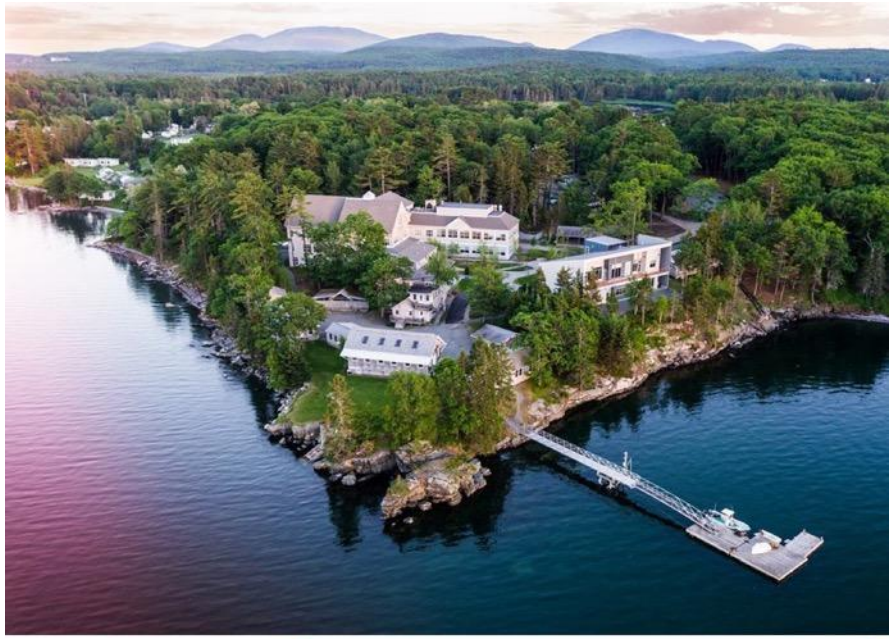

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Comparative Genomics & Data Science Core

Workflows in the Cloud

Joel H Graber, Director

Comparative Genomics and Data Science Core
MDI Biological Laboratory
02 May 2025

Agenda

- Bioinformatics Workflows
- NF-Core RNAseq Pipeline Setup & Launch
- Nextflow/NF-core
- Amazon Web Services (AWS)
- Memverge
- Lunch
- Issues, Questions, etc.
- Exploration of the Results
- Next Steps/Questions

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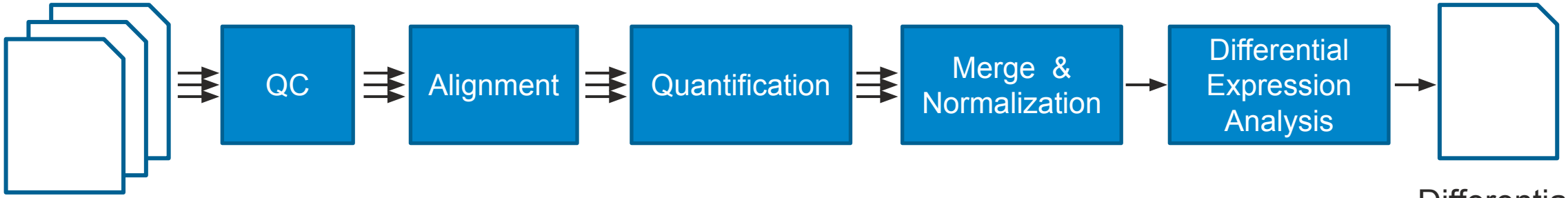
Outline

- What is a workflow? Why do we use them?
- What are the components and concepts of a workflow?
- Example: Bulk RNA-seq

What goes into the complete analysis of a genome-scale data set?

(using Bulk RNA-seq as an example)

- Most complex data needs multiple steps to go from raw data to ”answers”
- Example: RNAseq data to Differentially expressed genes



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Workflows are not necessary, but very useful

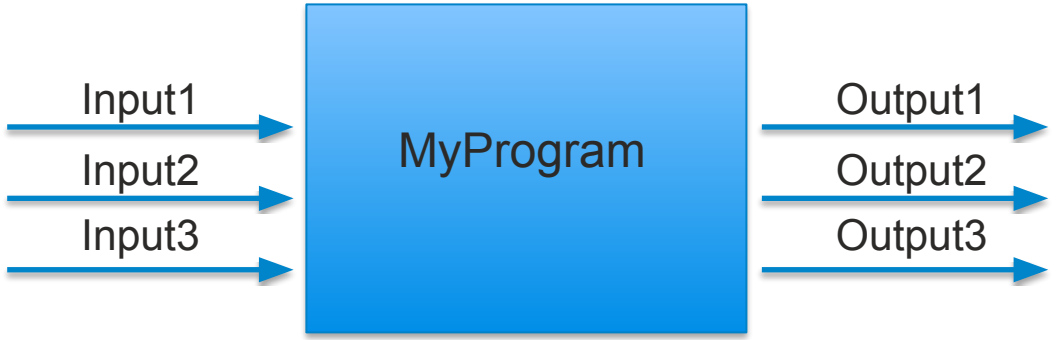
- Each of the steps is typically executed by a distinct program
- Early steps often must be run separately for each sample in an experiment
- These efforts can be performed manually, but
  - Can be tedious and time consuming
  - Unnecessary potential source of error or inconsistency
- A workflow system allows for
  - definition of steps and
  - flow of information between the steps

Workflows (pipelines) solve many issues

- The programmatic steps are run the same way every time.
- The output files can be named and placed in a consistent way
- If log files are generated, you have a record of what was done, including parameters and input data
- If you need to run the analysis again, the pieces are in place to do so.
- Reduced work in program installation and maintenance
  - (we will discuss why)

