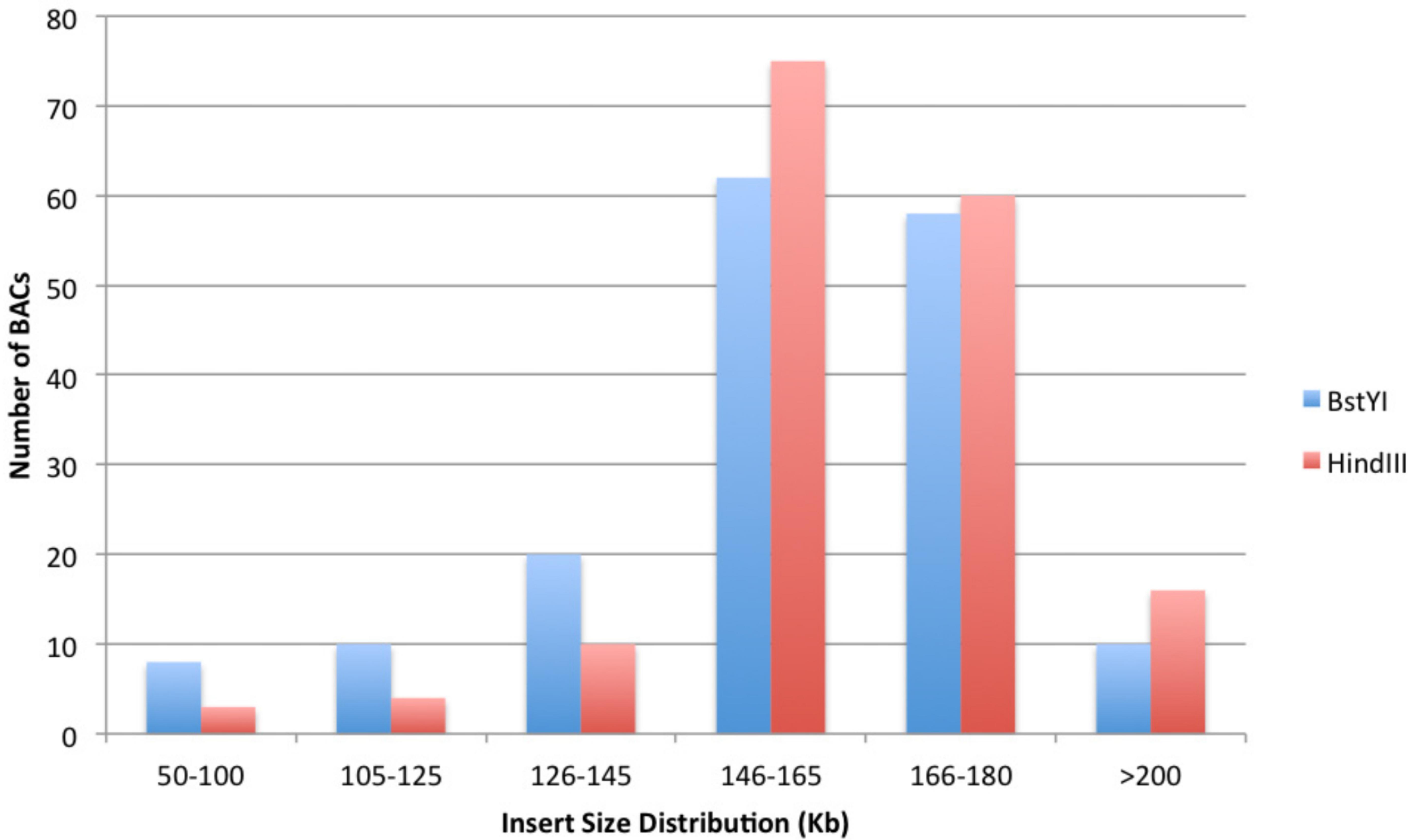


## Supplementary Figures

**Title:** Sub genome anchored physical frameworks of the allotetraploid Upland cotton (*Gossypium hirsutum* L.) genome, and an approach toward reference-grade assemblies of polyploids

