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April 10, 2023

Evolution of Centromeric Protein Coding Genes in the *Mus* Genus

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a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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Centromeres are chromosomal loci that facilitate error-free chromosome segregation during mitosis and meiosis to avoid aneuploidy and genetic instability, hallmarks of cancers and chromosomal disorders. Paradoxically, while the essential centromere function is strongly conserved, its components (centromeric DNA and associated proteins) are rapidly evolving. Centromeric components provide a unique system to understand evolutionary selection pressure acting on essential genomic components. Our lab has found that genes encoding centromeric proteins are under adaptive Darwinian selection in several *Mus* species. My study involves investigating the patterns of evolutionary changes in centromeric protein-coding genes in *Mus* species and how they contribute to the overall evolutionary selection pressure observed in the *Mus* genus. I found that centromeric proteins evolve differently in different phylogenetic branches of the *Mus* genus. Furthermore, centromeric proteins show different amounts of localization to centromeres in *Mus* species suggesting functional evolution in progress. Additionally, toward identifying possible copy number variations, which might be responsible for the rapid evolution of these genes, I have developed a cytological approach to determine the copy number of one of the centromeric protein-coding genes. Understanding the evolution of centromeric protein-coding genes will provide greater insight into centromere function and chromosomal disorders.

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Introduction

Centromeres determine where spindle microtubules attach on a given chromosome and are vital for chromosome segregation during cell division (Bloom and Costanzo 2017). Centromeres, with associated centromeric protein complexes, are essential for error-free chromosome segregation during mitosis and meiosis to avoid chromosome division errors such as aneuploidy and genetic instability, hallmarks of cancers and chromosomal disorders. Within eukaryotic chromosomes, DNA is packaged by histone proteins into chromatin. The basic unit of chromatin is the nucleosome, which consists of 146 bp long DNA wrapped around an octamer of histones (2 copies of each histone H2A, H2B, H3 and H4). At centromeres, nucleosomes are slightly different – histone H3 is replaced by a variant of H3 called CENP-A. CENP-A is the marker of centromere identity and function in most eukaryotes. CENP-A is deposited to centromeres by a specific chaperone called HJURP, which associates with chromatin during the late telophase/early G1 phase. Genes with essential functions tend to remain conserved because mutations disrupt their dedicated essential function. Therefore, given their essential function in chromosome segregation, it was previously assumed that centromeres and their associated components would be evolutionarily conserved. However, both centromeric DNA loci and several centromeric proteins were found to be rapidly evolving in eukaryotes. (Sanchez-Pulido et al. 2009, Kumon et al. 2021).

The centromere drive model, derived from the meiotic drive model, has been proposed to explain the rapid evolution of centromeric components. Female meiosis is asymmetric; out of four meiotic products, only one gives rise to the egg, while the other three are discarded as polar bodies. The meiotic drive model suggests that a variant gene that somehow can get access to the

egg is preferentially transmitted to future generations. Such an advantage in preferential transmission will allow the variant to spread across the population rapidly. For example, an increase in the size of the centromeric DNA locus can lead to increased microtubule binding, which could provide advantages to this centromere variant in segregating to the egg during female meiosis. However, such a change will pose fitness cost in males where meiosis is symmetrical. According to the centromere drive model, centromeric proteins evolve to suppress the harmful effects of centromeric DNA evolution in males. It remains to be proven whether centromeric proteins indeed evolve to functionally counter the evolution of centromeric DNA. Therefore, understanding evolutionary selection pressures and the functional consequences of the rapid evolution of centromeric protein-coding genes is of paramount interest to both evolutionary biology and functional genomics. Moreover, centromeric proteins, including HJURP, are overexpressed in several types of cancer (Dunleavy et al. 2009). A better understanding of HJURP protein evolution may also provide insight into the role of centromere mutations in cancer (Bloom and Costanzo 2017), especially alongside other centromeric proteins of the centromere complex.

My aim is to understand the how the evolution of essential centromeric proteins, including CENP-A, CENP-B, CENP-C, and CENP-T, affect centromere function in evolving species (Figure 1).

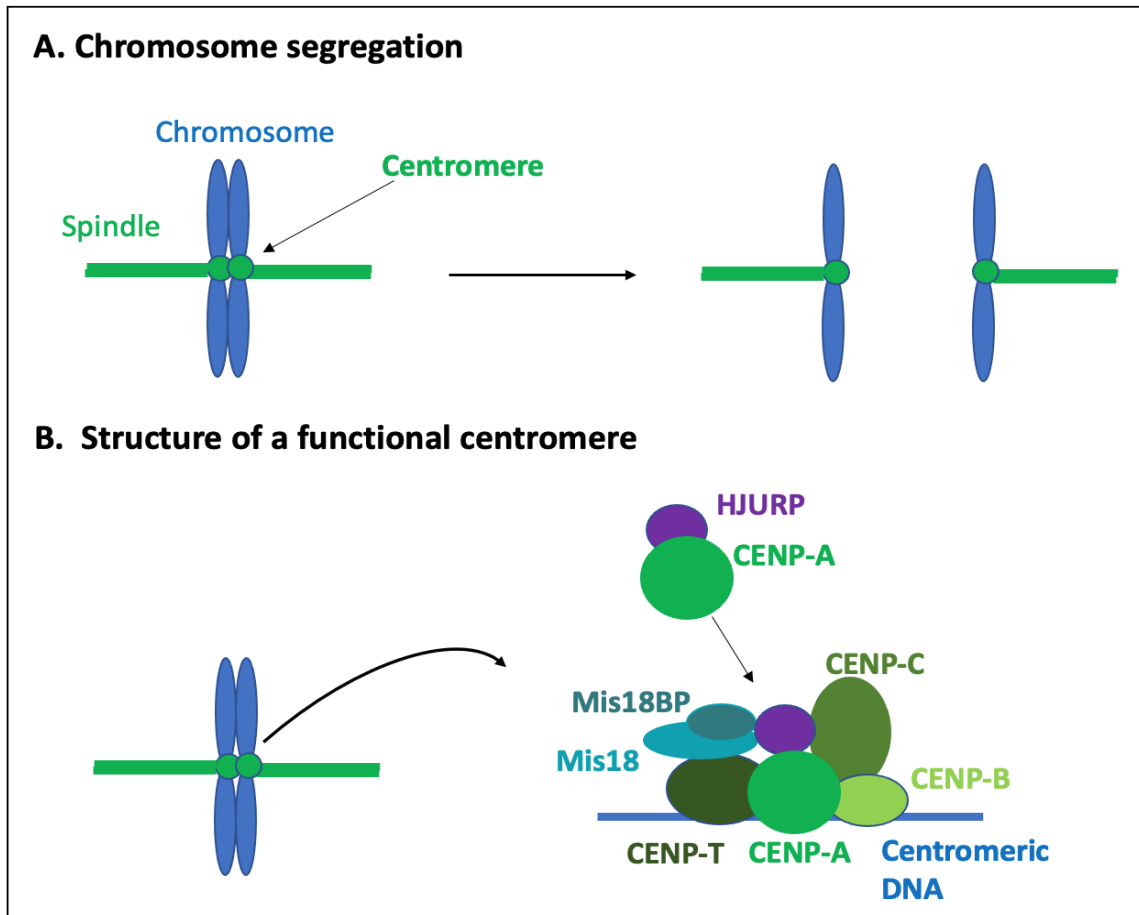


Figure 1. Functional centromeres facilitate chromosome segregation during cell division A. Spindle microtubules capture chromosomes by binding to functional centromeres and pull sister chromatid toward opposite poles to allow for chromosomes segregation. B. Components of a functional centromere. Centromeric proteins are localized to and bind centromeric DNA. HJURP localizes CENP-A to the centromeric DNA region, which allows for binding of additional proteins, including CENP-B, CENP-C, CENP-T, Mis18 and Mis18BP that are necessary for the attachment of the centromere to spindles. Together, centromeric proteins and the centromeric DNA form functional chromosome segregation machinery (Perpelescu, 2011).

Results

1. Centromeric protein-coding genes are evolving differently across different phylogenetic branches of the *Mus* genus.

The *Mus* genus is one of the best studied mammalian groups, and whole genome sequencing data is readily available for several closely related species. Various *Mus* lineages and species allow

for efficiently tracking centromeric protein evolutionary changes between species with varying but known evolutionary distances and relatedness (Figure 2).

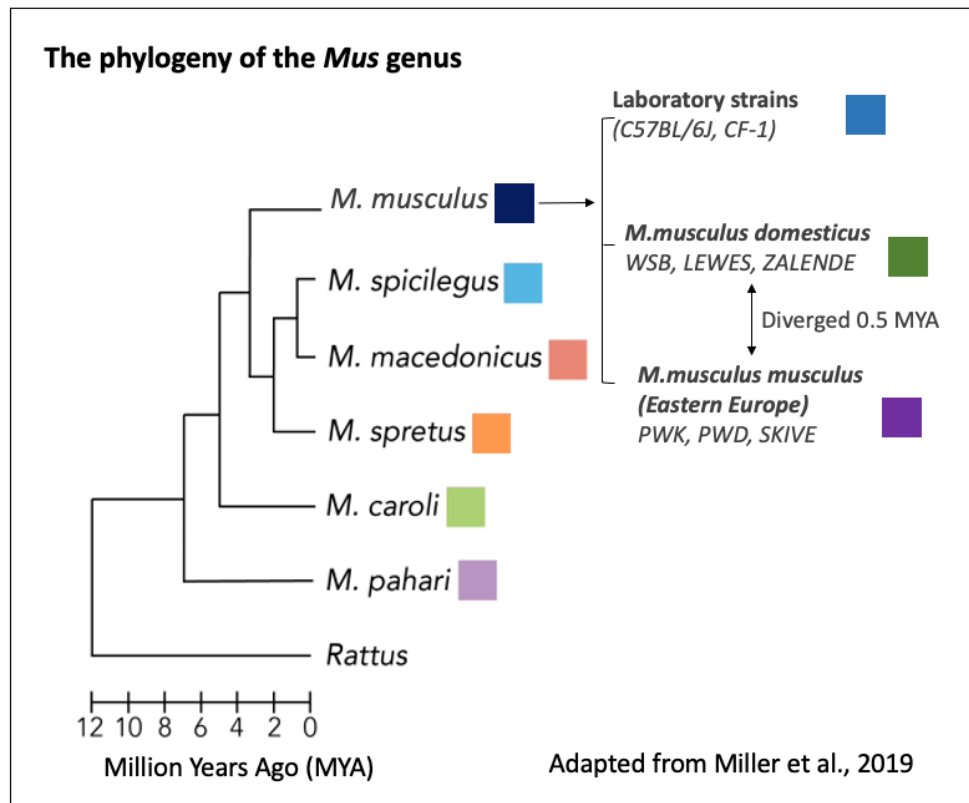


Figure 2. The phylogeny of the *Mus* genus. The *Mus* phylogenetic tree showing early diverging (separated by < 0.5 MYA) and late diverging lineages/species for which publicly available Whole Genome or transcriptome datasets are available for evolutionary analysis of protein-coding genes. Adapted from Miller et al. 2020.

Previous work from our lab found that HJURP and CENP-C are under long-term and rapid, short-term adaptive evolution in several *Mus* species (Morrison et al., unpublished). Adaptive evolution allows for the propagation of evolutionary changes that are beneficial to evolving species or lineages. Such adaptive and rapid evolution makes centromeric proteins excellent candidates for investigating evolutionary forces and their functional consequences on essential genes. However, two species, *Mus spicilegus* and *M. macedonicus* from one of the key *Mus* phylogenetic branches,

were not included in this study due to their absence from the assembled genome database at Ensembl.

My goal was to determine how centromeric protein-coding genes are evolving in the *M. spicilegus* and *M. macedonicus* phylogenetic branch with respect to the remaining *Mus* genus. Toward this, I extracted centromeric protein-coding genes and transcript sequences from publicly available whole genome and transcriptome datasets (Kodama, 2012 and SRA/Miller et al. 2020). I first extracted *M. spicilegus* transcripts for centromeric proteins based on homology to its close relative the Algerian mouse, *M. spretus* (phylogenetic distance shown in Figure 2) (Couger et al. 2018). Subsequently, by utilizing the constructed *M. spicilegus* transcripts, I extracted the centromeric protein transcripts for its close relative *M. macedonicus*. Finally, I compared centromeric protein-coding transcripts and found that evolutionary changes were concentrated in specific regions across centromeric protein-coding genes in *M. spicilegus* and *M. macedonicus* (Figure 3A).

