Supplemental Figure 1. *Fhb1*- (susceptible) spike 96 hours after inoculation with *F. graminearum*. Inoculated spikelets have been removed and the associated rachis is highlighted in the red box.

Supplemental Figure 2. RNAseq data analysis pipeline overview.

Supplemental Figure 3. Distribution of differentially expressed genes (DEGs) on the wheat subgenome A. Tracks a)-d) summarize DEGs with higher expression in the resistant genotype: Track a) illustrates DEGs for *F. graminearum*-infected spikelets at 96 hai, b) *F. graminearum*-infected rachis at 96 hai, c) DON infected spikelet at 12 hai, d) water inoculated spikelets at 12 hai. Tracks e)-h) summarize DEGs with higher expression in the susceptible genotype: Track e) illustrates DEGs for *F. graminearum*-infected spikelets at 96 hai, f) *F. graminearum*-infected rachis at 96 hai, g) DON infected spikelet at 12 hai, h) water inoculated spikelets at 12 hai. The heat map in the outer track illustrates the genetically anchored genes obtained for IWGSC (2014) with a maximum of 608 genetically anchored genes per Bin. Tracks a)-h) represent the relative amount of genes compared to the overall amount of genes in one bin (range from 0-100%).

Supplemental Figure 4. Distribution of differentially expressed genes (DEGs) on the wheat subgenome D. Tracks a)-d) summarize DEGs with higher expression in the resistant genotype: Track a) illustrates DEGs for *F. graminearum*-infected spikelets at 96 hai, b) *F. graminearum*-infected rachis at 96 hai, c) DON infected spikelet at 12 hai, d) water inoculated spikelets at 12 hai. Tracks e)-h) summarize DEGs with higher expression in the susceptible genotype: Track e) illustrates DEGs for *F. graminearum*-infected spikelets at 96 hai, f) *F. graminearum*-infected rachis at 96 hai, g) DON infected spikelet at 12 hai, h) water inoculated spikelets at 12 hai. The heat map in the outer track illustrates the genetically anchored genes obtained for IWGSC (in review) with a maximum of 608 genetically anchored genes per Bin. Tracks a)-h) represent the relative amount of genes compared to the overall amount of genes in one bin (range from 0-100%).

Supplemental Table 1. Samples collected for analysis and sequencing.

Supplemental Table 2. DON concentration and ergosterol concentration in deoxynivalenol and *F. graminearum* infected wheat spikes for the *Fhb1* NIL pair carrying either the resistant or susceptible allele.

Supplemental Table 3. Differentially expressed genes between the resistant (*Fhb1+*) and susceptible (*Fhb1*-) genotypes of spikelet tissue 96 hours after *F. graminearum* inoculation.

Supplemental Table 4. Differentially expressed genes between the resistant (*Fhb1+*) and susceptible (*Fhb1*-) genotypes of rachis tissue 96 hours after *F. graminearum* inoculation.

Supplemental Table 5. Differentially expressed genes between the resistant (*Fhb1+*) and susceptible (*Fhb1*-) genotypes of spikelet tissue 12 hours after DON inoculation.

Supplemental Table 6. Differentially expressed genes between the resistant (*Fhb1+*) and susceptible (*Fhb1*-) genotypes of spikelet tissue 12 hours after water inoculation.