Basic ideas of building a workflow: programs

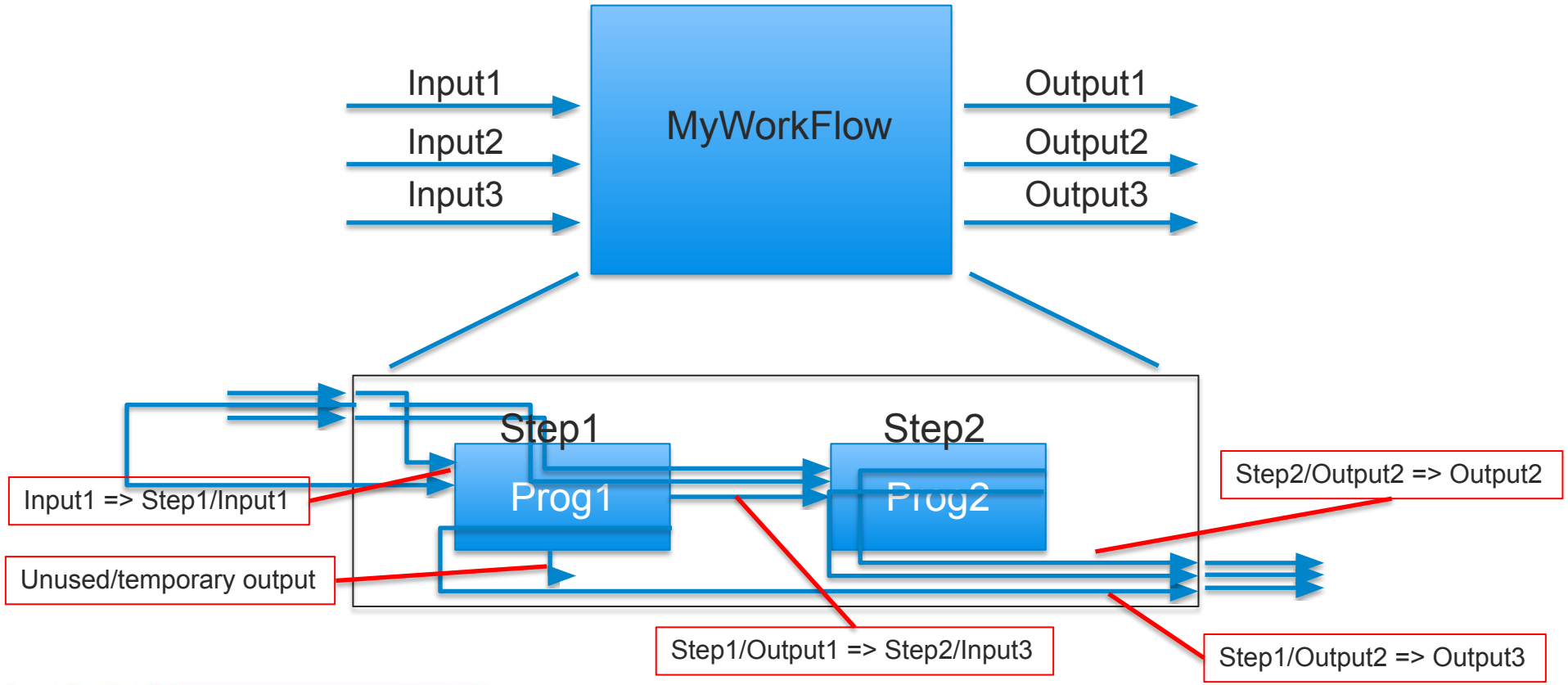
- At the base level is a single command, which has inputs and generates outputs



