# Supplementary Methods

## NimbleGen microarray validation experimental design and methodology

Earlier in our laboratory, homoeolog-specific probes were created by first assembling EST contigs from $A_2$, $D_5$, and $AD_1$ libraries and then identifying homoeolog-specific SNPs within these contigs. These SNPs represent nucleotide differences between the A-and D-genome orthologs, and offer the possibility of diagnosing the genomic origin of transcripts in the allopolyploid nucleus. Using this strategy, we have designed and implemented a novel NimbleGen microarray platform capable of measuring homoeolog-specific expression in *Gossypium* species (Udall et al., 2006). This microarray features two classes of probes, ~35mer probe-pairs differing by an A- or D-genome homoeolog-specific SNP, and ~60mer generic probes (not specific to either homoeolog). Thus this microarray platform has the ability to measure expression from both the homoeologs, detected by the corresponding ~35mer homoeolog-specific probes, and total gene expression, detected by the ~60mer generic probes designed in areas of common sequence between both homoeologs.

RNA samples from cotton fibers at early developmental stage (2dpa) in three biological replicates have been analyzed on this NimbleGen microarray platform for gene expression. The mean values for randomly selected 18 homoeologous genes pairs from microarray based results were compared with the estimates derived from homoeolog-specific Sequenom mass spectrometry for validating the homoeolog-specific expression ratios (Supplementary Figure 1). Briefly, the mass*ARRAY* technology amplifies A- and D-derived cDNA transcripts in parallel, and then quantifies relative homoeolog abundance based on matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry.

**Supplementary Figure 1a.** List of 18 homoeologous genes analyzed for validation of NimbleGen microarray values by massARRAY based homoeolog-specific expression measurements**.**

| **Contig** | **SNP position** | **domesticated *G. hirsutum* 2 dpa Fiber** | |
|---|---|---|---|
| | | NimbleGen %D expression | mass*ARRAY* %D expression |
| COTTON16_00001_452 | 1635 | 0.530 | 0.409 |
| COTTON16_00012_02 | 1315 | 0.660 | 0.654 |
| COTTON16_00056_02 | 720 | 0.420 | 0.431 |
| COTTON16_00922_01 | 949 | 0.000 | 0.007 |
| COTTON16_01121_02 | 632 | 0.143 | 0.017 |
| COTTON16_01189_01 | 1178 | 0.172 | 0.020 |
| COTTON16_01391_01 | 705 | 0.818 | 0.939 |
| COTTON16_01704_01 | 1415 | 0.616 | 0.480 |
| COTTON16_06427_01 | 504 | 0.458 | 0.615 |
| COTTON16_09095_01 | 1294 | 0.530 | 0.579 |
| COTTON16_15666_01 | 268 | 0.465 | 0.309 |
| COTTON16_19029_01 | 267 | 0.612 | 0.489 |
| COTTON16_19620_01 | 376 | 0.752 | 0.812 |
| COTTON16_22170_01 | 513 | 0.467 | 0.266 |
| COTTON16_26306_01 | 942 | 0.491 | 0.449 |
| COTTON16_32946_01 | 1145 | 0.487 | 0.567 |
| COTTON16_34102_01 | 940 | 0.635 | 0.625 |
| COTTON16_34627_01 | 1149 | 0.672 | 0.554 |

# Supplementary Figure 1 b.

domesticated *G. hirsutum* (2 dpa Fiber)