A two component Ac/Ds gene tagging platform for maize

February 1, 2006
Project Overview

Project goals

1. Distribute and precisely position 10,000 *Ds* elements throughout the maize genome.

2. Create 10,000 families of insertionally stable and unstable *Ds* elements.

3. Integrate *Ds* flanking sequence into annotated assemblies of maize genome.

4. Distribute genetic materials to the plant research community.

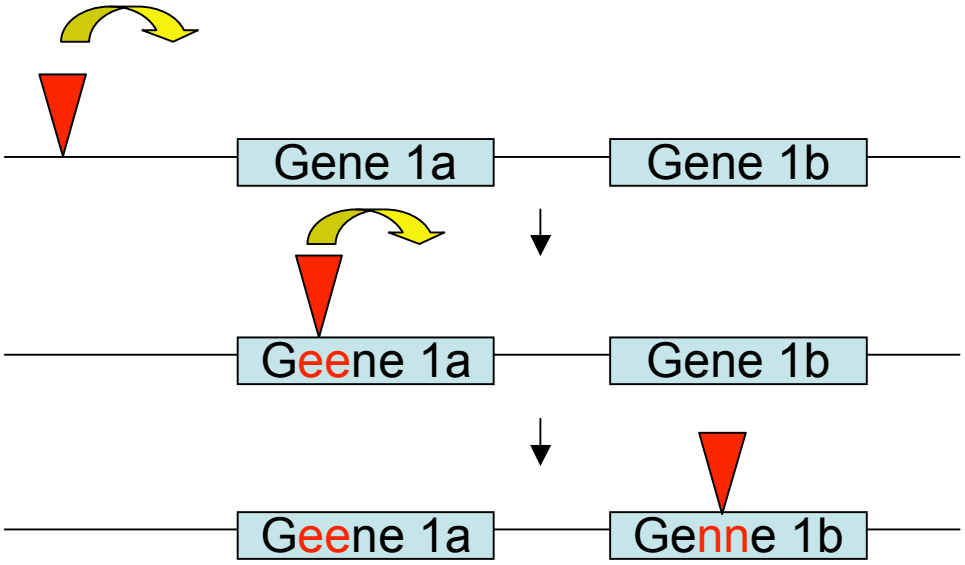
General features of *Ac/Ds*

- *Ac* - 4.6 kb autonomous elements encoding transposase
- *Ds* - non-autonomous deletion derivatives
- Usually transposes to linked sites (60%)
- Insert preferentially to hypomethylated regions of genome
- Displays negative dosage: increasing copies delays tnp

Ac *Ac/Ac* *Ac/Ac/Ac*

Ac/Ds of transpose to linked sites

- Directed gene tagging (Dellaporta et al. 1988)
- 'Reconstitutional mutagenesis' (Athma et al.1992; Moreno et al.1992; Alleman and Kermicle 1993)
- 'Hopscotch mutagenesis' (Tantikanjana et al.2004)



Adapted from Tantikanjana *et al* (2004) *Plant Physiol* 135:840-848

Ac/Ds tranpsose frequently in somatic tissues

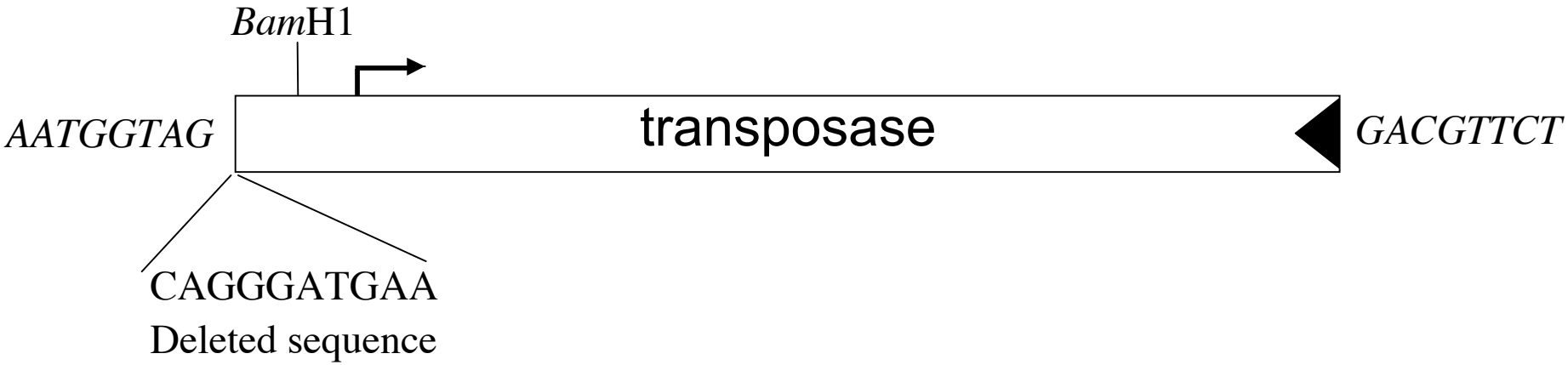
- Define autonomy/non-autonomy of gene action (McClintock1951)
- Lineage analysis (Emerson, 1917;Dawe and Freeling 1990)
- Reversion analysis to generate novel alleles (Wessler et al. 1986)
- '*Ac*-casting' to sequence regions flanking *Ac* (Singh et al. 2003)