- If we understand this, we can incorporate it into a workflow

Basic ideas of building a workflow: connections

- Workflows are built from multiple steps, with information passed along



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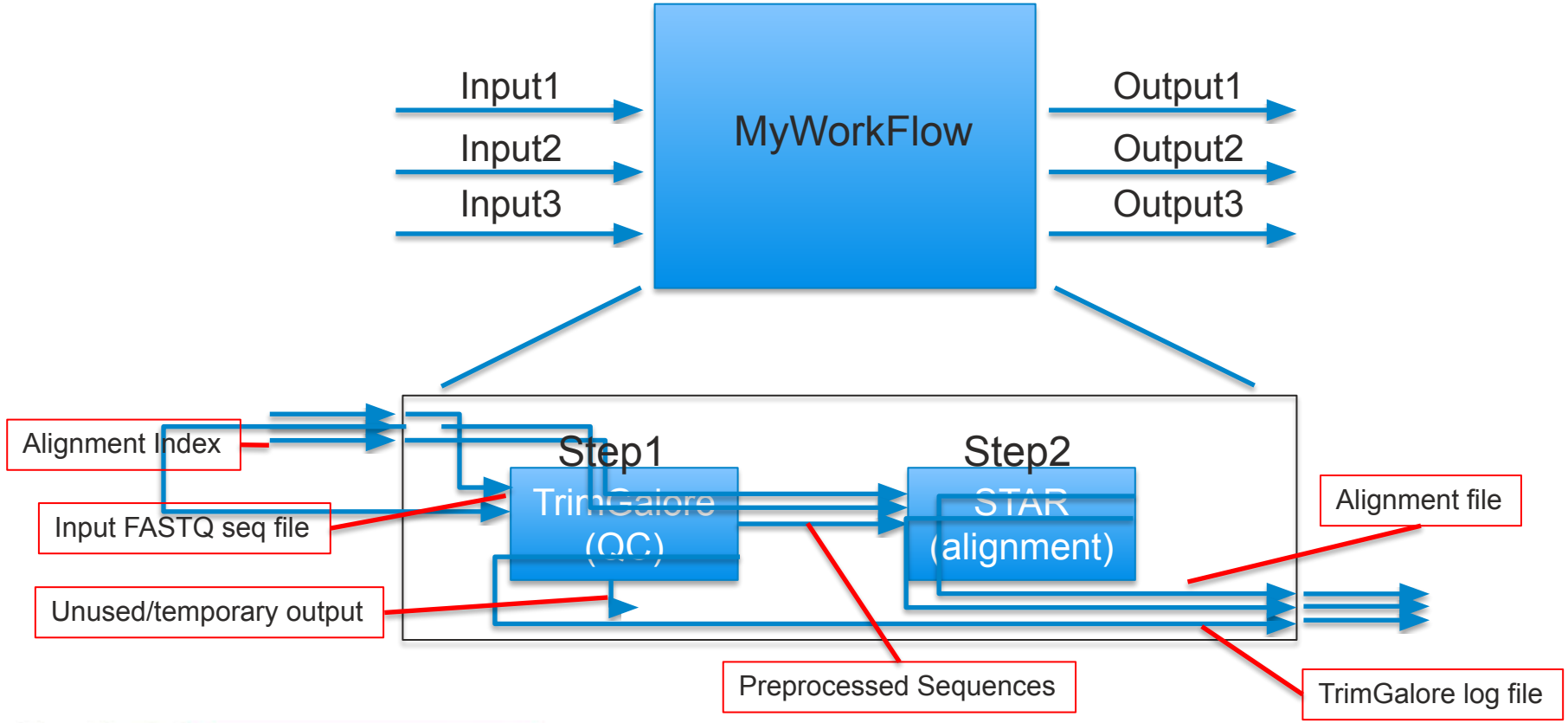

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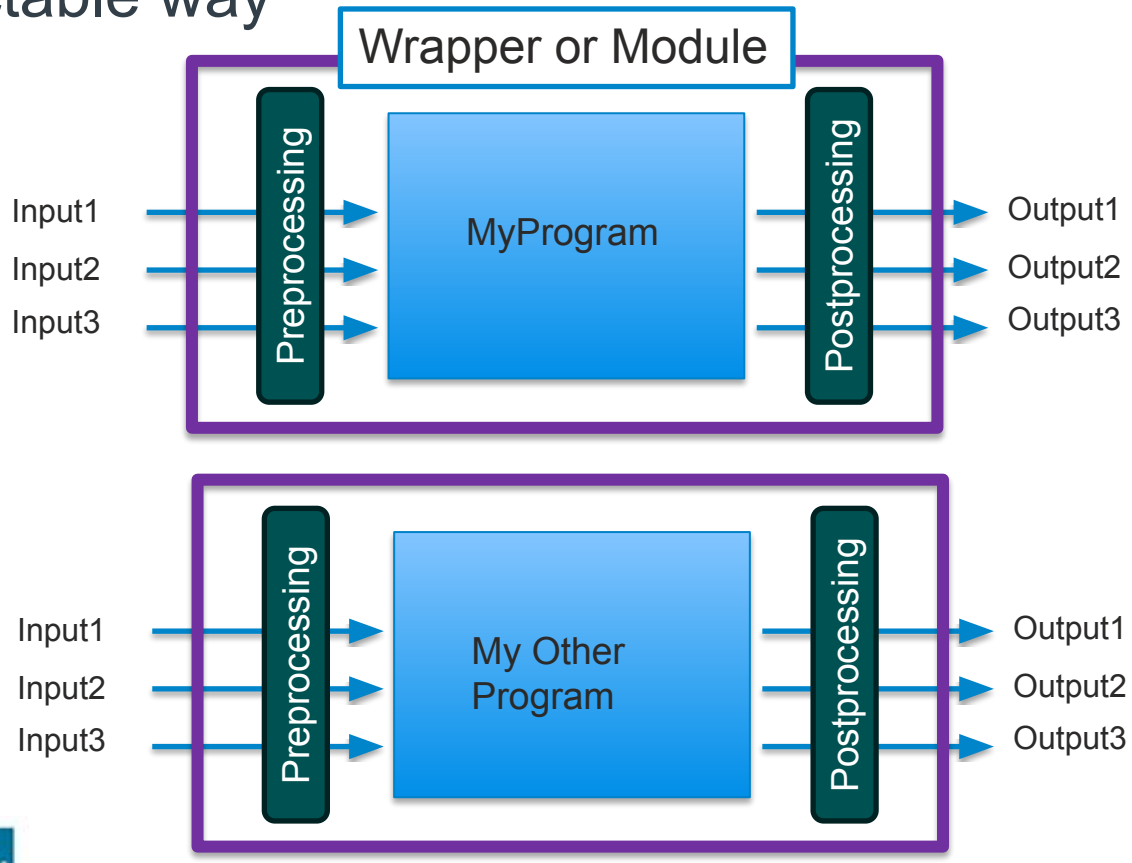
Workflows are best when flexible and adaptable

- In an ideal and unchanging world, with one set of programs, you would
  - Define your set of steps once and
  - Write a set of scripts that
    - Execute the tools you need
    - Pass the information correctly along
- But-
  - Nearly every step has multiple programs that can carry out the function
  - The alternative programs can use different parameters and produce different output
- Also-
  - Programs change, Libraries change
  - Either can break the program or the entire workflow

