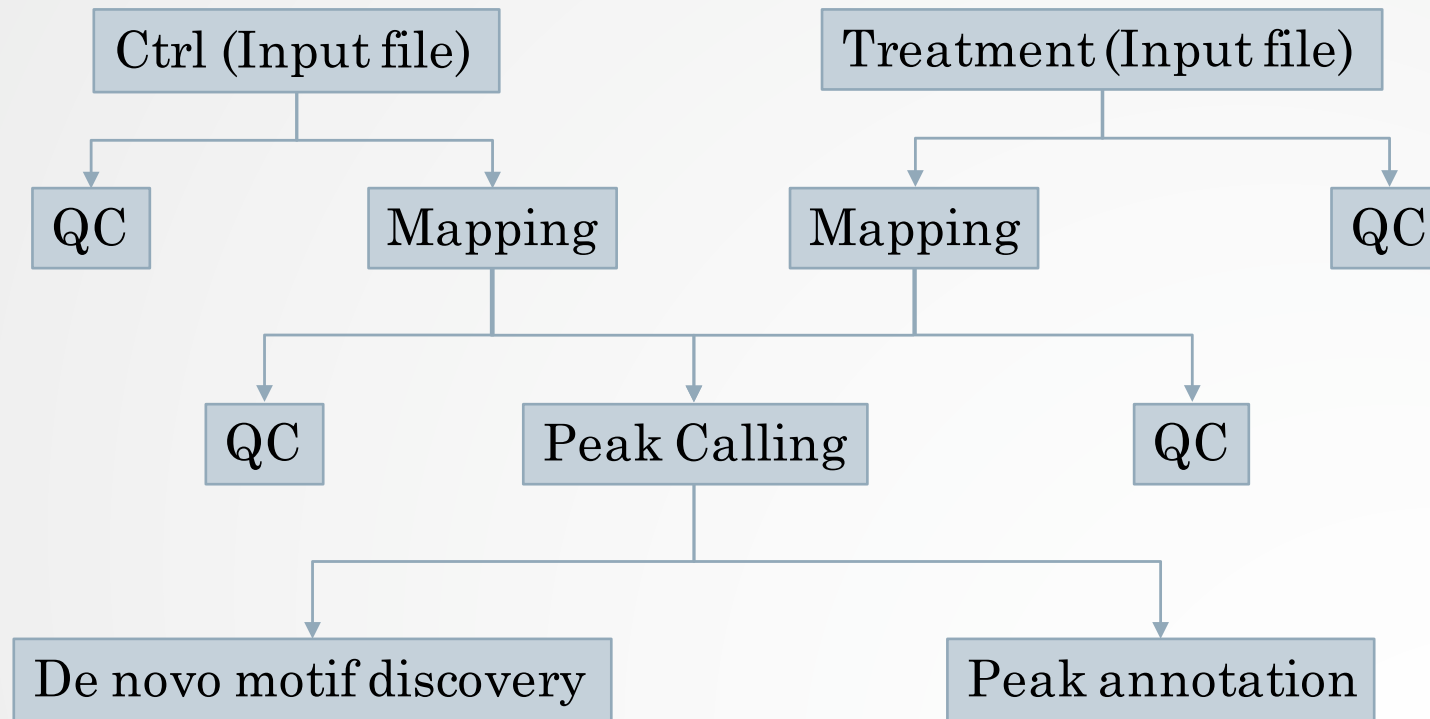


# NGS analysis automatization: Galaxy workflows (answers to questions)

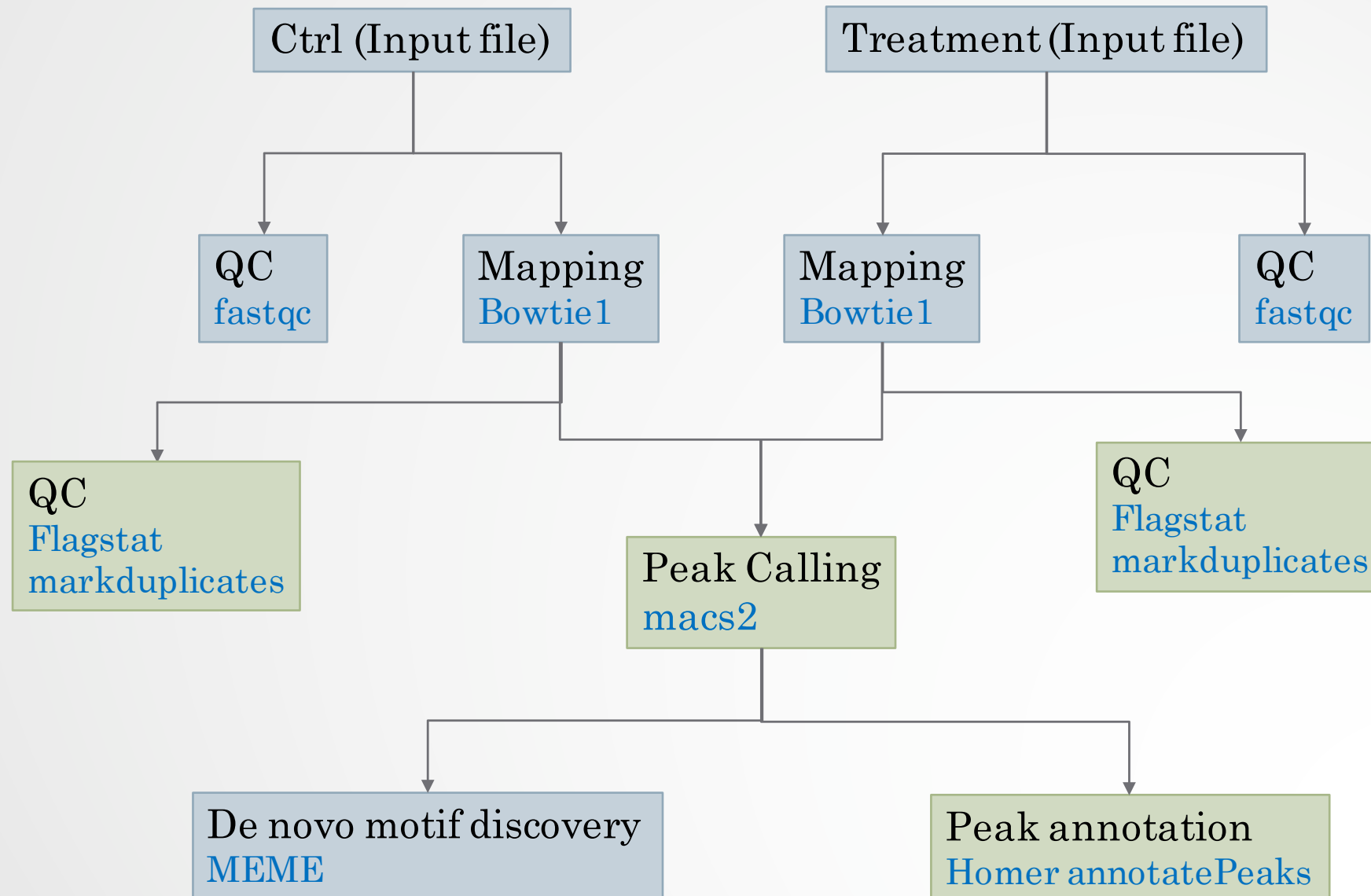
Stéphanie Le Gras  
([slegras@igbmc.fr](mailto:slegras@igbmc.fr))

# Exercise: your workflows for NGS data analysis


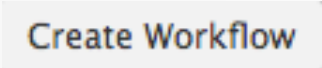
1.



