/CEN7::URA3		and
	CSE4/CSE4-TAP(NAT)		Sanyal
			2013)
J154	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	CEN7::URA3_T3	(Thakur
	∆his1::hisG arg4::HIS1/ARG4 CEN7/CEN7::URA3		and
	CSE4/CSE4-TAP(NAT)		Sanyal
			2013)
LSK416	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	1R_T1	This
	∆his1::hisG arg4::HIS1/ARG4 1R/1R::URA3		study
	CSE4/CSE4-TAP(NAT)		

LSK417	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	1R_T2	This
Lon	Δhis1::hisG arg4::HISI/ARG4 1R/1R::URA3	11(_12	study
	CSE4/CSE4-TAP(NAT)		study
LSK418	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	1R_T3	This
	Δhis1::hisG arg4::HIS1/ARG4 1R/1R::URA3		study
	CSE4/CSE4-TAP(NAT)		j
LSK419	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	2R_T1	This
	<i>Ahis1::hisG arg4::HIS1/ARG4 2R/2R::URA3</i>		study
	CSE4/CSE4-TAP(NAT)		j
LSK420	$\Delta ura3::imm434/\Delta ura3::imm434\Delta his1::hisG/$	2R_T2	This
	Ahis1::hisG arg4::HIS1/ARG4 2R/2R::URA3	_	study
	CSE4/CSE4-TAP(NAT)		j
LSK421	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	2R_T3	This
	Ahis1::hisG arg4::HIS1/ARG4 2R/2R::URA3		study
	CSE4/CSE4-TAP(NAT)		j
LSK422	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	3R_T1	This
	Δhis1::hisG arg4::HIS1/ARG4 3R/3R::URA3		study
	CSE4/CSE4-TAP(NAT)		j
LSK423	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	3R T2	This
2011.20	Δhis1::hisG arg4::HISI/ARG4 3R/3R::URA3	011_11	study
	CSE4/CSE4-TAP(NAT)		~~~~~
LSK424	$\Delta ura3::imm434/\Delta ura3::imm434\Delta his1::hisG/$	3R_T3	This
	∆his1::hisG arg4::HIS1/ARG4 3R/3R::URA3		study
	CSE4/CSE4-TAP(NAT)		j
LSK425	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	4R_T1	This
	Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3	—	study
	CSE4/CSE4-TAP(NAT)		5
LSK426	$\Delta ura3::imm434/\Delta ura3::imm434\Delta his1::hisG/$	4R T2	This
	∆his1::hisG arg4::HIS1/ARG4 4R/4R::URA3		study
	CSE4/CSE4-TAP(NAT)		5
LSK427	$\Delta ura3::imm434/\Delta ura3::imm434\Delta his1::hisG/$	4R_T3	This
	Ahis1::hisG arg4::HIS1/ARG4 4R/4R::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK427	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	5R_T1	This
	Δhis1::hisG arg4::HIS1/ARG4 5R/5R::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK428	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	5R_T2	This
	Δhis1::hisG arg4::HIS1/ARG4 5R/5R::URA3		study
	CSE4/CSE4-TAP(NAT)		
LSK430	Дига3::imm434/ Дига3::imm434 Дhis1::hisG/	CEN5_T1	This
	∆his1::hisG arg4::HIS1/ARG4 CEN5/CEN5-7		study
	kb_right :: URA3 CSE4/CSE4TAP(NAT)		
LSK431	Δura3::imm434/ Δura3::imm434 Δhis1::hisG/	CEN5_T2	This
	Δhis1::hisG arg4::HIS1/ARG4 CEN5/CEN5-7		study
	kb_right :: URA3 CSE4/CSE4-TAP(NAT)		

LSK432	Лига3::imm434/Лига3::imm434 Дhis1::hisG/	CEN5 T2	This
L3K432		CEN5_T3	
	Δhis1::hisG arg4::HIS1/ARG4 CEN5/CEN5-7		study
X (7X 100	kb_right :: URA3 CSE4/CSE4-TAP(NAT)		
LSK433	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	FAR URA_T1	This
	<i>Ahis1::hisG arg4::HIS1/ARG4 CEN7::URA3_127</i>		study
	kb farCEN/ CEN7 CSE4/CSE4-TAP(NAT)		
LSK434	Δura3::imm434/ Δura3::imm434 Δhis1::hisG/	FAR URA_T2	This
	∆his1::hisG arg4::HIS1/ARG4 CEN7::URA3_127		study
	kb farCEN/ CEN7 CSE4/CSE4-TAP(NAT)		
LSK435	Δura3::imm434/ Δura3::imm434 Δhis1::hisG/	FAR URA_T3	This
	∆his1::hisG arg4::HIS1/ARG4 CEN7::URA3_127		study
	kb farCEN/ CEN7 CSE4/CSE4-TAP(NAT)		
LSK436	<u>Лига3::imm434/ Лига3::imm434 Дhis1::hisG/</u>	MTW1-TAP IN	This
	∆his1::hisG arg4::HIS1/ARG4 MTW1/MTW1-	RM1000AH	study
	TAP(NAT)		staaj
LSK437	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	MTW1-TAP IN	This
LUITJ/	Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3	4L T1	study
		4L_11	study
L CIZ 420	MTW1/MTW1-TAP(NAT)		This
LSK438	$\Delta ura3::imm434/\Delta ura3::imm434 \Delta his1::hisG/$	MTW1-TAP IN	
	$\Delta his1::hisG arg4::HIS1/ARG4 4L/4L::URA3$	4L_T2	study
	MTW1/MTW1-TAP(NAT)		
LSK439	Δura3::imm434/Δura3::imm434 Δhis1::hisG/	MTW1-TAP IN	This
	∆his1::hisG arg4::HIS1/ARG4 4L/4L::URA3	4L_T3	study
	MTW1/MTW1-TAP(NAT)		
LSK440	∆ura3::imm434/ ∆ura3::imm434 ∆his1::hisG/	MTW1-TAP IN	This
	∆his1::hisG arg4::HIS1/ARG4 4R/4R::URA3	4R_T1	study
	MTW1/MTW1-TAP(NAT)		
LSK441	Δura3::imm434/ Δura3::imm434 Δhis1::hisG/	MTW1-TAP IN	This
	∆his1::hisG arg4::HIS1/ARG4 4R/4R::URA3	4R T2	study
		_	•
	MTW1/MTW1-TAP(NAT)		
LSK442	<i>MTW1/MTW1-TAP(NAT)</i> <i>Aura3::imm434/Aura3::imm434_Ahis1::hisG/</i>	MTW1-TAP IN	This
LSK442	Δura3::imm434/ Δura3::imm434 Δhis1::hisG/	MTW1-TAP IN 4R T3	This
LSK442	Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3	MTW1-TAP IN 4R_T3	This study
	Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW- TAP(NAT)	4R_T3	study
YJB867	Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW- TAP(NAT) Δura3::imm434/Δura3::imm434,		study This
	Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW- TAP(NAT) Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG,	4R_T3	study
YJB867 5	Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW- TAP(NAT) Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4	4R_T3 Cse4-GFP	study This study
YJB867	Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW- TAP(NAT) Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4 Δura3::imm434/Δura3::imm434,	4R_T3 Cse4-GFP 4L in Cse4-	study This study This
YJB867 5	$\label{eq:aura3::imm434/dura3::imm434 \Delta his1::hisG/\\ \Delta his1::hisG arg4::HIS1/ARG4 4R/4R::URA3\\ MTW1/MTW- TAP(NAT)\\ \Delta ura3::imm434/\Delta ura3::imm434,\\ \Delta his1::hisG/\Delta his1::hisG , \Delta arg4::hisG/\Delta arg4::hisG,\\ CSE4/CSE4-GFP-CSE4\\ \Delta ura3::imm434/\Delta ura3::imm434,\\ \Delta his1::hisG/\Delta his1::hisG , \Delta arg4::hisG/\Delta arg4::hisG,\\ \end{array}$	4R_T3 Cse4-GFP	study This study
YJB867 5 LSK443	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1	study This study This study
YJB867 5	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1 4L in Cse4-	study This study This
