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FISH transgene localization in hon102 and sgb101 transgenic maize

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My research project investigates the expression of transgenes within maize. Transforming maize with plasmid constructs that are intended to reduce chromatin gene expression by triggering RNA interference produces transgenic lines of maize. Little segments of chromatin genes are cloned as inverted repeats downstream of a constitutively expressed promoter, such that transcription of the inverted repeat leads to production of a double stranded RNA, which induces RNA-mediated degradation of mRNAs with homology to the double-stranded region. The plasmid construct also contains a second gene, the Bar gene, which confers resistance to the herbicide Basta. This allows the presence of the transgene to be followed by scoring the plants for resistance to this herbicide. However, a problem has arisen. Co-segregation of the transgene and Basta resistance that is seen in the first generation is lost in second generations in some lines. The expected 1:1 ratio that is expected between the two is lost, and a high proportion of Basta resistance is seen. A possible explanation for this, and what I am investigating, is due to silencing of the transgene due to its insertion into an unfavorable area of the genome, the untranslated and highly condensed heterochromatin. To do this, I localize the insertion points in the genome for some of these transgenes that tend to go silent and compare the locations to those from transgenes in lines where Basta resistance is stably maintained. Then, using a method called fluorescence in situ hybridization (or FISH), the maize chromosomes are fluorescently painted through the use of markers that are specific for known repetitive sequences within the maize genome. Markers corresponding to both the repetitive sequences that are found in heterochromatin and the transgenes in question are used to paint the chromosomes. If the transgenes tended to be near heterochromatin in the unstable lines more often than in the stable lines, this might lend credence to the hypothesis that the transgenes are being inserted into untranslated and so unexpressed heterochromatic areas.