Advantages of two-component system

- *Ds* insertions can be maintained as stable alleles
  - reverse genetic screens
  - physiological studies

- *Ds* insertions can be mobilized
  - footprint alleles
  - clonal analysis
  - transposition to linked sites

Ac-im, a stable source of transposase



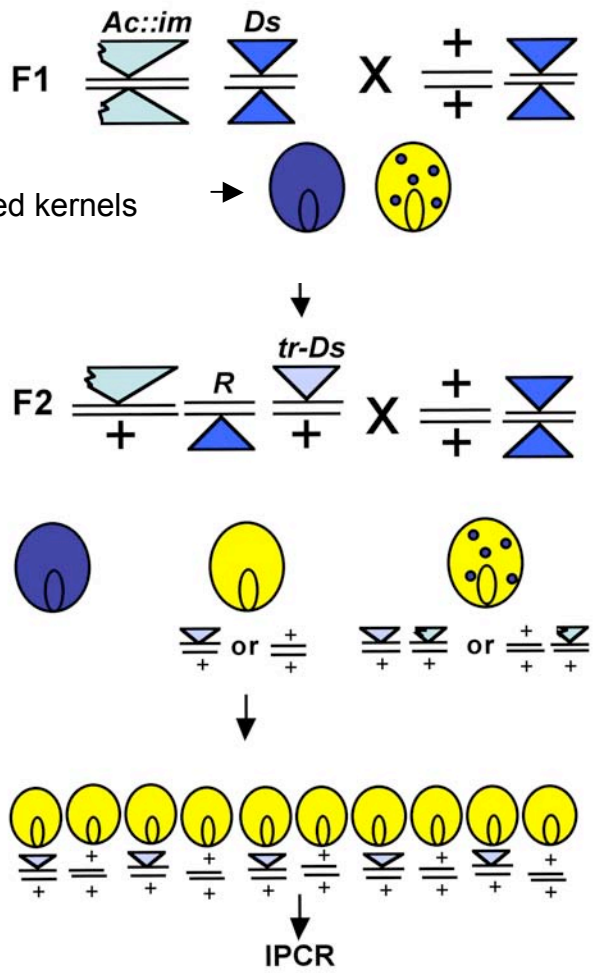
Ac *Ac/Ac* *Ac/Ac/Ac*

Genetic scheme to mobilize *Ds* insertions

1. *Ds* excisions selected as fully colored kernels

2. Plants are testcrossed to segregate away *Ac* transposase source from *tDs*

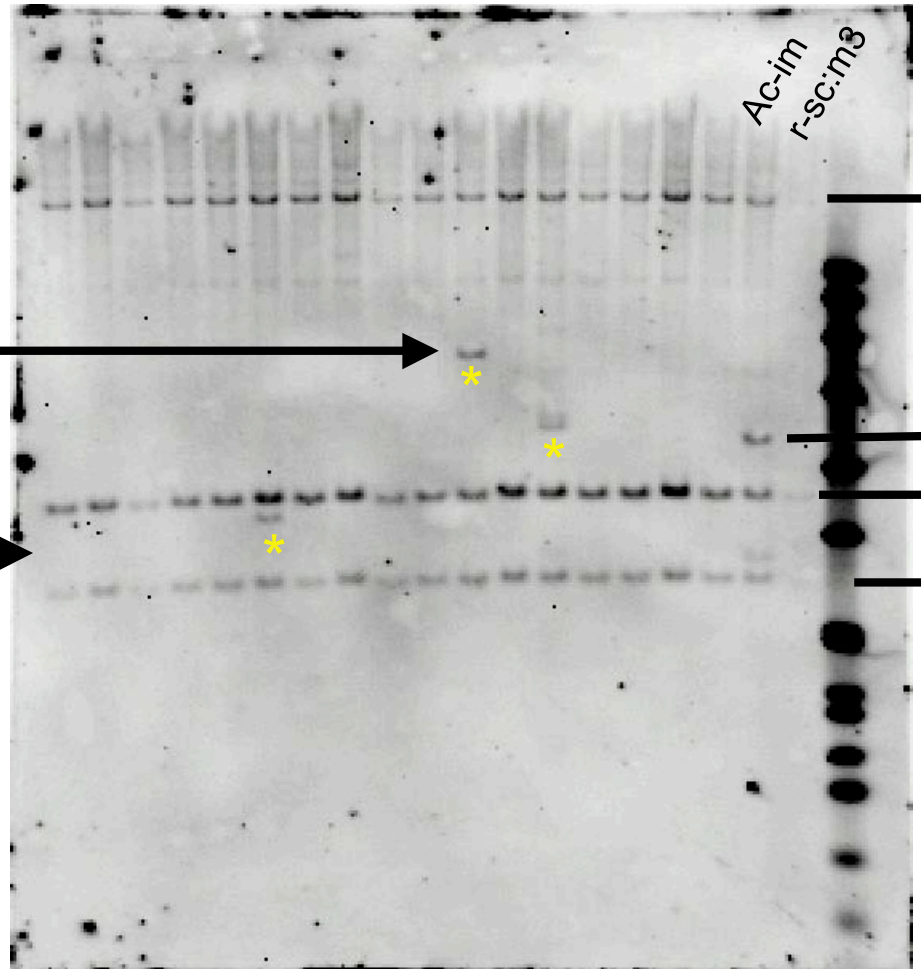
3. If *tDs* is linked to donor it will segregate with colored kernels. Therefore, colorless kernels selected and pooled to identify unlinked tDs elements in molecular assays.