YJB867 5 LSK443	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1	study This study This study
YJB867 5 LSK443	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1 4L in Cse4-	study This study This study This This
YJB867 5 LSK443	$\label{eq:alpha} \begin{split} \Delta ura3::imm434 \ \Delta his1::hisG \ \Delta his1::hisG \ arg4::HIS1/ARG4 \ 4R/4R::URA3 \ MTW1/MTW- TAP(NAT) \ \Delta ura3::imm434 \ \Delta ura3::imm434 \ \Delta his1::hisG \ \Delta his1::hisG \ \Delta his1::hisG \ \Delta arg4::hisG \ \Delta arg4::hisG, \ CSE4/CSE4-GFP-CSE4 \ \Delta ura3::imm434 \ \Delta his1::hisG \ \Delta his1::hisG \ \Delta arg4::hisG \ \Delta arg4::hisG, \ 4L/4L::URA3 \ CSE4/CSE4-GFP-CSE4 \ \Delta ura3::imm434 \ \Delta ura3::imm434 \ \Delta ura3::imm434, \ \Delta his1::hisG \ \Delta arg4::hisG, $	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1 4L in Cse4-	study This study This study This This
YJB867 5 LSK443 LSK444	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1 4L in Cse4- GFP_T2	study This study This study This study
YJB867 5 LSK443 LSK444	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1 4L in Cse4- GFP_T2 4L in Cse4-	studyThis studyThis studyThis studyThis studyThis studyThis studyThis study
YJB867 5 LSK443 LSK444	$\label{eq:alpha} \begin{split} \Delta ura3::imm434 \ \Delta his1::hisG/\\ \Delta his1::hisG \ arg4::HIS1/ARG4 \ 4R/4R::URA3\\ MTW1/MTW- \ TAP(NAT)\\ \Delta ura3::imm434/\ \Delta ura3::imm434,\\ \Delta his1::hisG/\ \Delta his1::hisG \ , \ \Delta arg4::hisG/\ \Delta arg4::hisG,\\ CSE4/CSE4-GFP-CSE4\\ \Delta ura3::imm434/\ \Delta ura3::imm434,\\ \Delta his1::hisG/\ \Delta his1::hisG \ , \ \Delta arg4::hisG/\ \Delta arg4::hisG,\\ 4L/4L::URA3 \ CSE4/CSE4-GFP-CSE4\\ \Delta ura3::imm434/\ \ \Delta ura3::imm434,\\ \Delta his1::hisG/\ \ \Delta his1::hisG \ , \ \ \Delta arg4::hisG/\ \ \Delta arg4::hisG,\\ 4L/4L::URA3 \ \ CSE4/\ \ CSE4-\ \ CSE4-\ \ \ \Delta ura3::imm434,\\ \Delta his1::hisG/\ \ \ \Delta his1::hisG \ \ \ \Delta arg4::hisG,\\ 4L/4L::URA3 \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$	4R_T3 Cse4-GFP 4L in Cse4- GFP_T1 4L in Cse4- GFP_T2 4L in Cse4-	studyThis studyThis studyThis studyThis studyThis studyThis studyThis study

	4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1		
LSK447	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^s , in <i>cis</i>)	study
LSK448	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^s , in <i>trans</i>)	study
LSK449	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^s , in <i>trans</i>)	study
LSK450	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^r , in <i>cis</i>)	study
LSK451	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^r , in <i>cis</i>)	study
LSK452	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^r , in <i>trans</i>)	study
LSK453	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	<i>∆his1::hisG/∆his1::hisG ,∆arg4::hisG/∆arg4::hisG,</i> <i>CEN7/CEN7::HIS1::URA3_7.7kb left CSE4-GFP-</i> <i>CSE4/CSE4</i>	(FOA ^r , in <i>trans</i>)	study
LSK454	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG, Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^r , in <i>trans</i>)	study
LSK455	Δura3::imm434/Δura3::imm434,	CEN7 del in 4L	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1	(FOA ^r , in <i>trans</i>)	study
LSK456	Δura3::imm434/Δura3::imm434,	4R in Cse4-	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4	GFP_T1	study
LSK457	Δura3::imm434/Δura3::imm434,	4R in Cse4-	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4	GFP_T2	study
LSK458	∆ura3::imm434/∆ura3::imm434,	4R in Cse4-	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4	GFP_T3	study

LSK459	Δura3::imm434/Δura3::imm434,	CEN7 del in 4R	This
2011.07	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	(FOA ^s , in <i>cis</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4	(- ,,	
	CEN7/CEN7::HIS1		
LSK460	Δura3::imm434/Δura3::imm434,	CEN7 del in 4R	This
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	(FOA ^s , in <i>cis</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4		
	CEN7/CEN7::HIS1		
LSK461	∆ura3::imm434/∆ura3::imm434,	CEN7 del in 4R	This
	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	(FOA ^s , in <i>cis</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4		
	CEN7/CEN7::HIS1		
LSK462	∆ura3::imm434/∆ura3::imm434,	CEN7 del in 4R	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	(FOA ^s , in <i>trans</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4		
	CEN7/CEN7::HIS1		
LSK463	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4R	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	(FOA ^s , in <i>trans</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4		
LOVACA	CEN7/CEN7::HIS1	ODN7 11:4D	
LSK464	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4R	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4	(FOA ^r , in <i>cis</i>)	study
	CEN7/CEN7::HIS1		
LSK465	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4R	This
LSK40J	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	(FOA ^{r} , in <i>cis</i>)	study
	4 <i>R</i> /4 <i>R</i> :: <i>URA3 CSE4</i> / <i>CSE4</i> - <i>GFP</i> - <i>CSE4</i>	$(\Gamma OA, \Pi Cis)$	study
	CEN7/CEN7::HIS1		
LSK466	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4R	This
2011100	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	(FOA ^r , in <i>cis</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4	(- ,,	
	CEN7/CEN7::HIS1		
LSK467	Δura3::imm434/Δura3::imm434,	CEN7 del in 4R	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	(FOA ^r , in <i>trans</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4		
	CEN7/CEN7::HIS1		
LSK468	∆ura3::imm434/∆ura3::imm434,	CEN7 del in 4R	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	(FOA ^r , in <i>trans</i>)	study
	4R/4R::URA3 CSE4/CSE4-GFP-CSE4		
	CEN7/CEN7::HIS1		
LSK471	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4L	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4	(FOA ^r , in	study
