

1   **Supplemental Table S1.** Number and percentage of sequencing reads mapped onto the  
2   allotetraploid cotton (AD) genome sequence.

Accession	Genome designation	Total reads	Clean reads	A		D		AD overlap		AD	
				Mapped reads	Mapped reads	Mapped reads	overlap	Mapped reads	percent	Mapped reads	percent
TM-1_1R	AD	55,702,587	47,324,516	23,110,443	23,607,780	5,176,813	41,671,862	88.10%			
TM-1_2R	AD	61,147,447	51,638,483	25,122,174	25,855,464	5,773,553	45,458,603	88.00%			
TM-1_3R	AD	53,827,026	46,268,794	22,069,500	22,830,235	5,636,361	39,664,962	85.70%			

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5 **Supplemental Table S2.** Number and expression levels of A and D homoeologous genomes

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Subgenome	Genes number	FPKM			
		R1	R2	R3	mean
A	16711	492777.76	466580.17	438941.09	466099.67
D	17400	514536.93	488100.39	457388.77	486675.36
total	34111	1007314.69	954680.57	896329.85	952775.04

9   **Supplemental Table S3.** Correlation coefficients between immunostaining and gene  
10 densities of homoeologous A chromosomes in *G. hirsutum* (TM-1).

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Gene \ H3K4me3	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12	A13
A01	0.846 <sup>a,*</sup>	0.514	0.648 <sup>a</sup>	0.148	0.549	0.734 <sup>a</sup>	0.730 <sup>a</sup>	0.721 <sup>a</sup>	0.366	0.682 <sup>a</sup>	0.716 <sup>a</sup>	0.334	0.097
A02	0.578	0.886 <sup>a,*</sup>	0.609 <sup>a</sup>	0.278	0.591	0.456	0.628 <sup>a</sup>	0.719 <sup>a</sup>	0.284	0.740 <sup>a</sup>	0.778 <sup>a</sup>	0.757 <sup>a</sup>	0.753 <sup>a</sup>
A03	0.802 <sup>a</sup>	0.720 <sup>a</sup>	0.824 <sup>a,*</sup>	0.047	0.692 <sup>a</sup>	0.767 <sup>a</sup>	0.080	0.730 <sup>a</sup>	0.278	0.804 <sup>a</sup>	0.812 <sup>a</sup>	0.532	0.232
A04	0.062	0.155	0.210	0.808 <sup>a,*</sup>	0.259	0.006	0.002	0.111	0.087	0.317	0.018 <sup>c</sup>	0.559	0.571
A05	0.832 <sup>a</sup>	0.506	0.645 <sup>a</sup>	0.101	0.943 <sup>a,*</sup>	0.801 <sup>a</sup>	0.747 <sup>a</sup>	0.690 <sup>a</sup>	0.307	0.608 <sup>a</sup>	0.840 <sup>a</sup>	0.218	0.096
A06	0.801 <sup>a</sup>	0.533	0.782 <sup>a</sup>	0.788 <sup>a</sup>	0.445	0.851 <sup>a,*</sup>	0.736 <sup>a</sup>	0.837 <sup>a</sup>	0.217	0.803 <sup>a</sup>	0.644 <sup>a</sup>	0.296	0.161
A07	0.790 <sup>a</sup>	0.898 <sup>a</sup>	0.728 <sup>a</sup>	0.090	0.767 <sup>a</sup>	0.703 <sup>a</sup>	0.949 <sup>a,*</sup>	0.787 <sup>a</sup>	0.027	0.818 <sup>a</sup>	0.869 <sup>a</sup>	0.759 <sup>a</sup>	0.666 <sup>a</sup>
A08	0.819 <sup>a</sup>	0.819 <sup>a</sup>	0.841 <sup>a</sup>	0.076	0.770 <sup>a</sup>	0.738 <sup>a</sup>	0.418	0.876 <sup>a,*</sup>	0.026	0.854 <sup>a</sup>	0.812 <sup>a</sup>	0.710 <sup>a</sup>	0.532
A09	0.398	0.218	0.292	0.700 <sup>a</sup>	0.519	0.483	0.244	0.211	0.894 <sup>a,*</sup>	0.067	0.384	0.352	0.456
A10	0.840 <sup>a</sup>	0.584	0.612 <sup>a</sup>	0.055	0.936 <sup>a</sup>	0.605 <sup>a</sup>	0.819 <sup>a</sup>	0.686 <sup>a</sup>	0.023	0.730 <sup>a,*</sup>	0.710 <sup>a</sup>	0.466	0.373
A11	0.598	0.458	0.622 <sup>a</sup>	0.124	0.801 <sup>a</sup>	0.829 <sup>a</sup>	0.694 <sup>a</sup>	0.664 <sup>a</sup>	0.410 <sup>a</sup>	0.585 <sup>a</sup>	0.842 <sup>a,*</sup>	0.131	0.005
A12	0.200	0.683 <sup>a</sup>	0.304	0.655 <sup>a</sup>	0.234	0.040	0.592	0.473	0.763 <sup>a</sup>	0.518 <sup>b</sup>	0.417	0.952 <sup>a,*</sup>	0.871 <sup>a</sup>
A13	0.393	0.645 <sup>a</sup>	0.422	0.505 <sup>a</sup>	0.319	0.234	0.729 <sup>a</sup>	0.507	0.657 <sup>a</sup>	0.677 <sup>a</sup>	0.517	0.942 <sup>a</sup>	0.919 <sup>a,*</sup>

