# Mapping Histone H2B-mCherry Genomic Insertion Sites Relative to DNA Replication Chromatin States in Maize

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## ABSTRACT

Chromatin dynamics are fundamental to understanding gene expression regulation and chromosome behavior during cell replication. Previous research from Dr. HW Bass' lab reported on the development of histone H2B-mCherry as a transgenic (introduced) reporter gene to study chromosomes in plants. In another project, they mapped DNA replication timing in maize, noting that some regions replicate early in S-phase of the cell cycle, while others replicate later, at middle-S or late-S. The early replicating regions are characterized as open chromatin. This project tests the hypothesis that the reporter gene preferentially inserts into the early S/open chromatin regions. To test this hypothesis, we have around ten independent histone H2B-mCherry transgene insertion lines of maize that will be used to map the location of the insertion. Once we map the genomic locations, we will know if they have preferentially inserted into genomic regions classified as early S/open chromatin or not. This new project involves isolating genomic DNA from the ten different insertion lines and using PCR cloning and sequencing to identify the exact site of insertion. The current status and results obtained will be presented.

## INTRODUCTION

This poster combines two different research projects to address the question of "just how random are transgene insertion sites in the maize genome". The first project involves the generation of multiple transgenic lines using T-DNA transformation with a reporter gene designed to make fluorescent chromosomes (Howe et a., 2012). These lines were made ten years ago, but the insertion sites are stable but they have not been mapped at the genomic level. The second project involves DNA replication timing, which is coupled to chromatin structure and nuclear architecture. We previously showed that early replicating genomic regions have characteristically more open chromatin compared to middle or late replication regions (Bass et al., 2015). Combining these two projects along with mapping the insertion sites, we can test the idea that insertion occurs randomly. The project involves relatively straightforward molecular biology requiring DNA isolation and sequence-specific PCR amplification. If successful, we will be able to test our hypothesis that the introduced transgenes preferentially insert into the early S/open chromatin regions, instead of randomly.

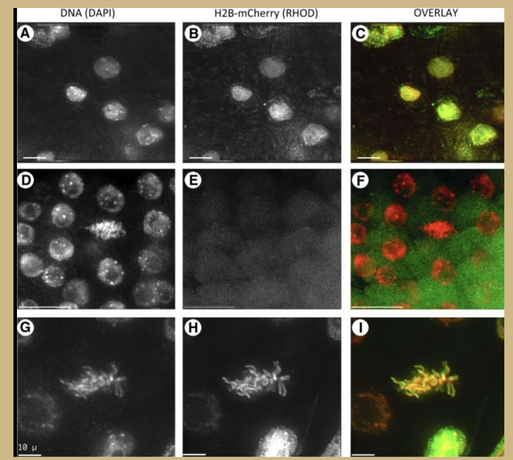


**Figure 1. Expression of Histone H2B-mCherry is seen by colocalization with DAPI stain for total DNA**. This figure shows DAPI images (1st column) of maize nuclei and Rhodamine images of fluorescent transgenes (2nd column), and two color overlay (3rd column). Each row is the same area on the microscope. The first and third row are expressing the fluorescent protein (panels B and H) and those plants should have transgene DNA for us to map. Figure is from Howe, Clemente, and Bass (2012) *DNA and Cell Biology* 31(6):925-938.

## RESULTS

1. Successfully identified multiple independent transgene insertion lines of maize for further study, providing a diverse set of samples for analysis.
2. Ground dried maize seeds to prepare DNA for extraction, ensuring a sufficient quantity for subsequent analysis.
3. DNA extraction is complete, and testing for PCR suitability is underway, while verifying the quality and integrity of the extracted DNA for amplification.
   - DNA integrity confirmation is important in order to find out whether or not the DNA is fragmented or degraded. Degraded DNA can lead to poor amplification by PCR.
   - PCR is highly sensitive to DNA quality and purity. Isolating DNA properly helps remove contaminants that could interfere with amplification. Poor-quality DNA can lead to failed reactions or inaccurate results.

## CONCLUSION

The significance of this project revolves around the exploration of how chromatin state influences transgene insertion and expression. This furthers the knowledge on gene regulation and chromosome behavior. By mapping transgene locations and analyzing expression changes, we will learn more about the genomic position effect, which has implications for genetic engineering and crop improvement. Additionally, the use of fluorescent histone reporters allows for innovate cytogenetic studies, building on established research in maize genome replication.

## METHODS

Identify independent transgene lines from existing seed stocks in the Bass lab

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Isolate genomic DNA from each line using ground seed powder

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Use TAIL PCR to amplify transgene-flanking genomic sequences

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Sequence the PCR products and align them back to the genome

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Determine which replication timing class (early, middle or late S) is at the insertion site

## REFERENCES

(**2015**) Bass HW, Hoffman GG, Lee T-J, Wear EE, Joseph SR, Allen GC, Hanley-Bowdoin L, and Thompson WF. "**Defining multiple, distinct, and shared spatiotemporal patterns of DNA replication and endoreduplication from 3D image analysis of developing maize (Zea mays L.) root tip nuclei.**" ***Plant Molecular Biology*** 89(4):339-351. [PubMed 26394866 | ONLINE | PDF++]

(**2012**) Howe ES, Clemente TE, and Bass HW. "**Maize histone H2B-mCherry, a new fluorescent chromatin marker for somatic and meiotic chromosome research.**" ***DNA and Cell Biology***. 31(6):925-938. (DOI: 10.1089/dna.2011.1514). [PubMed 22662764 | DNACellBio online | PDF |Supplemental 3D Image Movies ]