	<i>CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)</i>	cis) MTW1-TAP	
LSK472	$\Delta ura3::imm434/\Delta ura3::imm434,$	CEN7 del in 4L	This
-	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	(FOA ^r , in	study
	4L/4L::URA3 CSE4/CSE4-GFP-CSE4	cis) MTW1-TAP	
	CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)		

LSK475	Δura3::imm434/Δura3::imm434,	CEN7 del in 4R	This
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	(FOA ^r , in	study
	<i>4R/4R::URA3 CSE4/CSE4-GFP-CSE4</i> <i>CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)</i>	cis) MTW1-TAP	
LSK476	Δura3::imm434/Δura3::imm434,	CEN7 del in 4R	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	(FOA ^r , in	study
	<i>4R/4R::URA3 CSE4/CSE4-GFP-CSE4</i> <i>CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)</i>	cis) MTW1-TAP	
LSK301	Δura3::imm434/Δura3::imm434,	МСМ2	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	heterozygous null	study
LSK302	Δleu2::hisG/Δleu2::hisG, MCM2::NAT/MCM2 Δura3::imm434/Δura3::imm434,	(SN148) MCM2	This
LSK302	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	heterozygous null	study
	Δleu2::hisG/Δleu2::hisG, MCM2::FRT/MCM2	(SN148)	study
LSK303	Δura3::imm434/Δura3::imm434,	<i>mcm2</i> conditional	This
	$\Delta his1::hisG/\Delta his1::hisG,\Delta arg4::hisG/\Delta arg4::hisG,$	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG, MCM2::$		
	FRT/MET3prMCM2		
LSK304	$\Delta ura3::imm434/\Delta ura3::imm434,$	<i>mcm2</i> conditional	This
	Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2::	mutant (SN148)	study
	FRT/MET3prMCM2		
LSK305	$\Delta ura3::imm434/\Delta ura3::imm434,$	mcm2 conditional	This
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG, MCM2::$		
	FRT/MET3prMCM2		
LSK306	$\Delta ura3::imm434/\Delta ura3::imm434,$	mcm2 conditional	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	mutant (SN148)	study
	Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2 CSE4 TAP(HIS)/CSE4	CENPA-Prot A	
LSK307	$\Delta ura3::imm434/\Delta ura3::imm434,$	<i>mcm2</i> conditional	This
2011007	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	mutant (SN148)	study
	Δleu2::hisG/Δleu2::hisG, MCM2::	CENPA-Prot A	
	FRT/MET3prMCM2 CSE4 TAP(HIS)/CSE4		
LSK308	Δura3::imm434/Δura3::imm434,	mcm2 conditional	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	mutant (SN148)	study
	Δleu2::hisG/Δleu2::hisG, MCM2::	CENPA-Prot A	
LSK309	<i>FRT/MET3prMCM2 CSE4 TAP(HIS)/CSE4</i> Δura3::imm434/Δura3::imm434,	МСМ2	This
LUNJUJ	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	heterozygous null	study
	MCM2::NAT/MCM2 CSE4-GFP-CSE4/CSE4	(10118)	stady
LSK310	Δura3::imm434/Δura3::imm434,	МСМ2	This
	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	heterozygous null	study
	MCM2::NAT/MCM2 CSE4-GFP-CSE4/CSE4	(10118)	
LSK311	Δura3::imm434/Δura3::imm434,	mcm2 conditional	This
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	mutant (10118)	study
	MCM2::FRT/MET3prMCM2 CSE4-GFP-		
	CSE4/CSE4		

LSK312	A		This
LSK312	$\Delta ura3::imm434/\Delta ura3::imm434,$	<i>mcm2</i> conditional	
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	mutant (10118)	study
	MCM2::FRT/MET3prMCM2 CSE4-GFP-		
1.01/010	CSE4/CSE4		
LSK313	$\Delta ura3::imm434/\Delta ura3::imm434,$	mcm2 conditional	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	mutant (10118)	study
	MCM2::FRT/MET3prMCM2 CSE4-GFP-		
	CSE4/CSE4		
LSK320	∆ura3::imm434/∆ura3::imm434,	ORC4 heterozygous	This
	Δ his1::hisG/ Δ his1::hisG , Δ arg4::hisG/ Δ arg4::hisG,	null (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG, ORC4::NAT/ORC4$		
LSK321	∆ura3::imm434/∆ura3::imm434,	ORC4 heterozygous	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	null (SN148)	study
	Δleu2::hisG/Δleu2::hisG, ORC4::NAT/ORC4		
LSK322	Δura3::imm434/Δura3::imm434,	orc4 conditional	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG,$		
	ORC4::FRT/MET3prORC4		
LSK323	Δura3::imm434/Δura3::imm434,	orc4 conditional	This
	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG,$		study
	ORC4::FRT/MET3prORC4		
LSK324	$\Delta ura3::imm434/\Delta ura3::imm434,$	orc4 conditional	This
LUKJZ4	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG,$	mutant (SIV140)	study
	ORC4::FRT/MET3prORC4		
LSK325	$\Delta ura3::imm434/\Delta ura3::imm434,$	orc4 conditional	This
LSK323			
	$\Delta his1::hisG/\Delta his1::hisG, \Delta arg4::hisG/\Delta arg4::hisG,$	mutant (SN148)	study
	Δleu2::hisG/Δleu2::hisG, ORC4::	CENPA-Prot A	
1.01/20.6	FRT/MET3prORC4 CSE4 TAP(HIS)/CSE4	4 11.1 1	
LSK326	Δ ura3::imm434/ Δ ura3::imm434,	orc4 conditional	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG, ORC4::$	CENPA-Prot A	
	FRT/MET3prORC4 CSE4 TAP(HIS)/CSE4		
LSK327	∆ura3::imm434/∆ura3::imm434,	orc4 conditional	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	mutant (SN148)	study
	$\Delta leu2::hisG/\Delta leu2::hisG, ORC4::$	CENPA-Prot A	
	FRT/MET3prORC4 CSE4 TAP(HIS)/CSE4		
LSK328	∆ura3::imm434/∆ura3::imm434,	ORC4 heterozygous	This
	Δ his1::hisG/ Δ his1::hisG, Δ arg4::hisG/ Δ arg4::hisG,	null (10118)	study
			1
	ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4		
LSK329	0 0	ORC4 heterozygous	This
LSK329	ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4	<i>ORC4</i> heterozygous null (10118)	This study
LSK329	ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4 Δura3::imm434/Δura3::imm434,		
	ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4 Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG,Δarg4::hisG/Δarg4::hisG, ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4	null (10118)	
LSK329 LSK330	ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4 Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG,		study

LSK331	Δura3::imm434/Δura3::imm434,	orc4 conditional	This
	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	mutant (10118)	study
	ORC4::FRT/MET3prORC4 CSE4-GFP-CSE4/CSE4		