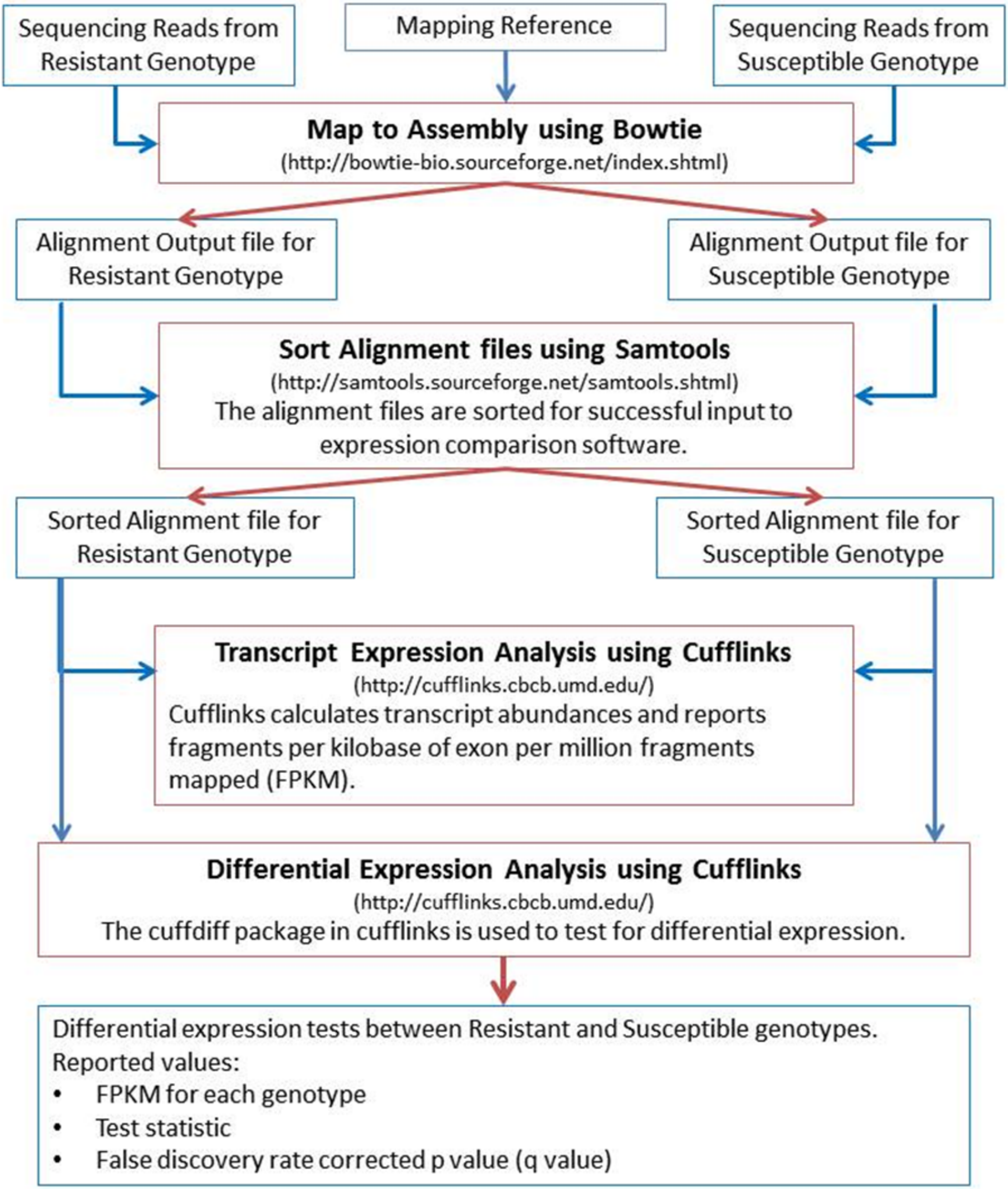
Supplemental Table 7. Differentially expressed genes between the resistant (*Fhb1+*) DON-inoculated and resistant (*Fhb1+*) water-inoculated samples of spikelet tissue 12 hours after inoculation.

Supplemental Table 8. Differentially expressed genes between the susceptible (*Fhb1*-) DON-inoculated and susceptible (*Fhb1*-) water-inoculated samples of spikelet tissue 12 hours after inoculation.

Supplemental Table 9. Differentially expressed genes betwenn the resistant (*Fhb1+*) and susceptible (*Fhb1*-) genotypes of spikelet tissues 12 hours after DON inoculation that are not differentially expressed in any of the other samples.

Supplemental Table 10. Differentially expressed *Fusarium graminearum* genes between the resistant (*Fhb1+*) and susceptible (*Fhb1*-) genotypes of spikelet tissue 96 hours after *F. graminearum* inoculation.

Supplemental Table 11. Differentially expressed *Fusarium graminearum* genes between the resistant (*Fhb1+*) and susceptible (*Fhb1-*) genotypes of rachis tissue 96 hours after *F. graminearum* inoculation.



