**Chromosome creation primers**

**AL1 C-terminus His tag:**

**5’ telomere addition Fw:**

CCC CAA AAC CCC AAA ACC CCA TGA GAG TTT GTG AAA AAT TAA GTT TG

**3’ telomere addition Rev:**

CCC CAA AAC CCC AAA ACC CCT ATA TTA AAT ATC AAG AAA AAG TAA AAA GAC AG

**AL1/Histidine tag overlap primer Fw:**

GGT GAT GGT GAT GAC CAC TTC CAC CTG ATC CTT CTT GAT TGG TCA AGT CTA CGG TGA TGG

**AL1/Histidine tag overlap primer Rev:**

AGG TGG AAG TGG TCA TCA CCA TCA CCA TCA CTG AGA CCA TCA ACA GAG ACT ATC CCT AAC

**Hsp70-GFP:**

**5’ telomere addition Fw:**

CCC CCA AAC CCC CAA ACC CCT GTA GTA TCA AAT ATG TAA G

**3’ telomere addition Rev:**

CCC CAA AAC CCC AAA ACC CCA TGA TAA ATA ATT TGA AGT AC

**5’ Hsp70 UTR/GFP overlap primer Rev:**

ATT CAT AAT TTT AGT GTT TAG TGT TTA ATT AAA AGT TAT AAT TAT TAC ATC ATT AAT CGC

**5’ Hsp70 UTR/GFP overlap primer Fw:**

CTT TTA ATT AAA CAC TAA ACA CTA AAA TTA TGA ATT CTA GAG GAG AAG AAC TTT TCA CTG

**3’ Hsp70 UTR/GFP overlap primer Rev:**

CAC TGA TTA GCT CAA TCT CCA GCA TGT TGG GCC ATA TG

**3’ Hsp70 UTR/GFP overlap primer Fw:**

CTG GAG ATT GAG CTA ATC AGT GAA AGC AAT CTC AGC TAA ATA TTC TTT ATG

**Chromosome detection primers**

**AL1-Histindine tag detection:**

**AL1 c-terminus primer Fw:**

GAC AAG GAT CAG AAG ATC AAC AAC AGT C

**AL1 c-terminus primer Rev:**

GTT AGG GAT AGT CTC TGT TGA TGG TCT C

**GFP detection:**

5’ Hsp70 UTR/GFP overlap primer Fw & 3’ Hsp70 UTR/GFP overlap primer Rev as listed above

