# Investigating and Characterizing Half Crossover Cascades in *Saccharomyces cerevisiae*

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## Abstract

We are examining the rate at which a genetic event, Half Crossover Cascade (HCC), occurs when normal genome maintenance is compromised. Half crossovers (HCs) occur when the intermediates of Break-Induced Recombination (BIR) get disrupted during double strand break (DSB) repair. The cell ends up with fused pieces of donor and recipient molecules and a destabilized donor molecule, that often gets lost. The cell can get caught in a cycle of break and repair, if HCs keep occurring, called Half Crossover Cascades (HCCs). HCCs are rare but can cause devasting changes to the genome from a single DSB, which could explain the genetic heterogeneity of some tumors in human cancer patients. During normal BIR and HCs, DNA is resected before homology search is performed, producing a single-stranded piece of non-homologous DNA (ssDNA), or tail, that is ultimately clipped off by Rad1 clipase protein later in DSB repair. We questioned whether the removal of this ssDNA tail by Rad1p is required for HCCs to proceed and whether the length of the tail will affect the frequency of HCCs observed. We engineered the *Saccharomyces cerevisiae* genome to include three homology regions (middle 2 kb of LYS2 gene, called YS region) on three different chromosomes (6, 9, 10) to make HCCs a viable option for DSB repair and to recover clones that may have gone through this process, by reconstituting the full *LYS2* gene. We used CRISPR/Cas9 to induce single-ended DSBs 4bp and 92bp away from the homology region on Chr6, thus causing cells to repair a DSB near one of the homology regions. Lys$^+$ clones recovered through this assay were quantified, then phenotypically characterized by patching onto various media for auxotrophic and drug resistance markers. We examined changes in the frequency of Lys$^+$ formation and the mutational spectra of Lys$^+$ colonies (i.e. DNA repaired by reciprocal crossovers vs. half crossovers) in *rad1Δ* and WT cells. In the *rad1Δ* mutant, when the DSB occurred 92 bp away from the repeat sequence, we observed a significant decrease in Lys$^+$ frequency compared to WT clones. In contrast, when the break occurred 4 bp away from the repeat sequence, we found that there was not a significant decrease in Lys$^+$ frequency between the two genotypes. We conclude from these findings that Rad1p is required to clip the ssDNA tail when breaks occur far from a homologous sequence, but less so when breaks occur closer in.

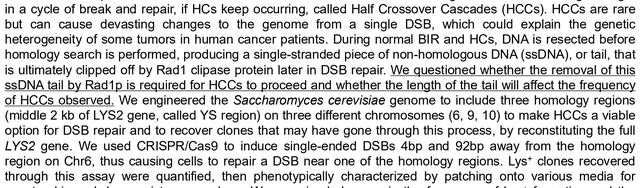
## Experimental HCC Detection

### Figure 1. HCC Detection

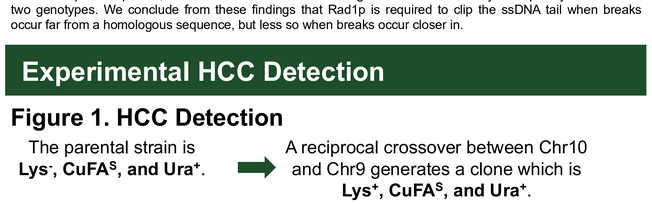
The parental strain is **Lys$^-$, CuFA$^S$, and Ura$^+$.** ➡ A reciprocal crossover between Chr10 and Chr9 generates a clone which is **Lys$^+$, CuFA$^S$, and Ura$^+$.**

Cas9 induces a DSB on Chr6R to the right of the YS sequence. The location of this break largely forces a single-ended DSB due to its proximity to the end of the chromosome. The DSB also results in the loss of *URA3*.


The single ended DSB on Chr6 may result in a half-crossover with Chr10, initiated through the shared homology between the YS regions (left). A half-crossover between Chr6 and Chr10 generates a Chr6/10 translocation product, as well as a single-ended DSB on Chr10, that now needs repair (right).



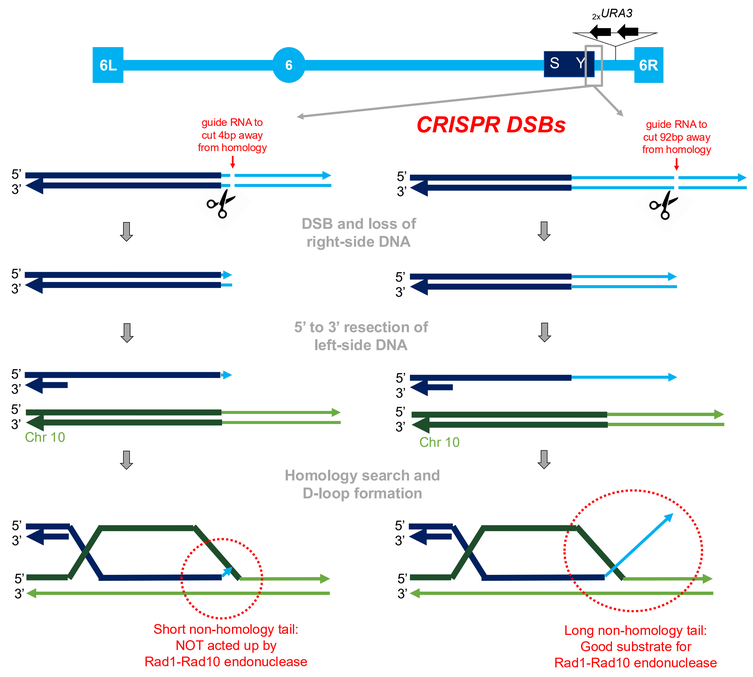
The single-ended DSB on Chr10 may be repaired through BIR (right), using Chr9 as a template, creating a Chr10/9 product (left). This final BIR step duplicates the CNV cassette and generates a complete *LYS2* gene.