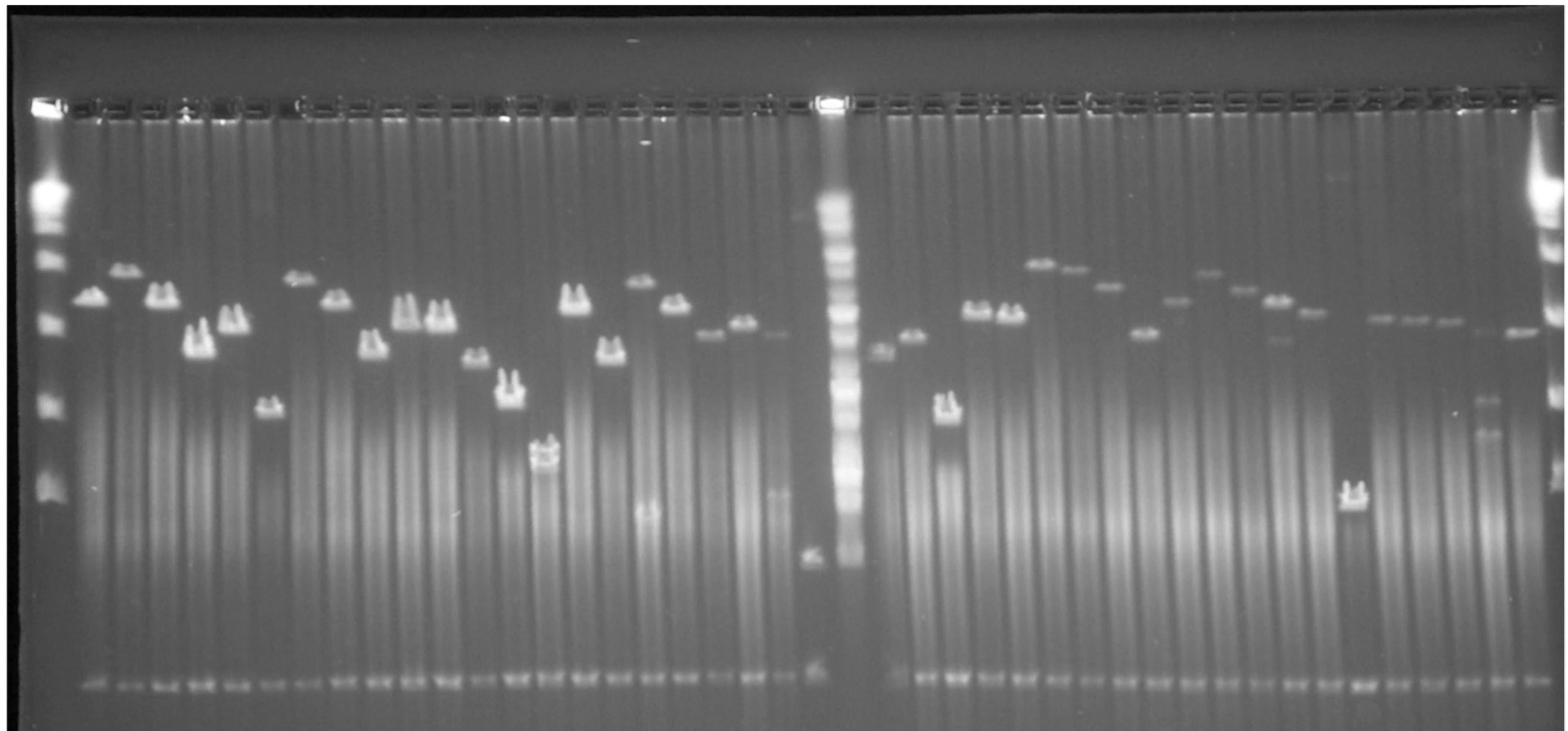
### Authors:

Christopher A. Saski<sup>1,a\*</sup>, Brian E. Scheffler<sup>2,a</sup>, Amanda M. Hulse-Kemp<sup>3,a</sup>, Bo Liu<sup>3</sup>, Qingxin Song<sup>4</sup>, Atsumi Ando<sup>4</sup>, David M. Stelly<sup>3</sup>, Jodi A. Scheffler<sup>5</sup>, Jane Grimwood<sup>8</sup>, Don C. Jones<sup>6</sup>, Daniel G. Peterson<sup>7</sup>, Jeremy Schmutz<sup>8\*</sup>, and Z. Jeffery Chen<sup>4,\*</sup>

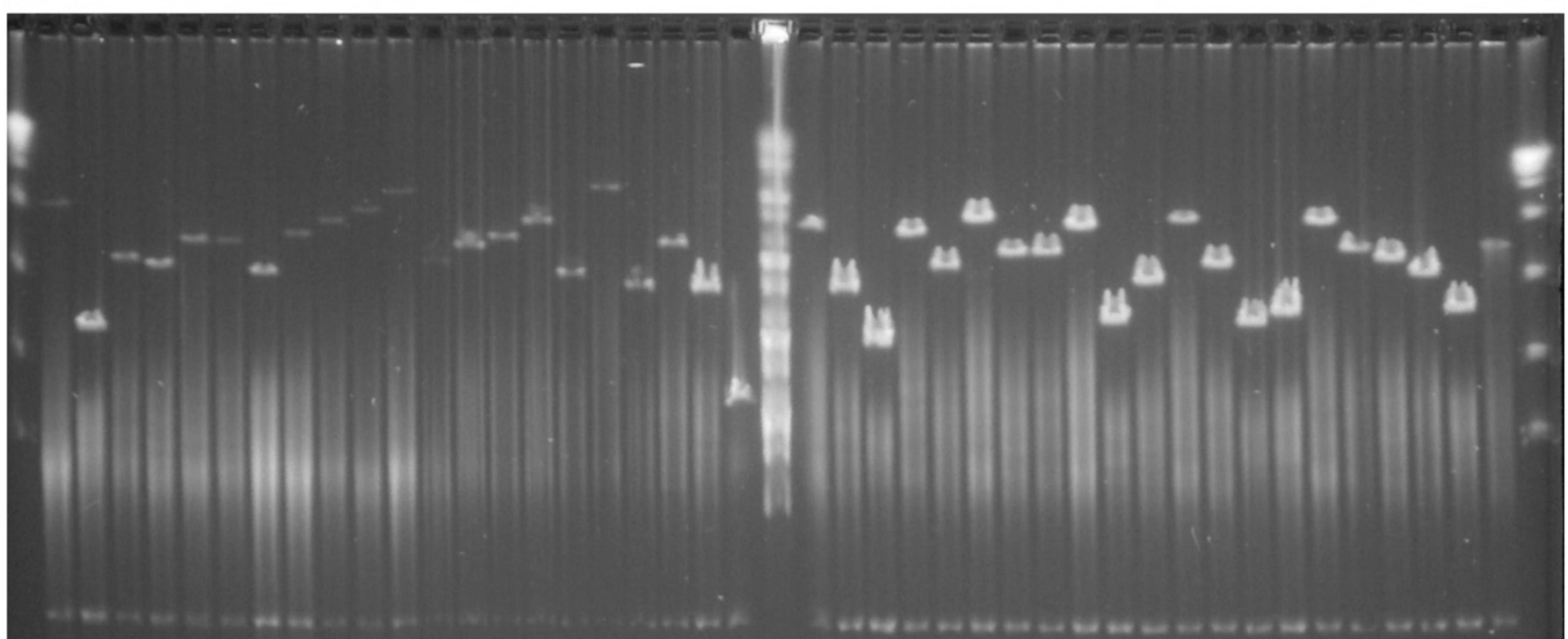


**Figure S1.** BAC insert size distribution of the