Chapter - 2

Materials and Methods
### 2.1 Yeast methods

#### 2.1.1 High efficiency yeast transformation

Yeast transformations with plasmids (including genomic library) or PCR products were done based on high efficiency LiAc protocol (Gietz and Woods, 2002). 

5x10⁶ cells from the overnight incubated primary culture were added to the 25ml broth and incubated for 4-5 hrs at 30°C with constant rotation. 1x10⁸ cells from the secondary culture were used for transformation. The cell pellet was washed in 1ml of 0.1 M LiAc and resuspended in 240µl of 50% Poly ethylene glycol (PEG). To this mixture, 36µl of 1M LiAc and 74 µl of transformation mix containing 40µl of salmon sperm DNA and 34µl of both plasmid/DNA fragment and sterile MilliQ water were added. Cells were vortexed briefly and incubated at 42°C for 40 minutes. Then cells were spun at 13k for 15 sec and cell pellet was resuspended in 200µl of sterile water and plated on selective dropout media. If the selection was on G418 plates, the cell pellet was resuspended in 1ml of YPD broth and incubated at 30°C for 10-12hrs to allow the expression of the gene and then plated on YPD media containing (200 µg/ml) G418 drug.

#### 2.1.2 Extraction of genomic DNA from yeast cells

##### 2.1.2.a. Zymolyase method

Cells grown overnight in 5ml of YPD or in selective broth were harvested by centrifuging at 3 k rpm for 5 min. The cell pellet was resuspended in 0.5 ml of 1M Sorbitol and 0.1M Na₂ EDTA (pH 7.5) and transferred into 1.5 ml microfuge tube. Cells were speroplasted by incubating the cell suspension with 20 µl of Zymolyase 100,000U (2.5 mg/ml) at 37°C for 60 min. Cells were centrifuged for 1 min at 13 k rpm and the cell pellet was resuspended in 0.5 ml of 50mM Tris-Cl (pH 7.4) and 20mM Na₂ EDTA (pH 8.0). 50 µl of 10% SDS was added to the cell suspension, mixed well and then incubated at 65°C for 30 min. 200 µl of 5M Potassium Acetate was then added to the cell suspension and placed in ice for 60 min. Cells were centrifuged for 5 min at 13 k rpm and supernatant was tranferred to a fresh microfuge tube. One volume (0.75 ml) of 100% isopropanol was added to the supernatant, mixed and allowed to sit at room temperatue for 5 min. Then centrifuged very briefly for 2 min at 13 k rpm and
supernatant was poured off. DNA pellet was air dried and resuspended in 0.3 ml of TE (pH 7.4) containing 20 µg/ml of Rnase A. DNA was incubated at 37°C for 30 min for degrading RNA. Then 30 µl of 3M Sodium Acetate (pH 7.0) was added to the DNA solution and mixed. To this, 0.2 ml of 100% isoproponal was added and mixed once again. DNA was recovered by centrifuging at 13 k rpm for 2 min. The supernatant was poured off, DNA pellet was air dried and resuspended in 30µl of TE (pH 8.0).

2.1.2.b. Rapid isolation of genomic DNA from yeast cells

Cells grown overnight in 5ml of YPD or in selective broth were harvested by centrifuging at 3 k rpm for 5 min. The cell pellet was washed in 0.5 ml of sterile distilled water and resuspended in 200 µl of breaking buffer. Glass beads (~ 200 µl volume) were added to the cell suspension and then 200 µl of phenol/chloroform/isoamylalcohol (25:24:1) was added and mixed. Cells were vortexed at high speed for 2 min. 200 µl of TE (pH 8.0) was added and once again vortexed briefly for 10 to 15 sec. Then the sample was centrifuged at 13 k rpm for 5 min at room temperature. The aqueous layer was transferred to a clean microfuge tube and 1 ml of 100% ethanol was added and mixed by inversion. DNA was recovered by centrifuging at 13 k rpm for 5 min. The supernatant was poured off, DNA pellet was air dried and resuspended in 30µl of TE (pH 8.0).