LSK332	∆ura3::imm434/∆ura3::imm434,	orc4 conditional	This
	$\Delta his1::hisG/\Delta his1::hisG$, $\Delta arg4::hisG/\Delta arg4::hisG$,	mutant (10118)	study
	ORC4::FRT/MET3prORC4 CSE4-GFP-CSE4/CSE4		
CAKS3b	Δura3::imm434/ Δura3::imm434 Δhis1::hisG/	CENPA depletion	(Sanyal
	$\Delta his1::hisG \Delta arg4::hisG / \Delta arg4::hisG$		and
	CSE4::PCK1prCSE4/ cse4::hisG:URA:hisG		Carbon
			2002)

Supplemental table S7. Primers used in the study.

Name	Sequence	Description
URA3 EXT	GTTTCAGAATCCGAAAAAGTGACGAAACTTATCAT	Cassette primers for
HSP2_FP	AATTGTACGAATATTCTTATCAAACACACCCTGAG	URA3 integrated 10 kb
	CTTCCGGATAATAGGAATTG	left of CEN7
URA3 EXT	GTTGCTCGAGGTTAGAGTCTATCTTGAAAAATTTT	
HSP2_RP	GTACATACAAACTGATATAACTCGACAATGGTCTT	
	AGAAGGACCACCTTTGATTG	
URA3 AT	CTCAAAAATACTTTAACAAACGGGTATATTGCTGA	Cassette primers for
HSP2_FP	TATTCTGATTAAAACATTTGATCGTTTTATGTGAGC	URA3 integrated 7.7 kb
	TTCCGGATAATAGGAATTG	left of CEN7
URA3 AT	CTTAACCCCAGACAGTTTTAACAATTTAGACACTA	-
HSP2_RP	CTACTAATTGCAACGTACTAACTAGTGAAACCCTT	
1101 2_14	AGAAGGACCACCTTTGATTG	
19.6520_AvrII F	AAACCCCCTAGGTTGCGAATATCTATTG	
	AAACCCAAGCTTACGCGTAATGGTCCCATCAGCAG	Construction of pFA-
II_MluI_R	TGCA	URA3-I-SceI-TS-Orf
19.6522_HpaI	CCCAAAGTTAACACGCGTCTGCCAACAAGAATGC	19.6520/65
MluIF	AACT	
19.6522_SacII	CCCAAACCGCGGTATATTTTTGTTGTATCAGAATC	
_R	CTACGCC	
L1_URA	CACATATTTTTACTTTCTGTATTATTCAGATCTTTA	Cassette primers for
INT_FP	CTCGTTGAAAAAAAATTTTTTTTTTTCAAAAGCTTCC	URA3 integrated 3.5 kb
	GGATAATAGGAATTG	left of CEN7
L1_URA	GATGTAGTTGTATCTTTAATATCACAGTTATGATA	
INT_RP	AGGGTCGTGTATATGTGAACATGGATTTGCTTAGA	
	AGGACCACCTTTGATTG	

PJ71	TGCTTACCATAATAGATGCTTAAAGCAACTAAAAT TAAGCTACTGGAAAGCTCCAGTGGTCCTAGATCCC GACTAATAGG	Cassette primers for URA3 integrated 1.7 kb left of CEN7
PJ72	ATTCGGGCAATTGTGTTCGTTATTGGTGGTAAATA ATGGTAAGACTACTTGGCACATGTATAGAAGGAC CACCTTTGATTG	
PJ67	ATTGATTGAATTTATAGCGGAAAATGGATGACAAT TAAAGGTTACGTGACGCTTTTTGCTCCTAGATCCC GACTAATAGG	Cassette primers for URA3 integrated 1.9 kb right of CEN7
PJ68	CTACATTTTCATGGACCAAACCCACTACAACACAT GCACCACACTGCACCTCCCCTAAAATAGAAGGAC CACCTTTGATTG	
19.6525_HpaI	CCCAAAGTTAACACGCGTGTCAATGCAGTCGTTGA	
_MluI_F 19.6525_SacII	ATAC	Construction of pFA-
_R		URA3-I-SceI-TS-Orf
19.6524_AvrII _F	AAACCCCCTAGGGAGTGATGATGAGATTAACCAG	19.6524/65
19.6524_HindI II_MluI_R	AAACCCAAGCTTACGCGTGCCTTATATGCCACCGA TGA	
R1_URA	GTCAGAAATTGATTTATGGACGAGATAAGACTAA	Cassette primers for
INT_FP	AATATGATTCTTCTAAAATCACATAATTAATTAGA GCTTCCGGATAATAGGAATTG	<i>URA3</i> integrated 6.5 kb right of CEN7
R1_URA INT_RP	GTGTAACAAAAATTTGCAATCACATCATTGACAGC CACCACAGTTTTTTTATAATAAGTGATATTGTTAG AAGGACCACCTTTGATTG	
РЈ70	TTGCTTTAAATGTTTCAAACCATAGGTATGAGTTT GGGTAGTATTTGGCGGAATTAATGTCCTAGATCCC GACTAATAGG	Cassette primers for URA3 integrated 10.4 kb right of CEN7
PJ71	ATCACTCTTGTCGTTTATTGTAGATCACTAAAAGT AATGGTTGTGTGAATAACTCCTGCTTAGAAGGACC ACCTTTGATTG	
URA3 AT	CAGTTTTAAGAAGGTTTACATTATTAGCCTACGAA	Cassette primers for
HSP3_FP	CAAAGACAGGTTATGATAGGAAACAGAGCTCCTG TTTTTATTCAGCTTCCGGATAATAGGAATTG	URA3 integrated 16.3 kb right of CEN7
URA3 AT HSP3_RP	GCAATCGATCGTAAACGCCACTCAAGCTAAACTG AAAACTACTACGCCTAGAAGGCTAATCGGTACCA ATTAGAAGGACCACCTTTGATTG	

URA3 AT	GATCACATATGATTCTAGTACCACTAAACATTATC	Cassette primers for
CRTL7_FP	AACAACTATCATCAATTAGTAGAATTACTCTGAGA	URA3 integrated 100 kb
	GCTTCCGGATAATAGGAATTG	left of CEN7
URA3 AT	CCACGTGGATTTTTAAAATCTCAATAGTTTCTATA	
CRTL7_RP	GTGGTGGTATACCACTACTACGACTGTGGATTCAT	
	TAGAAGGACCACCTTTGATTG	
URA3 at	CAATTCCTATTATCCGGAAGCTGTCGTGTAAGGCG	Cassette primers for
nCEN5-II_FP	GTAAATGGTTTTGGTGGGTTTATTTTTCTTTAAAAA	URA3 integrated 7.5 kb
III ta	TCCAGACATGTCTTGC	left of CEN5
URA3 at	CAATCAAAGGTGGTCCTTCTAACACACTATTTACT	
nCEN5-II_RP	TGTGGTAAACATACTATTGGTTGATAATGATGTTA	
CENT 2	GCAATGGGTTTATGCTTATTTAC	= DOD = f CEN7
nCEN7-3 nCEN7-4	GCATACCTGACACTGTCGTT AACGGTGCTACGTTTTTTTA	q-PCR of CEN7
NCEN7-4 URA3 RT1	TGTTGAAAGTTGCTGTAGTG	q-PCR for <i>URA3</i>
URA3 RT2	TGCAGGAAATAAGATTGC	promoter
URA3 RT2	TCATCAGTGGGATCATTAGCA	q-PCR for <i>URA3</i> ORF
URA3 RT4	CACGTTGGGCAATAAATCCA	q-i CK IOI UKAS OKI
CEN1 core		q-PCR for <i>CEN1</i>
RT1	CAATCTAGCATTTCCTTCACACA	
CEN1 core RT2	TGACGCAATGAAGTAGGTGAT	
CACH5F1	CCCGCAAATAAGCAAACACT	qPCR for CEN5
CACH5R1	TTCATGGAAGAGGGGTTTCA	
7S10 RTF	CTTGTAAATTTAATTGTCGCTGAGG	qPCR primers for neocentromere mapping
7S10 RTR	CGGATAATCGTCCAACATATGAC	
7S11 RTF	GTCTTCTGACCTACCCATCAC	
7S11 RTR	GAGGCGGAAGTTGGACC	
7S12 RTF	CGTTGTGGCAATTGTATTTATG	
7S12 RTR	GCCATAGCTTAGCAAATAACC	
7S13 RTF	CATGGCTAATCCAACAACACATG	
7S13 RTR	GCTGGCTCTTGTTCTTGTATC	-
421K RT1	CCTATCGCCACAAGGGAGA	1
421K RT2	CAACGACTGCATTGACTCTTT]
7S14 RTF	GGATGTTGAGTTCAAAGCCTG	
7S14 RTR	CCAGCCAAATAATCTAGCTGC	
4RTF	ATTTGTCCCATCCGTAATTGATTC]

JANKAATAGCTATATCAGTTGTCAGCTTAC17RTFAATGCTTGGCCCTCAGTTGTCAGCTTAC20RTFACTGAAGTCGGCGTGTGATC20RTFACTGAAGTCGGCCTGATTAGGCGAA16RTFACCAGGATAATCTAACTGGCAAC15RTRCAATAGCTCATCTAACTGGCAAC15RTRCAATAAGGTCATCCTTTTATACCT7RIRTGGAAGTGTAACTATTGAGCGGA7RIRTCAGTAAAGGTCATCCTTTTATACCT7RIRTGGAAGTGTAACTATTGAGCGGCA7RIRTCTGGTATTCAACAATGGAGCGGTGTGG7F3RTCTGGCGATATCAAATCAGGT7F4RTGGAGCTGGCGATCAATTGT7F4RTGGAGCGGCGAACAATTGT7F4RTCGAGCAGGCGGATTAATGGT7F4RTCCACACCTGTTGACGAG7F4RTCACCCCAATTGAAGGAGCAGT7F4RTCACCCCGATTAATGGG7F4RGTAGGCGGCGGATTAATGG7F4R RTCCACACCTGTTAGCGGG7F4R RTCCACAACCTCAAGGAGCAG7F4R RTCCACAACCTCAAGGAGGGG7F4R RTCTGCAAAACACACAAGGAGGAGG7F4R RTGCACAAAATCAGATACCAAGCC7R5 RTGCTTGGTCATACCAATGCGGGG7F6 RTCAAGGAAGTCATTTCTCAGAAGG7F6 RTCAAGGAAGTCATTTCTCAGAAGGGGAATTCC7F6 RTCAAGAAATGCCGCTAACAATCCAATACCATAATTCCTPTTGTCCCCAATGGAATCCTTACGATGGGAATTCC7R GRATCCTGGAGGATCCTTGCAGAGGGAATCCGGAGTAATGGTAAACATTTACTTGGAGGGAATTCCTPTTGTCCCGGGGTAAACAATCCAATCGAC0RC4 11CCGGGGTACCTGGATAACCATTCGC0RC4 16TCCCCCGGGGTATAACCATTCCATTTAGGTGC0RC4 11CGGGGTACCCTGGATTCAACCAATCCATTTGAGAGG0RC4 12AACTGCAGTGCATTAACCATTCTTTAAGGCG0RC4 14CC	3RTR	ACGTTTTACCAGCCTATGC	