tDs insertions unlinked to donor *Ac* identified by DNA blot analysis

1. *Ds* tranpsositions detected as unique bands using a *Ds* probe. Non-transposing cryptic *Ds* elements are also detected

2. Most lines carry linked *tDs* (no band in Southern) and are not selected

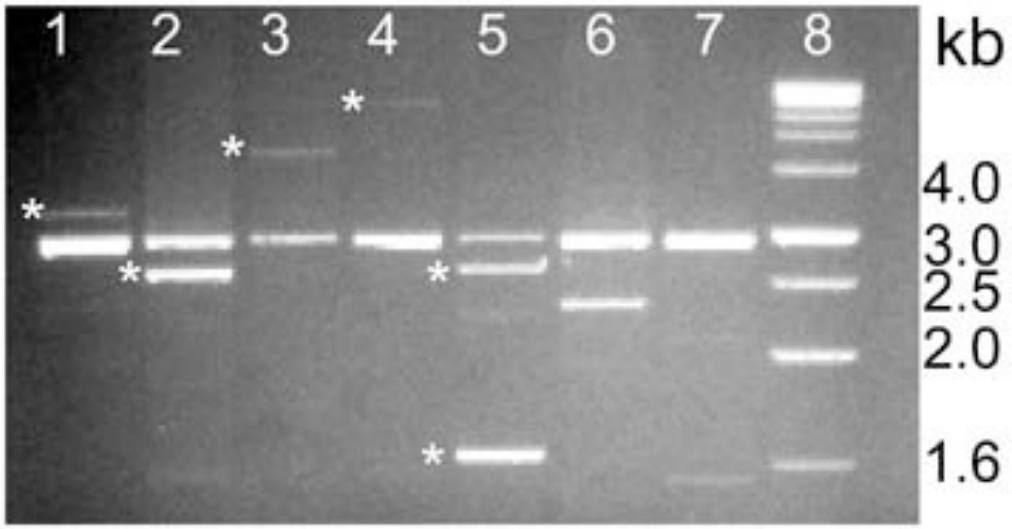


***Ds6* gene probe**

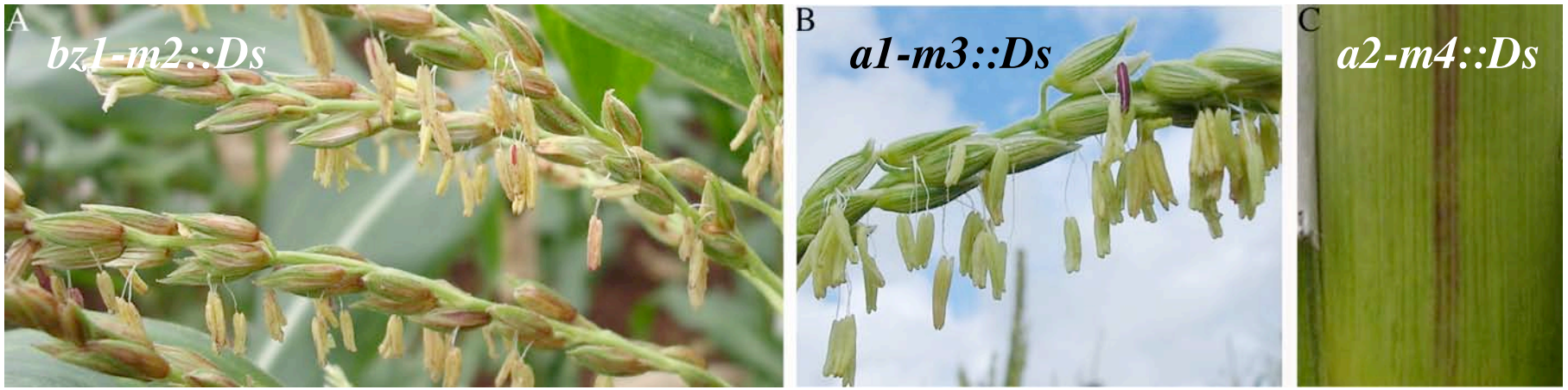
Inverse PCR used to clone sequences flanking *Ds* insertions