Next, I investigated the short-term early selection pressure by comparing the mutation rates between pairs of species. This was performed by calculating the dN/dS ratio that compares the rate of nonsynonymous amino acid changes (dN) to the rate of synonymous amino acid changes (dS) between closely related species (Booker et al. 2017). In neutrality, where selection is not occurring, dN/dS should be equal to 1. The dN/dS ratio greater than 1 indicates positive selection where a beneficial nonsynonymous mutation sweeps through the population and becomes fixed. This means that the frequency of this allele increases in the population. The dN/dS ratio less than 1 indicates negative selection in which the harmful amino acid changes are discarded i.e., purifying selection. I determined if centromeric protein-coding genes are undergoing positive or

negative selection in *M. spicilegus* and *M. macedonicus*, and other closely related *Mus* species (Figure 3B). I found that among centromeric proteins analyzed, only CENP-T was undergoing positive selection between *M. m. domesticus* and *M. macedonicus*. Next, I included *M. spicilegus* and *M. macedonicus* in the long-term adaptive selection analysis previously performed in the lab using CodeML software. The CodeML analysis demonstrates long-term evolutionary trends through a comparison of many selected species, with varying evolutionary distances and relatedness, in the *Mus* genus. As previously reported in the lab, I found that HJURP and CENP-C were undergoing positive selection (Figure 3C). These results suggest that the early evolution of CENP-T in the *M. macedonicus* and *M.m.domesticus* species pair is not adaptive across the long evolutionary distance in the *Mus* genus. Furthermore, the long-term adaptive evolution of HJURP and CENP-C observed in the *Mus* genus is absent in the *M. spicilegus* and *M. macedonicus* branch. Overall, these findings suggest that centromeric protein-coding genes are evolving differently across different branches of the *Mus* genus.

A. Clustering of evolutionary changes across centromeric protein-coding genes



B. Short-term selection between *Mus* species pairs

	<i>macedonicus</i> vs <i>Mmd</i>	<i>macedonicus</i> vs <i>Mmm</i>	<i>macedonicus</i> vs <i>pahari</i>	<i>macedonicus</i> vs <i>caroli</i>	<i>macedonicus</i> vs <i>spretus</i>	<i>macedonicus</i> vs <i>spicilegus</i>
HJURP	0.466	0.765	0.602	0.484	0.596	0.577
CENPA	n/a (dN=0)	n/a (dN=0)	0.299	0.141	n/a (dN=0)	n/a (dN=0)
CENPB1	0.207	0.177	0.117	0.207	0.177	0.311
CENPB2	0.310	0.271	0.143	0.310	0.271	0.310
CENPC	0.635	0.516	0.516	0.782	0.544	0.454
CENPT	1.051	0.843	0.452	0.410	0.777	0.341
Mis12	0.098	0.250	0.155	0.030	0.125	0.248
Mis18a	0.149	0.148	0.399	0.315	0.098	0.301
Mis18bp1	0.687	0.677	0.579	0.749	0.665	0.690

C. Long-term selection across the *Mus* genus

Species: *M.spicilegus*, *M.macedonicus*, *M.pahari*, *M.caroli*, *M.spretus*, *M.m.musculus* and *M.m.domesticus*

	M1 v M2 (p-value)	M7 v M8 (p-value)	Number of codons analyzed	Number of of positive sites	Percent positive sites
HJURP	0.0061658	0.004768	654	22	3.4
CENPA	0.7587	0.4992	132	5	3.8
CENPB	1	1	456	2	0.4
CENPC	1.15E-07	1.90E-08	870	32	3.7
CENPT	0.9737	0.9458	500	11	2.2
Mis12	1	1	207	0	0.0
Mis18a	1	1	203	0	0.0
Mis18bp1	1	1	847	0	0.0

Figure 3: Centromeric protein-coding genes are evolving differently across different branches of the *Mus* genus. A. Evolutionary changes in *M. spicilegus* and *M. macedonicus* centromeric protein-coding genes are concentrated at specific regions when compared to the reference *M. spretus* genes.

These identified domains may be categorized by specific function, with some domains possibly relevant to centromeric binding and targeting. These domains would provide a stronger link between evolution and centromeric protein function, especially if changes in critical domains are identified between multiple species, which would demonstrate a strong evolutionary trend. B. Short-term positive selection using pairwise analysis of *Mus* species pairs. dN/dS is the ratio of the rate of nonsynonymous amino acid (dN) and the rate of synonymous amino acid changes (dS) between two species that determines the nature of ongoing selection pressure. dN/dS = 1, neutral selection, dN/dS < 1, negative selection, dN/dS > 1, positive selection. A dN/dS value ≥ 1, indicating neutral or positive selection is highlighted in red. NA indicates a value that cannot be calculated due to either no synonymous (dS) or nonsynonymous (dN) changes

between the two compared sequences. **C.** Long-term positive selection analysis across several *Mus* species using Codeml analysis. Likelihood of models of neutral codon evolution (M1 or M7) were compared to models allowing positive selection (M2 or M8). Codons with insertions, deletions, and ambiguous alignments were excluded from the analysis. The number of positive selection sites is the number of codons with $p > 0.95$ Bayes Empirical Bayes (BEB) analysis from M8. A Likelihood Ratio Test (LRT) p -value < 0.5 indicating positive selection is highlighted in red.

2. Centromeric proteins exhibit differences in centromere localization in early and late diverging *Mus* species.

Following the characterization of the pattern of evolutionary changes in centromeric protein-coding genes, I investigated how the function of these centromeric proteins has changed during evolution. Localization of centromeric proteins to centromere determines their function in chromosome segregation. Using cytological visualization, I investigated how localization of these proteins to centromeres is different in *Mus* species. I first developed a protocol to prepare immunostaining grade mouse liver cell spreads. I tested several methods, including hypotonic treatment followed by fixation and dropping cells on the slide and a direct tissue spread method followed by fixation. The direct tissue spreading protocol produced high-quality spreads comparable to those produced from a cell line (Figure 4). Next, I standardized antibodies against centromeric proteins in 3T3 cell lines. CENP-A alone was stained with anti-CENP-A antibodies and several centromeric proteins were stained together with CREST antibodies (Supplementary Figure 1). CREST serum is derived from CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) patients and contains antibodies against several centromeric proteins, including CENP-A, CENP-B and CENP-C (Mayo Clinic, 2023).

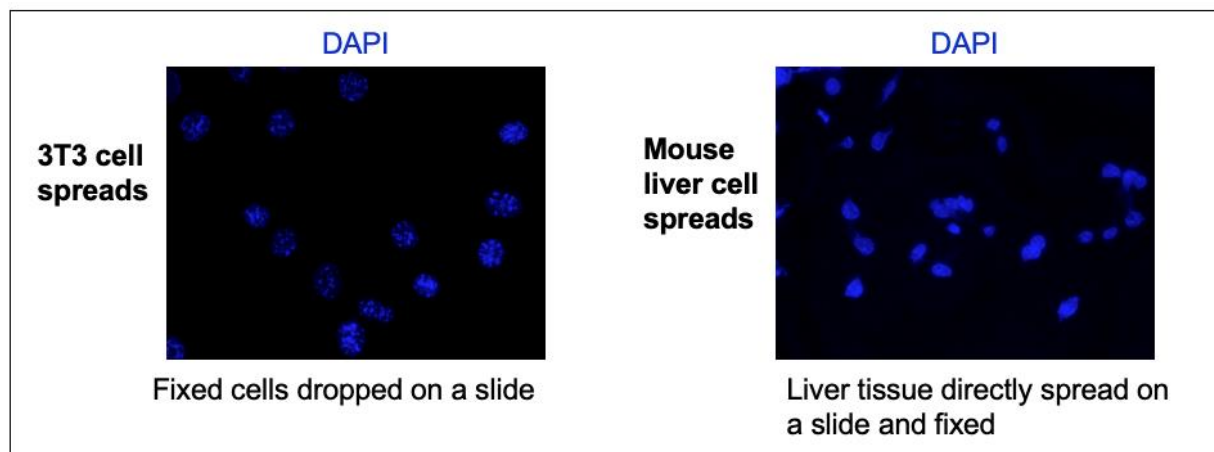


Figure 4. Preparation of cell spreads from mouse liver tissues. Direct spreading of liver tissue on slides results in high quality cell spreads comparable to those produced from a cell line. DAPI stained nuclei from 3T3 cell spreads using dropping method (Left) and mouse liver cell spread produced using the direct spreading method (Right).

Following the standardization of liver cell spreading protocol and antibody staining, I stained centromeric proteins in the liver cell spreads from various early and late diverging *Mus* species (Figure 5A). To determine the relative levels of centromeric proteins at centromeres, total cell fluorescent counts were analyzed for CREST and CENP-A (Figure 5B). I found that the relative intensity of both CENP-A and CREST staining differed in *Mus* species analyzed. These findings reveal specific functional differences at centromeres in the form of centromeric protein localization in early and late diverging *Mus* species and require further investigation, including replicates. These functional differences might have resulted from both early short-term and long-term evolutionary changes in the *Mus* genus.

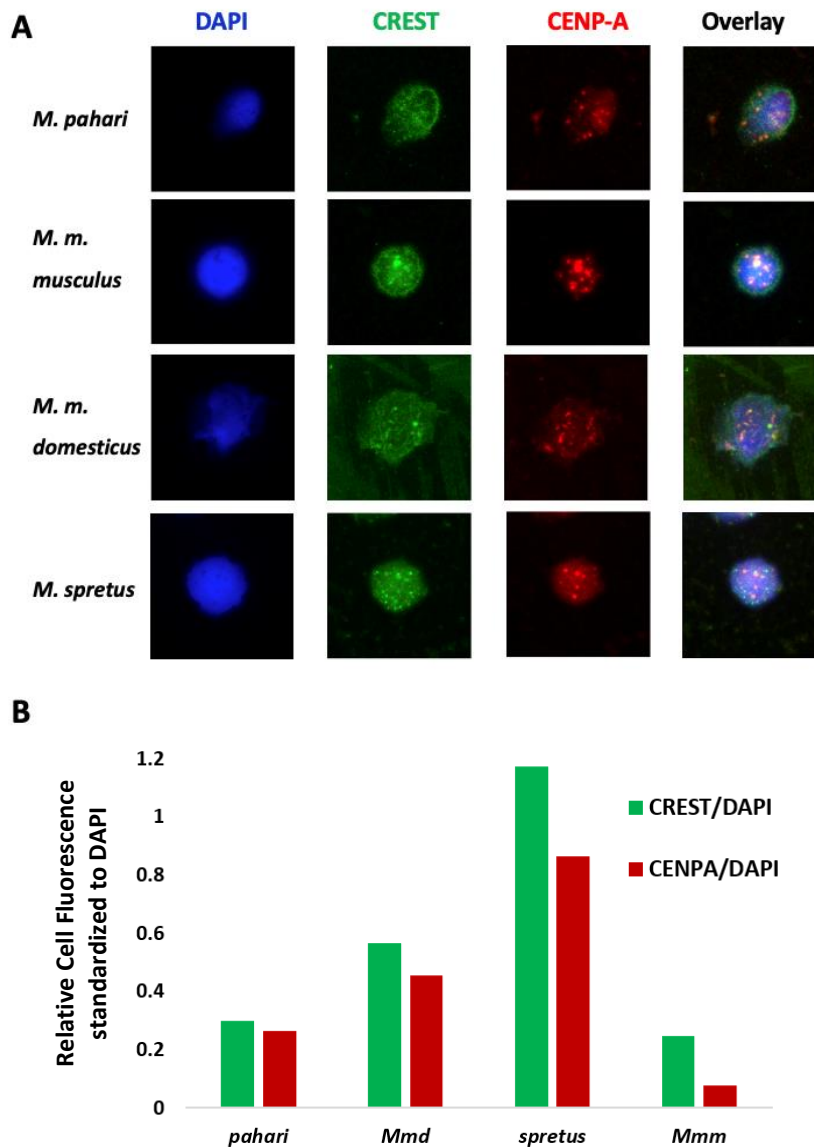


Figure 5. Differences in the localization of centromeric proteins in *Mus* species. A. Images showing DAPI, CENP-A and CREST immunostaining in *M. Pahari*, *M. m. musculus*, *M. m. domesticus* and *M. spretus*. B. Normalized relative cell fluorescence calculated by dividing total cell fluorescence of CREST and CENP-A, by the fluorescence of DAPI for the respective species.