2.1.3 Extraction of whole cell protein from yeast cells by Trichloroacetic acid (TCA) method

Cells grown overnight in 5ml of YPD or in selective broth were harvested by centrifuging at 3 k rpm for 5 min. The cell pellet was resuspended in 200 µl of 20% TCA and glass beads were added up to the meniscus and then cells were lysed by vortexing for 1 min. Cell suspension was transferred into a new microfuge tube. Glass beads were washed twice with 200 µl of 5% TCA and the washes were added to the previous suspension. Cell pellet was collected by centrifuging at 3 k rpm for 10 min and resuspended in 200 µl of 1x Laemmli buffer. The laemmli buffer turns red because of the low pH of cell pellet. Therefore, 50 µl of 1M Tris base (no pH adjustment) was added to turn blue. The sample was boiled for 3 min and centrifuged again at 3 k rpm.
for 10 min. Protein sample was transferred to a new microfuge tube and the pellet was discarded.

**2.1.4 Spore enrichment**

Diploid cells were grown on dextrose deficient YPK medium and incubated at 25°C for 4-5 days. Toothpick full of spores were resuspended in 500 μl of YPD broth. The cell suspension was vortex mixed and centrifuged at 13 k rpm for 10 sec. The cell pellet was resuspended in 1 ml of 100 μg/ml of zymolyase (100,000U) and incubated at 30°C for 20 min. 500 μl of cell suspension was aliquot in separate microfuge tube and centrifuged at max speed for 30 sec. Cell pellet was washed in 1 ml of sterile water and resuspended in 100 μl of sterile water. Cells were then agitated for 2 min in upright position using vortex mixer at max speed. Aqueous cell suspension was discarded and the tube was rinsed with sterile water for several times. The spores were resuspended in 1 ml of 0.01% Nonidet P-4. Appropriate volume of cell suspension was plated on YPD medium and incubated at 30°C for one day (refer Guthrie and Fink, 1991).

**2.1.5 Silencing assay**

Silencing assays were done to test the loss in silencing in yeast. For this, the yeast cells were initially grown in nutrient rich broth or selective broth dropped out for specific amino acids (for retaining plasmids) at 30°C with appropriate rotation for overnight and then the culture was subjected to 10 fold serial dilution for 5 times. 5 μl of each dilution was spotted on complete medium to check the total number of cells grown and on selective medium for measuring the loss in silencing of reporter gene. For example, loss in silencing of TRP1 reporter gene is tested by spotting on tryptophan dropout medium. In case, the reporter gene is URA3, then serially diluted cultures were spotted on the medium containing 1mg/ml of 5-FOA (5-Fluoro orotic acid). Expression of the URA3 gene (Orotidine-5’-phosphate decarboxylase) leads to the conversion of 5-FOA into 5-fluorouracil, a toxic compound. This indicates that strains expressing URA3 cannot grow in this medium and those repressing URA3 can grow. Therefore, 5-FOA serves as a good indicator of the expression status of the URA3 reporter gene. After spotting, plates are incubated at 30°C for 2-3 days and analyzed loss in silencing by observing growth of cells.
2.1.6 Construction of mutants and tags in yeast

Strains with gene deletion and tagging were done as described in (Longtine et al., 1998). For gene knockout, DNA fragment was PCR amplified so as to contain selectable marker flanked by around 30 bp of DNA of the region of the gene of interest. This fragment was transformed into yeast by high efficiency LiAc protocol (Gietz and Woods, 2002). The flanking regions recombine with the genomic region of the gene of interest by homologous recombination and insert the selectable marker in place of the gene. Therefore, the gene is replaced by selectable marker. For gene tagging, the forward primer was designed by taking sequence just upstream of the stop codon and in frame so that it does not disrupt the reading frame of the tag and selectable marker which are going to be inserted in the downstream of the gene. These insertions were confirmed by screening PCR which gives diagnostic 500bp fragment. The sequences of the primers used for gene deletion, tagging and screening PCR are given in Table 3.

The strains that showed positive in screening PCR were further confirmed by genomic southern analysis. Genomic DNA from these strains was subjected to restriction digestion with appropriate enzymes that gives different fragments at gene locus of interest in wild type and deletion/tagged strains. The digested genomic DNA was transferred to nylon membrane and subjected to southern hybridization with radio labelled probe. The blot was then exposed to X-ray film to obtain the autoradiogram.