12   <sup>a</sup> P<0.01; \* designated pair

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14   **Supplemental Table S4.** Correlation coefficients between immunostaining and gene  
 15   densities of homoeologous D chromosomes in *G. hirsutum* (TM-1).  
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H3K4me3 \ Gene	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12	D13
D01	0.850 <sup>a,*</sup>	0.615	0.256	0.771	0.463	0.749 <sup>a</sup>	0.832 <sup>a</sup>	0.807 <sup>a</sup>	0.027	0.787 <sup>a</sup>	0.788 <sup>a</sup>	0.673	0.824 <sup>a</sup>
D02	0.743 <sup>a</sup>	0.801 <sup>a,*</sup>	0.700	0.318	0.260	0.616	0.747 <sup>a</sup>	0.599	0.305	0.761 <sup>a</sup>	0.772 <sup>a</sup>	0.782 <sup>a</sup>	0.624
D03	0.439	0.639	0.862 <sup>a,*</sup>	0.726	0.103	0.260	0.334	0.552	0.666 <sup>b</sup>	0.395	0.348 <sup>c</sup>	0.120	0.687
D04	0.821 <sup>a</sup>	0.727	0.743	0.940 <sup>a,*</sup>	0.452	0.740	0.808 <sup>a</sup>	0.838 <sup>a</sup>	0.086	0.737	0.827 <sup>a</sup>	0.854 <sup>a</sup>	0.841 <sup>a</sup>
D05	0.200	0.195	0.179	0.429	0.924 <sup>a,*</sup>	0.272	0.553	0.146	0.830 <sup>a</sup>	0.398	0.678 <sup>b</sup>	0.378	0.163
D06	0.726 <sup>a</sup>	0.429	0.342	0.248	0.675	0.795 <sup>a,*</sup>	0.654	0.591	0.508	0.706 <sup>a</sup>	0.673 <sup>b</sup>	0.208	0.579
D07	0.776 <sup>a</sup>	0.369	0.252	0.674	0.495	0.624	0.819 <sup>a,*</sup>	0.692	0.058	0.606	0.798 <sup>a</sup>	0.509	0.585
D08	0.763 <sup>a</sup>	0.612	0.668	0.893 <sup>a</sup>	0.219	0.562	0.738 <sup>a</sup>	0.767 <sup>a,*</sup>	0.312	0.682	0.700 <sup>a</sup>	0.792 <sup>a</sup>	0.794 <sup>a</sup>
D09	0.147	0.040	0.636	0.113	0.792 <sup>a</sup>	0.579	0.160	0.049	0.966 <sup>a,*</sup>	0.322	0.438 <sup>c</sup>	0.694	0.312
D10	0.714 <sup>a</sup>	0.535	0.089	0.498	0.884 <sup>a</sup>	0.776 <sup>a</sup>	0.749 <sup>a</sup>	0.631	0.653	0.753 <sup>a,*</sup>	0.885 <sup>a</sup>	0.020	0.424
D11	0.824 <sup>a</sup>	0.699 <sup>a</sup>	0.163	0.729 <sup>a</sup>	0.805 <sup>a</sup>	0.822 <sup>a</sup>	0.867 <sup>a</sup>	0.802 <sup>a</sup>	0.426	0.884 <sup>a</sup>	0.919 <sup>a,*</sup>	0.308	0.641
D12	0.298	0.192	0.859 <sup>a</sup>	0.455	0.531	0.010	0.153	0.357	0.940 <sup>a</sup>	0.147	0.079 <sup>c</sup>	0.895 <sup>a,*</sup>	0.685
D13	0.779 <sup>a</sup>	0.627	0.668	0.809 <sup>a</sup>	0.152	0.550	0.739 <sup>a</sup>	0.818 <sup>a</sup>	0.426	0.676	0.621 <sup>b</sup>	0.855 <sup>a</sup>	0.874 <sup>a,*</sup>

17   <sup>a</sup> P<0.01; \* designated pair

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19   **Supplemental Table S5.** Number and percentage of sequencing reads of ChIP-seq mapped  
20   onto the allotetraploid cotton (AD) genome sequence.