# Exercise: your workflows for NGS data analysis

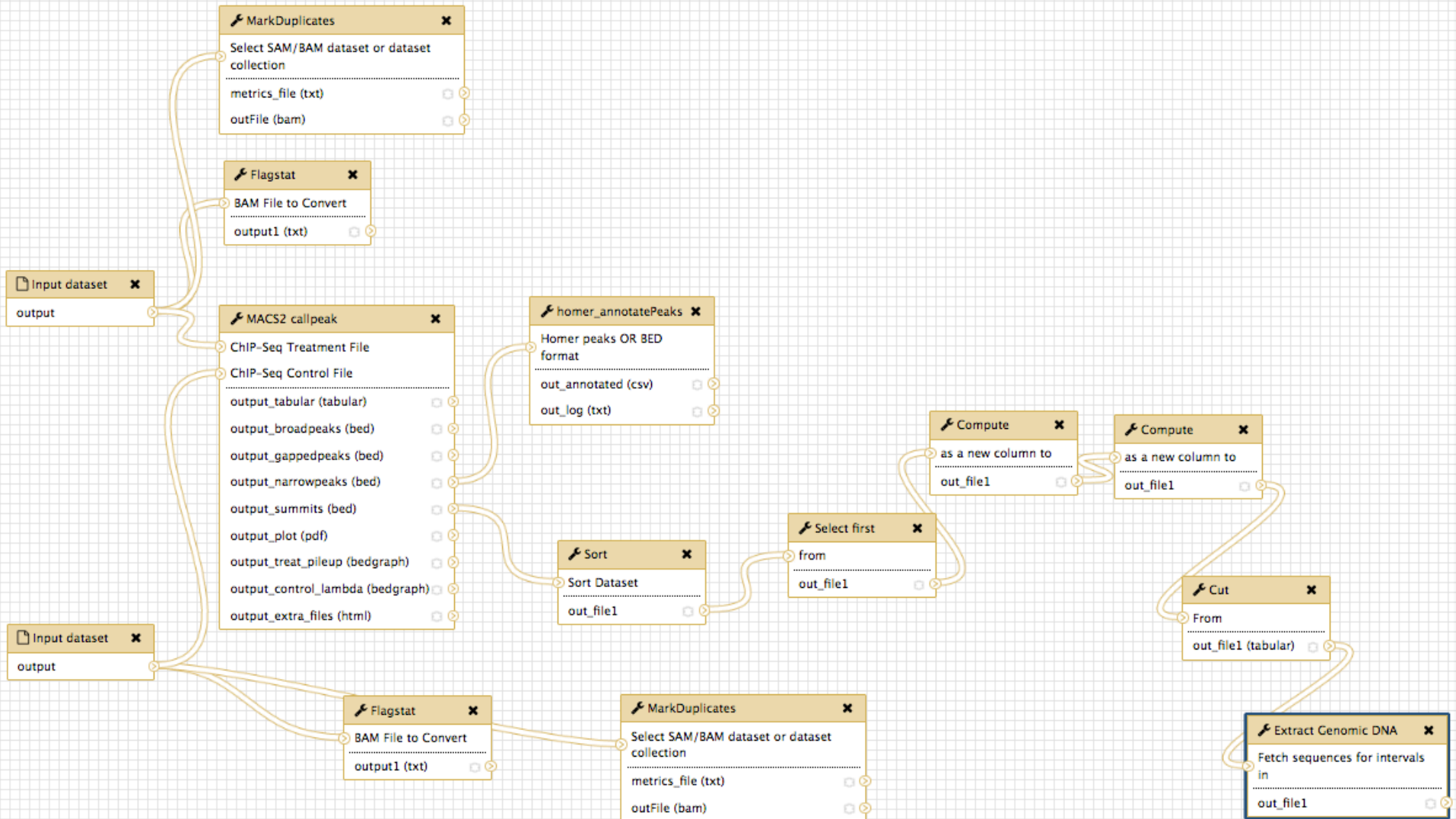


# Exercise: your workflows for NGS data analysis

- 2.
  - Go to the history in which you analyzed chIP-seq data (history named “ChIP-seq data analysis”)
  - Click on 
  - Select Extract Workflow
  - Enter the workflow name: “ChIP-seq data analysis”
  - Adapt the workflow steps to the needs:
    - Keep the second MACS2 run
  - Go back to top and click on 
  - Edit the workflow:
    - Click on Workflow (top menu)
    - Click on ChIP-seq data analysis > Edit

