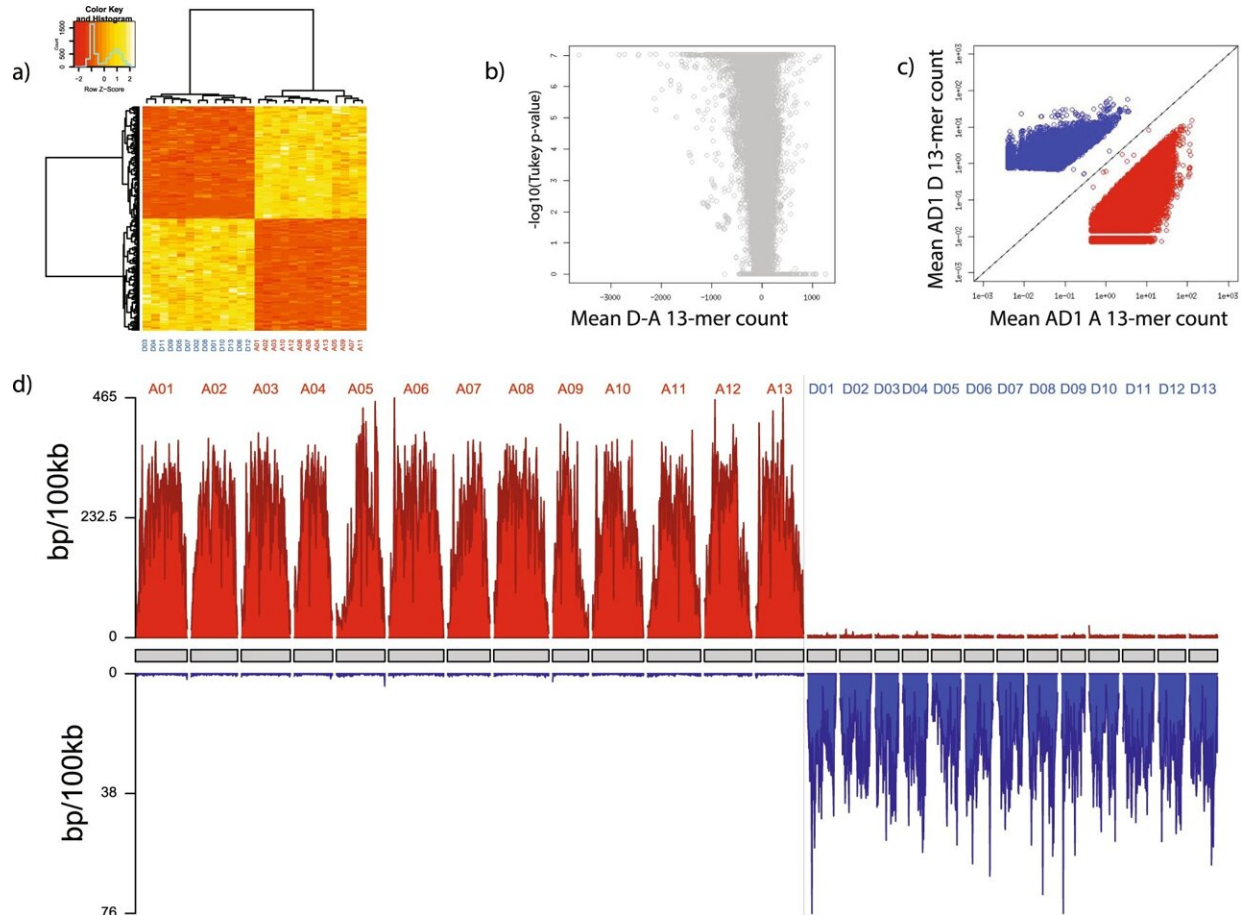


New method traces ancestry of hybrids

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Cotton allotetraploidy. Upland cotton (*Gossypium hirsutum*) is an AADD allotetraploid, where the A subgenome related to African diploids and the D subgenome is related to *G. raimondii*³³. It is called AD1 to differentiate it from the related tetraploid *G. barbadense* (AD2). We sought to identify k-mers that differentiate the subgenomes of *G. hirsutum* (Supplementary Note 2; for *G. barbadense* see Supplementary Fig. 3). **a** Heatmap showing 13-mer density as a function of AD1 chromosomes (columns, clustered on top) vs. a sample of 100 13-mers found to differentiate the A- and D-subgenomes (rows, clustered on

left). A chromosomes are indicated in red and D chromosomes are indicated in blue. **b** Volcano plot showing Bonferroni-corrected Tukey p -value (Bonferroni-corrected; $df = 24$) vs. mean 13mer count difference D-A between subgenomes of *Gossypium hirsutum*. Each point is a 13mer. By definition, all Tukey's HSD tests are one-sided. Effect size is shown on the x -axis (converted to 13mer count/chromosome) and 95% Confidence Intervals for each 13mer can be found in Supplementary Data 4. **c** Scatterplot showing mean A chromosome 13-mer count on x -axis, mean D chromosome 13-mer count on the y -axis. A-enriched 13-mers shown in red, D-enriched 13-mers shown in blue. Black line is $y = x$. Only the 13-mers found to differentiate subgenomes (Bonferroni-corrected p

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