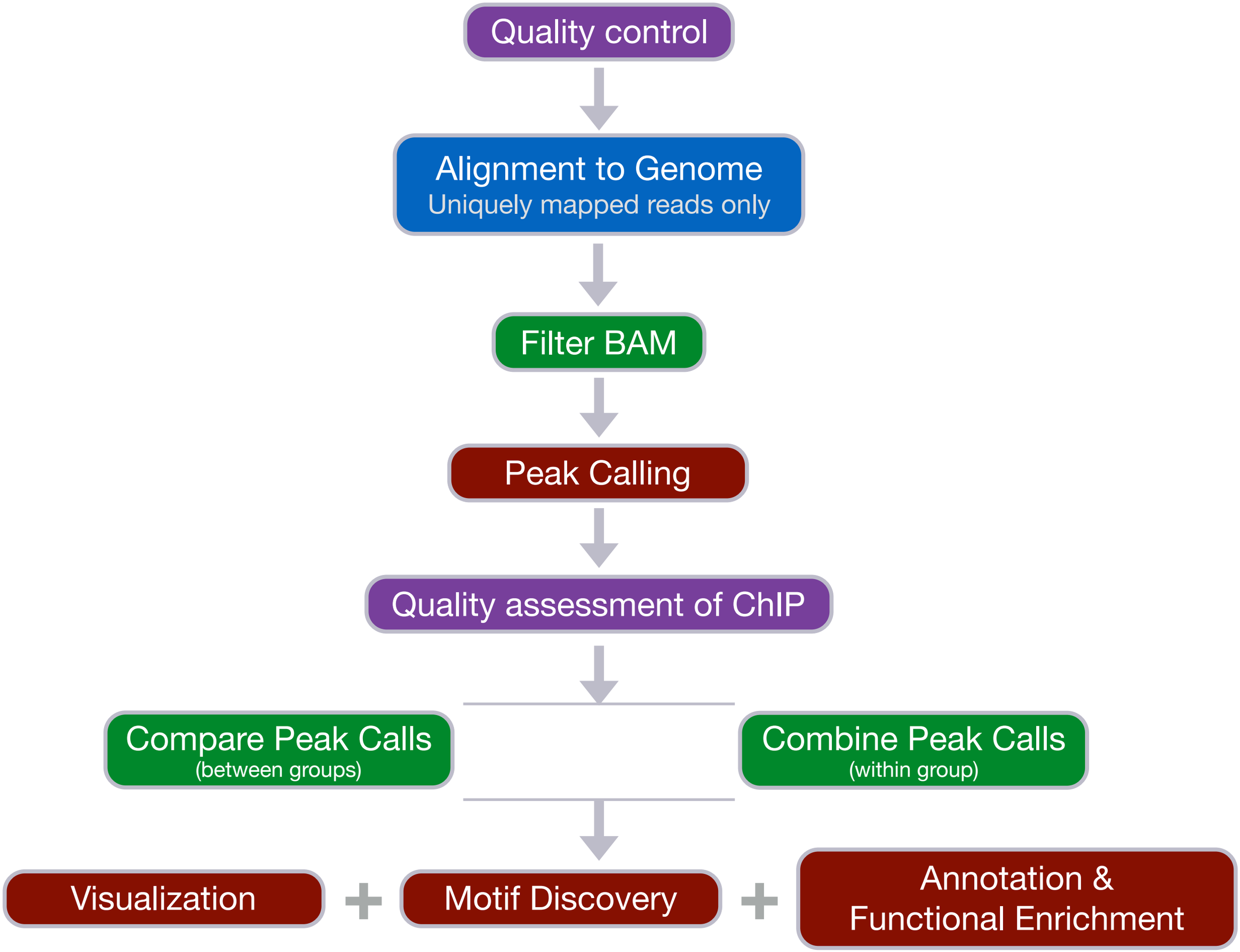