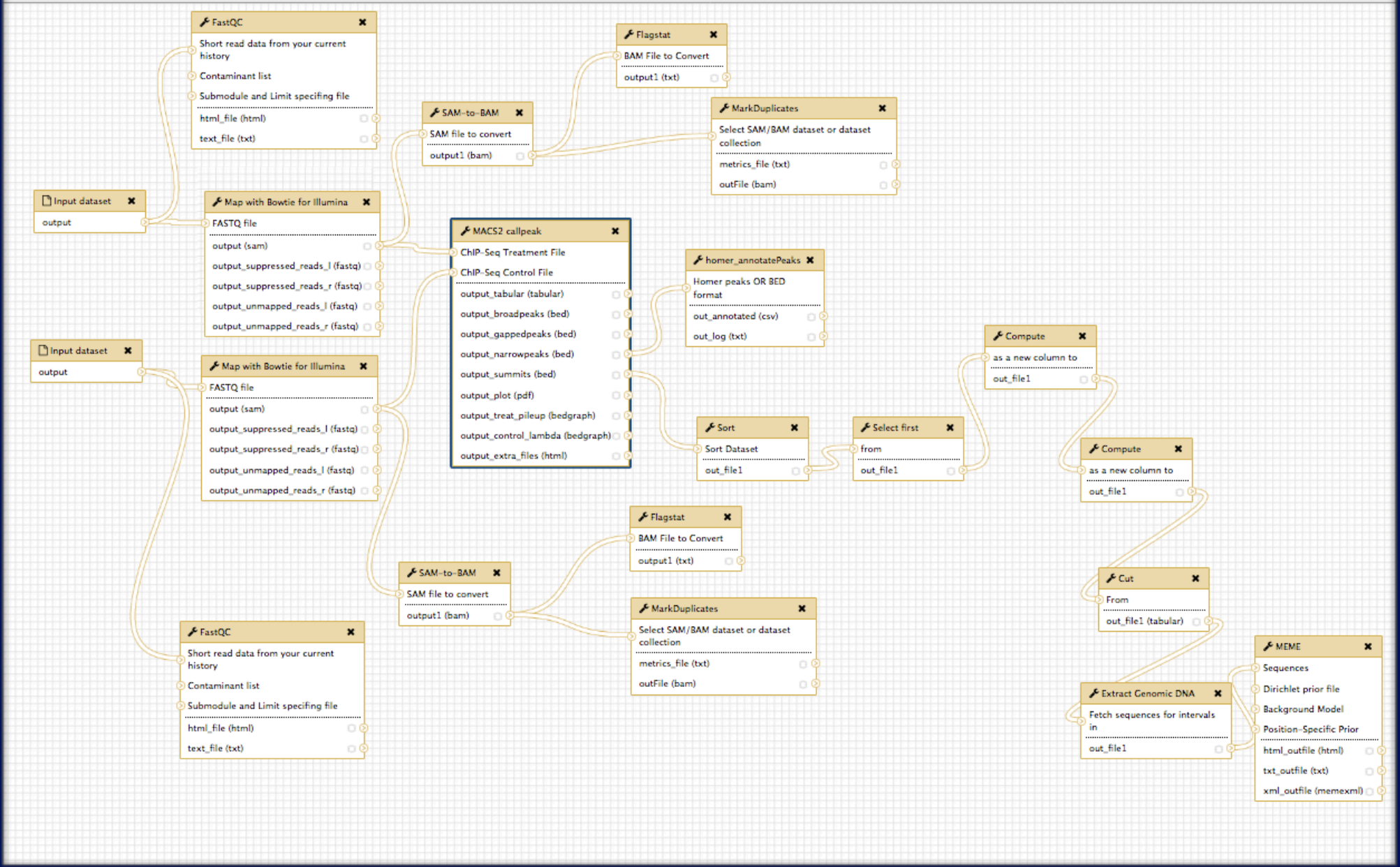
# Exercise

Workflow Canvas | Workflow constructed from history 'ChIP-seq data analysis'



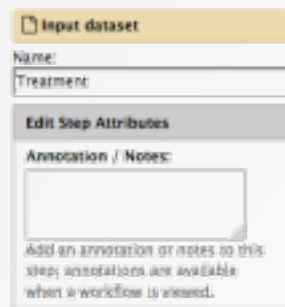
# Exercise


Workflow Canvas | CHIP-seq data analysis




# Exercise: your workflows for NGS data analysis

- Name the input datasets
  - Click on the input boxes and enter “Treatment” in the treatment input box and “Control” in the control input box (After having clicked on the input box, enter text in the right panel).