2.1.7 Tetrad dissection of yeast spores

Diploid cells were grown on dextrose deficient YPK medium and incubated at 25°C for 4-5 days. Tooth pick full of spores were resuspended in 500 μl of YPD broth. The cell suspension was vortex mixed and centrifuged at 13 k rpm for 10 sec. The cell pellet was resuspended in different concentration of 100 μg/ml of zymolyase (100,000U) and incubated at 30°C for few seconds to 2 minutes. Check under light microscope for tetrad which are intact and place 20μl of the suspension vertically from the middle of the YPD agar plate so that the suspension spread from one corner to other. Dissect the tetrad spores on YPD by using Olympus tetrad dissection microscope and plates were incubated for 2 days at 30°C and patch four individual colonies on YPD to test auxotrophic markers segregates as 2:2 ratio.
2.1.8 Quantitative mating assay in yeast diploid cells

Quantitative mating assay was performed on diploid wild type and deletion mutants. Initially cells were grown on YPD or selective media at 30°C for overnight. The mating tester either Mat α and Mat α strains (1 X 10⁸ cells) were grown in YPD and mixed with 2 X10⁶ diploid cells and collected on a nylon filter. Filters were transferred (cell side up) to YPD plates and incubated for 4 hrs at 30°C to allow cells to mate. Diploid cells that have lost the MAT α locus become competent to mate with the mating tester MAT α and vice versa. Cells from the filter were then resuspended in buffer saline and plated at several dilution onto the SD plates and incubated at 30°C until colonies were large enough to count. We also tested the mating locus mutant cells that have lost the either MAT α or MAT α by spot assay for this we incubated the diploid cells mix with the appropriate number of mating tester and incubated in liquid media for overnight and 10 fold serial dilutions were made and 5 μl of diluted samples spotted on synthetic deficient plates and YPD plates to test the lost of MAT locus.

We also performed quantitative mating assay on multiple heterozygous marker (MHM) wild type and deletion mutants to test the LOH events at MAT locus. To this we followed the similar protocol as described previously, but the exact number of MHM strains would be quantified by selecting the MHM strain in synthetic deficient supplemented with Histidine and Lysine, which permissive for growth of deletion mutants but not mating tester PT-1 cells.

2.1.9 Artificial chromosome (SUP11) loss assay

Artificial chromosome SUP11 (Ade+) was introduced into the kinetochore mutants initially which are Ade- by crossed them with wild type strain carrying the artificial chromosome SUP11 and tetrad dissection was performed to isolate mutants with SUP11 from the spores. Wild type and mutant with SUP11 initially selected them on Adenine drop out media for overnight at 30°C to retain the SUP11. Several dilutions were made and plated them on YPD agar plates with minimal Adenine concentration (6μg/ml) and incubated at 30°C until colonies become large enough (3- 4 days) to scored SUP11 lost with half sectored red and white colonies.
2.1.10 Half-sector (LOH) assay in MHM strains

Half sector assay was performed on each diploid multiple heterozygous marker (MHM) strain to calculate the rate of loss of heterozygosity (LOH). Initially, wild type and MHM deletion mutant strains were grown on selective media plates to maintain the heterozygosity. Two colonies from each deletion mutants and wild type isolates were picked and resuspended in PBS and several dilutions were made, plated about 150 cells on large plates contained lead nitrate media and incubated at 30°C for 3-7 days. The rate of LOH event per cell division was determined by obtaining the frequency of half-sectored colonies, either red/white or black/white among total colonies excluding fully those colonies that were completely coloured. The rate of LOH events calculates as

\[
\text{The rate of LOH} = \frac{\text{Half-sectored colonies}}{[\text{All colonies} - (2 \times \text{fully coloured colonies})]}
\]