17RTFAATGCTTGGCCCTCAGTATAAC20RTFACTGAAGTCGGCCTGTGATC19RTRGATAACTGGACTCATTAGGCGAA19RTRGATAACTGGACTCATTAGGCGAAC15RTRCTATTGCCCAATCAATAACCTT7FIRTCAGTAAACGTCATTAGGCGCAC7FIRTCAGTAAACGTCATTAGACTCC7RIRTGGAAGTGTAACTATTGGCCCC72RTATTAAATAGAATGCGGCAATACC7R3RTCTGGTATCAATTGAACTCC7R3RTGTCACCCCAATTCAAATCGGACGGT7R3RTGTCACCCCAATTCAAATCACGT7F4RTTCGGACTGGGACAATTGT7F4RTGGAGCTGGCGATCAATTGT7F4R RTCCACACTGGAGAGACCGTT7F4R RTCCACAACTGTGTGACGAG7F4R RTCCACAACTGTGTGGACCGG7F4R RTGCACAAACTCAAGGAGACCAGG7F4R RTGGACAAACTCAAGGAGACCAG7F4R RTGGCAAAACTCAAGGAGGAGG7F4R RTGGCAAAACTCAAGGAGAGCAG7F4R RTGGCAAAACCTCAAGGAGGAGG7F4R RTGGCAAAATCAAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7R6 RTCACAAGAACATCAAATTGGGG7F6 RTCCACAAATGGAGATCCTTACGATGGGGAATCC7R6 RTCACAAAATGGAGATCCTTACGATGGGGAATCC7F6 RTCACAAAAATGGCGCTAACAATACCAAGGGGGAATCC7F6 RTCACAAAAATGGCGCTAACAATACCAATACCATTAATTCCT7ACTCCATGTACAAATGCCGCTAACAATACCATTAATTCCT7ACTCCCATGTAAGGTTGATTTACCTTGAGGGGAATCC7GGAGTAATGGTAACAATTGCA9CACAAAAATGCCCGCTAACAATACCAATACCATTAATTCCT9CACAAAAATGGCCGCTAACAATACCAATACCATTAATTCCT9CACAAAAAGGCTGAACATTTGAACATTTGC9CACAAAAGGTGAACCATTGGAC </td <td></td> <td></td> <td>-</td>			-
20RTFACTGAAGTCGGCTGTGATC19RTRGATAACTGGACTCATTAGGCGAA16RTFACCAGGATAATCTAACTGGCAAC15RTRCTAATGCCCCAATCAATAACCTT7FIRTCAGTAAACGTCATCTCTTTTATACCT7RIRTGGAGTGTAACTATTGGCTCC7F2RTATTAAATAGAATGCGGCAATACC7R2RTATTTAAATAGAATGCGGCAATACC7R3RTGTGACCCCAATTCAATTAGGT7F4RTGGAGCTGGGGATCAATTGGT7R4RTGGACGGGGATCAATTGATC7R4RTTCACCACATGAGAGGACCGTT7F4RTCACACATGAGAGGACCGTT7F4RTCACACATGAGAGGACCGTT7F4RGTAGGCGCGGATTAATGTG7F4R RTCCACAACTGTTGGACGGG7F4B RTCCACACTGTTGGAGGGG7F4C RTGACAAACCTCAAGGAGCAGG7F4C RTGGACAAAACCACAAGGAGGGG7F4C RTGGACAAAACACTCAAGGAGCGGG7F6 RTCTCCAAGAACATCAAATTGGG7F8 RTGGACAAAACCTCAAATTGGAGGGG7F6 RTCTCCAAGAACATCAAATTGGG7F6 RTCCACACTGTTACCAATCCAAGGAGGAATTC7F6 RTCAAGGAAGTCATTTCTCAATTTACTTGAGGGCAPTGTGCGAATGGGAGTCCTGAGGATTCTCAATTTAGGGGGAATTCCGAATATTTATGAGAACCCGCTAACAATACCAATTAATTCCTPACTCCATGTACAAAATGCCGCTAACAATACCAATTATTCCTPACTCCATGTACAGAATAGCTTGTGAAAACTGTGTTTCPCGGGGTAATGGTTAAACATTTGCHIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGACAATTCGACORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 112AACTGCACGGCACATTCAACTTTTAACGTTGTTTAAGGCORC4 12AACTGCACGGGACATTTAACTTTTAAGGTGCTTTTAAGGCT			
IPRTRGATAACTGGACTCATTAGGCGAAI6RTFACCAGGATAATCTAACTGGCAACI5RTRCTATTGCCCCAATCAATAACCTTFIRTCAGTAAACGTCATCTCTTTTATACCT7RIRTGGAAGTGTAACTATTGAGCTCC7R2RTATTTAAATAGAATGCGCCAATACC7R3RTCTCGCTATTCACAATGGAACGGT7R3RTGTCACCCCAATTCAAATGACGGT7R4RTCGAGCTGGCGATCAATTGGT7R4RTCCACAACTGAGAGACGCGTT7R4RCCACAACTGAGAGACCGTT7R4RCCACAACTGTGACGAGG7R4RCCACAACTGTTGACGAG7R4RCCACAACTGTTGACGAG7R4RCCACAACTGTTGAGGAGGACCGT7R4RGTAGCCCCGGATTAATGG7R4R RTCCACAACTGTTGGATCG7R4R RTCCACAACTGAGGAGCAG7F4R RTGACAAACCTCAAGGAGCAG7F4R RTGACAAACACTCAAGGAGGAG7R4R RTCCAACTGATTGGAGGGG7F4R RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATGCGAG7F6 RTCAAGGAAGTCATTCTCAATTGAGGGGAATCC7F6 RTCAAAGGAAGTCATTCTCAATTGAGGGGAATCC7F6 RTCAAAGGAAGTCATTCTCAATTGAGGGGAATCC7F6 RTCAAAGAAGTCAATTCCAATTGCAATGCGAGGAATCCPACTCCCAATGGAATACCCAACATCCAATACCAATGCGAATCCPCAAAAAAGCCCGCTAACAATACCAATACCATATATTCTPACTCCATGTAACAATTCGAPCGGGTAATGGTTAACATTTGCPCGGGTAATGGTAACCTTGGAATACCCAACAGCTTIGATCPCGGGTAATGGTAACAATTCGACRC4 16CCCCCCCGGGGTATAAGTTGACTTTGAGTORC4 16TCCCCGCGGGGTATAAGTTGATTTAGGCORC4 16CCCCCCCCGGGGTATAAGTTGACTCTTTAAGGTCORC4 12			
16RTFACCAGGATAATCTAACTGGCAAC15RTRCTATTGCCCCAATCAATAACCTT7FIRTCAGTAAACGTCATCTTTATACCT7RIRTGGAAGTGTAACTATTGAGCTCC72RTATTTAAGAATGCGGCAATACC7R3RTCTGGTATCACAATGGAACGGT7R3RTCTGGTATCACAATGGAACGGT7R4RTGGAGCTGGCGATCAATTGT7F4RTGGAGCTGGCGATCAATTGT7F4RTCCACACATGAGAGGACCGTT7F4RTCACACATGAGAGGACCGTT7F4RTCACACCTGATGAAGCAGCAATG7R4A RTFCCACACCTGTTGACGAG7F4B RTCCACACCTGTTGGGATCTG7F4C RTGAACACCTCAAGGAGCAGG7F4C RTGACAAACACTCAAGGAGCAG7R4C RTCTGCAAATCAATAGATACCAAGGC7F5 RTGGACAAAATCAGATACCAAGGC7F6 RTCTCCAAGAACATCAAATGGGG7F6 RTCACAAGACATCAAATGGAGATCC7F6 RTCACAAAAGCCCAAAATCGAATCCAATGAGGGAATTC7FCGGAATATTTATGAGAAACCCCAACACCAAGAGCACT7FCTCCCAAGAACATCAAATGGGG7F6 RTCACAAAAATGCCGGCAATACCAATACCATTAATTGG7FCCCCCAATTGCAATTCCTAACAATACCATTAATTGT7AATTCCGGGGATCCTGGAG7CCCCCGGGGTAATGGTAAACATTCACAATCCCAATTGACT7GGAGTAATGCTAAACCATCAAAAATGCCGGAGAATCC7GGAGTAATGCTAAGCTAACAATACCAATACCATTAATTCCT7SCCCCCGGGGTAATAGGTTGAAAAATGTGTGTTTC7GGAGTAATGCTAAGGTTGAAAAATGTGTGTTTAAGC7GCGCGGTACCTTGGATGAAAATGCTGGAGA7CTCCCCGGGGTTATAGGTTGAAAAATGTGTGTTTAAGCC7GCGGATACCTGGTTGAAAAATGTGTGTTTAAGCC7GCGGGTACCTGGAAAAAGTTTAAGGTGCTTTAAGC7GGG			
ISRTRCTATTGCCCCAATCAATAACCTTFIRTCAGTAAACGTCATCTCTTTATACCT7RIRTGGAAGTGTAACTATTGAGCTCC7RIRTATTAAATAGAATGCGCAATACC7R2RTATTTAAGGATGCGCAATACC7R3RTGTCACCCCAATTCAAATCGAACGGT7R4RTTCACACATGAGAGAGCGTGT7F4RTCGAGCTGGCGATCAATTTGT7F4RTCACACATGAAGAGACGGT7R4RTTCACACATGAGAGACCGTT7F4RCGGATAATTGAAACCAGCAATG7R4RTCCACACCTGTTAGCGAG7F4RCCACAACTGGTGACGAG7F4RCCACAACTGTGTTAGTGTGGATCTG7F4R RTCCACAACTCAATGGAGGCGG7F4R RTCCACAACCTCAAGGAGCAGG7F4R RTCCACAACTCAATGGAGGTGG7F4R RTCCACAACATCAATTGGAGGTGG7F4R RTGGACAAAATCAGATACCAAGCC7F5 RTGGACAAAATCAGATACCAAGCC7F6 RTCCCCAAGGAACATCAAATCGAGGAG7F6 RTCACAGGAAGTCATTTCTTCAGAAGG7F6 RTCACAAGAAGTCATTTCTCAATTTACTTTGAGGGCA7F6 RTCACAAAAATGCCGCTAACAATACCAATACCATTAATTCCTPCGAATATTTAAGGAAAACCEN7DHIS_FGTAAACTTTTCGATTCCAATTACCAATAACATTAATTCCTPCACAAAAAGCCGCAAAATACCCAACAATCCAATACATTAATTCCTPCAAAAAGCCGAAATACCCAACATTCACAATACCATTAATTCCTPCAAAAAGCCGAAATACTTGGTTGAAAATGTGTTTCORC4 15CCCCCCGGGGTAATAGGTTGCTTTAAGGTCORC4 16TCCCCCCGGGGTAATGGTGAATTCACTTTAGGCORC4 12AACTGCAGTCATTAAGTTGAACATTCATTTAGGCORC4 12AACTGCAGTAATGCCATTTAACTCTTTAAGGCCMC12AACTGCAGTAATGCCATTTAACTCTTTAAGGCC	-		
TFIRTCAGTAAACGTCATCTCTTTATACCTTRIRTGGAAGTGTAACTATTGAGCTCCTRIRTGGAAGTGTAACTATTGAGCTCCTP2RTATTTAAATAGAATGCGCAATACCR2RTATTTAAGGATGAGAGGTGTGGTF3RTCTGGTATTCACAATGGAACGGTTRARTTCACCCCAATTCAAATCACGTTF4RTGGACCTGGCGGATCAATTTGTTR4RTTCACACATGAGAGGACCGTTTF4R RTCCACAACTGTGTGACGAGTF4A RTRCCACACCTGTTGACGAGTF4C RTGAGCCGGGATTAATGGAAGCAGCAATGTR4R RTCCACACCTGTTGATGGATCTGTF4C RTGACAAACACTCAAGGAGCAGCAGTF4C RTGACAAACACTCAAGGAGCAGTR4C RTCTGCAAAATCAGATACCAAGGCCTR5 RTGGACAAAATCAGATACCAATGCGGTR6 RTCCAAGGAAGTCATTCTCAATTGGGGTR6 RTCAAGGAAGTCATTCTCAATTAGCTGGGAATTCCCCEN7DHIS_FGTAAACTTTTTGCAGTACCAAATACCAATACCATGAGGAGATCCPCACAAAAATGCCGCTAACAATACCAATACCATTAATTCCTPCACAAAAATGGTGATATCCAAATACCAATACCATTAATTCCTPCACAAAAATGCCCGCTAACAAATACCAATACCATTAATTCCTPCACAAAAATGCCCGCTAACAAATCCAATACCATTAATTCCPCACAAAAATGCCCGCTAACAATTCCAACATPCACAAAAATGCTCGAACAATTCGACORC4 13CGGGTAATCGTAACAATTCCTTGAGTTGACORC4 14CCCCCCGGGTTATAGGTTGCTTTTAGTGCORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCCORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCC			