3. Developing a cytological approach to determine copy number variations for centromeric protein-coding genes.

In addition to nucleotide level changes, genomic loci can undergo copy number variations (CNVs) during evolution. CNVs are particularly interesting for rapidly evolving essential genes as the multiple gene copies provide additional opportunities for gene expansion and differentiation. I

hypothesize that CNV contributes to the rapid evolution of centromeric protein-coding genes in the *Mus* genus. CNVs are generally detected by computational methods that rely on the sequencing depth of the Whole Genome datasets and can report low confidence CNVs in datasets with a low sequencing depth, which is often the case with datasets from non-model organisms such as *Mus* species other than the reference *M. musculus* strain. Therefore, I decided to utilize a more direct and deterministic FISH (Fluorescent in-situ hybridization)-based Oligopaint method to determine CNVs by visualizing single-copy genomic loci (Beliveau et al. 2014). The Oligopaint-FISH bypasses the need to utilize bacterial artificial chromosome (BAC) probes, which are difficult to obtain. Instead, Oligopaint-FISH uses several short oligos spread across the genomic locus of interest, making it a versatile method for visualizing any genomic locus (Figure 6). Since HJURP emerged as one of the centromeric protein-coding genes that exhibited long-term adaptive evolution in the *Mus* genus, CNVs in *Mus* species might be responsible for HJURP evolution. Therefore, I decided to standardize HJURP Oligopaint. First, we constructed an oligo library (Supplementary Table 3) spanning the HJURP locus. Next, the library was amplified using fluorescently labeled primers and nicked to generate a single-stranded probe, which was then gel extracted and purified (detailed protocol in the methods section). The purified probe was used to visualize HJURP loci in 3T3 cells. HJURP Oligopaint probe produced four separate dot-like signals in 3T3 cells known to be tetraploids. These results demonstrated the success of the Oligopaint method for detecting HJURP gene copy numbers (Figure 7). In the near future, this HJURP Oligopaint probe will be used to determine the number of HJURP copies in the liver cell spreads from various *Mus* species.

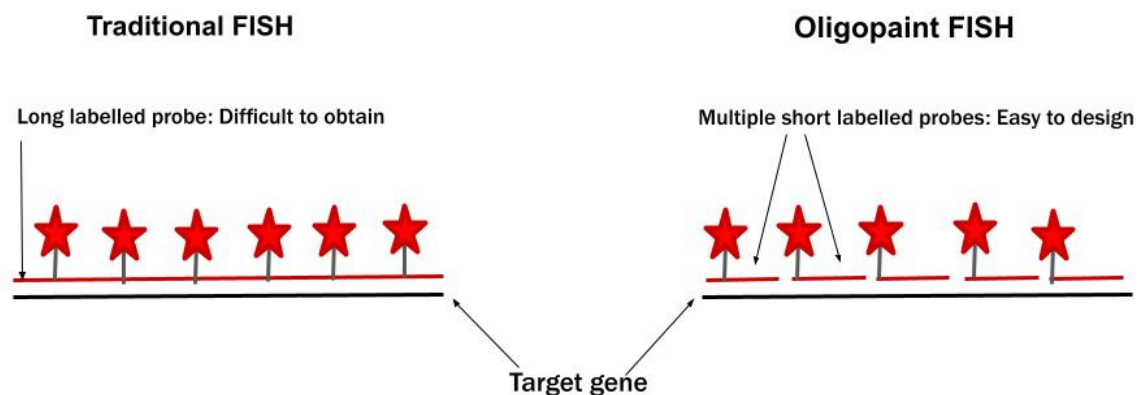


Figure 6. Oligopaint-FISH is a superior cytological visualization method compared to the traditional FISH bioinformatic approach. Traditional FISH involves using long labeled BACs as probes that are much more difficult to obtain than the short, labeled oligos used as probes in Oligopaint-FISH. Additionally, long labeled BAC probes bind to large genomic regions leading to non-specific signals, which can pose a serious problem while studying target genes undergoing evolutionary change. Oligopaint FISH probes bind to precisely defined sequences leading to the desired amount of fluorescence and specificity.

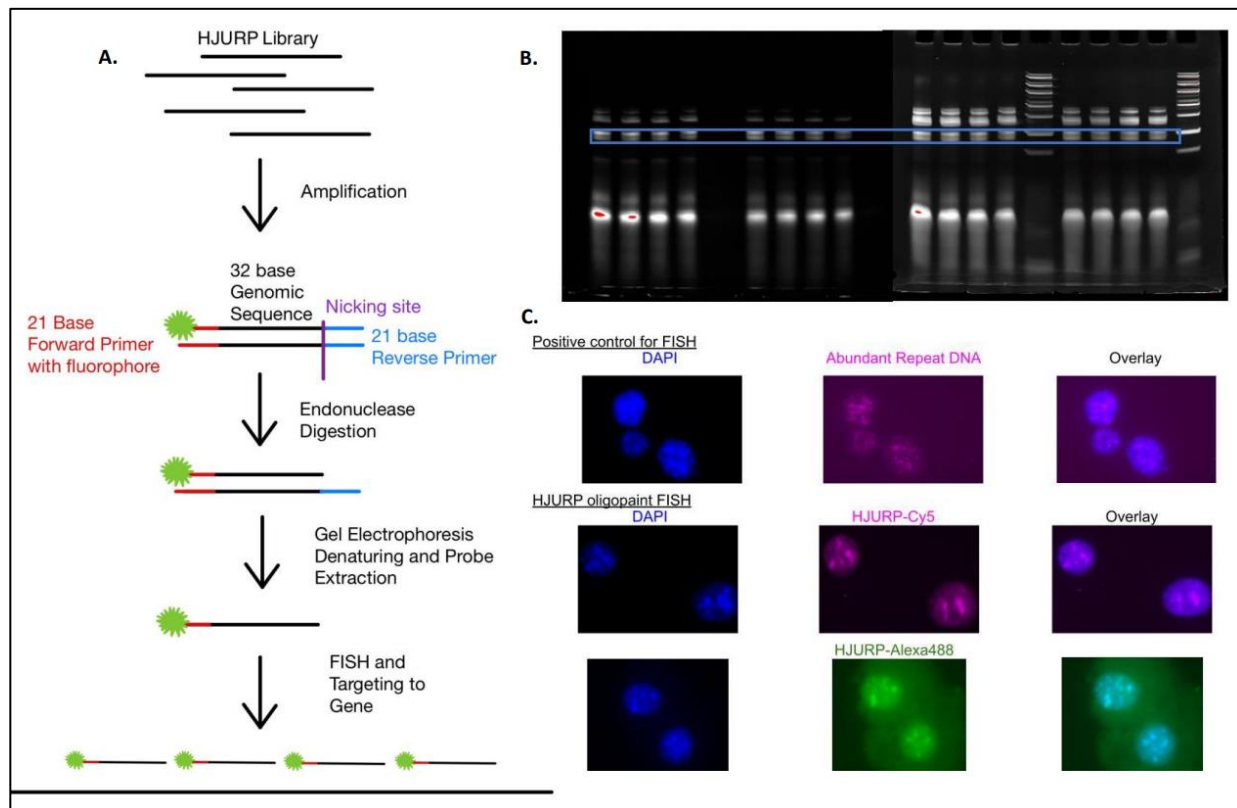


Figure 7. Standardization of Oligopaint FISH for HJURP gene in a cell line. **A.** A schematic of HJURP Oligopaint probe preparation. Two different fluorophores for the forward primer, Cy5 and Alexa488 were tested. The Alexa488 probe produced brighter signals as compared to the Cy5 probe. **B.** The 53 bp probe DNA band on TBE-Urea gel marked with the blue box was extracted and purified (Left- Alexa488 channel, right - ethidium bromide). **C.** Purified probe was used to perform FISH in 3T3 cells. Abundant repeat DNA was used as a positive control for the FISH experiment.

Discussion

Findings present in this study suggest that the pattern of selection pressure on centromeric protein-coding genes varies in different branches of the *Mus* phylogenetic group. While HJURP and CENP-C are subjected to long-term adaptive evolution, CENP-T shows only short-term

positive selection in selected phylogenetic branches. *M. spicilegus* and *M. macedoncius* display a unique pattern of centromeric protein-coding genes in the form of short-term selection on CENP-T. Furthermore, the long-term adaptive evolution of HJURP and CENP-C does not result from a common evolutionary selection pressure on all the *Mus* species. Future studies will focus on further investigating the differences in the localization of centromeric proteins in both early and late diverging lineages/species in the *Mus* genus, including replicating the results presented in Figure 6, as well as determining centromere localization of additional centromeric proteins for which the antibodies have been standardized (Supplementary Figure 1) in these *Mus* species. Additionally, several other *Mus* species available in the lab will be analyzed to determine if these functional differences in centromeric protein localization are common to the entire *Mus* genus. Furthermore, *M. macedonicus* mice will be obtained to determine if CENP-T, which shows short-term rapid evolution in these species, exhibits functional differences.

In addition to nucleotide changes, CNVs can significantly impact the evolution, especially the adaptive evolution, of essential genes. I have standardized the Oligopaint-FISH method for determining the HJURP copy number. In the near future, HJURP Oligopaint FISH will be used to determine HJURP CNVs in several species from the *Mus* genus. Furthermore, the Oligopaint method can be customized for other centromeric proteins of interest, such as CENP-A, by generating a new library pool to determine such CENP-A CNVs across species. Ultimately, this evolutionary work contributes toward our understanding how evolutionary changes occur and how they impact centromere function, which will provide insights into broader mammalian cell division function and organismal health.

Methods

Assembly, alignment, and analysis of centromere protein-coding gene transcripts

Transcriptome data for *Mus spicilegus* was downloaded from DRA search, and RNA sequence data for *M. macedonicus* was downloaded from NCBI SRA. Protein coding sequences for *M. Spretus* were downloaded from Ensembl. Protein coding genes analyzed included HJURP, CENP-A, CENP-B, CENP-C, CENP-T, Mis12, Mis18a, Mis18b, and Mis18bp1. Reference sequences (*M. spretus* or *M. spicilegus*) were utilized to generate a BLAST (Basic Local Alignment Search Tool) analysis of transcriptome data. BLAST sequences were aligned in DNADynamo and adjusted for accuracy. DNADynamo generated and exported consensus sequences for protein-coding genes. Mutations between consensus sequences were displayed in the DNADynamo program.

Pairwise Analysis of *Mus* Species

Protein coding sequences for *M. caroli*, WSB, PWK, and *M. pahari* were downloaded from Ensembl. Consensus protein coding sequences of seven species (WSB, PWK, *M. caroli*, *M. pahari*, *M. spretus*, *M. spicilegus*, and *M. macedonicus*) were aligned in DNA Dynamo, where gaps and affected codons were removed, creating an accurate primary alignment for all species. If a majority of species aligned well, but one or two species caused significant disruptions to the alignment, the excepting species were removed to create a secondary alignment with fewer codons removed. The secondary alignment was used to produce more accurate ratios between species, with the primary alignment filling in the gaps. The primary alignment was converted to PAML format and used to create a maximum relatedness evolutionary tree. This tree and the sequences were applied to CodeML, which identified sites of positive selection. Finally, the

primary and secondary alignments were run in SNAP, Synonymous Nonsynonymous Alignment Program, to generate dN/dS ratios between species. (Korber, 2000 and www.hiv.lanl.gov/).

Oligopaint HJURP Probe Preparation

HJURP Probe amplification primers

Custom 5' O F Primer: TGAATAGCAGCGGTGGCAAAC

Custom 3' O F primer: (containing Nb.BsrDI site): GTATCGTGCAAGGGTGCAATG

HJURP Oligopaint library. HJURP probe library sequences are given in Supplementary Table 3.

An example probe from the library is given below.

TGAATAGCAGCGGTGGCAAACTTTAATTTATTAGCAGTTTGCTGTCAGCAGCTTGGCTGGTTTCATTGCA
CCCTTGCACGATAC

Amplification and Precipitation. The 100 ul PCR reactions were set using 3.76 ul Forward primer with a fluorophore, 1.06 ul reverse primer, 1 ul HJURP library (0.44 pg/ul), 45.18 ul dH₂O, and 50 ul EmeraldAmp® GT PCR Master Mix. The reactions were then set in a thermocycler for 40 cycles with the following parameters: 98 °C for 10 seconds, 50 °C for 30 seconds, 72 °C for fifteen seconds, with cycles followed by 72°C for five minutes. The reactions were stored at 4 degrees between use. To precipitate the PCR products, 1-2 ul glycogen, 80 ul 2M ammonium acetate, and 1 mL of ice-cold 100% ethanol were added to 400 ul of PCR reaction, mixed thoroughly, and incubated at -80°C for 1 hr or at -20°C for at least 3 hours. After incubation, the mixture was centrifuged for 35 minutes at 4 °C and 13,000 rpm. The supernatant was decanted, and 1ml 70% ethanol was added to each tube. The mixture was centrifuged again for 20 min at 4 °C and 13,000 rpm. The supernatant was decanted, and the pellet was dried. The pellet was then resuspended in 40 ul ddH₂O.