1. * marks bands of predicted size based on DNA blot analysis. Common band represents parental *Ds* insertion from donor *Ds*

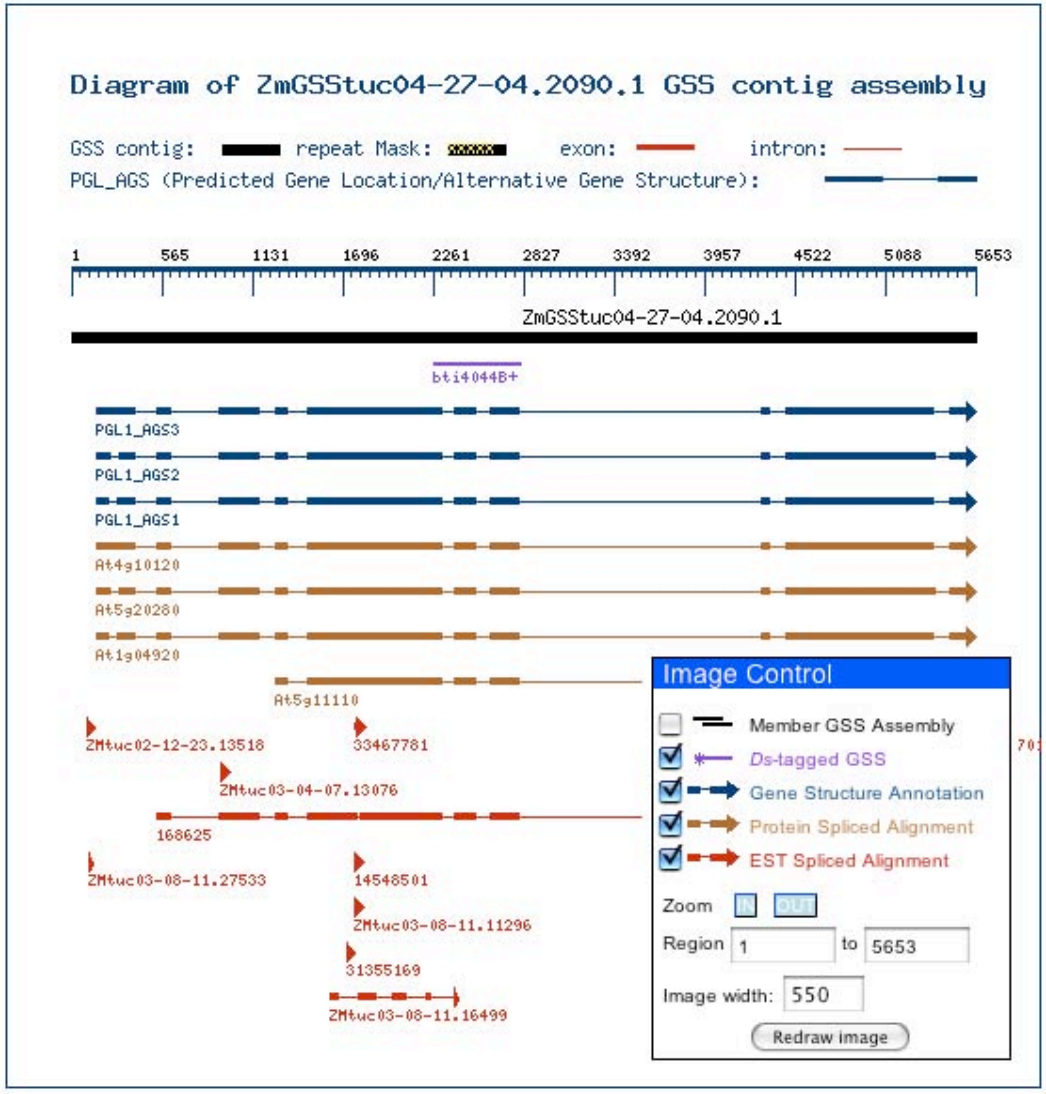
2. IPCR products are gel excised and sequenced to precisely determine *tDs* position in genome



Ac-im transactivates multiple *Ds* elements



Ds insertions will be integrated into GSS assemblies



Project Team

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