restriction-derived TM-1 BAC libraries, GH\_TBb and GH\_TBh.

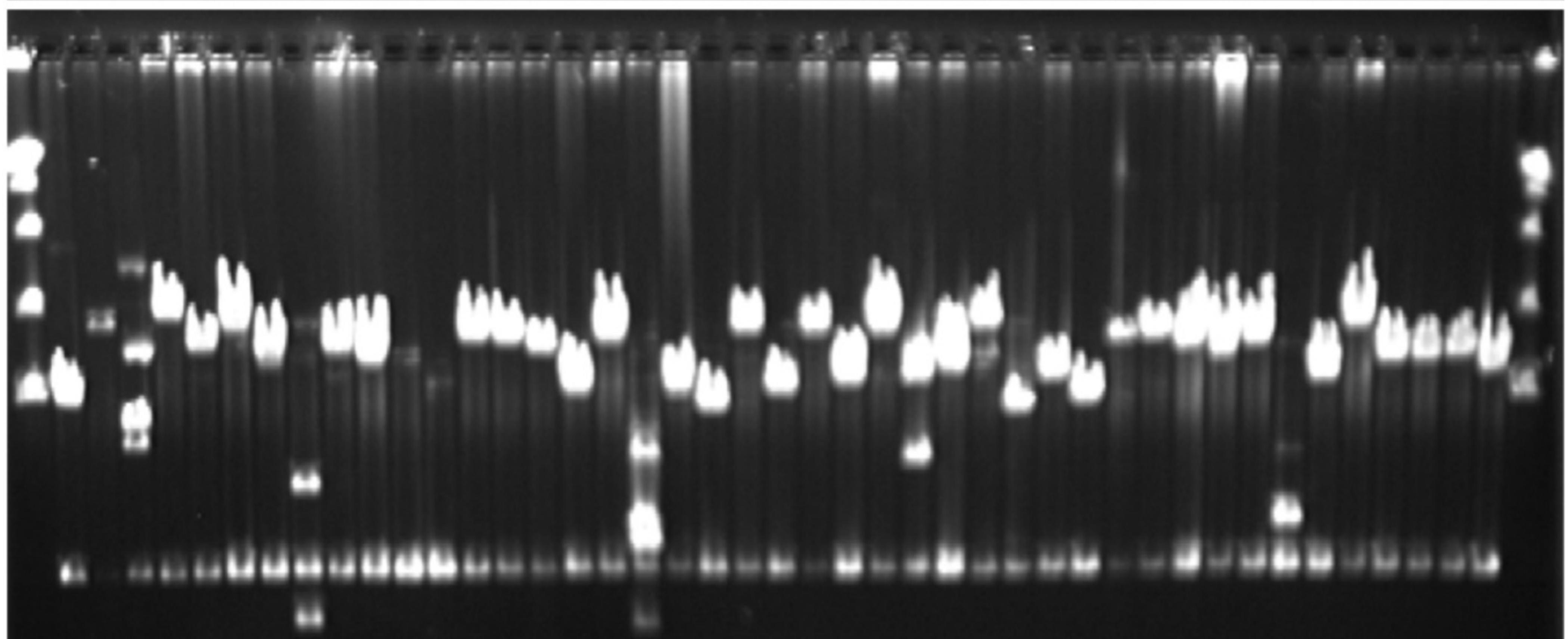
**GH\_TBb**



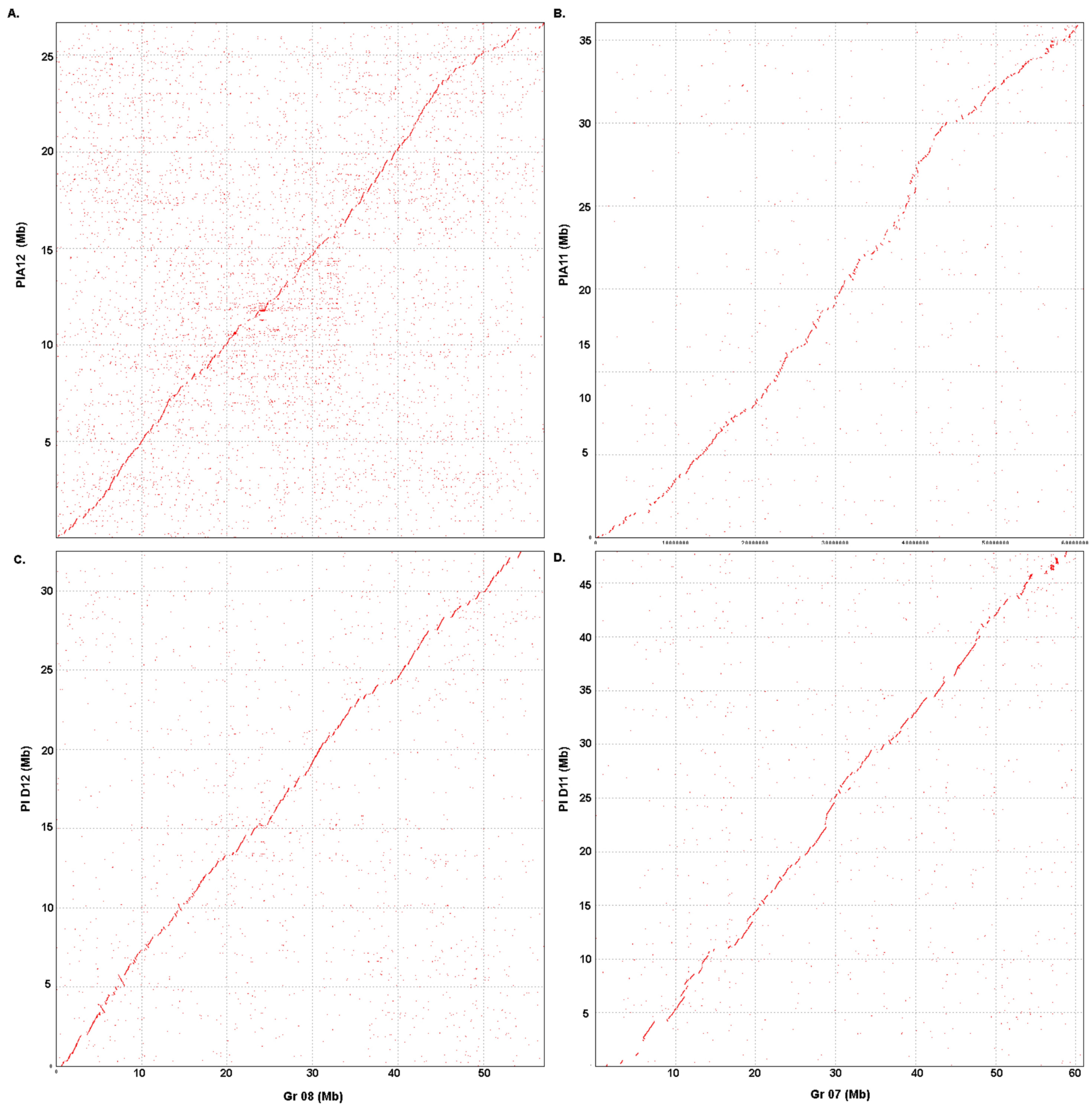
**GH\_TBh**



**GH\_TBr**

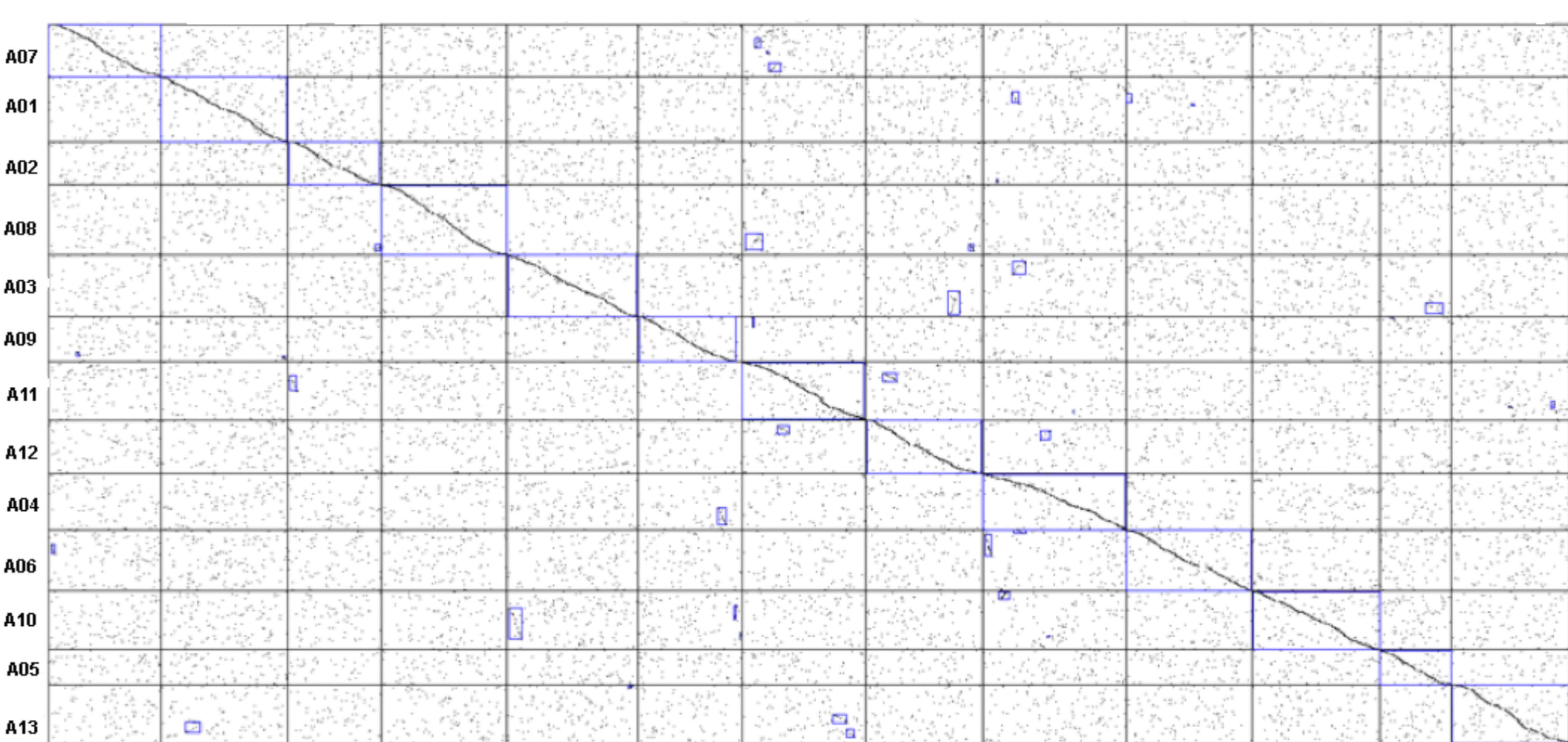


**Figure S2.** Figure S2 A-C. Pulsed-field gel electrophoresis of random *NotI* digested TM-1 BACs from GH\_TBb, GH\_TBh, and GH\_TBr. The 2 outermost lanes contain Lambda ladder PFG marker (NEB) and the middle lane contains Lambda midrange PFG marker (NEB).

