

Methods S1

Genomic *in situ* hybridization (GISH)

Seeds were germinated on moistened filter paper in petri dishes. Actively growing roots were removed from seedlings and placed in gas treatment for 2 h, fixed in 90% acetic acid and stored in 70% v/v ethanol. Chromosome spread preparation was performed as previously described (Han et al., 2006). Genomic DNA of *Th. elongatum* was isolated by a modified CTAB method (Kidwell and Osborn, 1992) and labeled with f ChromaTide Alexa Fluor 488-5- dUTP (Invitrogen) by the nick translation method and used as a probe. The probe was blocked with genomic DNA of “Chinese Spring”. Detection and visualization were performed as described (Han et al., 2009).

Supplemental References

- Han, F.P., Lamb, J.C., and Birchler, J.A.** (2006) High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. *Proc. Natl. Acad. Sci. USA*, **103**, 3238–3243.
- Han, F.P., Gao, Z., and Birchler, J.A.** (2009) Centromere inactivation and reactivation reveal both genetic and epigenetic components for centromere specification. *Plant Cell*, **21**, 1929–1939.
- Kidwell, K.K., and Osborn, T.C.** (1992) Simple plant DNA isolation procedures. In JS Beckmann, TC Osborn, eds. *Methods for Genetic and Physical Mapping*, Kluwer, Dordrecht, pp 1–13.