# Determining the Meiotic Stability of *HRAS1* Minisatellite Tract

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## Abstract 1

Minisatellites are repetitive DNA sequences with repeat units that range from 16 to 100 base pairs in length. These sequences are stable during mitosis but are highly unstable during meiosis with alteration in both length and sequence compositions. One class of minisatellite, rare alleles, have been correlated with cancers, including primary tumors of the brain, lung, ovaries, colon, bladder, and breast. Due to the difficulty of studying meiosis in humans, a novel minisatellite system in the yeast *Saccharomyces cerevisiae* using the common A1 allele, of the human *HRAS1* minisatellite integrated into its genome adjacent to the *HIS4* gene was used in this research. After the insertion of the *HRAS1* minisatellite into the yeast genome, it was found to exhibit the same phenotypes as in mammalian cells. Our strains have a rare *HRAS1*minisatellite allele isolated from breast cancer cells inserted into the promoter region of *HIS4*. Two strains, namely Δ*slx1* and Δ*slx4* mutants of this *his4-H10 HRAS1* rare minisatellite allele, were used to collect data. Data from other researchers showed that *SLX4* and *SLX1* play a vital role in resolving Holliday junctions recombination. Hence, to determine the factors involved in minisatellite stability, we determined the frequency at which the *HRAS1* minisatellite repetitive tract undergoes alterations during meiosis, and the nature of the alterations, as well as calculated recombination and crossover frequency of this minisatellite in the our mutants.

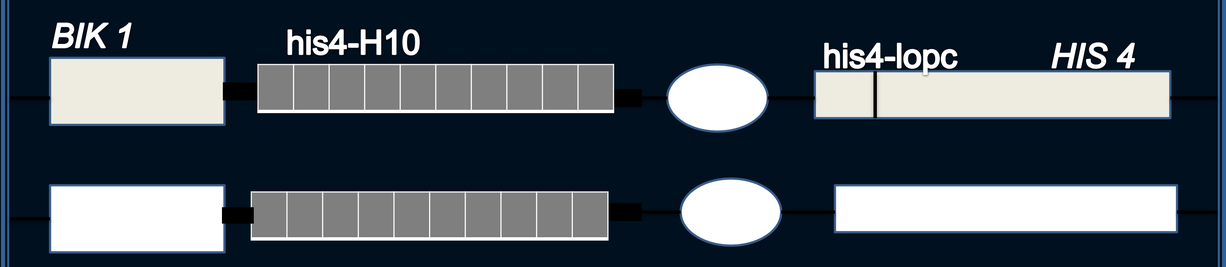
## Candidate Genes 4

We studied the effect of deletion of two genes namely SLX1 and SLX4 of HRAS1 minisatellite budding yeast cell on recombination and crossover of homologous chormosomes.

- Human homologous of SLX4 is called BTB12 and yeast SLX1 is called SLX1 in humans.
- SLX4 forms complex with SLX1 and other protein factors during DNA breaks.
- These complex act as resolvase for holliday junction formed during the double strand break.
- Both SLX1 and SLX4 influence recombination and cross over frequency during meiotic cell division.

## HIS4 Locus set up 2

Chromosome 3




- his4-H10 is the insertion of HRAS 1 minisatellite.
- Oval shape is TATA box.
- his4-lopc is 28 base pair insertion at HIS4 locus to see the recombination event at *HIS 4* locus.