Basic ideas of building a workflow: wrappers

- Wrappers are programs that act as interfaces, and activate the program in “generic” and predictable way



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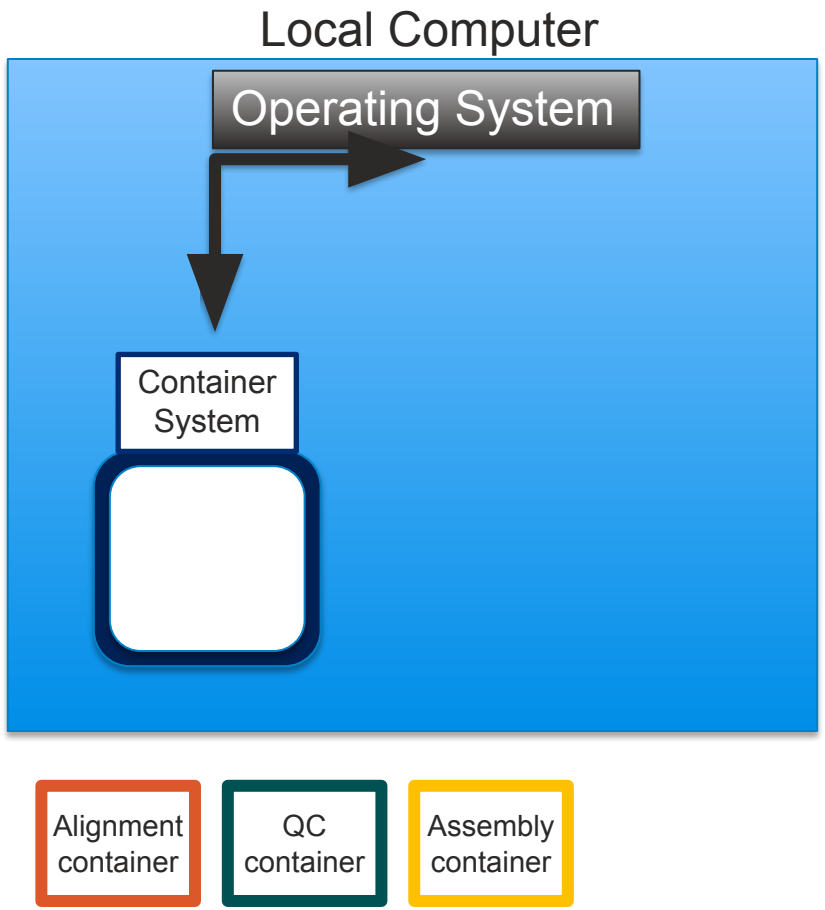
Basic ideas of building a workflow: Dealing with Change Using Containers

- Most programs are not created “from scratch”
- They are instead built from existing “functional components
  - System libraries: e.g., compression utilities like Gzip
  - Specialized libraries: e.g., a FASTQ file reader
  - The components are loaded and accessed by ”Application Interfaces”
- The components can change or even be deprecated and lost
- Containers provide a means of managing and maintaining functionality

Containers Simplify Software Installation/Maintenance

- A container system is a program that creates protected computing environments within a larger computer, passes information in and out
- Containers are constructed to include all necessary resources to run a specific program
- Benefits:
  - Programs with conflicting requirements can be run on the same computer by using container-based versions
  - Once a container is constructed it can be loaded and run on ANY computer that runs the container system
  - Repositories of containers are freely available



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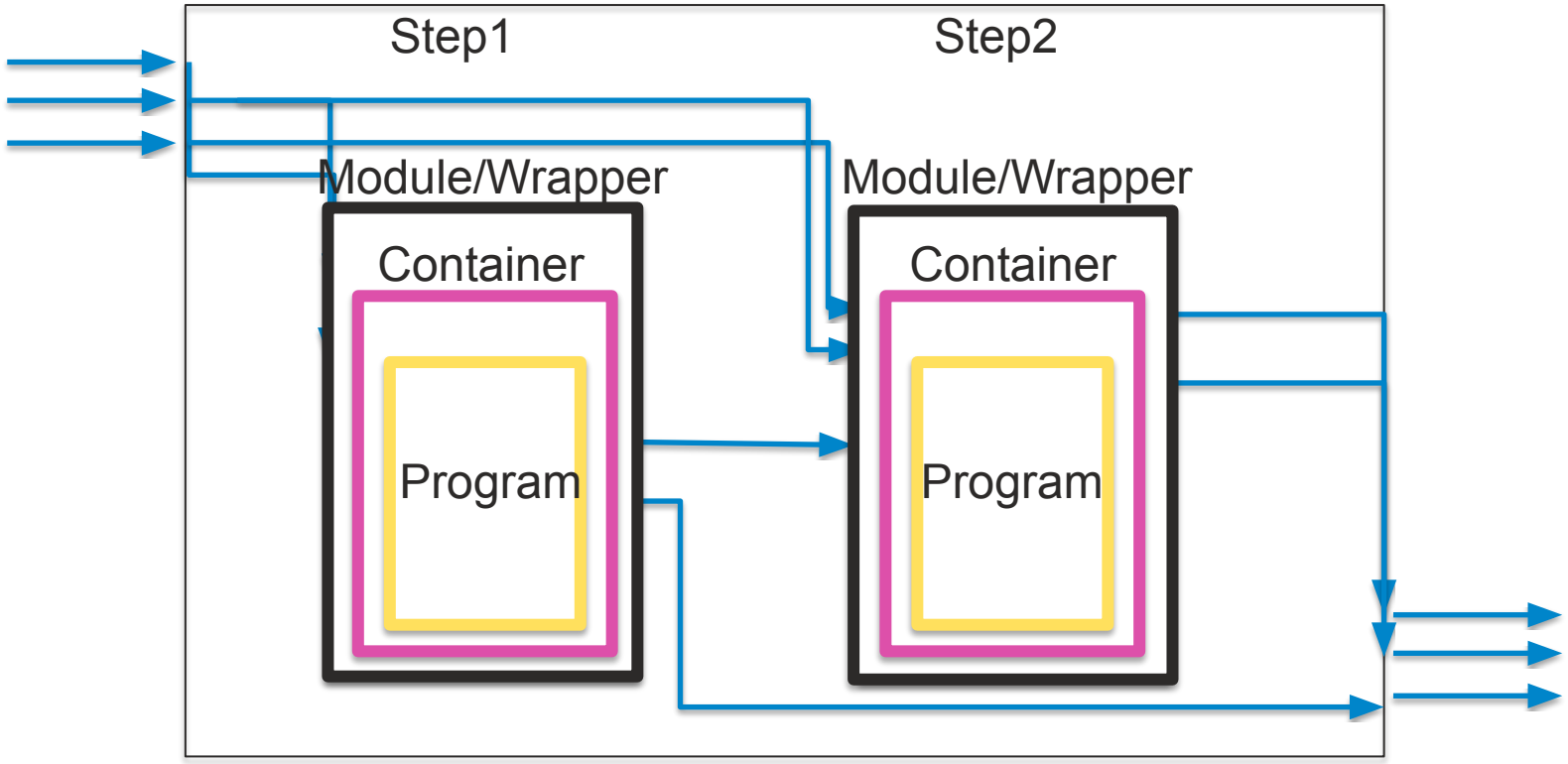
A modern workflow system uses wrappers and containers