TRIRTGGAAGTGTAACTATTGAGCTCCTF2RTATTAAATAGAATGCGGCAATACCTF2RTATTTAAGGATGAGAGGTGTGGTF3RTCTGGTATTCACAATGGAACGGTTR3RTGTCACCCCAATTCAAATCACGTTF4RTGGAGCTGGCGATCAATTGTTF4RTCGGACTGACGACAATGGAAGCAGGACCGTTTR4RTTCACACATGAGAGGACCGTTTF4A RTFCCGGATAATTGAAGCAGCAATGTR4A RTRCCACACTGTTGACGAGCT4B RTCCACACTGTTTAGTGTGTGGATCTGTF4C RTGGACAAACCTCAAGGAGCAGTR4C RTCCACACTGTTTAGTGGTGGGGGTR4C RTCTGCAAATCAATTGGAGGGGTR5 RTGGACAAAACCAAAATCAGATACCAAGCCTR5 RTGCTTTGGTCATACCAATACCAGTR6 RTCCAAGGAAGTCATTTCTTCAGAAGGCEN7DHIS_FGTAAACTTTTCGATTCTCAATTACTTGGAGGGAATTC CGGAATATTATGAGAAACPCACAAAAATGCGCGCTAACAATACCAACAGCACTPCGACTAAATGGTGAAACCATTACCAACATTCAACATTGGAGGAATCC CGGAATATTATGAGAAACPCACAAAAATGCCGGCTAACAATACCAACATCATAATCCCAPCACAAAAATGCGGAATCCCAACAATACCAACATTACTGAGGGAATCC CGGATATCTGGGGATCTGGAGPCACAAAATGCGAAAACCCAACATCAACATTACCATAACCATTAATPCACAAAATGCGTAAACATTTGCHIS ORF_1CAAAGAGCTGAACAATTCGACHIS ORF_1CAAAGAGCTGAACAATTCACAGGACCORC4 16TCCCCGCGGGTATAGGTTGTTTAAGTGCORC4 16CCCCCCGGGGTATAGGTTGCTTTAAGTGCORC4 12AACTGCAGTGCCATTAACCTTTTAACTCTTTAAGGCGORC4 12AACTGCAGTGCCATTAACCTCTTTAAGGCG			-
TP2RTATTAAATAGAATGCGGCAATACC7R2RTATTTTAAGGATGAGAGGTGTGG7R3RTCTGGTATTCACAATGGAACGGT7R3RTGTCACCCCAATTGAAATGAACCGT7F4RTGGAGCTGGCGATCAATTGTGT7F4RTCCACACATGAGAGGACCGTT7F4RCGGATAATTGAAAGCAGCAATG7F4RCGGATAATTGAAAGCAGCAATG7F4RCCACAACTGTTGACGAG7F4RGTAGGCGCGGATTTAATGTG7F4RGTAGGCGCGGATTTAATGTG7F4RGACAAACACTCAAGGAGCAG7F4RGACAAACACTCAAGGAGCAG7F4RGACAAACACTCAAGGAGCAG7F4RGGACAAAATCAGATACCAAGCC7F5 RTGGACAAAATCAGATACCAAGCC7F6 RTCTCCAAGAACACTCAAATTGGG7F6 RTCCCAAGGAAGTCATTTCTCAAATTACTTGAGGGCA7F6 RTCAAGGAAGTCATTTCTCAATTACTTGAGGGGCA7F6 RTCAAGGAAGTCATTTCTCAATTACTTGAGGGGCAPTGTGCCAAATGCAGAGATTCCTTACGATGGAGATTCCCGGAATATTTATGAGAAACWITH HIS1PCCCACAAAATGCCCCCTAACAATACCATACATTACTPCGGGATACTGGAAATGCCACACATGCTTGGATCPGGGGTACCTGGAACATTCGACNIS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTGGATTATAGGTGCTTTAGTGCORC4 14CCCCCCGGGGTATAGGTGGTTTAAGTGCORC4 15TCCCCCGCGGTATAAGGTGCTTTAGTGCORC4 12AACTGCAGTGCCATTAAGTTGCTTTAAGGCGORC4 12AACTGCAGGCCATTAAGTTGCTTTAAGGCGORC4 12AACTGCAGGCCATTAAGTTGCTTTAAGGCG			
TR2RTATTTTAAGGATGAGAGGTGTGGTF3RTCTGGTATTCACAATGGAACGGTTR3RTGTCACCCCAATTCAAATCACGTTR4RTGGAGCTGGCGATCAATTGTTF4RTCGGACATGGAGGACCGTTTF4A RTFCCGACAATGGAAGGACCGATGTR4A RTRCCACACTGTTGACGAGCCAACTTGTTAGTTGTGGATCTGTF4C RTGACAAACACTCAAGGAGGACGGTR4C RTCCACAACCTGATGAGAGGAGGAGTR4C RTCGGACAAAATCAGATACCAAGGCTR5 RTGGACAAAATCAGATACCAAGCCTR6 RTCTCCAAGAACACTCAAGAGAGTR6 RTCCACAAGCACTCAAATTGGGTR6 RTCAAGGAAGTCATTTCTCAGAAGCEN7DHIS_FGTAAACTTTATGAGAAACCEN7DHIS_RCAAAAAATGCCCGTAAACAATACCAATTACTGAGGGAATTC CGGAATATTTATGAGAAACCEN7DHIS_RCAAAAAATGCCCGCTAACAATACCAATGCAATTCCT PNACTCCATGTACAGAAATACCAACATTGACNIS ORF_2GGAGTAATGGTAAACAATTCGACORC4 13CGGGGTACCTGGTTGTAAAAATGTGTTTAAGTGC ORC4 14ORC4 16TCCCCGCGGGTATAAGGTGCTTTAAGTGC ORC4 12ORC4 12AACTGCAGTGCCAATTCACAATTCACAGGACCMET3 cassette cloning ORC4 12			-
TF3RTCTGGTATTCACAATGGAACGGT7R3RTGTCACCCCAATTCAAATCACGT7R4RTGGACCCGCGATCAATTGT7R4RTTCACACATGAGAGGACCGTT7F4A RTFCCGAAATTGAAAGCAGCAATG7F4A RTFCCGACAACCTGTTGACGAG7F4B RTGTAGGCGCGGATTTAATGTG7F4C RTGACAACACTCAAGGAGCAG7F4C RTGACAAACACTCAAGGAGCAG7F4C RTGGACAAAATCAGATACCAAGCC7F6 RTGGACAAAATCAGATACCAAGGCC7F6 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7F6 RTCAAGGAAGTCATTTCTCAGAAGCEN7DHIS_FGTAAACTGCAATGCGGAATTCCTTACGATGGGAATTCPACACAAAATGCCGCTAACAATACCAATACCAATACCATAATTCCTPACACAAAATGCCGGTAACAATACCAATACCAATATCCTPGAGTAATGTAACAATTGGAHIS ORF_1CAAAAAATGCCGGAACATTCAACAATGCGACINS ORF_2GGAGTAATGGTAAACATTTGCORC4 13CGGGGTACCTTGGTTGAAACATTCGACORC4 14CCCCCGCGGGTTATAGGTGGTTTAAGTGCORC4 12AACTGCAGTGCCATTAACCATTATAGTGCORC4 12AACTGCAGTGCCATTAACCATTAACTCTTTAAGGCG			
TR3RTGTCACCCCAATTCAAATCACGT7F4RTGGAGCTGGCGATCAATTGT7F4RTTCACACATGAGAGGAGCCGTT7R4R TTCACACATGAAAGCAGCAATG7R4A RTRCCGATAATTGAAACCAGCAATG7F4B RTGTAGGCGCGGGATTTAATGTG7F4B RTCCAACTTGTTTAGTTGTTGGATCTG7F4C RTGACAAACACTCAAGGAGGAGG7R4C RTCGCAAACTCATTGGAGTGG7F5 RTGGACAAAATCAGATACCAAGCC7F6 RTCTCCAAGAACATCAAATTGGG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAAGCTCATTTCTCAATTACATTGAGGGCAAPGTAAGCTATTTAGGAGATCCTTACGATGGGAATTC CGGAATATTTATGAGAAACPCACAAAATGCCCCCTAACAATACCAATACCATTAATTCCT PACTCCATGTACAGAATCCCAACATACCATCATTAATTCCT PCACAAAATGGTGATACCCAACATGCATTGTTTC CGAGTAATGGTTAAACATTTGCHIS ORF_2GGAGTAATGGTTAAACATTCGAC CGGGATACTGGAGAATCCGGGAGORC4 13CGGGGTACCTTGGTTTGTAAAAATGTGTGTTC ORC4 14ORC4 14CCCCCGCGGGTTATAGGTTGCTTTAGTGC ORC4 12ORC4 12AACTGCAGTGCCATTAACATTCACTTTAAGGCGVACTGCAGGGCACCTTGATTTAACTCTTTAAGGCGVC4			
TF4RTGGAGCTGGCGATCAATTTGTTR4RTTCACACATGAGAGGACCGTTTF4A RTFCGGATAATTGAAAGCAGCAATGTR4A RTRCCACAACCTGTTGACGAGTF4B RTGTAGGCGCGGATTTAATGTGTR4B RTCCAACTTGTTTAGTTGTTGGATCTGTF4C RTGACAAACACTCAAGGAGGAGTR4C RTCTGCAAATCTATTGGAGTGGTR5 RTGGACAAAATCAGATACCAAGCCTR6 RTCTCCAAGGAAGTCATTCTCAATTAGTGGGGATR6 RTCTCCAAGAACATCAAATGGAGAGAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTACTTGAGGGCAPCTGCCAAATGGAGAGATCCTTACGATGGGAATCCCGGATATTTATGAGAAACCEN7DHIS_RCACAAAATGCCCCTAACAATACCATCATTACTTGAGGGCAPCACAAAATGCCCCCTAACAATACCATCATTACTTGTGATCAATTCCCGGGATCCTGGAGWITH HIS1HIS ORF_2GGAGTAATGGTAAACATTTGCINS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTCORC4 14CCCCCGCGGGTTATAGGTTGCTTTAGGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTAGGCORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCGMC4MET3 cassette cloningORC4 12AACTGCAGTGCCATTAACTTTAACTCTTTAAGGCG			