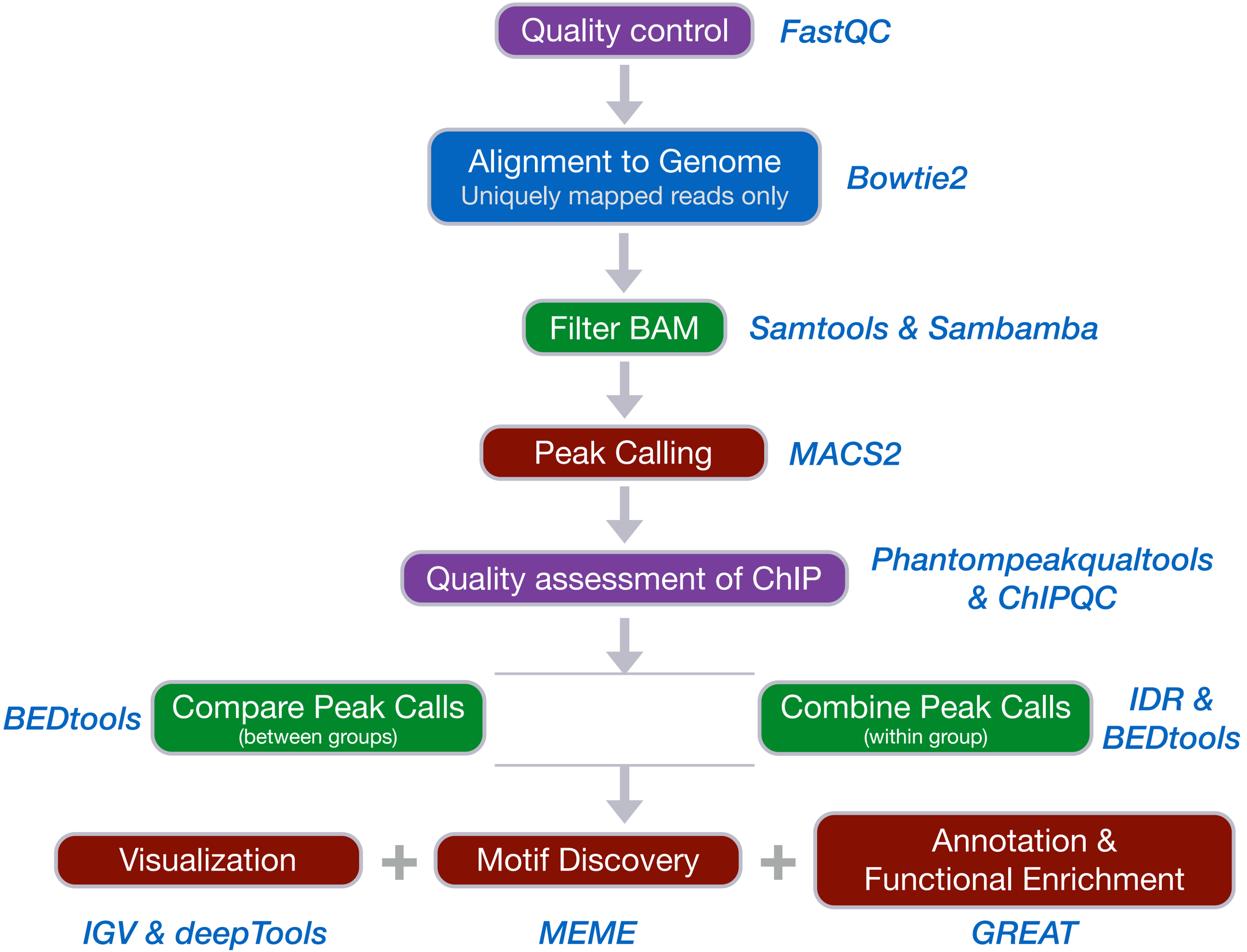
Workshop scope