Nicking Endonuclease Digestion and Concentration. A reaction mixture containing 20 ul amplified DNA, 10ul Nb.BsrDI Digestion enzyme, 10 ul Cut Smart Buffer, and 60 ul ddH₂O was prepared. Incubated the tubes at the following program in a thermocycler: 65°C for 4 hours, 80°C for 20 minutes, 4°C for one hour. Precipitated as described previously, with the final pellet resuspended in 60 ul ddH₂O. Incubated at 37°C for 30-60 minutes to ensure proper DNA suspension.

Electrophoresis, Gel Extraction, and Recovery. Preparing 15% TBE-UREA polyacrylamide gel: Make 10 ml TBE-UREA-PAGE solution containing: 5 ml 30% acrylamide solution, 1 ml 10X TBE, and 4.8g UREA. Added dH₂O to make the final volume 10 ml. Mixed the solution thoroughly and then added the following: 100 ul of 10% APS, 10 ul of TEMED (flammable). A 10 mL solution is sufficient for 2 gels. Mixed thoroughly after addition. The gel starts polymerizing as soon as APS and TEMED are added, so those reagents were added last. Poured the solution into gel plates without waiting. The solution was poured, the comb was inserted, and the gel was allowed to solidify.

Setting up the electrophoresis system: Heated 1L of 1X TBE buffer for approximately 30 mins at 60-65 °C to prevent renaturation. Ensured buffer was not boiling to avoid any crack. Filled the tank with the hot 1X TBE buffer. Ran the gel (preloading) at a constant 20 W.

Sample preparation and running: Added 15 ul 2X TBE-Urea sample buffer to 15 ul pooled digestion product or 15 ul DNA ladder. Vortexed and incubated at 95°C for 5 minutes in a thermocycler, then kept on ice. Loaded the samples into the gel. Ran the gel for approximately 1 hour at a constant 100 W, or until the blue sample buffer reached the bottom of the gel.

Staining and extracting the ssDNA: Removed the gel from the plastic casing and stained with 10 ul ethidium bromide for 5 min, de-stained with ddH₂O for 5 min and visualized the gel on a UV

box. Stain and de-stain steps occur in sufficient 1x TBE buffer such that the gel is completely covered and can agitate. Imaged gel in Ethidium Bromide channel and a channel appropriate for the fluorophore to help determine the necessary band. Cut out the approximately 53 bp band using a razor blade (Figure 5c). Added each gel slice to a 2 ml Eppendorf containing 3x the mass volume of Diffusion buffer and crushed the gel. Diffusion buffer: 0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA, 0.1% SDS at pH 8.0. Incubated overnight at 55°C. The next day, transferred the liquid from each tube to a fresh 2 ml Eppendorf using a syringe. Added 1-2 μ l glycogen and 3 times the volume of ice-cold 100% ethanol to each tube, vortexed vigorously and precipitated as described before. Added 10 μ l of ddH₂O to each pellet. Incubated at 37°C for 30-120 minutes to ensure DNA suspension. Quantified DNA using a tapestation or Nanodrop.

Liver Tissue Cell Spread Production

Direct Spreading of Snap Frozen Tissue: Frozen mouse liver tissue was removed from -80°C, and a small piece, approximately 0.5 grams, was extracted. The tissue extract was washed twice in 1X PBS. After washing, a portion of the tissue, approximately 0.1 grams, was placed on a standard microscopy slide with 60 μ l of 1X PBS. Using curved forceps, tissue was finely minced until the PBS liquid became cloudy. Additional PBS was added until liquid was evenly distributed on the slide. A standard glass coverslip was gently placed on the slide, and the slide was placed in liquid nitrogen for approximately one minute. The slide was removed from the liquid nitrogen and the coverslip was removed with a fresh scalpel. The slide was immersed in a chilled jar containing acetone for three minutes.

Homogenized Cell Spread Method: Obtained homogenized frozen mouse liver cells from -80°C, approximately 10 million cells in a 1.5 ml sample. Defrosted and washed cells in 5 ml PBS. Added

5 ml of prewarmed to 37°C 75 mM KCL dropwise while gently shaking. Incubated cell solution for 15 minutes at 37°C. Freshly prepared sufficient methanol: glacial acetic acid (3:1) fixative solution. Added 5 drops of freshly prepared fixative, invert, and spun cell solution at 2000 rpm for 10 minutes. Decanted supernatant leaving 250 µl of hypotonic solution. Resuspended cell pellet by tapping and added 5 ml of fixative dropwise for the first 1 ml while gently shaking. Incubated for 20 min at 4°C. Spun down cells at 2000 rpm for 10 min, and decanted leaving 250 µl of solution. Resuspended cells, and continued the process of adding 5ml of fixative, incubating, and spinning down cells approximately 3-6 times, with subsequent incubation times lasting 10 minutes, until cell pellet was colorless and sufficiently washed. Resuspended the pellet in 500 µl of fixative to create a cloudy cell solution. Drew the cell solution into a glass dropper and from a height greater than four feet, dropped the cells onto standard microscopy slides. Allowed the slides to air dry for three hours before use.

Oligopaint FISH

Obtained microscopy slides with fixed cells and ensured slides were at room temperature. Incubated slides in 2X SSCT + 50% (v/v) formamide for 5 minutes at 82°C in a prewarmed coplin jar. Removed lids from jars to allow them to reach temperature. Checked that solution was at the appropriate temperature with a thermometer. Transferred slides to a coplin jar containing 2X SSCT + 50% formamide at 60°C and incubated for 20'. Removed the slides from the 60°C coplin jar and allowed them to cool to room temperature. Created 25 µl Hybridization cocktail (per slide) in thin-walled PCR tubes. The hybridization cocktail consists of 2x SSCT (0.1% Tween) 50% formamide solution with 10% Dextran Sulfate, containing 10-20 pmole of Oligopaint Probe DNA, and 1 µl RNase A. Cut the bottom of PCR tubes and squeezed 25 µl of a hybridization cocktail to

a 22x22 coverslip. Inverted slides onto the cocktail-containing coverslips such that the area containing the cells is covered. Sealed with rubber cement. Allowed the rubber cement to air-dry for 5' at room temperature. Denatured for 5' at 82°C by placing slides top of a water-immersed heat block inside a water bath or using the thermocycler. Transferred slides to a humidified chamber and hybridized overnight at 37°C. The next day, removed the coverslip carefully and washed slides in a pre-warmed coplin jar containing 2X SSCT at 60°C for 15 minutes. Transferred slides to a coplin jar containing 2X SSCT at RT and incubated for 10 minutes. Transferred slides to a coplin jar containing 0.2X SSC at RT and incubated for 10 minutes. Removed slides from the coplin jar, remove extra liquid, and mounted with 2X DAPI and applied a coverslip. Sealed each slide using nail polish and ensured dry before imaging.

Immunostaining and Analysis

Seeded cells in 4-well slide at 150,000-300,000 cells a well. Let grow overnight. Checked cells for confluency, around 70% is ideal. Fixed cells with acetone by adding 250 ul of cold acetone to each well. Incubated for 5 minutes at -20°C. Fixed cell spreads can serve as a substitute for seeded slides. Discarded acetone, and incubated in 1% BSA 1X PBST (0.1% Triton X) for 30 minutes at room temperature. For all subsequent steps, used 250 ul solution per well for seeded slides, and 500 ul solution per fixed cell spread slide. Discarded liquid, and incubated for at least three hours at 4°C in 1% BSA 1X PBST containing primary antibody. Discarded antibody solution, and washed slides 3x for 5 minutes in appropriate volumes of PBST. Incubated slides with secondary antibody in PBST for one hour at room temperature. Discarded solution, and washed slides 3x for 5 minutes in PBST. Mounted slides with 2X DAPI solution and a coverslip. Sealed each slide using

nail polish and ensure dry before imaging. Slides remain stable for several months when stored at 4°C in dark.

Fluorescence intensity analyses

Using ImageJ, converted images to 16-bit grayscale and selected threshold intensity range from DAPI stained image. Utilized this threshold image to analyze other particles in other channels representing antibodies. Loaded fluorescent antibody image and outlined a cell of interest with a selection tool. Measured area integrated intensity and mean grey value. Selected an area of outside of the cell with no fluorescence for the background and took measurements. Used the following formula to find CTCF (Corrected Total Cell Fluorescence): $CTCF = \text{Integrated Density} - (\text{Area of selected cell} \times \text{Average fluorescence of background})$. CTCF values were then normalized to DAPI.

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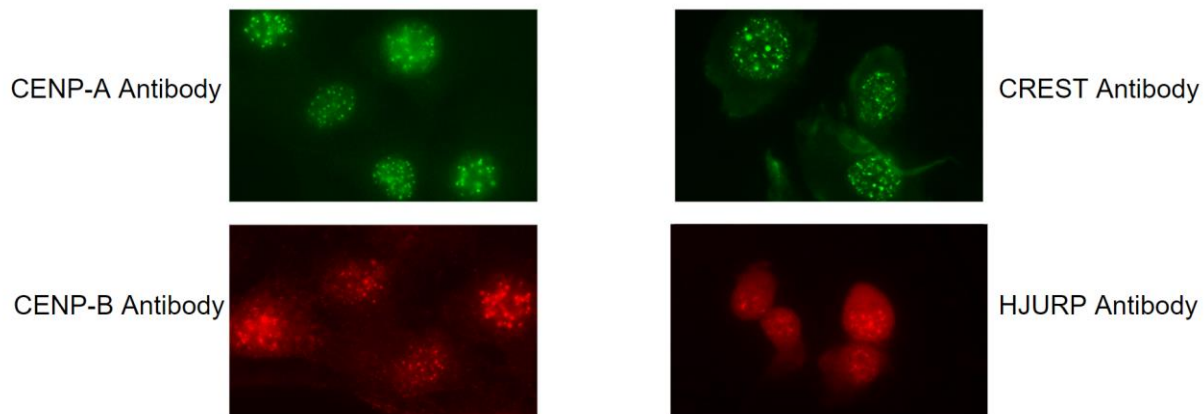
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Supplementary Information

Standardization of antibodies against centromeric proteins for immunostaining.

To demonstrate the feasibility of staining various centromeric proteins, antibodies for the centromeric proteins HJURP, CENP-A, CENP-B, and CREST, an anticentromere antibody, have been standardized in the mouse 3T3 cell line (Figure S1).



Supplementary Figure 1. Standardization of antibodies against centromeric proteins in **3T3 Cell line**. CREST antibody - 1:500 dilution, Anti-CENPA antibody - 1:500, Anti-CENPB antibody - 1:1000, Anti-HJURP antibody -1:250.

