Appendix
A 1.1 Construction of EST2 clone in Yeplac181 vector (CKM205)

EST2 gene was sub cloned into Yeplac181, 2µ vector carrying LEU2 and ampicilin resistance markers. 2a genomic library plasmid was digested with BamHI and SalI restriction enzymes and DNA fragment of size 3.8 kb bp containing full length EST2 gene along with its promoter region was ligated into BamHI and SalI digested Yeplac181 (CKM6) vector listed in Table 2. The resulting plasmid was confirmed by digesting with BamHI and SalI restriction enzymes. Yeplac181 vector backbone contains one BamHI site at the polycloning region and insert contains one SalI site. Therefore, upon digestion, the clones give two fragments of size 5.7 kbp and 3.8 kb as seen in Figure A1.C.

Figure A1: Confirmation of EST2 clone in Yeplac181 vector. A). EST2 insert from 2a library plasmid. B). Gel eluted DNA fragments BamHI & SalI digested YEplac181 plasmid of size 5.7 Kb in lane 4 and BamHI & SalI released DNA fragment of size 3.8 kb containing EST2 gene along with its promoter from 2a genomic library plasmid in lane 1-3. C). Confirmation of EST2 clone by digesting with BamHI & SalI enzymes that releases three fragments of size 5.7 kb and 3.8 kb.
A1.2 Construction of NKP2 clone in Yeplac181 vector (CKM204)

NKP2 gene was cloned in multi copy Yeplac181 vector. NKP2 PCR product digested with EcoRI & HindIII and 1.1 kb DNA fragment containing NKP2 gene (453bps) and its promoter region (700bp) was cloned into EcoRI & HindIII digested Yeplac181 vector. The resulted plasmid (CKM204) was confirmed by digesting with EcoRI & Hind III restriction enzyme. Yeplac181 vector backbone contains EcoRI & Hind III sites at poly cloning region flanking the NKP2 insert and the insert does not contain sites for these enzymes. Therefore upon digestion with EcoRI & HindIII enzymes, the clone gives two fragments of size 5.7 kb and 1.1 kb as seen in Figure A2.

![Figure A2: Confirmation of NKP2 clone in Yeplac181 vector (CKM204). NKP2 PCR product and YEplac181 empty vector were digested with EcoRI & HindIII restriction enzymes. Panel A shows NKP2 PCR product digestion gives single fragment of size 1.1kb (insert containing NKP2 gene with promoter) whereas empty vector digested with same EcoRI & HindIII to become linear. In panel B Lane 1 is empty vector showing single band of size 5.7kb. Lane 2 & 3 are NKP2 clones showing fragment of size 1.1 kb.](image-url)

(Appendix)
A 2.1 Construction of nkp2::his5+

**PCR amplification of DNA fragment for NKP2 gene deletion.** nkp2 null mutant was constructed by knocking off complete gene with his5+ marker by using PCR based homologous recombination method (Longtine et al., 1998). The forward primer for deleting NKP2 gene was designed by selecting 30 bp just upstream of start codon and reverse primer by taking 30 bp sequences downstream of stop codon. The sequences of the primers used are listed in Table 3. Plasmid E337 (listed in Table 2) is used as template for amplifying his5+ marker. The Figure A3.A shows the DNA fragment of size 1403 bp amplified by PCR. This DNA was transformed into competent yeast cells by LiAc method. It gets integrated into the genome by homologous recombination. The transformants were selected on histidine dropped out medium. Then single colonies were picked up and the genomic DNA was extracted by phenol:chloroform:isoamylalcohol (25:24:1) method.

**Screening PCR for nkp2 null mutant.** Screening PCR was done for nkp2 null mutant by using the forward primer (around 19 bp) that gets annealed within the deletion module and the reverse primer (19 nt) that anneals with the unaltered downstream region of the NKP2 gene. The sequences of the primers are given in Table 3. The diagnostic PCR product of around 565 bp in Figure A3.B shows that NKP2 gene has been replaced by his5+ marker by homologous recombination.

**Southern confirmation for nkp2 null mutant.** Genomic southern was done for further confirmation of nkp2 null mutant. Genomic DNA from yeast strains that showed positive for NKP2 knockout in screening PCR was isolated by zymolyase method. This genomic DNA was subjected to 6hrs restriction digestion with XhoI & SalI enzymes that gives 0.7 kb fragment at NKP2 locus in WT strain and 2.2 kb fragment in nkp2 null mutant. The digested genomic DNA was run on 0.8% Agarose gel and transferred to nylon membrane. The bands were detected by southern hybridization of the blot with alpha p-32 radiolabelled NKP2 probe. NKP2 probe was made by digesting the multi copy 2a library plasmid (CKM204) with SalI & XhoI restriction enzymes. The DNA fragment of size 654 bp having part of C-terminus of NKP2 gene and its downstream region, was taken as template and radiolabelled using BRIT random radiolabelling kit.
The blot was washed and exposed to autoradiogram. Figure A3.C shows the presence of 2.2 kb band confirming that the particular yeast strain is nkp2 null mutant.