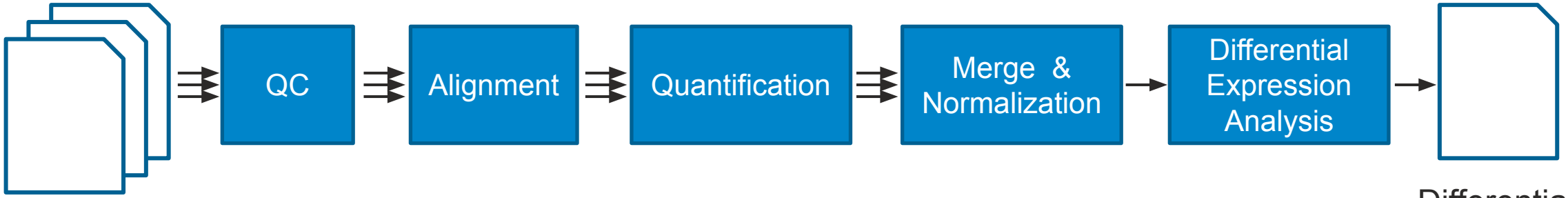
Basics of Workflow Systems

- A workflow system consists of
  - A language capable of describing the process that captures dependencies and computational complexities
  - A program (“engine”) capable of
    - Reading and executing the workflow description
    - Requesting/allocating the necessary computational resources to carry out the work
- The power of these systems is that workflows
  - Can be run on any system for which an engine has been programmed and set up
  - Can be rerun for new data sets and/or analysis by changing a simple text-formatted parameter file

What goes into the complete analysis of a genome-scale data set?

(using Bulk RNA-seq as an example)

- Most complex data needs multiple steps to go from raw data to ”answers”
- Example: RNAseq data to Differentially expressed genes



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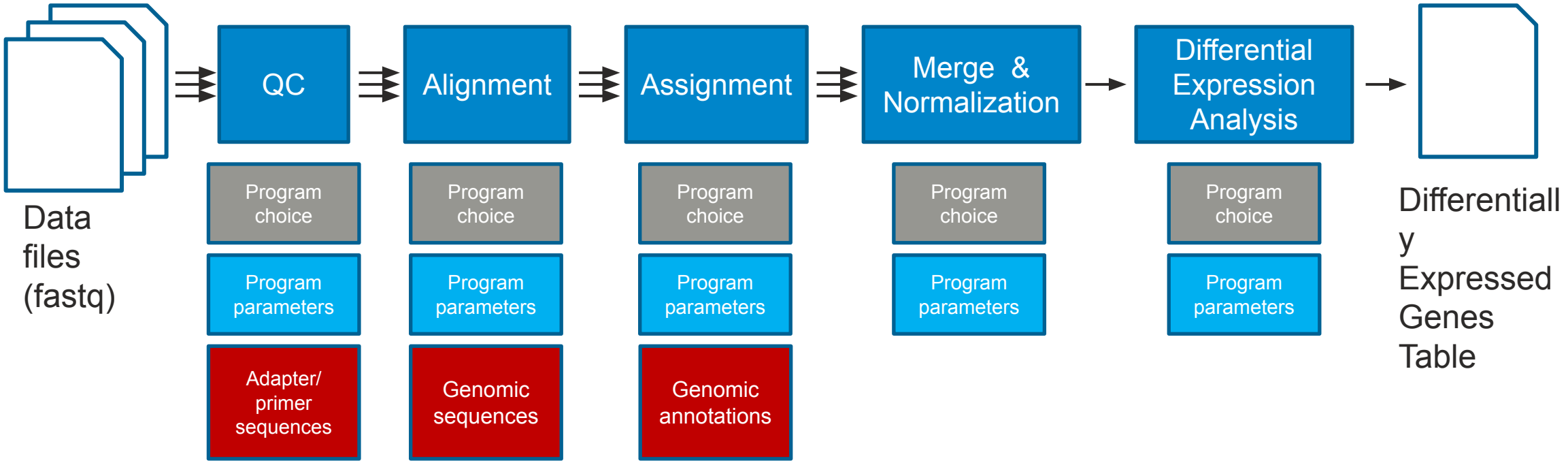
What goes into the complete analysis of a genome-scale data set?