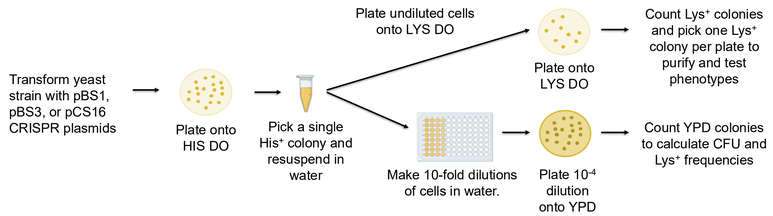
The final single HCC clone is **Lys$^+$, CuFA$^R$, and Ura$^-$.**

## Methodology and Results

### Figure 2. CRISPR Plasmid Cut Sites and Rad1-Rad10 endonuclease role




### Figure 3. Method to induce and measure frequency of Lys$^+$ colonies.




### Figure4. Expected phenotypes for Lys$^+$ clones.

| Class of Rearrangement | Growth on Media | | | | |
|---|---|---|---|---|---|
| | Lysine Drop Out | Uracil Drop Out | Tryptophan Drop Out | Nutrient Rich + Geneticin | Synthetic Complete Drop Out + Copper (200μM) & Formaldehyde (2.75mM) |
| None – Parent | No Growth | Grows | Grows | Resistant | Sensitive |
| Reciprocal Crossover | Grows | Grows | Grows | Resistant | Sensitive |
| Single HCC | Grows | No Growth | Grows | Resistant | Resistant |
| Double HCC | Grows | No Growth | Grows | Resistant | Sensitive |
| Other Rearrangement | Grows | Grows | Grows | Resistant | Resistant |

### Figure 5. Median Lys$^+$ Frequency and Chromosomal Rearrangement Classes in Lys$^+$ Clones

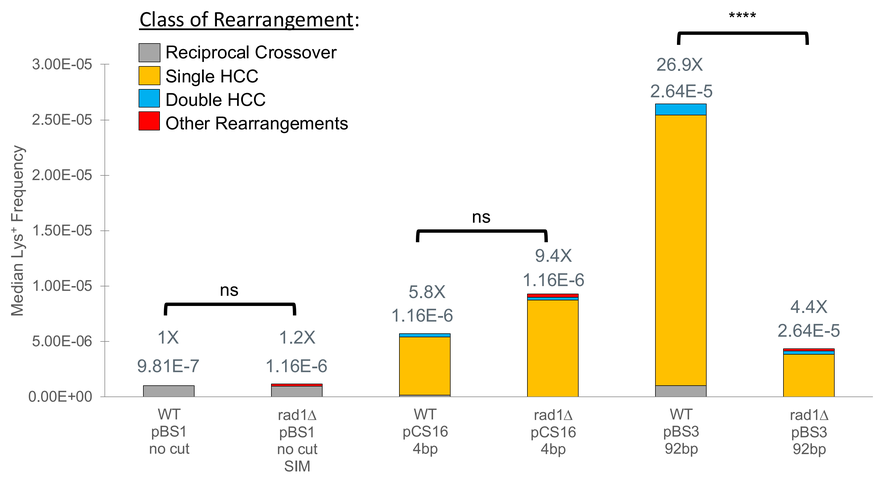



Figure 4 Legend: 30-40 Lys$^+$ clones were quantified and phenotyped for each plasmid and genotype combination. Height of bars represent median Lys$^+$ frequencies and color coding indicates the class of rearrangement detected. "Other rearrangements" were Lys$^+$ clones with CuFA resistance acquired through means other than HCCs. Fold increases were calculated by comparing to WT pBS1 Lys$^+$ frequency. We calculated simulated data for *rad1Δ* pBS1 to show the phenotypic breakdown of these clones because the median Lys$^+$ frequence was zero. Simulated Lys$^+$ frequency ($1.16x10^{-6}$) was calculated using the median number of YPD colonies (36 colonies) from the $10^{-4}$ dilution from several previous experiments and using 1 Lys$^+$ colony per culture. Statistical significance was determined using the Wilcoxen Rank Sum Test for genotype comparisons for each plasmid (**** indicates $p < 0.0001$).

## Conclusions

- There was no difference in Lys$^+$ frequency nor chromosomal rearrangement classes between WT and *rad1Δ* when DSBs occurred spontaneously (pBS1, which contains Cas9, but no guide RNA) ($p = 0.201$, when comparing WT to simulated *rad1Δ* pBS1 data). Additionally, all Lys$^+$ clones came about through RC or Other rearrangements in both genotypes.
- There was no difference in Lys$^+$ frequency nor chromosomal rearrangement classes between WT and *rad1Δ* when DSBs occurred 4bp away from YS region (pCS16), forming a short non-homologous ssDNA tail ($p = 0.227$). Both genotypes formed Lys$^+$ clones through HCCs.
- There was a significant decrease in Lys$^+$ frequency and amount of HCCs when DSB is 92bp away from YS region (pBS3), forming a long non-homologous ssDNA tail ($p = 1.86x10^{-11}$). Yet again, both genotypes formed Lys$^+$ clones through HCCs.
- We conclude that *RAD1* is required to deal with long ssDNA tails, but less so for short ssDNA tails. We hypothesize that other DNA repair enzymes could make up for the lack of Rad1p when the ssDNA tail is so short.
- We also conclude that *RAD1* is not required for HCCs to proceed because we observed HCCs in both WT and *rad1Δ* mutants.

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