Supplementary Table 1. *M. spicilegus* constructed centromeric protein coding gene transcripts

Name	Sequence
CENP-A	ATGGGCCCCGTCGCAAACCGGAGACCCCAAGGAGGAGACCTCCAGCCCGGCGCCTGGACCCTCGCGACAGAGCTCCAGTGTAGGCTCTCAGACACTGCGCAGAAGACAGAAATTCATGTGGCTTAAGGAAATCAAGACCCTGCAGAAGAGCACAGACCTCTTGTTCAGGAAGAAGCCTTTCAGCATGGTTGTTAGAGAAATATGTGGGAAGTTCAGCCGTGGTGTGGATTTATGGTGGCAAGCCCATGCCTTGTGGCCCTTCAGGAGGCA GCAGAAGCTTTCCTCGTCCACCTCTTTGAGGACGCCTACCTCCTCTCTTACATGCTGGTCGGGTACGCTTTTCCCAAAGACATTCAATTGA CCAGGAGAATCCGAGGCTTCGAGGGCGGACTCCCTAA
CENPB	ATGGGCCCCAAGCGGGCGGCAGCTGACGTTCCGGGAGAAGTCGCGGATCATCCAGGAGGTGGAGGAGAACCCGGACCTACGCAAGGGCGA GATCGCGGGCGCTTCAACATCCCGCGCTCCACGCTGAGCACCATCCTGAAGAACAAGCGGCCATCCTGGCGTCGGAGCGCAAGTACGGA

	GTGGCCTCCACCTGCCGAAGACCAACAAGCTGTCCCGTACGACAAGCTGGAGGGGCTTCTCATCGTTGGTTCCAGCAGATCCGCGCCG CCGGGCTGCCGGTCAAGGGCATCATCTCTGAAAGAGAAAGCGCTGCGGATAGCGGAGGAGCTGGGCATGGACACTTCACTGCTTCCAACG GCTGGCTGGATCGCTTCCGAGGCGCCACGGTGTAGTGGCTGACGCGCGTGACCCGTTCTCGGGCGCGAAGCTCTGCCCTCGGGCCCC AGCGGCACCTGCCGGCCAGCCACGGTCCCTCCGAGGGCAGCGGTGGAAGCACACCGGGCTGGCGCACGAGGGAGGAGCAGCCGCCGT CGGTAGCTGAGGGCTACGCTCGCAGGACGTGTTACGCGCCACCGAGACCAGCCTGTGGTACGACTTCTGTCCGACGAGCCTCAGGGCT CTGGGGAGGTGATGGACCGCGCGCCAGGCCACCGAGCCTTAGCGTCTTGTGTGCGCCAACGCCGATGGCAGCGAGAAGCTTCCCC ACTGGTCGAGGCAAATCTGCCAAGCCCCGTGACGGGCAAGGTGGTCTGCCCTGCGACTACACTGCCAACTCGAAGGGTGGAGTACCACC CAGGCTCTGGCTAAGTACTTGAAGCTCTGGACACTCGAATGGCAGCAGAATCTCGTCGGGCTCTTCTGTGGCCGGCGCTGTGGTGCCCA GTCCTTGGACACTCGGGCTCGGCACGTGCAGCTGGCCTTCTTCCCTCTGGCACCCTGCATCCTTTGGAGCGAGGAGTGGTCCAACAGG TGAAGGGCCACTACCGCCAGCGATGTTGCTCAAGGCCATGGCAGCACTCAGGGGTAGGATCCCTCAGGCTTGCACCTGGGCTTGTGGA GGCCTGCACTTTGTGGCTGCAGCCTGGCAGGCACTGGAGCCCTCGGACATAGCAACTTGTCTTCTGAGGCGGGTTTTGGAGGTGGTCTT AATGCCACTATCACCATTCTTCAAGAGTGAGGGAGAGGAAGAGGAGGAAGAGGAGGAGGAAGAGGAGGAGGAGGAAGGGAGGGTG AAGGGGAAGAGGAAGAGGAAGAAAGAGGGGAGGAGGAAGAAAGAGGGGAAGGAGAGGAGGTAGGAGAGGAAGAGGAAGAGGTAGA AGAGGAGGGTGAGGTTGATGATAGTGAAGAGGAGGAAGAAAGTCTCTGAGGGTTAGAGGCTGAAGACTGGGCCAGGGAGTA GTGGAAGCCAGTGGTGGTTTTGGGGTTATAGTGTCCAGGAAGAGGCCAGTGCCTTCCATTTCTGGAAGGAGGAGAGGACTCT GACTCAGACAGTGATGAAGAGGAGGACGATGAAGAGGAGGATGAGGATGATGATGATGAGGATGATGATGAGGATGGTATGAGGTCC CTGTGCCAGCTTTGGGGAGGCCATGGCTTACTTTGCCATGGTCAAGAGGTACCTGACCTCTTCCCATCGATGACCGAGTGCAGAGTCAC ATCCTTCATTTGGAACACGATCTGGTCCACGTGACTAGGAAGAACCATGCCAGGCAGGCGGGAGTTCGGGGTCTTGACATCAAAGCTGA
CENP-C	GGGACGCCTCCGTCCCAATCTTACTAGTCTCTGGAACCTTGGCCGGCTTGGGTTTCCCTCTCAGGCCTCCGTTTGACCTAGTGGGCGG GAACCATGGCCTCGTTCCATCTGGATCATCTAAAGAACTACCACAGAAGATATTGCCGGCCTTCAGAGCACCACATTCATACAAAAA GGCCAGAACTGCTGGAATCTTACAAGATTGTTTTGAAGTACAGATAAGGCTTCATTTCTGATGATTTTACAGATGACTTCACTTCTCA ACAAAAAAGAAAGCTAATTATAGTCAATCATCAAGCAAAAGTGCCAGAGTCACATTCCAACCAGTTCAGTGTCTTCAAGGACGG GAGAACTTCTTGAAGCGTCTGCAGAGCCGAGTGAAGCCGCTGGTGGATCAGTTCAGGCTAATGAAGTTCACCATGGTGTGTCTGATGA ATTGGACTATGTGTTCTGCTCCCTGTGGTCTCTGGATGCAATGTCAACACGTTACAGAAAGCCGCTTCACTGTCTGGCCAAAAGAGAG TGGCTTCTGTCAAGGAGTCCAGTAGATAGGCAGGCTTCAAATAAAACATTTCTTTAAAACAGGAAAAGGTTAACTTTGAAGATAAA GTTACTTCGAGCAGCAGAAACAGAGAACAGTGTGTACAAGTAGAGGATAACTTATCCAAGGGACAAGAAGGACATCATCAAGAAATC ACTCAGAAAAGAGACGATCTGAGTTCTGATGTCCAATCACGTTCTAAGAAGAAATTTTCGGAGTTGTTTTAGAGACTGTCAAGAGGAAAAG TAAATCCAGTCTGTGGTCAGGCACACAGCAGCTTCCATCTTCTCCGCTCTCCAAGTGACATGAAATATTAGAAGATGAATTTATTAT TGATCGGTGAGATAGAAGTTTTCAAGTCGACTTTGGGTGATGATACCAAGCAAAGACAGACATCTGAGCGCCCAAGCCATCTCTGAAA ACACTGCACTGCTTCAAGTAAGAAGTCAAGAGAGAAATCTCATAGTTTATCTGCAATGACGTTTGCAAGAAACACAGCTGTATAAGGT CACCAATAGAGGAAGCTCAGCTCTGTGTAAGAAAACCTGGGAACCTACTGTACAGATGAGTTGGAATGATTGTGATCTCTGAAAA TAAATGCAATCTGAGACTGCCAAAACCCACCGCATGGGAAAGGACTACGAAACAGAACAGGAGGAGTGTCTAAGCTTAAAGCAGC TGAAGAGCTCAGGAAGGACAGAGTAGCTGTGAAAATAGCAATGTGTCAAACTGGCCAAGACAAGCTACAGATCACTCGAAAAGAAA TATGAAGGACTGTGAAGAGGTGAGGAATGAGCCTACTCCAGAAACAGAAAGCCAGCTCTTGAAAAAAGAAAAAAACAATAGCACCCA AACCAATAAGGAAAAATCTGGAAGAAATTTTTCTGGTGGGTCCAAGAACAAATTTGTTCCAAAAAAGTAACTTTGACTTCCAGGAGAA GTTGTAGAATTTCTCAGCGTCCATCTGACTGGTGGAGGGTAAAATCAGATGAAAGTTCTGTTGATAGAAATCCTTCAAAAGAAAAATACTCA CAGTGGTATATCCCAATAAAAGAAAGCAGACTAAGAGAAATCATGTATCTAAGAGGGCTGGGAAGAAACCTGGTCCATCAAAAAGACAG AAGACAGAGATGAGCCCAAGAGTACAGAAGTCTTAAATGTAAGGATTCTGGAGGAAGTGTAGTGGCCATGATGACACTTCTCGTTCCC AGAGGAAGCCATTGAAAAACATTGAGGCAGACCCAACTCAGAAGAGCCTTGCTATTCTCGACCTAAAAGAGGCTGTAAGTATCGAAACAA TGTTATGACATCACCGAATGTTCAATTAAGTCTCATACTGAGGAATATACAGCAAGACACAAATGGAGTCTGCTTCAAATTCAGAAGTGT CTAAACGTTCAGTGTGGGAAGAAAGTGGACCTTCCAGGTTCAAGAATTATGAAATGCCAGGGAGCAGTAATCTGAGATGGGTGATGAAC AGGACCAGAAAGATTTGCATTTTACAACAAGAAGTTTAAATATGGTACCAGATAAAAAATTACATCAAAATTAGTACTGCCCTCAAACCTAC CAAATGTTCTGCTGATCTAATAGAATACGTTTGAACCTCTGGAGTATTGGCAGGGGAAACGGGTAGATTATCAAGAAAGTTCTGCTGGGACA ACTTGTGCTTGAAATAATATCCCCAGCTTCACTACCCAGAAAATAAAGGCACAAAGAAACCTCGGCAAGTCAACAAAAAAGTTACCAAGA AGCAGACCCATCTCAATAGCCATGAAAAGGCTAAGATGGAAGTGGCTCTGGATATGCATCTGGGAGATCCCTTTAGGCAACATTGGCGAA GGACCCAGAAACCGCAGAGCTTGTCCCATGGATCTTAAAGTCCACGAGATACACACCGCTTTTTTGTGAGCAACATGGTTTGAAAGTTTT TAAACATTGGATACAATATATTTTCTACTGGAATTTGGTTTTAGGACCTATGAAGAGAAAGGAAAAACAGCATGTTGGCCAAGATATAT TGTTTTTATGTTAACTTTGGTGACCTTTATGTACATTACATGAACACCTTATAAGTTAACTACTGGGGATTCACTCATGTCTTCTCAGGT AATCATTACAACATTAACCTCTTGAATGTGGAAAGTAGTCTTCTTTTACTCAAATAAAAGGTGAAATAAATGTGTATGTATATAAAAT ACGTTGTTTATATGTACATTTAATAACTGATTATTTTAAA
CENP-T	AGTTAGGAGGGGTGGGGTCTGAACATGGCGGACCTCAGCTTTTCTGACGGCGATCCGACTGTACGCACGCTTTTGGCGCGCTGCTGGAG ACGGCGGATTGCGCACTCCGATGAGACGCCGGAGCACTCGGATCAATGCACAAAGAGACGGTCTCCAACGCCATACTCTAACAGGCAG GCTAGCCAAACAAAGACAAGTGTAGGAGGCAATCCCATGGAGCTAGGTGAGTGGCAGATGACTCGTGTCAAGGTCTGGGCGCCTG GAGGAACAGACACCTCGACTCTGCTAAGGAATATCTTAACTGCCCCAGAATCGTCCACTGTGATGCCAGACCCAGTGGTGAAGCCAG CACAAGTACCAGAAGTGGCCGGTCTCCAGACGGGAGAGCAGTGGGGGAAGCCTGGAGCTACACCTTCTGAACTGGAGCCCCCTCAAC CCTGGCTCCGGGTCTAACAGCCCTGGCAAAAGGAACAGAACTGAGATTATCCGTATTTAGCAGGAAAGTGAACAGGGGTGGCTCTC TCCAAGAGCCACGTAGGAGTAGGAGTGTGATGTTTATCCTTGGCCAGCTCTTCAATCTCACCTTTGTTTACCTGGTCAGCCAGAGACA GTGGAGAGACCTGGTCTGGCTCGAAGACGTCCATCCGCCAGCTTGTAATGCTGGTGTCTTTGCAGGATCTGGAAGACAATTCCTTAGC CTCTGCTTCCAGGTGACAGTACAGACACACCTGTGCTGCTCTGCGGATGATGAGGCTTGAAGACACACAGCTTCTCAGACCTT GGCTGCTTTTCCCTTAGTGGGAACATTCTGTCCAGCCCTTCTGCGCTGGGGTTGAAGATGTTGAAAGAGTGTGGGCCACCAAGCT CGGGGACAGGCTGCAAGCCGCATGAGCAGAACTGGCTGTGCTGCTCTCTTCCCTTCTGAGGCCACAGCCTCAGCTGCAGAACCG AGAGAAGCAGTAGGATCCAACGAGGCTGCGAGGCCAAGGATCAAGAAGGATCTCAGGGTATGAGGAACTTCTGCTAGGCCAGCAAGT GGAGAATTATCTCAGCACACATGACTACTCCAGCTGAACGCCACCCCTGTACCTGGAGTTGCAGTCTATCTCAGAGCCCTGGA GTCTGTGACAGCAAGTGCCTATCCAGGACCCAACTGCAAGCTTAGGCGCCGCAAGACCCCAAGACCCCAAGAGCTGAGTGTG AAGTTCTCAGCTTCTGTACTAAGATGCCCGTGGAGAAGACGGCTCTGAGATAGTAGAGAAGTGCCTAGACAAGTATTTCCAGCACCTTTG CAATGACCTGGAGGTATTTGCTGCTATGCTGGCGTAAGATTGTGAAGCCAGAGGACTTATTGCTGCTGATGCGACGGTAGTGANTAGAA