(using Bulk RNA-seq as an example)



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“Rigor and Reproducibility”

- Every choice outlined in the last slide can impact results of analysis
- Recording, monitoring, and sharing these factors is now recognized as critical in genomics analysis
  - A required aspect of all NIH grant proposals
  - Also required by many journals
- Resource: Karl Broman (Wisconsin)
  - http://kbroman.org/steps2rr/
  - http://kbroman.org/dataorg/pages/resources.html

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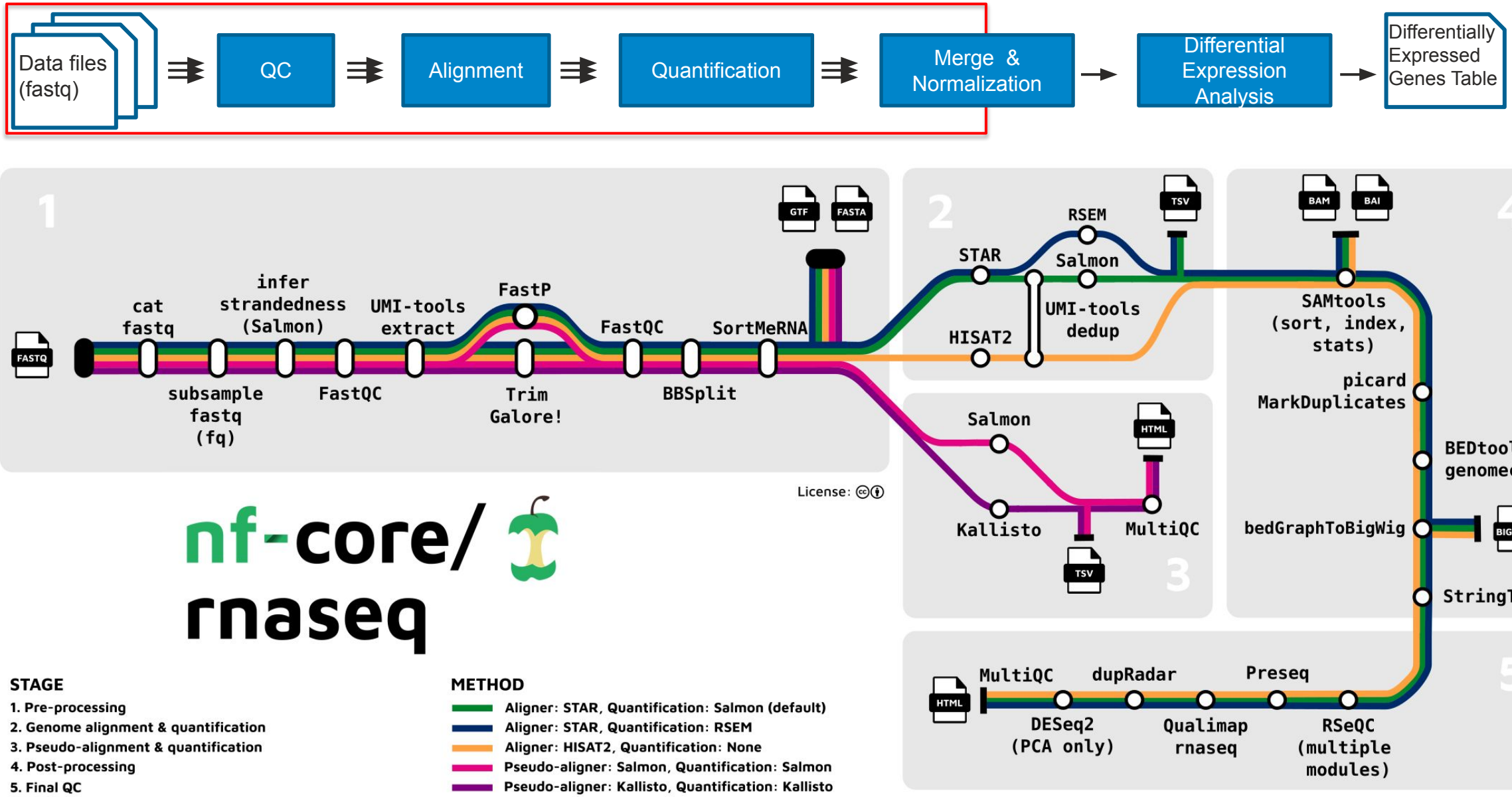