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Sample ID	Clean Reads	Mapped Reads	Mapped Rate (%)	Uniquely Mapped Reads	Uniquely Mapped Rate (%)
TM-1_1R-input	25,182,189	24,632,041	97.82	15,950,480	63.34
TM-1_1R-IP	25,028,831	24,219,563	96.77	15,854,549	63.35
TM-1_2R-input	26,470,323	25,861,008	97.70	16,764,139	63.33
TM-1_2R-IP	27,365,259	26,410,617	96.51	17,557,866	64.16

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23   **Supplemental Table S6.** Correlation coefficients of immunostaining densities (enriched with  
 24   H3K4me3) in the distal portions between A and D homoeologous chromosomes in *G.*  
 25   *hirsutum* (TM-1).

A \ D	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12	A13
D01	0.938 <sup>a,1</sup>	0.898 <sup>a</sup>	0.855 <sup>a</sup>	0.760 <sup>a</sup>	0.446	0.737 <sup>a</sup>	0.658	0.878 <sup>a</sup>	0.512	0.892 <sup>a</sup>	0.413	0.735 <sup>a</sup>	0.829 <sup>a</sup>
D02	0.499	0.824 <sup>a</sup>	0.839 <sup>a</sup>	0.809 <sup>a</sup>	0.190	0.664 <sup>a</sup>	0.573	0.476	0.648	0.657	0.126	0.272	0.662 <sup>a</sup>
D03	0.791	0.962 <sup>a,2</sup>	0.867 <sup>a</sup>	0.728	0.211	0.849 <sup>a</sup>	0.725	0.852 <sup>a</sup>	0.097	0.763 <sup>a</sup>	0.108	0.543	0.892 <sup>a,2</sup>
D04	0.800 <sup>a</sup>	0.817 <sup>a</sup>	0.408	0.929 <sup>a,1</sup>	0.723	0.741	0.462	0.511	0.475	0.865 <sup>a</sup>	0.680	0.061	0.738
D05	0.559	0.334	0.013	0.196	0.955 <sup>a,1</sup>	0.351	0.683	0.121	0.721 <sup>a</sup>	0.593	0.600	0.428	0.101
D06	0.566	0.724 <sup>a</sup>	0.717 <sup>a</sup>	0.590	0.612	0.885 <sup>a,1</sup>	0.643	0.499	0.183	0.784	0.627	0.321	0.641
D07	0.760 <sup>a</sup>	0.808 <sup>a</sup>	0.803 <sup>a</sup>	0.655	0.617	0.821 <sup>a</sup>	0.681 <sup>a</sup>	0.610	0.134	0.828 <sup>a</sup>	0.599	0.377	0.722 <sup>a</sup>
D08	0.450	0.733 <sup>a</sup>	0.796 <sup>a</sup>	0.853 <sup>a</sup>	0.184	0.641	0.470	0.909 <sup>a,1</sup>	0.625	0.710 <sup>a</sup>	0.117	0.858 <sup>a,2</sup>	0.832 <sup>a</sup>
D09	0.035	0.301	0.681	0.800 <sup>a</sup>	0.671	0.228	0.177	0.730	0.974 <sup>a,1</sup>	0.061	0.712	0.920 <sup>a</sup>	0.529
D10	0.552	0.789 <sup>a</sup>	0.584	0.467	0.858 <sup>a</sup>	0.755	0.436	0.450	0.107	0.871 <sup>a,1</sup>	0.870 <sup>a</sup>	0.195	0.628
D11	0.893 <sup>a</sup>	0.930 <sup>a</sup>	0.892 <sup>a,2</sup>	0.630	0.692	0.827 <sup>a</sup>	0.835 <sup>a,2</sup>	0.812 <sup>a</sup>	0.350	0.947 <sup>a,2</sup>	0.958 <sup>a,1</sup>	0.634	0.885 <sup>a</sup>
D12	0.185	0.485	0.782 <sup>a</sup>	0.869 <sup>a</sup>	0.461	0.431	0.021	0.825 <sup>a</sup>	0.954	0.160	0.489	0.52 <sup>a,1</sup>	0.672
D13	0.582	0.807 <sup>a</sup>	0.617	0.516	0.142	0.758 <sup>a</sup>	0.482	0.492	0.700	0.682	0.094	0.249	0.876 <sup>a,1</sup>

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 27   <sup>a</sup> P<0.01; <sup>1</sup> designated pair (marked in yellow); <sup>2</sup> highest value but not designated pair  
 28   (green); <sup>3</sup> low correlation coefficients of the designated pair (pink).  
 29