2.2 Recombinant DNA methodology

2.2.1 Preparation of ultra competent DH5α cells

Ultra competent cells of DH5α strain of E.coli were prepared by Inoue method described in (Sambrook and Russell, 2001). A single bacterial colony was inoculated in 25 ml of SOB/LB broth and incubated at 37°C with constant rotation around 150-200 rpm for 6-8 hrs. This primary culture was then inoculated (4ml, 3ml, 2ml, 1ml) into four 250 ml conical flasks containing 100 ml of SOB broth and incubated at 18-22°C with moderate shaking for overnight. Incubation was stopped when the OD reached 0.55 at 600nm and cells were harvested by centrifuging at 2500g for 10 min at 4°C. Supernatant was poured off and centrifuge tube was stored open on a stack of paper towel for 2 min to drain away the broth completely. Cell pellet was resuspended in 32 µl of inoue transformation buffer for 100 ml of initial culture. Cells were harvested again by centrifuging at 2500g for 10 min at 4°C. Supernatant was discarded and centrifuge tube was stored open on a stack of paper towel for 2 min to drain away the solution completely. The cells were then suspended in 2 ml of ice cold inoue transformation buffer and 0.15 µl of DMSO was added and mixed by swirling and stored in ice for 10 min. Bacterial suspension was aliquot into microfuge tubes and immediately snap froze
by immersing the tightly closed tubes in liquid nitrogen. Then the tubes were stored at -70°C until needed.

2.2.2 Bacterial transformation

Ultra competent *E.coli (DH5α* strain) cells were thawed and aliquot into a sterile microfuge tube. 2.5µl of plasmid DNA of concentration around 10µg was added to 50µl of competent cells or 10 µl of ligation mixture was added to 100µl of competent cells. The tubes were stored in ice for 30 min and then transferred to 42°C for exactly 90 sec. Then they were rapidly transferred to ice and kept for 1-2 min. 1ml of LB broth was added to each tube and incubated at 37°C for 45 min. The samples were centrifuged at 5000 rpm for 1 min and the cell pellet was resuspended in 200µl of LB broth and plated on LB medium containing 100µg/ml of ampicillin or 50 µg/ml of Kanamycin. The plates were allowed to dry and incubated at 37°C for overnight.

2.2.3 Alkaline lysis minipreparation for plasmid extraction from bacterial transformants

Plasmid DNA was isolated from bacterial transformants by alkaline lysis miniprep method described in (Sambrook and Russell, 2001). Single bacterial colony was inoculated in 5 ml of LB broth containing 100µg/ml of ampicillin and incubated at 37°C and 160 rpm for overnight. 1.5ml of bacterial culture was transferred in a microfuge tube and centrifuged at 13 k rpm for 1 min. The cells were resuspended in 200µl of solution I and then solution II was added and mixed by gently inverting the tube for 5 times till the solution is turned clear and viscous. Ice cold 200µl of solution III was added and mixed immediately by inverting several times and left on ice for 5 min and the solution turns into a white precipitate. Then the sample was centrifuged for 8 min at 13 k rpm at 4°C. Supernatant was gently pipette and transferred to another clear microfuge tube. To this solution 420µl of 100% isopropanol (0.7 volume) was added and mixed by inverting. DNA was precipitated by centrifuging at 13 k rpm for 10 min and the pellet was washed with 500µl of 70% ethanol and once again centrifuged at 13 k rpm for 2 min. Supernatant was discarded, DNA pellet was air dried and resuspended in 30-50 µl of TE pH8.0 containing 30 µg/ml of RNaseA.
2.2.4 Construction of plasmids

EST2 clone in multi copy plasmid (CKM206) was constructed by digesting 2a genomic library plasmid by BamHI and SalI enzymes and DNA fragment of size 3.8 kb containing full length EST2 gene along with its promoter region was ligated into BamHI and SalI digested multi copy vector, Yeplac181 (CKM6) listed in Table 2. Full length NKP2 gene along with its promoter was amplified by PCR digested with EcoRI & HindIII and cloned into EcoRI and HindIII digested Yeplac 181multi copy vector (CKM204). Sequences of the primers used for cloning NKP2 were given in Table.3. The other plasmids used in this work are listed in Table.2. Plasmids used as templates in PCR to generate yeast knockouts and tags are described in (Longtine et al., 1998).