TRARTTCACACATGAGAGGACCGTTTF4A RTFCGGATAATTGAAAGCAGCAATGTF4A RTRCCACAACCTGTTGACGAGTF4A RTRCCACAACCTGTTGACGAGTF4B RTGTAGGCGCGGATTTAATGTGTF4B RTCCAACTTGTTTAGTTGTGGATCTGTF4C RTGACAAACACTCAAGGAGCAGTR4C RTCTGCAAATCTATTGGAGGTGGTF5 RTGGACAAAATCAGATACCAAGCCTF6 RTCTCCAAGAACACTCAAATACCAGTF6 RTCTCCAAGAACATCAAATTGGAGTR6 RTCAAAGGAAGTCATTTCTCAATTACCTTGAGGGCAPGTAAACTTTTTCGATTCTCAATTACCTTGAGGGCAAPCTCCAAGAACTCAGAATCCAAAATCCAATACCATTAATTCCTPCAAAAATGCCGCTAACAATACCAATACCATTAATTCCTPACTCCCATGTACAGAATCCGAACATTCAGATGGCAGAHIS ORF_2GGAGTAATGGTAAACATTGACAORC4 13CGGGGTACCTGGAAATACCTAAATTCGACORC4 14CCCCCGCGGGTTATAGGTTGCTTTAGAGTGCORC4 11CGCGGATCCATGAATTCACAGAACCORC4 12AACTGCAGTGCCATTAACTTTTACTTTAAGGCGORC4 12AACTGCAGTGCCATTAAACTTTTACCTTTTAAGGCGORC4 12AACTGCAGTGCCATTAAACTTTTAACTCTTTAAGGCGORC4 12AACTGCAGTGCCATTAAACTTTTAACTCTTTTAAGGCGORC4 12AACTGCAGTGCCATTAAACTCTTTTAAGGCG			
TF4A RTFCGGATAATTGAAAGCAGCAATG7R4A RTRCCACAACCTGTTGACGAG7F4B RTGTAGGCGCGGATTTAATGTG7F4B RTGTAGGCGCGGATTTAATGTG7R4B RTCCAACTTGTTTAGTTGGTGGATCTG7F4C RTGACAAACATCAAGGAGCAG7R4C RTCTGCAAATCTATTGGAGGTGG7F5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGGG7R6 RTCAAGGAAGTCATTTCTCAATTACTTTGAGGGCAPGTAAACTTTTTCGATTCTCAATTACCATGAGGAAATTCCPCAAAAATGCAGAATGAGAATCCAAATCCAATACCATTAATTCCTPCACAAAAATGCAGAATCCAAATACCAACATCAAATTCGAGCEN7DHIS_RCACAAAAATGCCGCTAACAATACCAATACCATTAATTCCTPACTCCATGTACAGAATACCAAAATCCAACATCGATGCTTGTATCGATTCCGGGGATCTGGAGACENTOLHIS ORF_2GGAGTAATGGTAAACATTTACTTGAGCORC4 13CGGGGTACCTGGAAATAGCTTTCTAAAAATGTTGTTCORC4 14CCCCCGCGGGTTATAGGTTGCTTTAAGTGCORC4 15TCCCCGGGGGTATAAGGTTGCTTTAGTGCORC4 16CCCCCGCGGGTTATAGGTTGCTTTAAGGCORC4 11CGCGGATCCATGAATTCACAGGACCORC4 12AACTGCAGTGCCATTAAACTTTTAACTCTTTAAGGCGORC4 12AACTGCAGTGCCATTAAACTCTTTTAAGGCGORC4 12AACTGCAGTGCCATTTAACTCTTTTAAGGCG	-		
TR4A RTRCCACAACCTGTTGACGAG7F4B RTGTAGGCGCGGATTTAATGTG7F4B RTCCAACTTGTTTAGTTGTGGATCTG7F4C RTGACAAACACTCAAGGAGCAG7R4C RTCTGCAAATCTATTGGAGGTGG7F5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTCAGAAG7R6 RTCAAGGAAGTCATTTCTCAATTACCAATGGAGATTC CGGAATATTATGAGAAACPTGTCCGAAATGGAGATTCCTACGATGGGAATTC CGGAATATTTATGAGAAACCEN7DHIS_FGTAAACTTTTTCGATTCCAATTACCATAATTCCT PACTCCATGTACAGAATACCCAACATCAATACCATTAATTCCT PCACAAAAATGCCCGCTAACAATACCATTAATTCCT PHIS ORF_2GGAGTAATGGTTAAACATTTGCA CGGGATACTGGTTAAACATTCGACNRS ORF_2GGAGTAATGGTTAAACATTTGC CAGGGTACCTGGGTTGTAAAAATGTTGTTC ORC4 13ORC4 14CCGCCGCGGGTTATAGGTTGCTTTAGTGC ORC4 14ORC4 15TCCCCGCGGGTTATAGGTTGCTTTAGTGC ORC4 14ORC4 16TCCCCGCGGGTTATAGGTTGCTTTAGTGC ORC4 12ORC4 12AACTGCAGTGCCATTTAACTCTTTAACTCTTTAAGGCG			
7F4B RTGTAGGCGCGGATTTAATGTG7R4B RTCCAACTTGTTTAGTTGTTGGATCTG7F4C RTGACAAACACTCAAGGAGCAG7R4C RTCTGCAAATCTATTGGAGGTGG7R4C RTCTGCAAATCAGATACCAAGCC7R5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTACCATTGAGGGCAPTGTCGCAAATGGAGATTCCTTACGATGGAATTCCGGAATATTTATGAGAAACWITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCTPACTCCATGTACAGAATACCCAACAATACCATTAATTCCTPGGAGTAATGGTTAAACATTTGCHIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGAACAATACCAATAGCTTTGATCORC4 13CGGGGTACCTTGGTTGTAAAAATGTTGTTCORC4 14CCCCCGCGGGTTATAGGTGGCTTTAGTAGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTAGTGCORC4 16TCCCCCGCGGGTTATAGGTGCTTTTAGTGCORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGGCCATTTAACTCTTTAACTCTTTAAGGCG	-		
TR4B RTCCAACTTGTTTAGTTGTTGGATCTG7R4 RTGACAAACACTCAAGGAGCAG7R4 C RTGACAAATCTATTGGAGGTGG7R4 C RTCTGCAAATCTATTGGAGGTGG7F5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTACTTGAGGGCA TTGTCGCAAATGGAGATTCCTACGATGGGAATTC CGGAATATTATGAGAAACCEN7 DELETION WITH HIS1CEN7DHIS_RCACAAAAATGCCGCTAACAATACCATTAATTCCT PCACAAAAATGCCGCTAACAATACCATTAATTCCT PCEN7 DELETION WITH HIS1IS ORF_2GGAGTAATGGTAAACAATTCGAC GAATTCCGGGGTACTGGAACAATTCGAC ORC4 13Deletion cassette for ORC4ORC4 14CCGCCGCGGGTTATAGGTTGCTTTAGTGCC ORC4 12AACTGCAGGGCAATTCAAAGGACAC	-		-
TF4C RTGACAAACACTCAAGGAGCAG7R4C RTCTGCAAATCTATTGGAGGTGG7R4C RTGCGCAAAATCAGATACCAAGCC7F5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA CGGAATATTTATGAGAAACCEN7 DELETION WITH HIS1PCTCCCATGTACCGATACCAATACCAATACCATTAATTCCT CGGAATATTTATGAGAAACCEN7 DELETION WITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT ACTCCATGTACAGAATAGCCACAATACCAATAATTCGACCEN7 DELETION WITH HIS1HIS ORF_2GGAGTAATGGTAAACATTTGC GAATTCCGGGGATCCTGGAGDeletion cassette for ORC4 13ORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC ORC4 14CCGCTCGAGAAATAGTTAGGTTGCTTTAGTGC ORC4 15Deletion cassette for ORC4 16ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloning for ORC4		GTAGGCGCGGATTTAATGTG	
7R4C RTCTGCAAATCTATTGGAGGTGG7F5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA TGGCGAATATTTATGAGAAACCEN7 DELETIONPTTGTCGCAAAATGGAGATTCCTACGATGGGAATTCC CGGAATATTTATGAGAAACWITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT PCACAAAAATGCCCGCTAACAATACCATGATTGTATC GAATTCCGGGGATCCTGGAGCEN7 DELETIONHIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC ORC4 14Deletion cassette for ORC4ORC4ORC4 16TCCCCGCGGGTTATAGGTGCTTTTAGTGCORC4ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloning for ORC4	7R4B RT	CCAACTTGTTTAGTTGTTGGATCTG	
Tig CIGCAAATCIATIGGAGGTGG7F5 RTGGACAAAATCAGATACCAAGCC7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA TGTCGCAAATGGAGATTCCTCAGTAGGAAGCCCEN7 DELETIONPACTCCATGTACAGAATAGCACACATACCAATACCATTAATTCCT CGAATATTTATGAGAAACWITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT GAATTCCGGGGATCCTGGAGWITH HIS1HIS ORF_2GGAGTAATGGTTAAACATTTGCDeletion cassette for ORC4 13ORC4 14CCGCTCGAGAAATAGGTTGCTTTAAGGTGCORC4ORC4 15TCCCCGCGGGTTATAGGTTGCTTTAGTGCORC4ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloning for ORC4	7F4C RT	GACAAACACTCAAGGAGCAG	
7R5 RTGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA CGGAATATTTATGAGAGAACCEN7 DELETION WITH HIS1PTTGTCGCAAATGGAGATTCCTTACGATGGGAATTCC CGGAATATTTATGAGAAACCEN7 DELETION WITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT PWITH HIS1HIS ORF_2GGAGTAATGGTTAAACATTTGC GGAGTACCTGGAGCADeletion cassette for ORC4 13ORC4 13CGGGGTACCTTGGTTGTAAGGTTGCTTTAGTGTC ORC4 16Deletion cassette for ORC4 16ORC4 11CGCGGATCCATGAATTCACAGGACC ORC4 12MET3 cassette cloning for ORC4	7R4C RT	CTGCAAATCTATTGGAGGTGG	