	ATGGGAGTCCGAGCATGGGCGGGCTGGTGCTTATCTGCCCTTGGAGTACCGGCAACAACCTATCCCNNGTGTGCATNNAGNCGCNGATT CNNNCNCTCCGATGAGACGCCGGAGCGCTCGGATCAA
HJURP	ATGGAGTCTATGGGTGCGGAGGACCCGAGGCTGCATCAGCAGCTTAATGAAAGCAGTAGCCGCTCCAGACGTTAATGAAGCGGCTGATA GCGAAGTACAACAGCCCTTTGAGGACGACCCGTTGGTGGAGATGACGACGTTGACCTACGAGACGCCAGGGGTTGAGAGTTTGGGA GGAAAGCTGATGAAGAAGGAAGACAAGGAATACACTCAGGTGATTGACAGATTAAATGGGCAAGCTCCAGAAGGTGATTGACAGAGCAG CGGTGCGGATACAAGCTTAGAGGAGAACTGGCCATCTTGACGCTCAGCGATGAGAGAAGCCTCTGGTGATCCTAGGCAGCGTCAGCCTGC AGTGCCCGGAAACACCTTGGAAGCTGATTTAAGAAGGAAGTACTTGACCCAGGTGGATATATTGCTTCAAGATGAAGAGTATTTAAGAAC GCAGAGAAGAGAGGTGGAAGACACAGTCATGACTTGGGTTCTTCCGTGACCTCATCTGTACACCAGCCTCTGGATGCCAGGATGCTA TCTCTGCAAGAGCTCTGGTGGTCTGGAGTATCTGCTTGTCTCTAGAGGCCAGGGTCCCTCATACCCTTGCCAGCTGACATGGCCATTG TGGCTGAAGTGATGGCTCTCCTTACTTGGGACCAGCAGCAACAGCGTAAGCAGCCAGTCCTTTGAGGTTGATGACCTTTGCAATGTGACAA TCAGCGATTTGTATGAAGGCATGATGCATTCCATGAGTAGGCTGCTGCGCTCAAAGCCATCCTGCATCATCTCCACCAAGACCTACATCAACC AGAGCTGGAAGCTCCGGAGGAGACCCTCAGCAAGCAGGGGCTGCACAAGAAGCAGGACAACCTGCCCGGAGCAAGCCCTCCAGAGGA GTGCCAGGAAGGGGCTGCGCCCTGCTCAGAGCCCGGGAAGAGGCCGGAATATTAAGAGACTACGGGAATTTACTGCAGCTTGCTCCCC ACAAGACAGGCTTAGAACTGAAAAGCGTTTCTTGAAGAAGCAACGCCAAGTCCATAAATCAGTCCAGCTTGAAGGAGCTCCAGATG ATGCCGCAAGGACTTGGACTTAAACCGTGAGCGAGAAAATAGGTTGATGACATTACAGTGGCTAATTTACCCATCAAGGTGGTTCCCA GACCAAGGATGCTTCCAAGTCAAGTAGAGAAAGTGGTATAGAGAGATTAAATCAAATTTGACAAGCTTCATCAGGAATATTGCTGTCTTCT GGGAAACAGCCTCACCTGACTGGCCCTACAGAGTCTTGGGTTGTCGATGTGTACAGAAGTGGTTCTAAGAGCCAGGCAGCCGTCGGGATG TAGAAACCTGCAGGCCAGTTACCTTTGCGCAGAGAAAAGACAGAAAGGCCAGGTGAAGCTTTAGAAGACCTGAGGGGAAATGGAAGT CTGTCAAAACAAGAGCTGTCTTCTAAGGAGCTGTCCCTCTCCAGAGGGCAGCCGCTCAGGAGCCCGGCCATTCTCAGTGAAGCTGTGGC CTTCAAGAGCACAATCTGAACCTACTGGTAAGGCAGTGTGGCCAGTACAGCTATTTACAGCACCATGCATAGGCTCTCCAGGCTGTGGGA GGATAAATCTGATGAGCTTAAAAAAGAAATTCACAGACTTTATCAGAACTATTGCTGTCAACGAGCGGGGAGTGTGCTGTGGC AGAGTATCGCCAATGAAAGCTGCTGCAGCTCTTCCCTGTCAATCAGAGCACTTGAAGAGACTAAATCCAGACTCCCCACAACAGAGTTCCCA GAAACGGTCAATCTCTCTGGCTGTCACAGAAGAGTCTGCGAGTCACTGACAGACTGCTAGTACACTGTGAGAGAGCCTTGGCTGC CAACAAAGAGGCGCAAGTTGCATACCCGGTAGCTTGTGCTACCAGGCCAAATCTCAATACCTCAGGAGCATCAGGCTGGCCCTGAGT GTCATGAGTGCTGATTGGGTGTTTGTGGACATGATGCTTATGTAGTCAGCAGCTGCTTGA
Mis12	ATGTCCTGTGGATCCGATGGCTATGAGGCCAGTCTTTGGCTTACACCCACAGACTTGCTGCTGAGGATCTACGTAGCATTTCAAGACCAC TGTTTTGAAGTGATGCAGGCTGTTGAACAGGTTATCCTAAAGAAGCTGGAGGACATCCCAAATGTGAGATACCCCTGTCCAGACTCGGA AATGCACAGAGAAGTTTCTTGCTTATGAAAGGGCGCTTCGATAACCTTTTTGGCAAAATGGAGCAGCTGATTTGCACTCGATTTGTGTA TTCCCCCAAACATCCTGCTTCTGAAGACAAGTGCAGGAGATGAATCCTTTCAAGTGAAGAAAACTCCAGCTTCTCAACAGGAAATCAAA GAGTTACAGGAGAAATACAAGGTTGAGTTGTGCACTGAGCAGGCCCTTCTGCAGAATTAGAGGAACAGAAAATGTTAAGGCCAACTCA GAGAGACCTTAACTTTCTTGATGAGCTTGAACATCGGCAGATATCAAGGAAGTGAATCTTAGGGAGAGTTGGCATCCCTGGTCCAG AGTGCGAGAAAATTCAGAGCAATTAGAGACAATGTAGAAAAGAAAGACGAGAGACTGGAACACAGTGA
Mis18	ATGTCGAGCGAGTCCCCCTGCTGGAGAAGCGTCTGTCGGAAGACTCGAACCGCTACCTGCGGCTGCAGAAGTGGGCAAAATGTCGAGC GCAGACGCTTAGGGCTAGAGAAGGAACGGCCTGAGGAGAAGGCGCGCGGAGAGAACCCGCTGGTGTCTTGTGCCGCTGTGCG CCGGCCACTGGGCGACTCGCTCACCTGGGTGGCCAGCCAGGAGGACCAACTGCATCCTGTTGCGCAGTGTCTCTGTAACGTCTCTGTG GATAAGGAACCGAACTGTCAAGTGCAGAGATGAAGACGTTGCTCCTTGAAGGCGCTGACTGCACGGGCTGCTCCCTCAGCCTTGGCT ATGTGTACAGATGCACTCCTAAGAACCTGGACTACAAGCGCAGTGTCTGCTCAGCGTTGAAGCCGTTGAAAGTGTAGCTTTAGGTTCC TCTGAGAAGCAATCTGTGTCAGAAGACAAGGAGCTTTCAACCTTGAAGCCGAGTTGAAATAGAGAAGTCTATAAGCAGATGGAAGAA GTCCTGACAGCCCTGCAAAAGAAGCTACGGGAAGTTGAATCTAACTGTCTTGGCCAGCCGGGCGAGCTGA
Mis18b p	ATGATTGTAACACCTTTGAAGCATTAGGAATTCATTGTCTTCTGGAACATTGCAAGAAGAAATATGCCTCTAGATGCAGTCTTTATTGAC AGCATCCCTTCAGGCACACTACTCCTTTGAAAGATTGGTAAAATATCAGAAGTCTCTCTAAAAGTTAATGGTCATAAAAAGAATCAGTTA TTAGAAAATAAGAACTTCTAACAATAAAGATCTATTTCAATCCACCATGTTATCAGAGGCTACCCCTTCAAAACCTCTCTTGATATCAGTGTTA TAAAAAAGCCCAACATGGATAGATTAAAGAAATGAAATGATTTATGAATCACCAGGTAAAATCTTTCAAAGATGAAGGATACAGCG TGACAAAACAAGAACTGACAAGGAGCAGTAGCATGTTGGGATCACCACAGGGTGAACACAGCAAAAGACTTTCTCTAACACGGATAAG AAAGCTCAGTTGCAACAAACCTATATCTGTGAGGAAAAACAAACGTCGGTCCAATCCAATGACCTTCATTGGGAGATCTCCAATTCTGAA CCAAGAACAAAAAATGTTTCAGCTTCGTGCATTCAAAGAAGGCATTAACTAGAGCCAGTTTGGCGGACAAGTTCTTATTCAAAGAGA GTCCTGTTAGGATCACTGTGTCAAAAAGAACACATTTGTTTTGGGAGGCATTGATTGTACTTATGAAAAGTTTAAAAATCTGATGTTAA CCATTAGTTCTCTTTGTGTTCTATTAAAGAAATCATAGCCAATCTATTACTTCTGATAATGATGTGACAACAGAAAGGACTGAAAAAGAGGATA TTACAGAACCAAAATGAAGAGATGATATCCAGAAGGACTATTCAAGATCCCAATAAAGAAATACATCTAAAAATTAACGTTCAAGTCCAAGACT AATTTAACACTATCTGGCCGTCTCAAAAGAAATGTACAAAGCTTGAAGCTGTTGTAAGAAAGTAAAAAATATCAGGCAGTCCAGCTACA GGAATGGATGATTAAAGTCATCAATAATAACTGCTATATGTGTAAGAGAAAGCTGGTAGATATGACTGATGTTATTGGCATAGCAATG TAATTATAGAGCGGATTAACACAATGAACCTTAGGACCTTATCAGGCAACATTTATATCTTAAAGGATTGATAGACTCGGTCTCCATGAAA GAAGCAGGATATCCCTGTTATCTACAAGAAAATTTATGTTTGGATTCCCCACAACCTGGAAGGAACACATTGATAAATTTCTAGAACAATTA AGGGCTGAAAAAAGAAACAAGACCAGACAGGAAACAGCAAGATCCAAAGAAAAACAAAAATCAAAAAAAGAGATGCAAGAGATAAAGA AACTTATGCTCTGCAAAAGGCCAGCATCACGTATGACCTGAATGATAATAGCGTAGAGAGAAGTGAAGTACCCACTGATCCCTTGAACCTAC TGGAACAGCTACCTCCGGCAAAGAAAGAACACCCGCTTCTCAGTCAGAAGAGAGCTTATGTTTTAATAACACCACTAGAAAACAAAA GTTGATAGAGCAAAAGATGATAGACTACAGTGTCTTATTGAAGGAATATCGGACTTTTTCAAAGCAAAGCATCAAGAAGAAAGTGACTCA GATATACATGGAACCTCAAGTTCTACAGTAAGTCTCAAGAGACCTTTGAACATAGAGTGGGATTGAAGGCAATACCAAGGAGGACTGCA ATGAATGTGACATAATCACTGCCAGACATATTCAGATACCTTTGCCGAAAGTAAACAAATGCTCACCATGATTTTTATGAAAAAGAACCAAG TTGCCCTCAAACTGCAGAAAATGAAAGTCAAATAGGTGATCACAGTATTGCCAGTCTCATCACATTTGTCAAGTGAAGAGAATGAAGT AGAAATTAAGTGAACCAAGAGCAAGAAATACAAAAGAAAGGCTGAATCGGGAGAGAGAAAAACACAACCATCACAAAAGATATCCT CCTCATTTAGAAAAGTGAAGGTGAGAGAGCTTGTATATCACACCAAGAGACCTCGATCTTCTGCTAAAGAAATCACATTACAAATCTGCTGT TAGCAAGGACTTTCTATTGAAGGAAAGGCATCTGATAGAAGTACGAGACAGCTGTAGACCATCTACCTGGTTAAACCGATGATGAAGAAT GGAGTGAGCAAGATTACAGAAGCTACATTGTGCTTTACATCTCTTCAAGACACAACCTGGCTTTTGGTCAGATGTGGCTATGGCAGTA GGTTCTGAACTGCTGATGAGTGCCAGAAAGAAATACACAGAAGAACCCCAAGGCCAAGGATCCCGAAAAACATGTCTTAAGAAAAAGCAA GCCAACAAAGGTCCAAATGGTGAGAAAGACAGTGTGATGCCAAGACTATTAAGATAACTGCCAAAGTGGGAAGCTTAAAAAGGAAACGA

[illegible]