2.3 Methods in yeast cell biology

2.3.1 Western blot

KRY549 (NKP2-13xmyc) and ctf19 subcomplex mutants with NKP2-13xmyc strains was grown in liquid media for overnight at 30°C. Whole cell protein from the tagged strains was extracted using standard TCA protocol described in (Lewis et al., 2007). Equal amounts of protein were loaded in two separate 10% polyacrylamide gels. After electrophoresis, proteins were transferred to two PVDF membranes and blocked with 5% non fat dry milk in TBST (150 mM NaCl, 10mM Tris-HCl pH8.0 and 0.1% Tween 20) buffer for one hour at RT. Then the membranes were incubated with anti myc antibody, (1:30,000 in 1% BSA in TBST buffer) for Nkp2-myc. The membranes were washed thrice in TBST buffer for 10 min each and incubated with secondary anti-rabbit-HRP antibody (1:10,000 in 1% BSA in TBST buffer) for Nkp2-myc for 1 hour at RT. Membranes were washed thrice with TBST for 10 min each and BioRad detection reagents and BioRad versadoc instrument were used for detecting the protein of interest as directed by manufacturers instructions. Same blots was stripped with stripping solution and probed with anti-Sir2 antibody (1:2,000 in 1% BSA in TBST buffer) for Sir2 for 2 hours at Room Temperature and incubated with secondary anti-rabbit-HRP antibody (1:10,000 in 1% BSA in TBST buffer) for Sir2 for 1 hour at RT to check the loading consistency.
2.3.2 Immunofluorescence

Immunofluorescence was done as described in (Gotta et al., 1996). Briefly, diploid yeast strains KRY109 (Sir4-13xmyc) was transformed with either empty vector CKM6 or EST2, NKP2 multi copy plasmids listed in Table 2 and the transformants were grown in SC-Leu broth and KRY549(.NKP2-13xmyc:his) and other Ctf19 subcomplex mutants with NKP2-13xmyc strains listed in Table 1 were grown in liquid YPD Overnight grown 5ml cultures were fixed with 0.5 ml of formaldehyde and incubated at 30°C in a shaker for 20 min. Cells were then washed thrice with sterile water and resuspended in 200 µl of 0.1M EDTA-KOH and 10mM DTT and incubated at 30°C for 10 min. Cell suspension was centrifuged at 3 k rpm for 5 min. The cells were spheroplasted by resuspending in 200 µl of YPD broth containing 1.2M sorbitol and one-tenth volume of zymolyase (2.5mg/ml) and incubated at 30°C for 30 min. Spheroplasts were washed thrice with 500 µl of YPD sorbitol and resuspended in 100 µl of YPD sorbitol. Spheroplasts were spotted on multi-well slides coated with polylysine. They were further permeabilized with methanol and acetone by incubating for 5 min and 1 min respectively at -20°C. Spheroplasts were blocked with 1% bovine serum albumin and incubated with appropriate primary antibody dilutions (mouse NSP1 antibody 1:500, rabbit myc antibody 1:12,000, rabbit SIR2 antibody 1:2000) for overnight at 4°C. Cells were then thoroughly washed thrice with PBST buffer for 5 min each and incubated with recommended dilutions of fluorescently labelled secondary antibody (alexa fluor tagged secondary anti mouse antibody 1:500, Cy3 tagged secondary anti rabbit antibody 1:500) in dark at room temperature for 45 min. Cells were thoroughly washed thrice with PBST buffer for 5 min each. After washes, slides were mounted in mounting medium containing DAPI and then viewed and photographed in a Leica confocal microscope. Images were processed using the same software.
Tables
Table 1: List of the yeast strains used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
<th>Source/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRY1</td>
<td>W303-1A/W303-1B MAT a/MAT a</td>
<td>(Thomas and Rothstein, 1989)</td>
</tr>
<tr>
<td>KRY2</td>
<td>W303-1A (leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 rad5-535) MAT a</td>
<td>(Thomas and Rothstein, 1989)</td>
</tr>
<tr>
<td>KRY3</td>
<td>W303-1B (leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 rad5-535) MAT a</td>
<td>(Thomas and Rothstein, 1989)</td>
</tr>
<tr>
<td>KRY9</td>
<td>yku70::Kanmx6 hmrAeb:: 3xUASg TRP1 + gal4::Leu2 (Leu+)</td>
<td>(David Shore)</td>
</tr>
<tr>
<td>KRY12</td>
<td>adh4::URA3-TelVII L MAT a</td>
<td>(Gotschling et al., 1990)</td>
</tr>
<tr>
<td>KRY28</td>
<td>hmrΔaeB :: 1XUASgURA3 MAT a</td>
<td>(Sternglanz)</td>
</tr>
<tr>
<td>KRY30</td>
<td>hisI- MAT a (mating tester)</td>
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</tr>
<tr>
<td>KRY31</td>
<td>hisI- MAT a (mating tester)</td>
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<tr>
<td>KRY33</td>
<td>SIR4-13xmyc:KanMx6 MAT a</td>
<td>(David Shore)</td>
</tr>
<tr>
<td>KRY38</td>
<td>W303-1B ADE2+ MAT a</td>
<td>(Susan Wente)</td>
</tr>
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<td>KRY48</td>
<td>hmr ΔAeb::3x UASg TRP1 MAT a</td>
<td>(YSB35b)</td>
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<td>KRY62</td>
<td>hmr ΔAeΔb::3x UASg TRP1 MAT a</td>
<td>(Chien et al., 1993)</td>
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<td>KRY109</td>
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<td>nkp2::his5+ MAT a</td>
<td>This study</td>
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<td>KRY311</td>
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<td>This study</td>
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<td>KRY313</td>
<td>nkp2::TRP1 adh4::URA3-TelVII L MAT a</td>
<td>This study</td>
</tr>
<tr>
<td>KRY314</td>
<td>nkp2::his5+ adh4::URA3-TelVII L MAT a</td>
<td>This study</td>
</tr>
<tr>
<td>KRY315</td>
<td>nkp2::TRP1/ nkp2::his5+ MAT a/MAT a</td>
<td>This study</td>
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<tr>
<td>KRY501</td>
<td>W303 ade2 can1 leu2 his3/cyc1 LacI::URA3</td>
<td></td>
</tr>
</tbody>
</table>