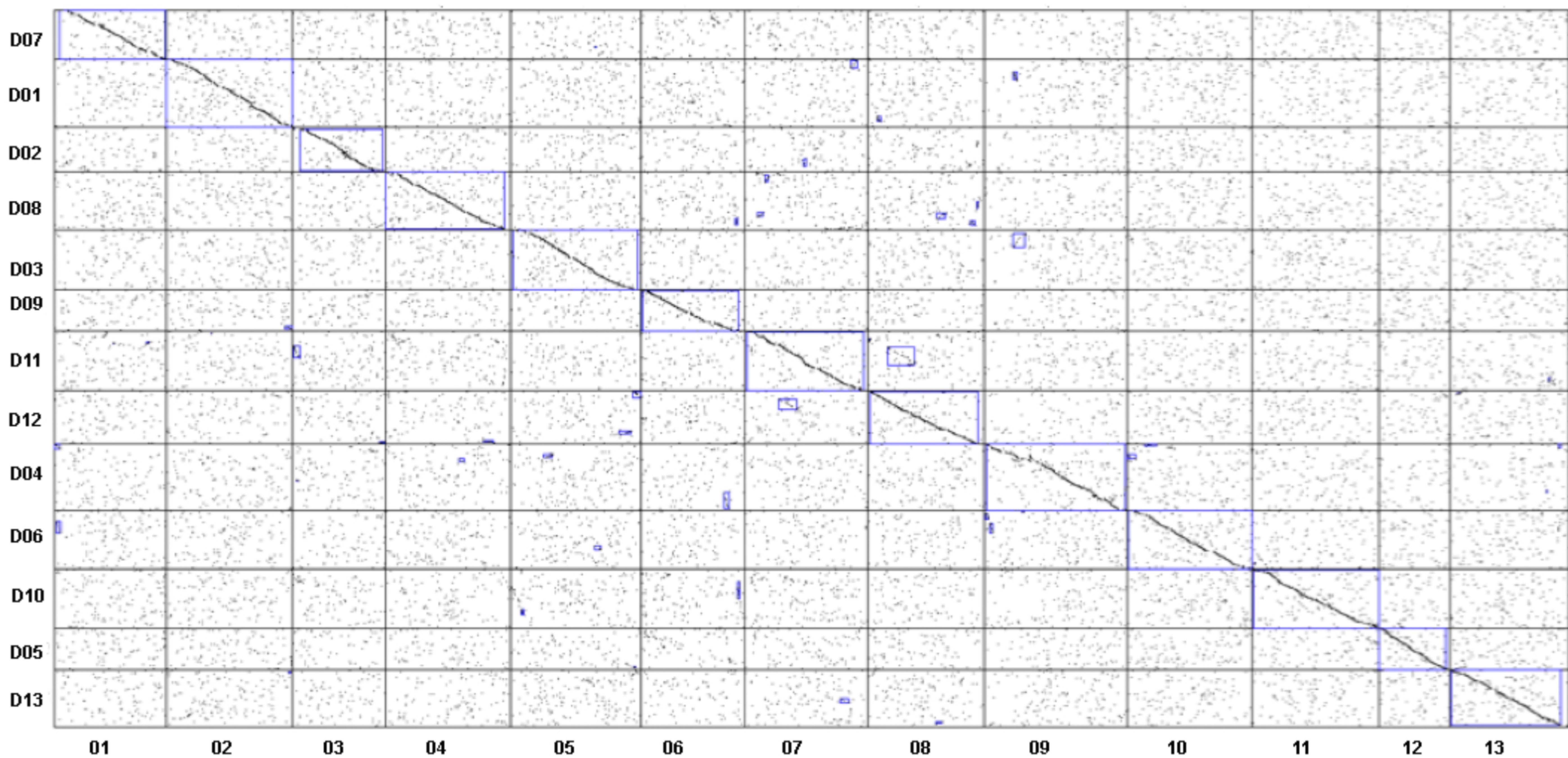


**Figure S4.** A. Dot plot alignment between *Gossypium hirsutum* chromosome A12 and *Gossypium raimondii* Chromosome 08. B. Dot plot alignment between *Gossypium hirsutum* D12 and *Gossypium raimondii* Chromosome 08.