	TGTTGATAGAAATCCTTCAAAAGAAAATAACTACCAGTGGTATATCCCAATAAAAAAGAAGCAGACTAAGAGAAATCATGTATCTAAGAG GGCTGGGAAGAAACCTGGTCCATCAAAAAGACAGAAGACAGAGATGAGCCCAAGAGTACAGAAGTCTTTAAATGTAAAGGATTCTGGGA GGAACCTGTTGGTGGCCATGATGACACTTCTCGTCCCAGAGGAAGCCATTGAAAAACATTGAGGCAGACCCAACCTCAGAAGAGCCTTGC TATTTCTCGACCTAAAAGAGGCTGTAAGTATCGAAACAATGTTATGACATCACCGAATGTTTCATTTAAAGTCTCATACTGAGGAATATACA AGCAAGACACAAATGGAGTCTGCTTCAAATTCAGAAAGTGCTTAAACGTTTCAGTGTGGGAAGAAAGTGGACCTTCCAGGTTCAAGAATTA TGAAATGCCAGGGAGCAGTAATCTGAGATGGGTGATGAACAGGACCAGAAGAGTTTGCAATTTTACAACAAGAAGTTTAAATATGGTAC CAGATAAAAAATTACATCACAAATTAGTACTGCCCTCCAACCTACCAGTTCGTCGATCTAATAGAATACGTTTGAAACCTCTGGAGTA TTGGCGAGGGGAACGGGTAGATTATCAAGAAAGTTCGTCGGGACAACCTTGCTTGAAATAATATCCCCAGCTTCAGTACCCACGAAAA TAAAGGCACAAAGAAACCTCGGCAAGTCAACAAAAAGTTACCAAGAAGCAGACCCATCTCAATAGCCATGAAAAGGCTAAGATGGA GCTGCCTCTGGATATGCGTCTGGGAGATCCCTTTCAGGCAACATTGGCGAAGGACCCAGAAACCGCAGAGCTTGTCCCATGGATCTTAT AAGTCCACAAGATACATACCGCTTTTTTGTGAGCAACATGGTTGAAAGTTTTTAAACATTGGATACAATATATTTTTCTACTGGAATAAT TGGTTTTAGACCTTATGAAGAGAAAGGAAAAACAGCATGTTGGCCAAGATATATTGGTTTTTATGTTAACTTTGGTGACCTTTTATGTAC ATTACATGAACACCTTATAAGTTAACTACTGGGGATTCACTTATGTTCTTCAGGTAATCATTACAACATTAATAATCTCTGAATGTGG AAAGTAGTCTCTTTTTACTCAAATAAAAAGGTGAAATAAATGTGTATGTATATAAAATACGTTGTTTATATGTACATTTTAACTGATT ATTTTTAA
CENP-T	GGGACGCTCCGCTCTCAATCTTACTCTAATCCTCCTGGAACCTGGCCGGCTTGGGTTTCCCTCTCAGGCTCCGTTTGACCTAGTGGGC CGGAACCATGGCCTCGTCCATCTGGATCATCTAAGAAGTACCACAGAAGATATTGCCGGCCTTCCAGAGACCCAACATTATACAAAA AAAAGGCCAGAACATGCTGGAATCTTACAAGATTGTTTTGAAGATCAGAGTAAGGCTTCATTTCTTGATGATTTTACAGAGTCATTGAC TTCTTCAACACAAAAAAGAAAGCTAATTATAGTCAATCATCAAGCAAAAAGTGCCAGAGTCACATTCAAAACAGTTCAGTGCTTCA AGGAGGGGAGAAGCTTCTGCAAGCGTCTGCAGAGCCGAGTGAAGCCGCTGGTGGATCAGTTCAGGCTAATGAAGTTCCACCTGGTG TGCTGTGTAATTGGACTTATGTGTTCTGCTCCCTGTGGTTCTCTGGATGCAACGTTCAACACGTTACAGAAAAGCCGCTTCACTGCTGG CCAAAAGAGAGTGGCTTCTGTCTCAAGGAGTCCAGTAGATAGGCAGGCTTCAAATAAAAACATTTCTTTTAAACAGGAAAAAGGTTAA ACTTTGAAGATAAAGTTACTTCGAGCACAGCAGAAACAGAGAACAGTGTGTTACAAGTAGAGGATAACTTATCCAAGGACAAGAAGG GACATCATCAGAAATCACTCAGAAAAGAGACGATCTGAGTCTGATGTCCAATCAGGTTCTAAGAAGAAATTTTTCGGAGTTGTTTTAGA GACTGTCAAGAGGAAAAAGTAAATCCAGTCTGTGGTCAGGCACACAGCAGCAGTTCATCTTCCGCTCCTCCAAGTGACATGAAAT ATTAGAAGATGAATTTATTATTGATCGGTGATAGAGTTTTCAGTGCAGCTTGGGTGATGATACCAAGCAAGACAGACATCTGAG CGCCCAAGACCATCTCCTGAAAACACTGCACTGCTCAAGGTAAGAAGTCAAGAGAGAAATCTCATAGTTTATCTGCAATGACGTTTGC AAGAAACACAGTCTGATAAGGCTCACCAATAGAGGAAGCTCAGCTCTCTGTTGAAGAAAACCTGGGAACCTACTGTACAGATGAGT TGGAAATGATTGTGATCTCTGAAAATAAATGCAATCTGAGACTGCCAAAACCCACCCGATGGGAAAGGACTACGAAACAGAAC CAGAGGAGAGTGTCTAAGCCTAAGCAGCTGAAGAGCTCAGGAAGGGACAGAGTAGCTGTGAAAATAGCAATGTGTCAAACACTGGCC AAGACAAGCTACAGATCAACTCGAAAAGAAATATGAAGGACTGTGAAGAGGTGAGGAATGAGCCTCATCCCAAGAAACAGAGCCAGC TCTTGAAAATAAGAAAAAACAATAGCACCCAAACCAATGAAGGAAAAATTCGGAAGAAATTTTCTGGTGGGTCGAAGAACAATTT TGTTCCCAAAAAGTAACTTTGACTTCCAGGAGAAGTTGTAGAATTTCTCAGCGTCCATCTGACTGGTGGAGGGTAAAATCAGATGAAAG TTCTGTGTATAGAAATCCTTCAAAGAAAAATACTACCAGTGGTATATCCCAATAAAAAGAAGCAGACTAAGAGAAATCATGTATCTAA GAGGGCTGGGAAGAAACCTGGTCCATCAAAAAGACAGAAGACAGAGATGAGCCCAAGAGTACAGAAGTCTTTAAATGTAAAGGATTCT GGAGGAAGTGTGGTGGCCATGATGACACTTCTCGTCCCAGAGGAAGCCATTGAAAAACATTGAGGCAGACCCAACCTCAGAAGAGCCT TGCTATTTCTCGACCTAAAAGAGGCTGTAAGTATCGAAACAATGTTATGACATCACCGAATGTTTCATTTAAAGTCTCATACTGAGGAATAT ACAAGCAAGACACAAATGGAGTCTGCTTCAAATTCAGAAGTGTCTAAACGTTTCAGTGTGGGAAGAAAGTGGACCTTCCAGGTTCAAGAA TTATGAAATGCCAGGGAGCAGTAATCTGAGATGGGTGATGAACAGGACCAGAAGAGTTTGCAATTTTACAACAAGAAGTTTAAATATGG TACCAGATAAAAAATTACATCACAAATTAGTACTGCCCTCCAACCTACCAGTTCGTCGATCTAATAGAATACGTTTGAAACCTCTGGA GTATTGGCGAGGGGAACGGGTAGATTATCAAGAAAGTTCGTCGGGACAACCTTGCTTGAAATAATATCCCCAGCTTCAGTACCCACGA AAATAAAGGCACAAAGAAACCTCGGCAAGTCAACAAAAAGTTACCAAGAAGCAGACCCATCTCAATAGCCATGAAAAGGCTAAGAT GGAGCTGCCTCTGGATATGCGTCTGGGAGATCCCTTTCAGGCAACATTGGCGAAGGACCCAGAAACCGCAGAGCTTGTCCCATGGATC TTATAAGTCCACAAGATACATACCGCTTTTTTGTGAGCAACATGGTTTGAAAGTTTTTAAACATTGGATACAATATATTTTTCTACTGGA AAATTGGTTTTAGGACCTATGAAGAGAAAGGAAAAACAGCATGTTGGCCAAGATATATTGGTTTTTATGTTAACTTTGGTGACCTTTTAT GTACATTACATGAACACCTTATAAGTTAACTACTGGGGATTCACTTATGTTCTTCAGGTAATCATTACAACATTAATAATCTCTGAAT GTGGAAAGTAGTCTCTTTTTACTCAAATAAAAAGGTGAAATAAATGTGTATGTATATAAAATACGTTGTTTATATGTACATTTTAACTGATT GATTATTTTTAA
HJURP	ATGGAGTCTATGGGTGGGAGGACCGCAGGCTGCATCAGCAGCTTAATGAAAGCAGTAGCCGCTTCCAGACGTTAATGAAGCGGCTGA TAGCGAAGTACAACAGCCCTTGGAGGACGACCGTGGTGGAGATGACGACGTTGACCTACGAGACGCCCCAGGGGTTGAGAGTTTG GGGAGGAAAGCTGATGAAGAAGGAAGACAAGGAATACACTCAGGTGATTGACAGATTAAATGGGCAAGCTCCAGAAGGCGATTGAGA GAGCAGCGGTGCGGATACAAGCTTAGAGGAGAAGTGGCCATCTTGAGCTCAGCGATGAGAGAAGCCTCTGGTGATCCTAGGCAGCGT CAGCTGCAAGTGGCGGAAACACCTTGGAACTGATTTAAGAAGGAAGTACTTGACCGAGGTGGATATATTGCTTCAAGATGAAGAGTA TTTTAAGAAGCAGAGAAGAGAGGTGGAAAAGACACAGTATGACTTGGGTTCTTCCGTGACCTCGTGTGACACCAGCCTCTGGAT GCCAGGATGCTATCTGCAAAAGAGCTGTGGTGGTCTGGAGTATCTGCTTGTCTCTAGAGGCCAGGGTCCCTCATACCCTTGCCAG CTGACATGGCCATTGTGGCTAGAAGTATGGCCTCTCTTACTTGGGACGAGCAGCAACAGCGTAAGCAGCCAGTCCCTTGGAGTTGAT GACCTTTGCAATGTGACAATCAGCAATTTGATGAAGGCATGATGCAATCCATGAGTAGGCTGCTGCGCTCAAAGCTATCCTGCATCATCT CCACCAAGACCTACATCAACAGAGCTGGAAGTCCGGAGGAGACCTCAGCAAGCAGGGGCTGCACAAGAACAGGACACACTGCC CCGAGCAAGCCCTCCAGAGGAGTGGCAGGAAGGGGCTGCGCCTGCTCAGAGGCCAGGAAAGGGGCGGAATATTAAAGAGACTA CGGGAATTTACTGCACGTTGCTCCCAAGACAGGCTTGAAGTGAAGAGCGTTTCTTTGAAGGAAGCAACGCCAAGTCCATAAAT CAGTCCAGCTTGAAGGAGCTCCAGATGATGCCGAGAAGGACTTGGACTTAAACCGTGAGCGAGAAATAGGGGGATTGACATTACAG TGGCTAATTTACCCGTCAAGGTGGTTCAGACCAAGGATGCTTCAAGTCAAGTAGAGAAGTGGTATAGAGAGATTAATAATCAATTT GACAAGCTTACAGGAATATTGCTGTCTTCTGGGAAACAGCCTCACTGACTGGCCCCACAGAGTCTTGGGCTGTGATGTGTACAGA AGTGGTTTAAAGGCCAGGCAAGGCTCGGATGTAGAAGCTGAGGACCAAGTTCACCTTTCGGGCAAGAAAGACAGAAAGGCCAG GTGAAGCTTTAGAAGACCTGAGGGGAAATGGAAGTGTGTCAAAACAAAGAGCTGTCTTAAAGGAGCTGCTCTCCAGAGGGCAGC CCGTACAGGAGCCCCGCCATTCTCAGCTGAGCTGTGGCTTCAAGAGCACAACTTGAACCTACTGTAAGGAGTGTGGCCAGTACA

