

ConditionA\_rep1

ConditionA\_rep2

ConditionB\_rep1

ConditionB\_rep2



### **Read alignment**

Align to reference and spike-in genomes, and filter common reads  
Estimate size factors for condition replicates  
Remove PCR duplicates



### **CMS measurement construction**

Build genome scan windows  
Tabulate read counts and mean WIG sums



### **DHMR detection**

Filter low-depth windows  
Negative binomial, *t*-test, Chi-squared, and *G*-test  
Independent filtering  
Merge detected adjacent differentially hydroxymethylated regions