**PI A**

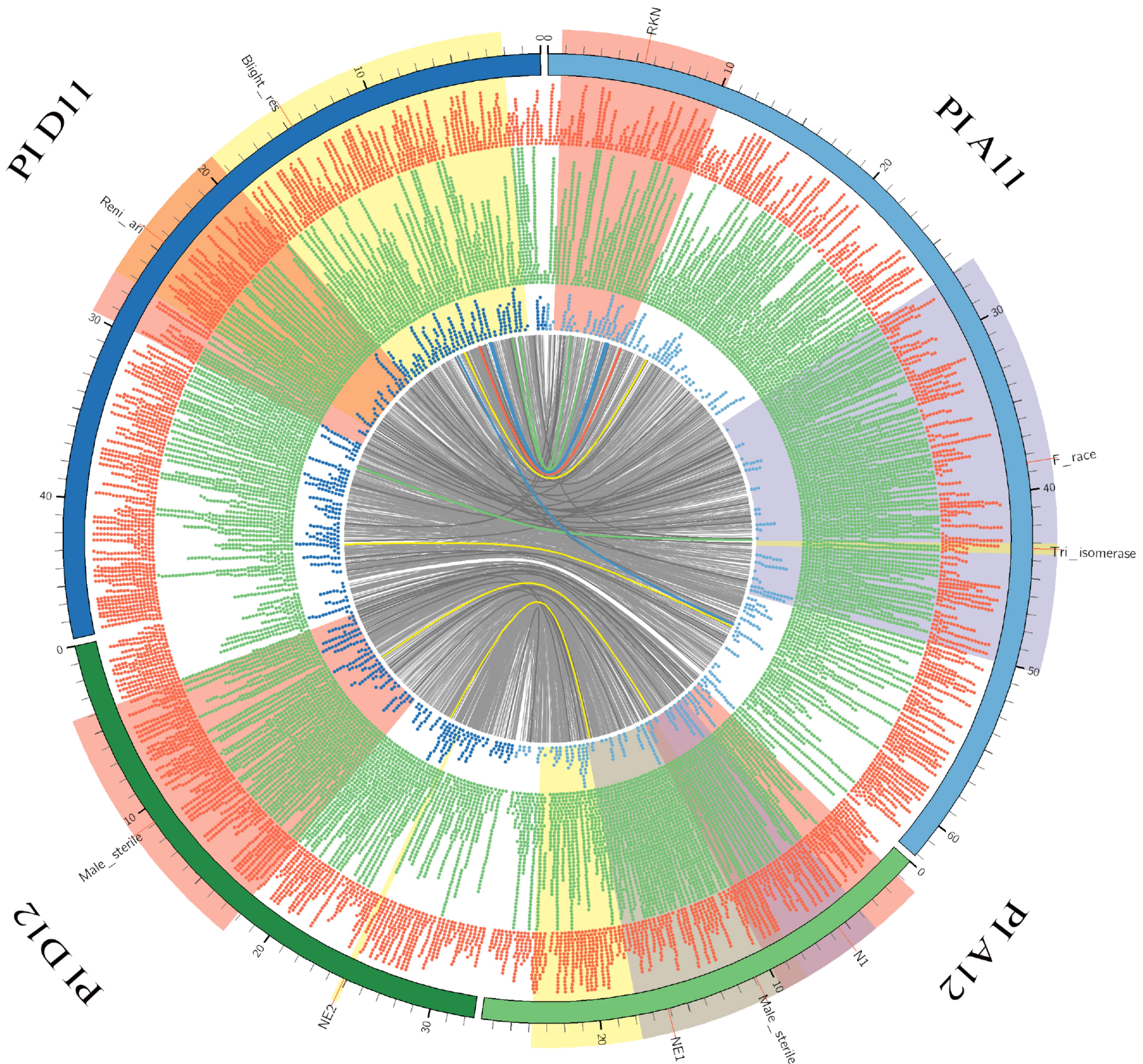


**PI D**



### ***G. raimondii* reference**

**Figure S3.** A dot plot alignment of the *G. hirsutum* TM-1 subgenome specific BES aligned to the *G. raimondii* extant diploid progenitor reference assembly. The BES alignments are relatively colinear and evenly distributed.



**Figure S5.** Pseudomolecule alignments of pilot chromosomes A11/D11 and A12/D12 as aligned as homeologous pairs by the inner links. Blue glyphs (internal) are retroelements, green glyphs are *Gypsy* elements, and red glyphs are *Copia* elements. Highlights denote SSR qtl boundaries of qualitative traits.