**TEBP-β (Contig22260.0):**

**TEBP-β Fw:**

GAG CAA ATC ACA ACA AGT TCA ACA ACA AAG CG

**TEBP-β Rev:**

GCT CAT TTC TTG GTT GAT CTC TTT GAG GCC

**qPCR primers**

**Mito Cox1:**

**qPCR Fw:**

GCC GTG TTT ACG CTT ATT TAC A

**qPCR Rev:**

CGT CTA GGC ATA CCA GCA TAT C

**AL1 (Contig20822.0):**

**AL1 qPCR Fw:**

AAA GTC TGG TTC TCG TGG C

**AL1 qPCR Fw:**

TCT GAA GCT TGT CCT TGG ATG

**AL1-Histidine tag qPCR Fw:**

GAG GCC ATC CAA CAA GAT CAC

**AL1-Histidine tag qPCR Rev:**

ATG ACC ACT TCC ACC TGA TCC

**Hsp70 (Contig18685.0):**

**Hsp70 qPCR Fw:**

TCC CCA ACG ATC AAG GAA AC

**Hsp70 qPCR Rev:**

ATA AGT CTC TTG GCG TCG AAG

**GFP:**

**GFP qPCR Fw:**

TGC CAT GCC AGA AGG ATA TG

**GFP qPCR Rev:**

ACT TCA GCT CTT GTC TTG TAG TT

**Otiwi1 (Contig16116.0):**

**Otiwi1 qPCR Fw:**

CCT GAG ATT TGA GAC CAT CGG

**Otiwi1 qPCR Rev:**

AGT CTA GCA TCA AAT CCA GGC

**AL2 (Contig15169.0):**

**AL2 Fw:**

GAA TGG ACC TGT GAC TGA GAA G

**AL2 Rev:**

GAT ACG ATT GCT GAA CTT GCT

**TEBP-α (Contig22209.0):**

**qPCR Fw:**

CCA CAG AGC CAC CAT CAG ACT TTA

**qPCR Rev:**

GAG AAT GGT ACG AGA TCG CTA GTA GC

**170 Repeat:**

**170 Repeat qPCR Fw:**

TGA CCA TCA CTT GAA AAT GCA TG

**170 Repeat qPCR Rev:**

CCA CGT ATC TGG TGA GTT GAG

**Telomeric PCR**

**AP12C4A4 (1st round):**

GTA ATA CGA CTC ACT ATA GGG CAC GCG TGG TCG ACG GCC CGG GCT GGT CCC CAA AAC CCC AAA ACC CCA AAA

**GFP-specific primers (1st round):**

Same as 3’ Hsp70 UTR/GFP Rev & 5’ Hsp70 UTR/GFP Fw from Hsp70-GFP creation primer set

**AP2 (2nd round):**

ACT ATA GGG CAC GCG TGG T

**GFP-specific Fw primer (2nd round):**

CTG CTG CTG GAA TTA CAC ATG GCA TGG ATG

**GFP-specific Rev primer (2nd round):**

TAA GTT GCA TCA CCT TCA CCC TCA CCA GAG

**Supplementary figure 1:** **Sanger sequencing of artificial nanochromosomes shows no changes in telomere location**

A) Diagram of the Hsp70-GFP construct transformed along with GFP specific primers (dark green triangles) used in the first or nested, second round of telomeric PCR. Telomeres are marked schematically as red rectangles, the GFP open reading frame highlighted in green, and the non-coding regions from the Hsp70 nanochromosome in which GFP replaced the Hsp70 gene are shown in yellow. B) Agarose gel of second round telomeric PCR from the GFP transformants with both 3’ and 5’ telomeric PCR products. Ladder is 1 KB+ (Thermofisher Scientific). Product sizes are 388 bp (3’) and 514 bp (5’). C) Sanger sequencing of the telomeric PCR products in panel B after gel extraction. Sequencing is aligned with respect to the GFP construct depicted in panel A. Grey bars at the bottom represent the Sanger sequenced region (along with details of the PCR from which they were generated on the left). The sequenced regions perfectly matched the designed construct in both cases, indicating correct retention of telomere addition sites.

**Supplementary figure 2:** **Validation of successful translation of the histidine tagged AL1 protein**

Western blot with Anti-rabbit Anti-histidine tag antibody (RM146) against cell lysate for JRB310 x JRB510 (wild type) and AL1 His-tag transformant x JRB510 mating at 18 hours post mixing. The band shown is approximately 70 kilodaltons in size (expected size of the tagged product is 65 kD).

**Supplementary figure 3:** **Gel electrophoresis quality control of the histidine tag immunoprecipitation**

SDS-PAGE gel of the Anti-histidine tag immunoprecipitation with the AL1 band, the heavy chain and light chain of the antibody labeled along with bands on the ladder. Lanes contain various fractions (as labeled) from the immunoprecipitation on the cell lysates from 18 hours post mixing of AL1-histidine tag mating (AL1-HT transformant x JRB510) and wild type mating (JRB310 x JRB510).

**Supplementary figure 4:** **Tagged AL1 associates with numerous RNAs**

Quantitation via qPCR of RNA from immunoprecipitation with an Anti-histidine tag antibody (RM146) of the histidine tagged AL1 mating (AL1-His tag x JRB510) normalized to levels in the immunoprecipitation of a wild type mating (JRB310 x JRB510). Targets quantified are RNA from the micronuclear-limited 170bp repeats, various macronuclear genes (AL2, Otiwi1, and TEBP-α), and the mitochondrial Cox1 gene (Mito Cox1). Error bars represent standard deviation of the technical replicates.