- Bowtie 1 parameters:
  - Select a reference genome : **set at runtime**   **Select a reference genome**
  - Bowtie settings to use: **Full parameters list**
  - Whether or not to make Bowtie guarantee that reported singleton alignments are 'best' in terms of stratum and in terms of the quality values at the mismatched positions (--best): **Use best**
  - Whether or not to report only those alignments that fall in the best stratum if many valid alignments exist and are reportable (--strata): **Use strata**
  - Suppress all alignments for a read if more than n reportable alignments exist (-m): **1**

# Exercise: your workflows for NGS data analysis

- MACS2
  - Build Model: **Build the shifting model**
- Select the box of the tool **cut**
  - Click on **configure Output: out\_file1**
  - Change datatype: **interval**
- MEME parameters:
  - Options Configuration: **Advanced**
  - Number of different motifs to search: **2**
  - Min width of motif to search: **6**
  - Max width of motif to search: **12**
  - E-value to stop looking for motifs : **1**
  - I certify that I am not using this tool for commercial purposes: **Yes**
- Click on  and Save

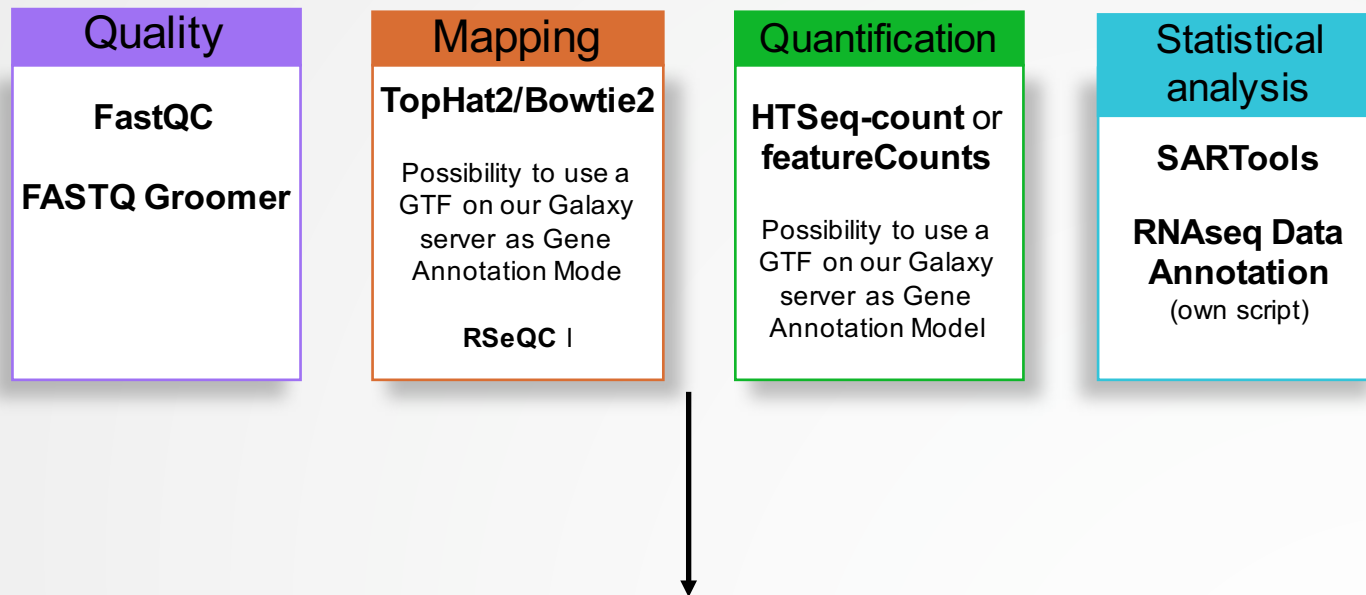


# Exercise: your workflows for NGS data analysis

- 3.
  - Click on Analyze Data (top menu)
  - Go to Shared data > Data Libraries > CNRS training > ChIPseq > workflow and add the two datasets to your history.
    - Tick the two boxes beside the sample names
    - Click on the button To history
    - Add the datasets to the new history “ChIP-seq test workflow”
  - Click on Workflow (top menu)
  - Click on the workflow “ChIP-seq data analysis” and select Run.
    - Treatment: chr10\_mitf\_2.fastq
    - Control: chr10\_ctrl2\_1.fastq
    - Step 4: Map with Bowtie for Illumina:
      - Select a reference genome: hg38
    - Step 5: Map with Bowtie for Illumina
      - Select a reference genome: hg38
    - Click on Run workflow

# Exercise: your workflows for NGS data analysis

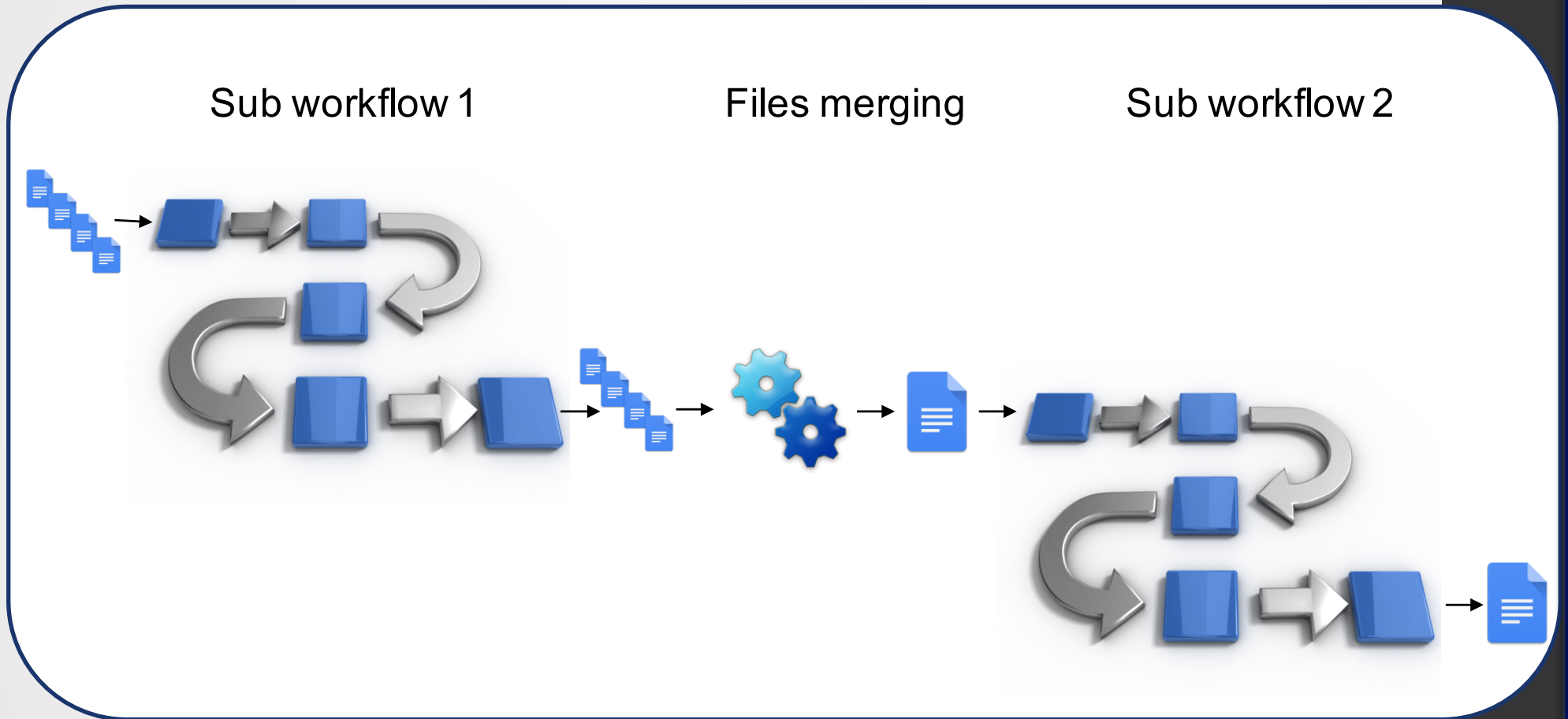
- 4.