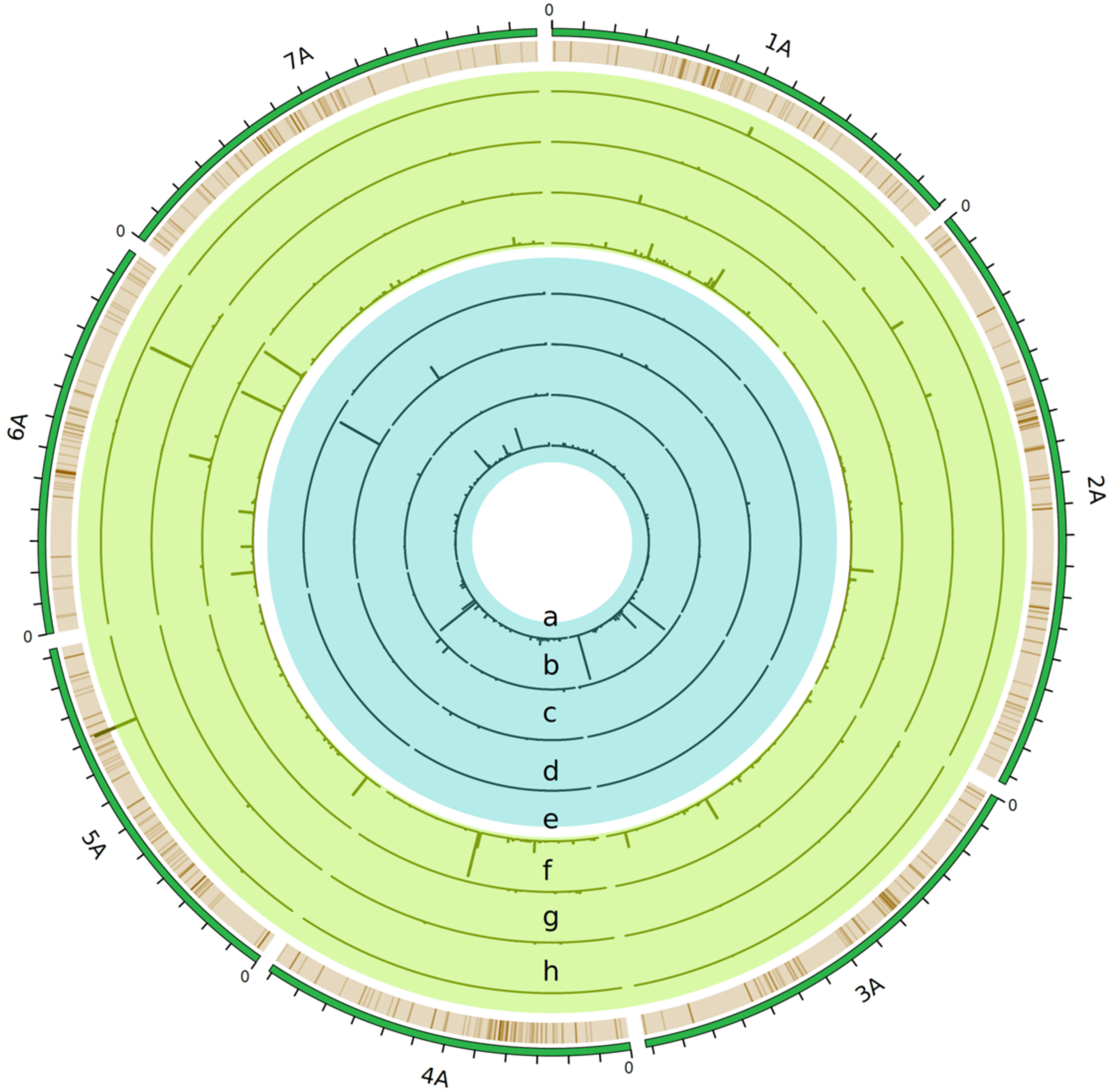
The alignment files are sorted for successful input to expression comparison software.

Cufflinks calculates transcript abundances and reports fragments per kilobase of exon per million fragments mapped (FPKM).

The cuffdiff package in cufflinks is used to test for differential expression.

Differential expression tests between Resistant and Susceptible genotypes.
Reported values:

- FPKM for each genotype
- Test statistic
- False discovery rate corrected p value (q value)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1A
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3A
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4A
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5A
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7A
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