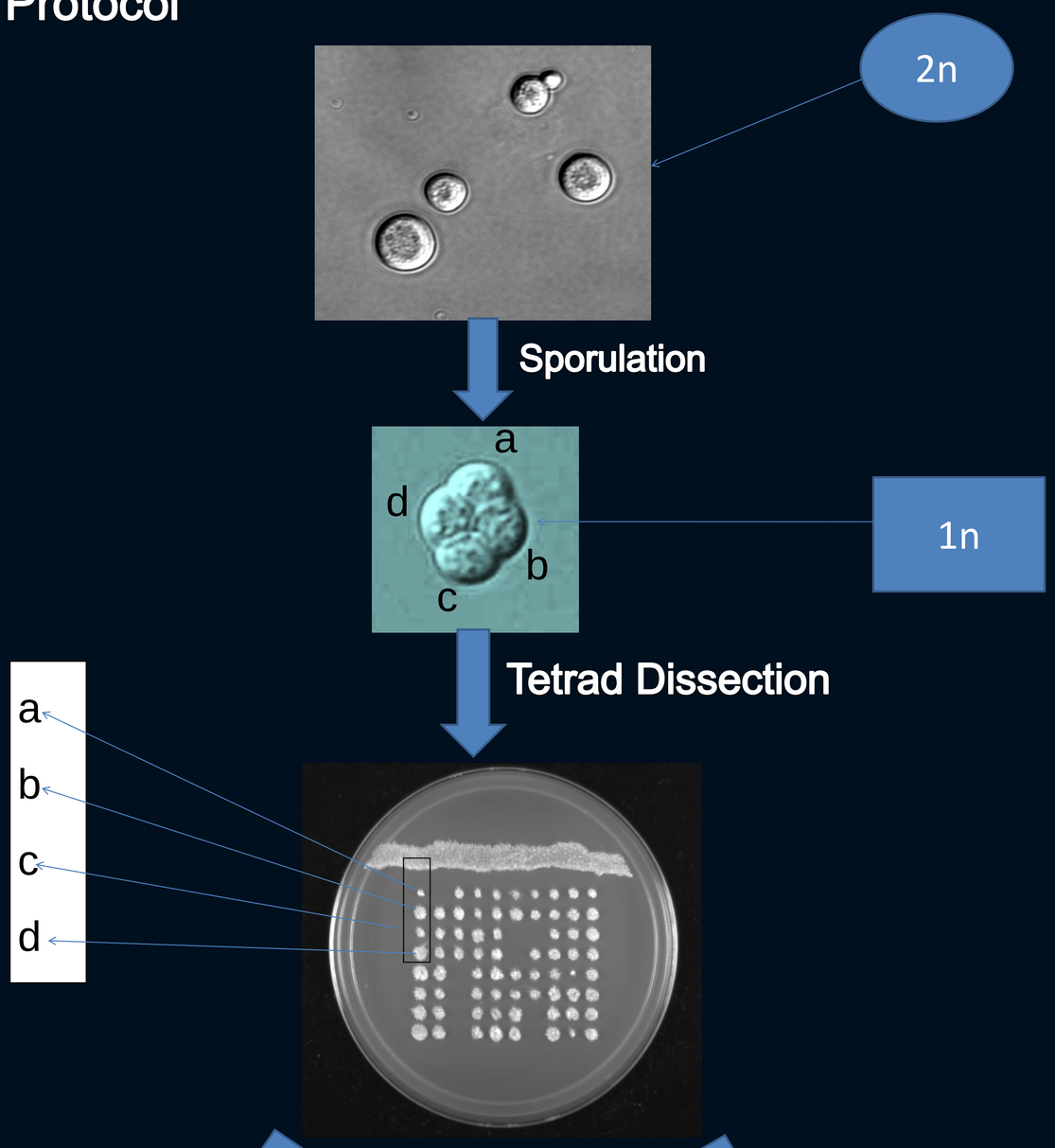
## Analysis of minsatellite tract length 5

| Stains | Relevant Mutation | Total tetrads | % total altered | % of tetrads with change | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 spore increase | 1 spore decrease | 2 altered | 3 altered | 4 altered |
| DTK 751* | + | 205 | 22 | 5 | 16 | 1 | 1 | 0 |
| DTK 1460 | Δslx1 | 50 | 33 | 4 | 14 | 12 | 2 | 0 |
| DTL 1487 | Δslx4 | 43 | 72 | 9 | 12 | 7 | 0 | 0 |

*Data from Andrea R. LeClere

- The data shows us there is high increase in spore alteration with Δ*slx4* than mutants with Δ*slx1* (72 % vs. 33%).
- Comparing with wild type we see that there is more alteration with two spores than single spores.

## Protocol 3




## Minisatellite stimulated recombination 6

| Strains | HIS4 promoter | Relevant mutation | Total tetrads | Ab. Seg. % | HIS4-LEU2 interval | |
|---|---|---|---|---|---|---|
| | | | | | cM | % of WT |
| DTK 751* | his4-H10 | WT | 867 | 48 | 32 | - |
| DTK 1460 | his4-H10 | Δslx1 | 121 | 52 | 34 | 106 |
| DTK 1487 | his4-H10 | Δslx4 | 43 | 49 | 12 | 37 |

*Data from Andrea R. LeClere

- The data shows the abberant segregation at HIS4 locus.
- From the data, we see that deletion of *slx1* and *slx4* cause 52 and 49% abberant segregation of HIS4 gene
- Also deletion of *slx4* have higher centi morgan distance in comparison to deletion of *slx1* (34 cM vs. 12 cM). The low number of mutants with Δ*slx4* comparison with Δ*slx1* may be the reason we see the difference in distnace between HIS4 and LEU2 distance.

## Conclusions 7

- We concluded that the minisatellite tract is instable during meiosis cell division.
- There is high abberant segragation with both Δ*slx4* and Δ*slx1* mutants close to 50%.
- The length of spores alterations conclude that the minisatellite stablity changes from generations to generations.

## Referecnes:

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