Transposition of a Maize *Mariner* Element in Yeast

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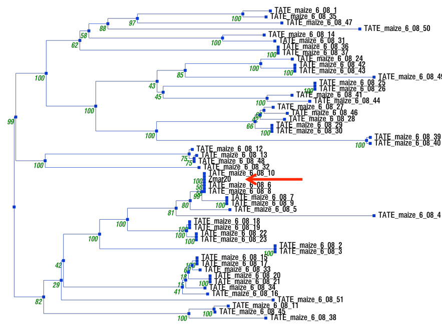
Abstract

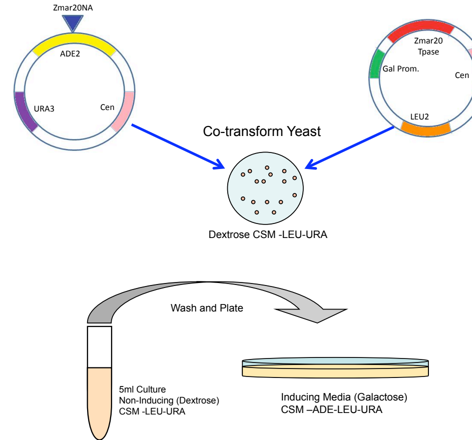
Computational analysis of the newly released genomic DNA from *Zea mays* identified three nearly identical copies of a *Mariner*-like element (*ZMar*20) with an intact coding region, suggesting recent transposition. To determine whether this element is capable of transposition we modified a previously developed yeast transposition assay. We found that a nonautonomous version of *ZMar*20 (*ZMar*20NA) can excise from *ADE2* when the *ZMar*20 TPase was expressed from a *CEN/ARS* plasmid (at a frequency of 7.2 events per $1x10^8$ cells). We hypothesized that the transposition frequency might be higher if the *ZMar*20 TPase was expressed from a *2µm* (high copy) plasmid. However, we observed approximately three-fold fewer excision events with this plasmid. Analysis of the site of *ZMar*20NA excision from *ADE2* revealed traces of the element (transposon footprints), suggesting that the TPase cuts the DNA within the element ends. These results demonstrate that *Zmar20* encodes an active transposase and that the combination of computational analysis followed by functional assays in yeast is likely to uncover more active maize transposable elements.

Computational Analysis



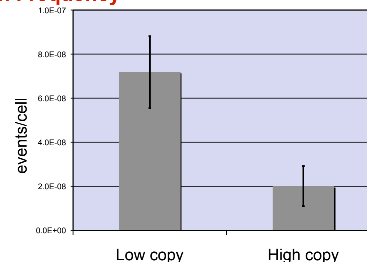
The TATE software package (see poster by Yujun Han) was used to infer the phylogenetic relationships of *Mariner*-like elements in the *Zea mays* genome. We observed that the clade with *ZMar*20 had three virtually identical elements indicating recent activity. This element was cloned into a yeast expression vector and tested with the reporter construct shown in the next figure.

Experimental Design



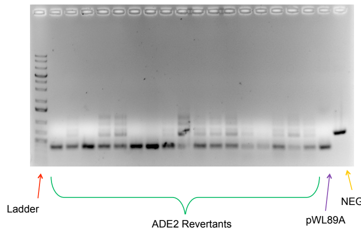
Yeast cultures were co-transformed with a reporter plasmid pWL89A (*ADE2* gene with *Zmar*20NA insert) and a plasmid encoding the transposase and cells containing both plasmids were selected on appropriate medium. Individual colonies were picked and grown in liquid non-inducing selective medium. The cultures were spread on galactose (induces TPase expression) plates lacking adenine to select for excision events of *ZMar*20NA from *ADE2*. After about two weeks of incubation at 30ºC, colonies were counted to determine excision frequency.

Excision Frequency



Two TPase encoding vectors were tested: a *CEN/ARS* (low copy) vector and *2µm* (high copy) vector. The graph depicts the average excision frequency (9 replicates) for these vectors ± standard error. Surprisingly, the low copy vector results in three times higher excision frequency than the high copy vector. The error bars do not overlap indicating that these two treatments are statistically different.

Excision Site Analysis



ADE2 TCTGTT------------AACGGT
8/26 TCTGTTTACTC---AGTAAACGGT
5/26 TCTGTTTACTCCGGAGTAAACGGT
4/26 TCTGTTTACGG---AGTAAACGGT
4/26 TCTGTTTACTCC---GTAAACGGT
1/26 TCTGCTTACTCC---GTAAACGGT
1/26 TCTGTCTACTC---CGTAAACGGT
1/26 TCTGTTTAC------------GGT
1/26 TCTGTTTACCCCGGAGTAAACGGT
1/26 TCTGTTTACTTCGGAGTAAACGGT

Footprints

We analyzed the excision sites of the *ADE2* revertants using PCR and sequencing. The *ADE2* revertants had an additional lower band. These bands were then sequenced and organized into catagories. The majority of the footprints retained sequence from *Zmar*20NA.

Discussion

This experiment revealed that *ZMar*20NA transposes in yeast, suggesting that *ZMar*20 is potentially activity in *Zea mays*. Contrary to our expectations, we found that the excision frequency was higher for the lower copy number vector. This could be due to the higher stability of the low copy vector (*CEN/ARS*) compared to the high copy vector (*2 µm*) or the potential deleterious effects of excess TPase. We suggest that a low copy vector be used for future experiments.

The footprints observed in yeast following *ZMar*20NA excision are very similar to the footprints observed for other *Mariner*-like elements in plants (i.e., *Osmar5*). These footprints contain traces of the element suggesting that the TPase cuts the DNA within the element itself. The diversity of footprints observed maybe caused by different amounts of exonuclease activity during repair of the double strand break. All footprints maintained the reading frame of the *ADE2* reporter, suggesting that only a fraction of the actual excision events were recovered.

Based on the success of this experiment we have tested additional maize elements. Specifically, we used the yeast assay system to test miniature inverted repeat transposable elements (MITEs) with ends homologous to the ends of *ZMar*20. Preliminary results suggest that the *ZMar*20 TPase does not mobilize three of these nonautonomous elements.