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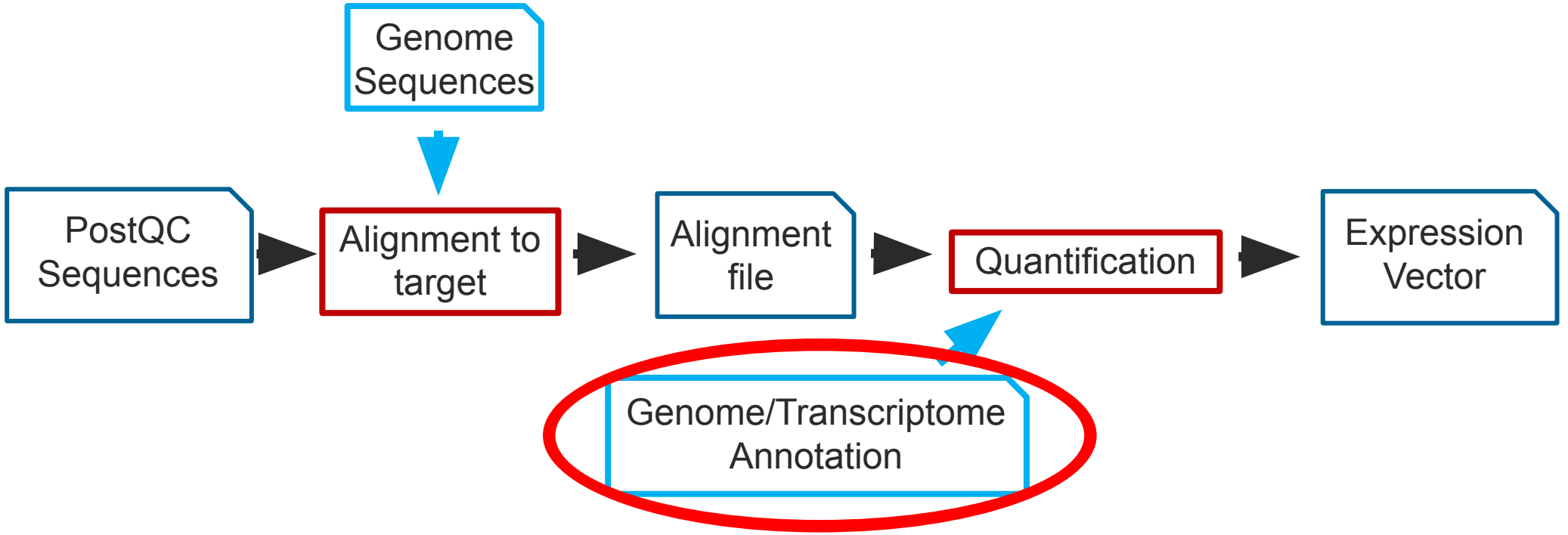
Alignment Approach to Quantification

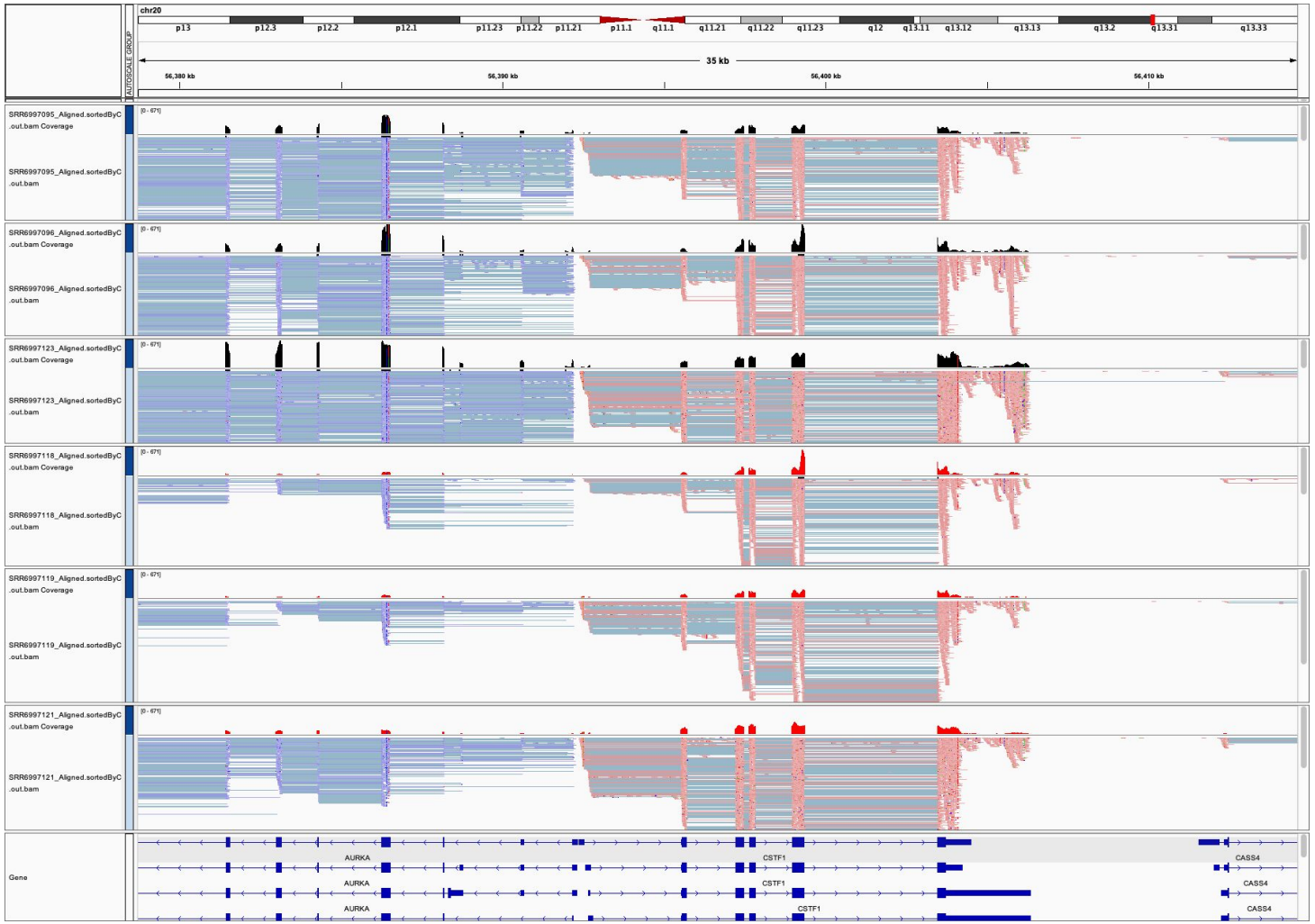


Gene information must be provided (e.g., GFF)