	GCTATTTTCAGCACCATGCATAGGCTCTCCAGGCTGTGGGAAGGATAACTATGATGAGCTTAAAAAGAATTCAACAGACTTTATCAGAAG TATTGTCTGTCAACCGCAGCGGGCGAAGGTGACTTCGTGTGGCAGAGATATCGCCAATGAAAGCTGCTGCAGCTCTTCCCTGTCAATCAGAA CACTTGAAGAGACTAAATCCAGACTCTCCACAACAGAGTTCAGAAACGGTCAATCTCTCTGGCTGTACAGAAAGTGTCTGCAGGAT TCAACTCCACAGACTGCTAGTACACTTGTGAGAGAGCCTTGGCTGCCAACAAAGAGGCGCAAGTTGTATACCCGGTAGCTTGTGCTCAC CAGGCGAAATCTCACAATACCTCAGGAGCATCAGGCTGGCCCTGAGTGTCATGAGTGCTGATTGGGTGTTTGTGGACATGATGCTTATG TGTAGTCAGCAGCTGCTTTGA
Mis12	ATGTCTGTGGATCCGATGGCCTATGAGGCCAGTTCCTTGGCTTCACACCACAGACTTGCCTGCTGAGGATCTACGTAGCATTTCAGACC ACCTGTTTGAAGTGATGCAGGCTGTTGAACAGGTTATCCTAAAGAAGCTGGAGGACATCCCAACTGTGAGATCACCCCTGTCCAGACTC GGAAATGCACAGAGAAGTTTCTTGTCTCATGAAAGGACGCTTCGATAACCTTTTCGGCAAAATGGAGCAGCTGATTTTGCAGTCGATT TGTGTATTTCCCCCAAACATCCTGCTTCTGAAAGACAAGTGTCAGGAGACGAATCCTTTTCAGTGAAGAAAACTCCAGCTTCTCCAAACAGG AAATCAGAGAGTTACAGGAGAAATACAAGGTTGAGTTGTGCACTGAGCAGGCGCTTCTTGCAGAATTAGAGGAACAGAAAACTGTTAA GGCCAACTCAGAGAGACCTTAACCTTCTTGTGATGAGCTTGAACATCGGCAGATATCAAGGAAGTGTAACTTTAGGGAGAGTTGGC ATCCCTGGTCCAGAGCTGCAGAAAACCTCAGAGCATTAGAGACAATGTAGAAAAAGAAAGCAGGAGACTGGAACACAGTGA
Mis18a	ATGTCGAGCGAGTCCCCCTGCTGGAGAAGCGTGTGTCGGAAGACTCGAGCCGCTACCTGCGGCTGCAGAAGTGGGCAACATGTCGA GCGCAGACGCTTAGGGCTAGAGAAGGAACGGCTGAGGAGAAGCGCGCGGCGGAGAACCCGCTGGTGTCTTTGTGCCCGCT GTCGCGGCCACTGGGCGACTCGCTCACCTGGGTGGCAGCCAGGAGGACACCAACTGCATCCTGTTGCGCAGTGTCTCCTGTAACGTC TCTGTGGATAAGGAACCGAACTGTCCAAGTGCAGAGATGAAGACGGTTGCATCCTTGAGGCACTGTACTGCACGGGCTGCTCCCTCAG CCTTGGTTATGTGTACAGATGCACTCCTAAGAACCTGGACTACAAGCGCAGCTTGTCTGCTCAGCGTTGAAGCGTTGAAAGTTATAC CTTAGGGTCTCTGAGAAGCAAATCGTGTGAGAAGACAAGGAGCTTTCAACCTTGAAGCCGAGTTGAAATAGAGAAGTCTATAAAGC AGATGGAAGAAGTCTGACAGCCCTGCAAAAGAAGCTACGGGAAGTTGAATCTACACTGTCCTTGGCCAGCCGGGCGAGCTGA
Mis18bp 1	ATGATTGTAACACCTTTGAAGCATTGAGGAATCTTTGTCTTCTTGGAAACATTGCAAGAAGAAATATGCCTCTAGATGCACTCTTTATTG ACAGCATCCCATCAGAGGCTACCCTTCCAAACCTCTCTTGATATCAGTGTATAAAGCCCAACATGGATAGATTAAGAAATGATATGAT TTATGAATCACCAGGTTAAATCTTTCTAAGAATGAAAGCAAAAGTACAGCGTGAATAAGAAGAGAACTTTCTCCAATTCTGANCCAAG AACAAAAAATGTTTCAGCTTCGTGCANTTCAAAGAAGGCATTAACTAGAGCCAGTTTGCGGACAAGTTCTTCATTCAAAGAGAGTCT CTGTTAGGATCACTGAGTCCAAAAAGAACACATTTGTTTGGGAGGCATTGATTGTACTTATGAAAAGTTTCAAATACTGATGTTAACAC CATTAGTTCTTTGTGTTCTATTAAGAATCATAGCCAATCTATTACTTCTGATAATGATGTGACACAGAAAGGACTGCAAAAGAGGAT ATTACAGAACCAATGAAGAGATGATATCCAGAAGGACTATTCAAGATCCCATAAAGAATACATCTAAAATTAACGTTCAAGTCCAAAG CCTAATTTAACTACTCTGGCCGGTCTCAAAGAAATGTACAAAGCTTGAAGTGTGTAAGAAAGTAAAGAAATATCAGGCAGTCCAG CTACAGGAATGGATGATTAAAGTCATCAATAATAACTGCTATATGTGTAGAAGGAAAGCTGGTAGATATGACTGATGTTATTGGCAT AGCAATGTAATTATAGAGCGGATTAACACAATGAAGTCTAGNACCTTATCAGGCANCATTATATCTTAAAGGATTGATAGACTCGGTC TCCATGAAAGAAGCAGGATATCCCTGTTATCTCACAAGAAATTTATGTTTGGATTTCACCAACTGGAAGGAACACATTGATAAATTT TAGAACCAATTAAGGGCTGAAAAAAGAACAGACGAGCAAGGAAAGAGTCCAAAGAAAAACAAAAATCAAAAAAAGAT GCAGAAGATAAAGAAACGTATGCTCTGCAAAAGGCCAGCATACGATGACCTGAATGATAATAGCGTAGAGAGAACTGAAGTACCCA CTGATCCCTTGAACCTACTGGAACAGCCTACCTCGGCAAGAAAGAACACCCGCTTCTCAGTCAGAAGAGAGCTTATGTTTAAATAA CACCATTAGAAACAAAAAGTTGATAGAGCAAGATGTATAGACTACAGTGTCTCTATTGAAGGAATATCGGACTTTTCAAAGCAAGC ATCAAGAAGAAAGTGAATCAGATATACATGGAAGTCCAAGTCTACAGTAAGTCTCAAGAGACCTTTGATCATAGAGTGGGATTTGAA GGCAATTACCAAGGAGGACTGCAATGAATGTGACATAATCACTGCCAGACATATTAGATACCTTGCCTGAAAGTAAACAATGCTCAC CAGTGATTTTATGAAAAAGAACAGTTGCCCTCAAAGTGCAGAAAACTGAAAGTCAAATAGGTGTATCACAGTATTGCCGGTCTCATC ACATTTGTCAAGTGAAGAGAATGAAGTAGAAATTAAGTGAAGCAAGCAAGAAATACAAAAGAAAGGCTGAATCGGGAGAGAGA AAACACAAACCATACAAAAGATATCCTCTCATTTAGAAAGTGAAGGTGAGAGAGCTTGTATATACACCAAAGAGACCTCGATC TTCTGCTAAGAAATCACATTACAAATCTGCTGTTAGCAAGGACTTTCTCATNGAAGNAAAGGCATCTGATAGAAGTACAGACAGCTGTT AGACCATCTACCTGGTTTAAACGATGATGAAGAATGGAGTGAGCAAGGTTACAGAAGCTACATTGTCTTTTACATCTCTTCCAAAGCA CAAACTGGCTTTTGGTCAGATGTGGCTATGGCAGTAGGTTCTCGAACTGCTGATGAGTGCCAGAGAAATACACAGAAAGACCCCAAG GCCAAGGATCCCGAAAACATGTCTAAGAAAAAGCAAGCCAAAGGTCCAAATGGTGAGAAAGACAGTGTGATGCCAAGACTAT TAAGATAACTGCCAAAGTGGGAACTTTAAAGGAAACGACAAATGAGGGATTGCCTAGAACATTTGGCAAAAGACAACCATGATGATT TTTTCACTGCAACACCTTGCAAGAAACAAAGATTAGTTGCCAACTTTCAATACAGNCAAGATGATGATTTTCTGCTAGATATGGACAG GGATCCAGCATCTCATCATCAATTATTACTTACCATTGAGAAGCACTACTNNNNANTGTGGGCACTTTAGCCCNAGCATGNTAGNCGC TATATAAAGGAATAACTGTGATAGATATGTTTACCAATGCAAAAAATGCTAAAAAATATGGCAAGAGTAATGGTGGCTTGTCTGGG GCAACATTAGGAAAAAACAGTTAAACTGATCTCTCATCTCCACCACCAACACGAAAGCCCTGTTTAAACAAAGATTAGGTAACAAACN CTGATATTTCAAAGTANTTCATTGATGATACAGAATCAGATGAAGAAGAGAAAGATTATTACTTTTCCAATTCTGACTGA

Supplementary Table 3. HJURP Oligopaint library sequences

Library name	Sequence
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTTGGCCAGATGATGGTCAACTGTTACAGCTGACTTTTCATTGCACCCTTGACAGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAGGAACCATTTGAAGGGAAGGACAGGAGAAAAGCCTTTTCATTGCACCCTTGACAGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAGATAGTTGGTCACTTGAGTTCTGTTACCACCCACGTTTCATTGCACCCTTGACAGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTACTAGCTATTGGCAACTAGACACCCTCAAGTAGCATTTTCATTGCACCCTTGACAGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGCCTCGTGGGTGCTGCATGGCTGCAAGAACTTTTCATTGCACCCTTGACAGATAC

HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAAAAGCATCAAGACCTTGGGATTTCTTCTTGTTGGGTGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAAGCTCAGGCAGTTTCAGCTGGCTGTCTCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAAAACACAAAATGTGGAGCATACTCTAGACGGCATGCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCAGAGTCAGAGAAGACAAACAGGTCCGCAGGGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAACCATATACCTTTATTGTGAAAACCACCCAGGCATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTTTTCCACGGTACCAGCTGCCCTGGAGCATGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGAGGTGCATCTGATGCCAGCAAGGTGATCGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGACAGAGACCCCTTCTAAAAGCAGAACTAGAGGCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTTTTTCATCCTGACTGCCAGTGTAACCCCGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCTCCTGTTGTCAAAGAACTCAAGGCACACTACGGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGCAGTTTGCCCTGGGTATGTGAGCCCAATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAAGAGAGACTGCCACATCACAAAGGGTCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAAAAGCCTTACTGGTCTAGCACAAAGGAGGAAGAACTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCTCTCATGTTGTCACTGAATGTAGAGACCTGGTCCCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAATGTGGCTCAAGATGGAAGTGAAGAGGATCTGGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTTAAAGGCCCTAAGGATAGGCTAGTAAGTCCAGGGGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAGTGGTGAGGAACTCTGGGTACCAGGGAATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAGTAGTTGGGCAAGCGAGGTCTCCGGGCTGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCAGGAAAAGCAAGGTTTTCTCCAGGTAGAGTGCCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCCAGATGGGTGGGCAAGGGACACAGCTGTTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTATCTTGGGACAGCTGGGGACACAGGCTGGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTCCACTCTCACAGTCACTTCCATAGTGATTTCTGGAGCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAGGCCACCAAGGCACACCACGATAGCAGTTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGGGGCTCACGAGGTTGAGGCAGCTAGACAATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTACAGCTCAAGGCCAAGAGCAAGGGAGATAGCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCACACCAGGCAGCCAGAGACCCAGTTCAACTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCCCAACAACAGCGCTCTTAAGACTAAGCCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGAGAGGTGTGAACAGCGTTCTGTGATGACTCAACTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCACCTACTCAGAAGCAGAAGTGAGGAAGGTGAAGATGTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTTCATGTTAAGAGGAAAAGTCCACTGAGAATCCCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTATAAGATAAAGAGACACAGTAATGGCCAAGGCTGGCTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAGAGAAGCCCAAGGCACCAACATCCACAATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTCAGGGAGCTGCAATGGAAGACTGCTCAGTACTTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTACAGGATGCCCTGGGGACCGTCTCCAAAATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTGGAGAAACAAAAGTCTGTGAGGTCTTCTCCTCATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTTAAGCAGCTGCTGACTACACATAAGCATCATGTCCATTTTCATTGCACCCTTGACGATAC
HJURP_pool	TGAATAGCAGCGGTGGCAAACCTTCGTGAAACTTGGCCTGGTGAGCACAAGCTATTTTCATTGCACCCTTGACGATAC
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