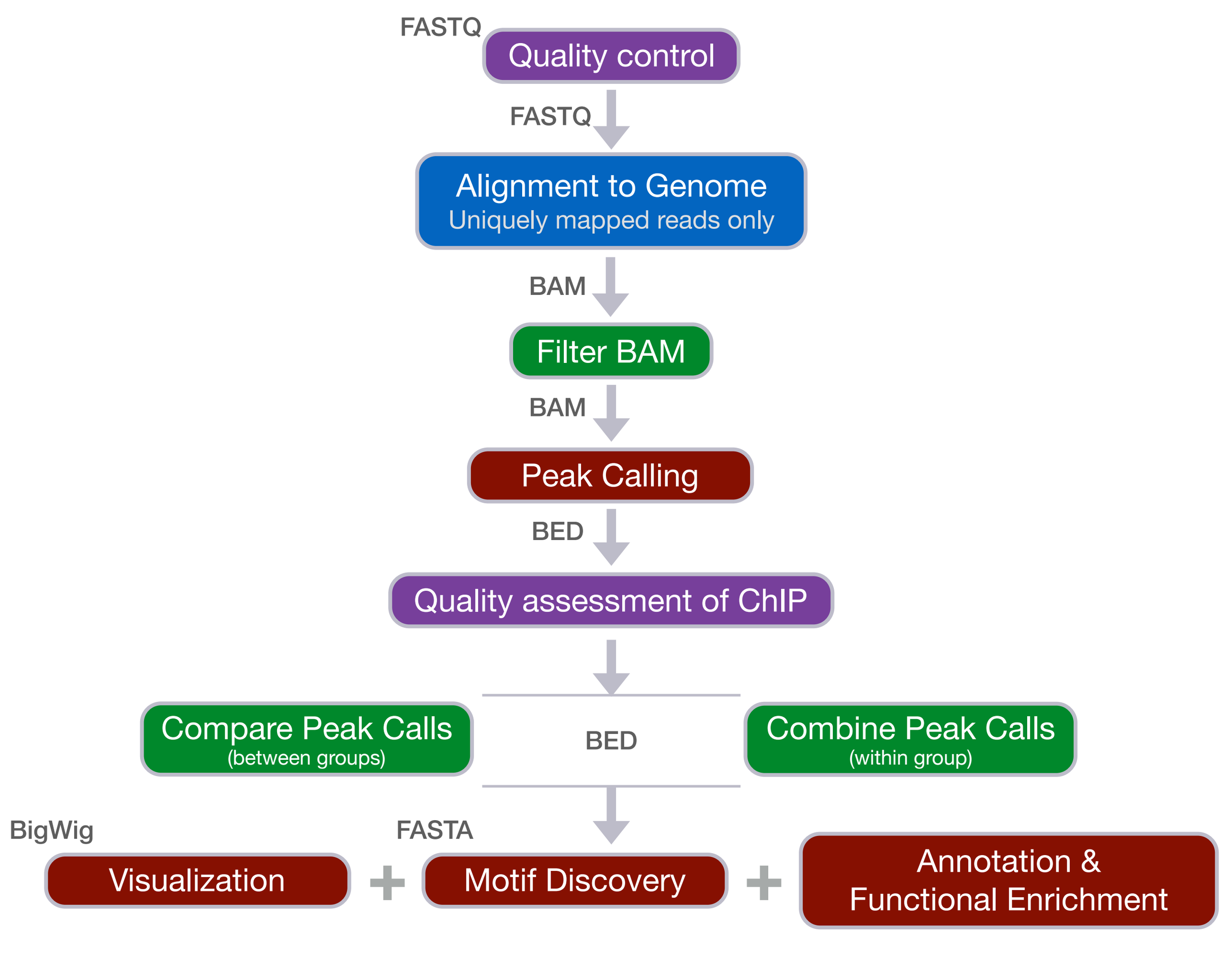
the workflow


Quality control
Alignment to Genome
Uniquely mapped reads only
Filter BAM
Peak Calling
Quality assessment of ChIP
Compare Peak Calls
(between groups)
Combine Peak Calls
(within group)
Visualization
Motif Discovery
Annotation &
Functional Enrichment