All strains used in this study were isogenic to W303.
**LacO::TRP1(CENIV)/LACO::TRP1**

*spo13::hisG/Spo13* (diploid)  
(AW Murray 1996)

KRY509  
W303 ade2 can1 leu2 his3/cyc1 *LacI::URA3*  
*Kry509*  
W303 ade2 can1 leu2 his3/cyc1 *LacI::URA3*

KRY510  
*LacO::TRP1(CENIV) MAT a*  
This study

KRY520  
*ade2::hisG his3Δ leu2Δ lysΔ met15:: TRP1 trp1Δ63*  
*ura3Δ sam2::ADE2 NatMx on Chr IVL MAT a*  
(Gottschling 2008)

KRY521  
*ade2::hisG his3 Δ 200 leu2 Δ lys2 Δ MET15*  
*trp1 Δ 63 ura3 Δ sam2::URA3 HPmMx on Chr IVL MAT a*  
(Gottschling 2008)

KRY525  
*sam2::ADE2/sam2::URA3 met15::TRP1/MET15*  
*NatMx4 on Chr IVL/HpMx4 on Chr IVL* (diploid)  
This study

KRY526  
KRY525 except *csm3::KanMx4/csm3::KanMx4* (diploid)  
This study

KRY528  
KRY525 except *ctf19::KanMx4/ctf19::KanMx4* (diploid)  
This study

KRY532  
KRY525 except *nkp2::KanMx6/nkp2::KanMx6* (diploid)  
This study

KRY549  
*NKP2-13xMyc:his5+ ADE2 MAT a*  
This study

KRY950  
KRY2 *SUP11*  
This study

KRY951  
KRY950 *SUP11* except *ctf3::KanMx4 MAT a*  
(Marston 2009)

KRY952  
KRY950 *SUP11* except *nkp1::KanMx4 MAT a*  
(Marston 2009)