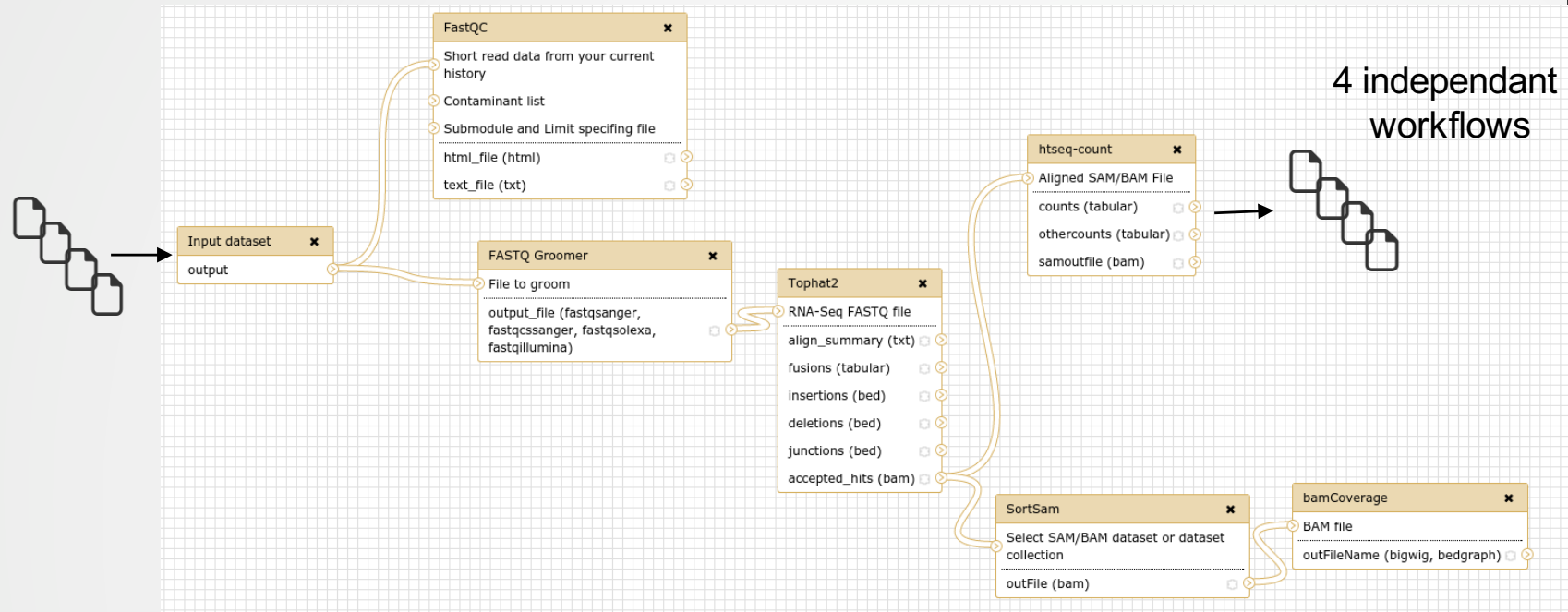
Problem : all steps can't be in a same workflow

# RNAseq workflow : limits

Main workflow



# RNAseq workflow : limits



HTSeq-count outputs



Merge



SARTools