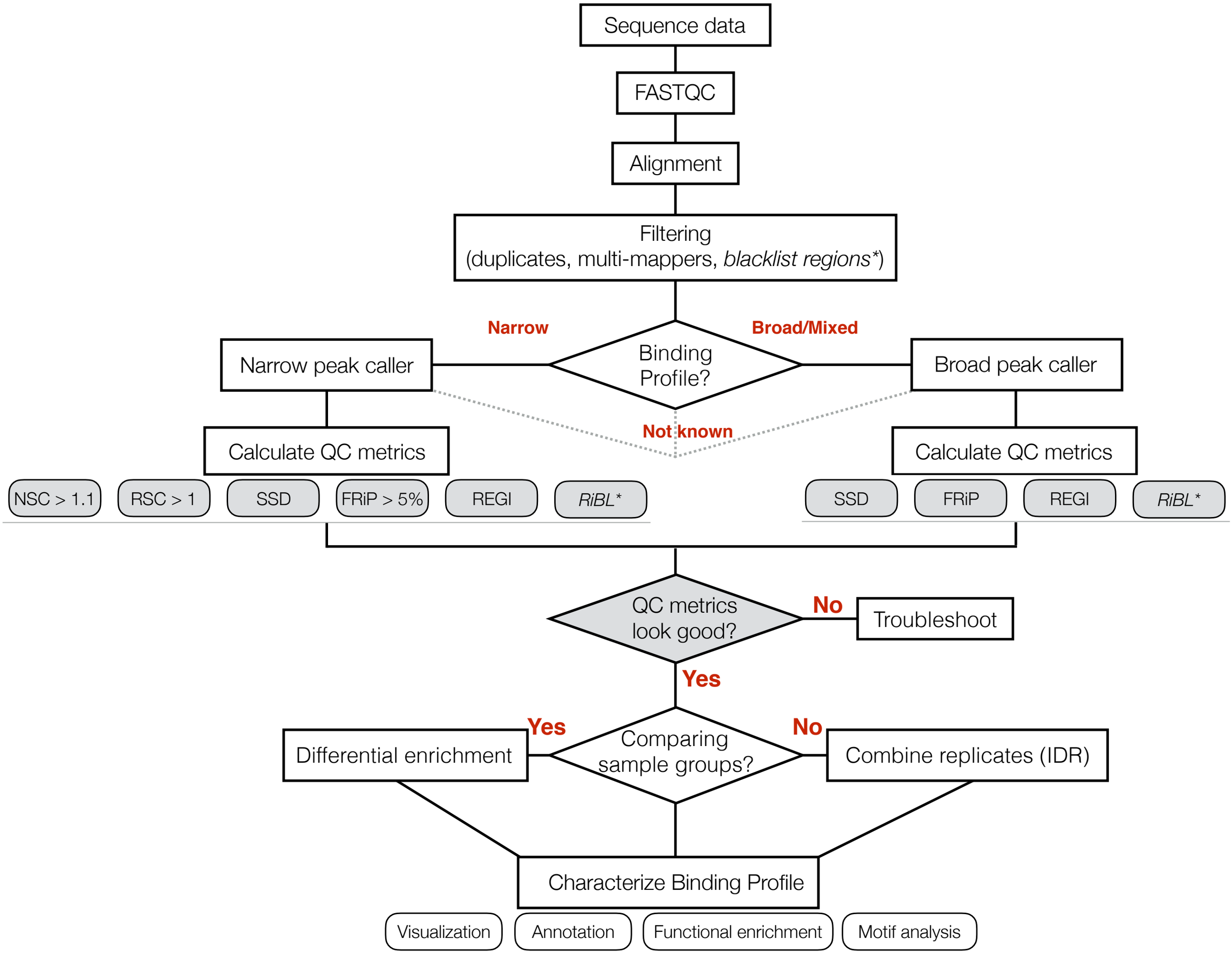
the toolkit


Quality control
FastQC
Alignment to Genome
Uniquely mapped reads only
Bowtie2
Filter BAM
Samtools & Sambamba
Peak Calling
MACS2
Quality assessment of ChIP
Phantompeakqualtools
& ChIPQC
BEDtools
Compare Peak Calls
(between groups)
Combine Peak Calls
(within group)
IDR &
BEDtools
Visualization
Motif Discovery
Annotation &
Functional Enrichment
IGV & deepTools
MEME
GREAT

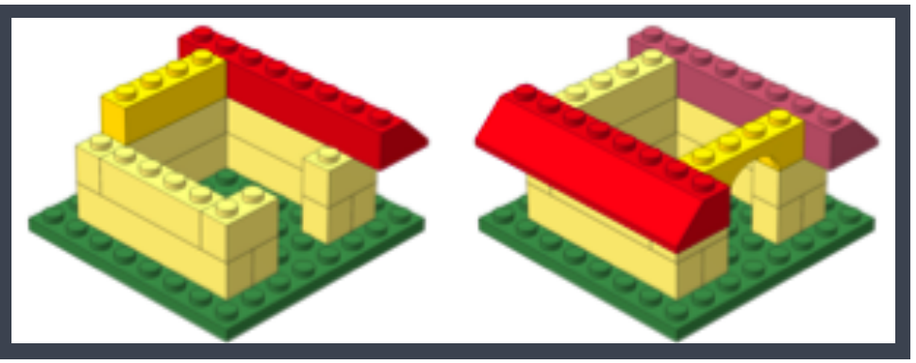
plug and play approach


FASTQ
Quality control
FASTQ
Alignment to Genome
Uniquely mapped reads only
BAM
Filter BAM
BAM
Peak Calling
BED
Quality assessment of ChIP
Compare Peak Calls
(between groups)
BED
Combine Peak Calls
(within group)
BigWig
FASTA
Visualization
Motif Discovery
Annotation &
Functional Enrichment

the process


Sequence data
FASTQC
Alignment
Filtering
(duplicates, multi-mappers, *blacklist regions**)
Narrow
Broad/Mixed
Binding
Profile?
Narrow peak caller
Broad peak caller
Not known
Calculate QC metrics
Calculate QC metrics
NSC > 1.1
RSC > 1
SSD
FRiP > 5%
REGI
*RiBL**
SSD
FRiP
REGI
*RiBL**
QC metrics
look good?
No
Troubleshoot
Yes
Yes
Comparing
sample groups?
No
Differential enrichment
Combine replicates (IDR)
Characterize Binding Profile
Visualization
Annotation
Functional enrichment
Motif analysis

Learning Objectives



- ✓ Describe best practices for designing an ChIP-seq experiment
  - ✓ antibody characterization, replicates, sequencing depth, qPCR
- ✓ Describe steps in a typical ChIP-seq analysis workflow
  - ✓ the appropriate tools and configurations
  - ✓ decisions to consider at every step in the workflow
- ✓ Use HMS-RC's O2 compute cluster to efficiently run the ChIP-seq workflow from sequence reads to peak calls, including QC and visualization.