KRY953  
KRY950 *SUP11* except *nkp2::his5+*  
This study

KRY954  
KRY950 *SUP11* except *mcm16:: KanMx4*  
This study

KRY955  
KRY950 *SUP11* except *mcm17:: KanMx4*  
This study

KRY956  
KRY950 *SUP11* except *mcm21:: KanMx4*  
This study

KRY957  
KRY950 *SUP11* except *mcm22:: KanMx4*  
This study

KRY958  
KRY950 *SUP11* except *iml3:: KanMx4*  
This study

KRY959  
KRY950 *SUP11* except *ctf19:: KanMx4*  
This study

KRY960  
KRY950 *SUP11* except *nkp2::his5+ ctf3::KanMx4*  
This study

KRY961  
KRY950 *SUP11* except *nkp2::his5+ nkp1::KanMx4*  
This study
<table>
<thead>
<tr>
<th>Name of the plasmid</th>
<th>Old Name</th>
<th>Brief Description</th>
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<tbody>
<tr>
<td>CKM6</td>
<td>YEplac181</td>
<td>2µ plasmid containing LEU2 marker</td>
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<tr>
<td>CKM17</td>
<td>p RAP1-GBD in pRS313</td>
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<tr>
<td>CKM18</td>
<td>p RAP1-GBD – Rap1 in pRS313</td>
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<tr>
<td>CKM32</td>
<td>CEN plasmid containing HIS3 marker</td>
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<td>CKM51</td>
<td>GBd -Yif1 in pMA424</td>
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<td>CKM57</td>
<td>E94</td>
<td>p RAP1-GBD – Sir4 in pRS313</td>
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<td>CKM67</td>
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<td>pFA6a-kanMX6 (gene deletion)</td>
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<td>E336</td>
<td>pFA6a-TRP1 (gene deletion)</td>
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<td>pFA6a-His3MX6 (gene deletion)</td>
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<td>CKM74</td>
<td>E342</td>
<td>pFA6a-13Myc –His3MX6 (C-terminus tagging)</td>
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<tr>
<td>CKM90</td>
<td>E358</td>
<td>pFA6a-13Myc-kanMX6 (C-terminus tagging)</td>
</tr>
<tr>
<td>CKM202</td>
<td>2a Library plasmid</td>
<td>Consisting of Chr XII fragment (764622-768636) i.e., NKP2 &amp; EST2</td>
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<td>CKM204</td>
<td>YEplac181+ NKP2</td>
<td>PCR product of NKP2 digested with HindIII and EcoRI inserted into CKM6</td>
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<td>CKM205</td>
<td>YEplac181+ EST2</td>
<td>BamHI &amp; SalI cuts EST2 from 2a library plasmid</td>
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Table 3: List of primers and their sequences used for PCR in this study

<table>
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<th>Name of the primer</th>
<th>Primer sequence (5’ to 3’)</th>
<th>Purpose</th>
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<td>F1 \textit{NKP2}</td>
<td>\text{GACAATTGTGCTCCCAATCTTGCTTTGAAATTACCATTATGAAACTCggatccgggttaattaa}</td>
<td>\textit{NKP2} gene deletion</td>
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<td>F2 \textit{NKP2}</td>
<td>\text{GCCATTCTGGGCCATTACAAATTATACATTTATATATAAATTATTTCAAggatccgggttaattaa}</td>
<td>\textit{NKP2} deletion/C-terminal tagging</td>
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<tr>
<td>R1 \textit{NKP2}</td>
<td>\text{GGAATATCCTGTGGGGCAGGTTGACCAGGGATCTGCTGCTAGTTTACgaattcgagctcgtttaaac}</td>
<td>\textit{NKP2} gene C-tagging</td>
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<tr>
<td>Scr \textit{NKP2}</td>
<td>\text{GAAGATGGCAGAAACTCG}</td>
<td>Screening PCR</td>
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<td>5N1 \textit{NKP2}</td>
<td>\text{CGAAGCTTCAATGGATCGGCGAGGAATATC}</td>
<td>Cloning full length \textit{NKP2} gene with promoter</td>
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<tr>
<td>3N1 \textit{NKP2}</td>
<td>\text{AGGAATCGGAAGGAGAAATCTAGAG}</td>
<td>Cloning full length \textit{NKP2} gene</td>
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<td>Yep13 F</td>
<td>\text{GCTACTTGGAGCCACTATC}</td>
<td>DNA Sequencing</td>
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<tr>
<td>Yep13 R</td>
<td>\text{CCAGCAACCGCACCTGT}</td>
<td>DNA Sequencing</td>
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