ActionGCTTTGGTCATACCAATACCAG7F6 RTCTCCAAGAACATCAAATTGGG7R6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA TTGTCGCAAATGGAGATTCCTTACGATGGAAATTC CGGAATATTTATGAGAAACCEN7 DELETION WITH HIS1CEN7DHIS_RGACAAAAATGCCCGCTAACAATACCATTAATTCCT PKUTH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT GAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAAGATACCAACAATGCTTGTATC GAATTCCGGGGTACCTGGAGADeletion cassette forORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC CGCCTCGAGAAATAGTTTTACTCTTGAGTTAGCDeletion cassette forORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCGfor ORC4	7F5 RT	GGACAAAATCAGATACCAAGCC	
TR6 RTCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA TTGTCGCAAATGGAGATTCCTTACGATGGGAATTC CGGAATATTTATGAGAAACCEN7 DELETION WITH HIS1PTTGTCGCAAATGGAGATTCCTTACGATGGGAATTC CGGAATATTTATGAGAAACWITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT GAATTCCGGGGATCCTGGAGWITH HIS1PACTCCATGTACAGAATACCAACAATGCTTTGTATC GAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAACATTTGCGGGGTACCTGGAGCADeletion cassette forORC4 13CGGCGGTCCAGGAATAGTTGCTTTAGTGCORC4ORC4 16TCCCCGCGGGTTATAGGTTGCTTTAGTGCORC4ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloning for ORC4	7R5 RT	GCTTTGGTCATACCAATACCAG	
CAAGGAAGTCATTTCTTCAGAAGCAAGGAAGTCATTTCTTCAGAAGCEN7DHIS_FGTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA TTGTCGCAAATGGAGATTCCTTACGATGGGAATTC CGGAATATTTATGAGAAACCEN7 DELETION WITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT PACTCCATGTACAGAATACCCAACATGCTTTGTATC GAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAACATTTGCGAATTCCGGGGATCCTGGAGIntersectionHIS ORF_2GGAGTAATGGTTAAACATTTGCDeletion cassette forORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC ORC4 14Deletion cassette for ORC415ORC4ORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloning for ORC4	7F6 RT	CTCCAAGAACATCAAATTGGG	
PTTGTCGCAAATGGAGATTCCTTACGATGGGAATTC CGGAATATTTATGAGAAACWITH HIS1CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCT ACTCCATGTACAGAATACCCAACATGCTTTGTATC GAATTCCGGGGATCCTGGAG	7R6 RT	CAAGGAAGTCATTTCTTCAGAAG	-
CGGAATATTTATGAGAAACCEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCTPACTCCATGTACAGAATACCCAACATGCTTTGTATCGAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAACATTTGCORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTCORC4 14CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11CGGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCG	CEN7DHIS_F	GTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCA	CEN7 DELETION
CEN7DHIS_RCACAAAAATGCCCGCTAACAATACCATTAATTCCTPACTCCATGTACAGAATACCCAACATGCTTTGTATCGAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTCORC4 14CCGCTCGAGAAATAGTTTACTCTTGAGTTAGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCG	Р	TTGTCGCAAATGGAGATTCCTTACGATGGGAATTC	WITH HIS1
PACTCCATGTACAGAATACCCAACATGCTTTGTATC GAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC ORC4 14Deletion cassette for ORC4GGGTTATAGGTTGCTTTTAGTGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11CGCGGATCCATGAATTCACAGGACCORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCG		CGGAATATTTATGAGAAAC	
GAATTCCGGGGATCCTGGAGHIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTCORC4 14CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11CGCGGATCCATGAATTCACAGGACCORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCG	CEN7DHIS_R	CACAAAAATGCCCGCTAACAATACCATTAATTCCT	
HIS ORF_2GGAGTAATGGTTAAACATTTGCHIS ORF_1CAAAGAAGCTGAACAATTCGACORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTCORC4 14CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11CGCGGATCCATGAATTCACAGGACCORC4 12AACTGCAGTGCCATTTAACTCTTTAAGCG	Р	ACTCCATGTACAGAATACCCAACATGCTTTGTATC	
HIS ORF_1CAAAGAAGCTGAACAATTCGACDeletion cassette forORC4 13CGGGGTACCTTGGTTTGTAAAAAATGTTGTTTCDeletion cassette forORC4 14CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4ORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4ORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTAAGGCGfor ORC4		GAATTCCGGGGGATCCTGGAG	
ORC4 13CGGGGTACCTTGGTTTGTAAAAATGTTGTTTCDeletion cassette forORC4 13CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4ORC4 14CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4ORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4ORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTTAAGGCGfor ORC4	HIS ORF_2	GGAGTAATGGTTAAACATTTTGC	
ORC4 14CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGCORC4ORC4 15TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4ORC4 16TCCCCGCGGGTTATAGGTTGCTTTTAGTGCORC4 11ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTTAAGGCGfor ORC4	HIS ORF_1	CAAAGAAGCTGAACAATTCGAC	
ORC4 15 TCCCCGCGGGTTATAGGTTGCTTTAGTGC ORC4 15 TCCCCGCGGGTTATAGGTTGCTTTTAGTGC ORC4 16 TCCCCGCGGGTTATAGGTTGCTTTTAGTGC ORC4 11 CGCGGATCCATGAATTCACAGGACC ORC4 12 AACTGCAGTGCCATTTAACTCTTTTAAGGCG	ORC4 13	CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC	Deletion cassette for
ORC4 16TCCCCGCGGGTTATAGGTTGCTTTAGTGCORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTTAAGGCGfor ORC4	ORC4 14	CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGC	ORC4
ORC4 11CGCGGATCCATGAATTCACAGGACCMET3 cassette cloningORC4 12AACTGCAGTGCCATTTAACTCTTTTAAGGCGfor ORC4	ORC4 15	TCCCCGCGGGTTATAGGTTGCTTTTAGTGC	
ORC4 12 AACTGCAGTGCCATTTAACTCTTTTAAGGCG for ORC4	ORC4 16	TCCCCGCGGGTTATAGGTTGCTTTTAGTGC	
	ORC4 11	CGCGGATCCATGAATTCACAGGACC	MET3 cassette cloning
MCM2_13 CGGGGTACCCTAATCCCATTTTGTTATGAATAT	ORC4 12	AACTGCAGTGCCATTTAACTCTTTTAAGGCG	for ORC4
	MCM2_13	CGGGGTACCCTAATCCCATTTTGTTATGAATAT	

MCM2_14	CCGCTCGAGGGTTGATTAAATAGTAATGTAATTAA	Deletion cassette for
	TAAAG	MCM2
MCM2_15	TCCCCGCGGGTGATTAGTGGGTTATGG	
MCM2_16	CGGAGCTCTGCATTCCAGATTATTTTCTG	
MCM2_11	CGCGGATCCATGTCAAGTCCACCAGCTG	N term of Mcm2 (For
MCM2_12	AACTGCAGGCGTCTTCATCTTCATCATCGTC	MET3pr cloning)

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