**Figure A3: Construction of nkp2::his5+.** A) PCR product of size 1.4 kb used for replacing NKP2 gene with his5+ marker. B). Screening PCR product of size 0.56 kb in lanes 1-3 shows that NKP2 gene has been replaced with his5+ marker in these strains. C). Genomic southern confirmation for nkp2::his5+ mutants. 2.2 kb band in lane 2-4 represents wild type strain and 0.7 kb band in lane 1 confirms nkp2::his5+ mutant.

**A 2.2 Construction of nkp2::KanMx6 and nkp2::TRP1**

nkp2 knockout with KanMx6 and TRP1 markers was done by following the same method used for knocking out nkp2 gene (Appendix 2.1). The sequences of the primers used for deleting nkp2 gene are given in Table 3. Plasmids E335 and E336 listed in Table 2 were used as templates to amplify KanMx6 and TRP1 markers respectively for replacing NKP2 gene. The Figure A4.A shows the PCR product of KanMx6 (1.4 kb) and TRP1 (1 kb) DNA fragments respectively. This DNA was transformed into yeast strains. The strains transformed with KanMx6 marker were selected on YPD plate containing...
200 µg of G418 drug and those with TRP1 marker were selected on tryptophan dropped out medium. These strains were further confirmed by genomic southern by hybridizing with NKP2 gene specific probe. The genomic DNA from these strains was digested with XhoI & SalI enzyme. WT strain gives band of size 0.7 kb, nkp2::KanMx6 mutant gives band at 2.2 kb and nkp2::TRP1 gives band at 1.8 kb. The template for NKP2 probe was made from 2a library. The DNA fragment of size 654 bp having part of C-terminus of NKP2 gene and its downstream region was taken as template and radiolabelled using BRIT random radiolabelling kit. The result of the autoradiogram of southern blot in Figure A4.B & C shows 0.7 kb band in WT strain, 2.2 kb band in nkp2::KanMx6 mutant and 1.8 kb band in nkp2::TRP1 mutant confirms that these yeast strains are nkp2 null mutants.

![Figure A4: Construction of nkp2::KanMx6 and nkp2::TRP1. A). PCR product of size 1.4 kb used for replacing NKP2 gene with KanMx6 marker and PCR product of size 1kb used for replacing NKP2 gene with TRP1 marker. B). Genomic southern confirmation of nkp2::KanMx6 mutant. 2.2 kb band in lane 2 and 4 confirms nkp2::KanMx6 mutant and C) 1.8 kb band in lane 7 confirms nkp2::TRP1 mutant. 0.7 kb band in other lanes shows that the genomic DNA in these strains was unaltered at NKP2 locus.](image-url)
A 2.3 Construction of NKP2-13x-myc strain

Nkp2 was tagged with 13x myc epitope at its C-terminus by PCR based homologous recombination method (Longtine et al., 1998). 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 myc epitope and selectable marker HIS3Mx6 which is going to be inserted in the downstream of the gene. The sequences of the primers are given in Table 3. Plasmid E342 (listed in Table 2) was used as template for PCR to amplify 13x myc-HIS3Mx6 DNA fragment. Figure A5.A shows the PCR product of 13x myc-HIS3Mx6 DNA fragment of size 2.2 kbp which is transformed into yeast strain by following high efficiency LiAC transformation protocol. The transformants were selected on histidine dropout medium. The colonies that grew on histidine dropout medium were subjected to screening PCR. The sequences of the primers used for screening PCR are given in Table 3. The diagnostic screening PCR product of 565 bp in Figure A4.B shows that NKP2 is tagged with myc epitope at its C-terminus.

These strains were further confirmed by genomic southern by hybridizing with NKP2 gene specific probe. The genomic DNA from these strains was digested with Xhol and SalI restriction enzymes by incubating at 37ºC for 6-8hrs. WT strain gives band of size 0.7 kb whereas NKP2-13xmyc-HIS3Mx6 strain gives band of size 2.8 kb at NKP2 locus. NKP2 probe was made by digesting the multi copy 2a library plasmid (CKM202) with SalI & Xhol restriction enzymes. The DNA fragment of size 654 bp having part of C-terminus of NKP2 gene and its downstream region, was taken as template and radiolabelled using BRIT random radiolabelling kit.. The result of the autoradiogram of southern blot in Figure A5.C shows 0.7 kb band in lane 1 represents WT strain and 2.8 kb band in lane 3 confirms that the yeast strain is tagged with 13myc epitope at C-terminus of NKP2.
Figure A5: Construction of NKP2-13xmyc tag. A). 2.2kb PCR product used for tagging 13x myc at C-terminus of NKP2 gene. B). Screening PCR product of size 560bp in lanes 1-3 shows that NKP2 gene has been tagged with 13x myc-HIS3Mx6 at its C-terminus in these strains. C). Genomic southern confirmation of NKP2-13xmyc-HIS3Mx6 strain. 0.7 kb band in lane1 represents wild type strain and 2.8 kb band in lane2-4 confirms NKP2-13xmyc-HIS3Mx6 strain.