GFF example of a gene and its graphical representation

chr1	tool	gene	11218	15435	.	+	.	ID=gene1
chr1	tool	mRNA	11218	15435	.	+	.	ID=transcript1;Parent=gene1
chr1	tool	exon	11218	13000	.	+	.	ID=exon1;Parent=transcript1
chr1	tool	exon	13800	14002	.	+	.	ID=exon2;Parent=transcript1
chr1	tool	exon	15000	15360	.	+	.	ID=exon3;Parent=transcript1
chr1	tool	exon	15384	15435	.	+	.	ID=exon4;Parent=transcript1
chr1	tool	UTR5	11218	12000	.	+	.	ID=UTR5a;Parent=transcript1
chr1	tool	CDS	12801	13000	.	+	0	ID=CDS1;Parent=transcript1
chr1	tool	CDS	13800	14002	.	+	0	ID=exon1;Parent=transcript1
chr1	tool	CDS	15000	15234	.	+	0	ID=exon1;Parent=transcript1
chr1	tool	UTR3	15234	15360	.	+	.	ID=UTR3a;Parent=transcript1
chr1	tool	UTR3	15384	15435	.	+	.	ID=UTR3b;Parent=transcript1

RNA-seq analysis: alignment/quantification



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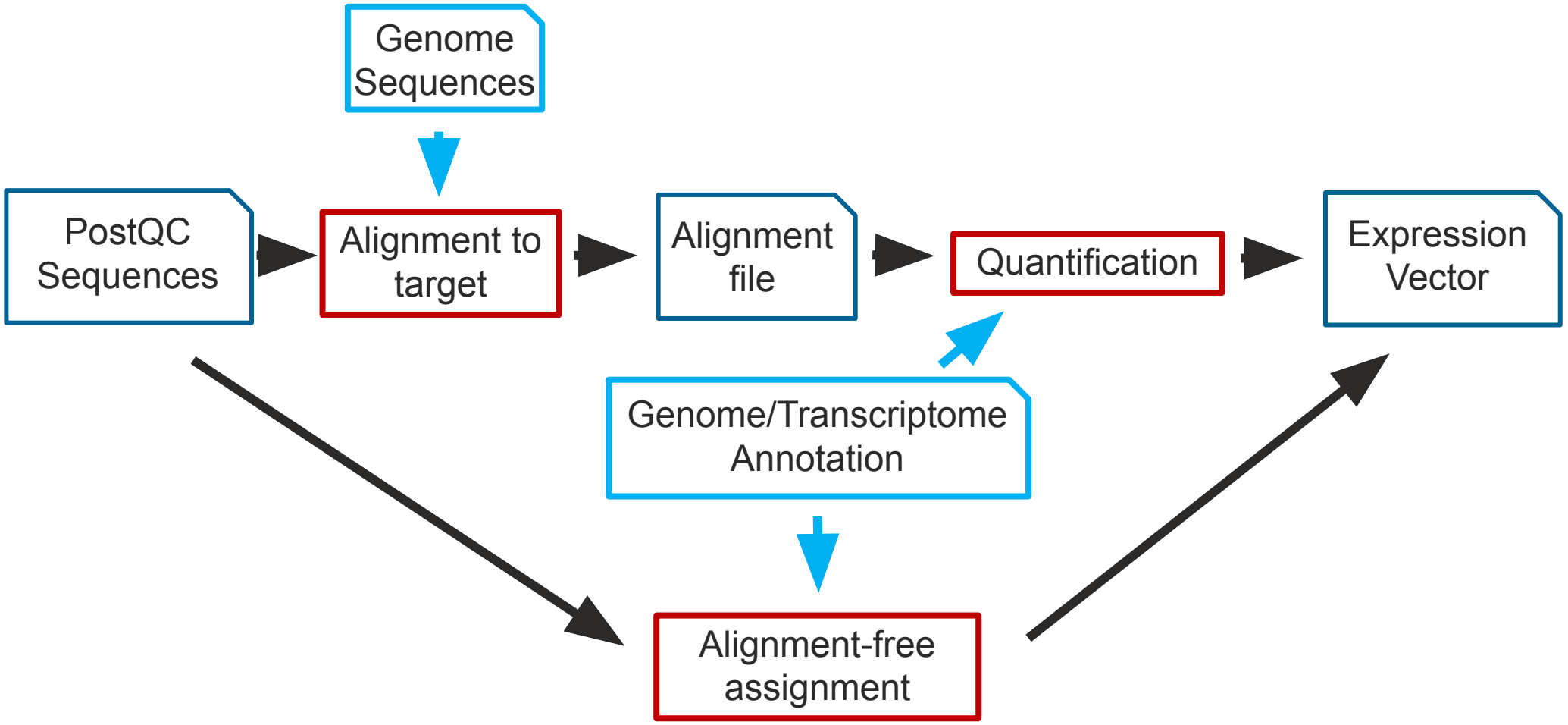
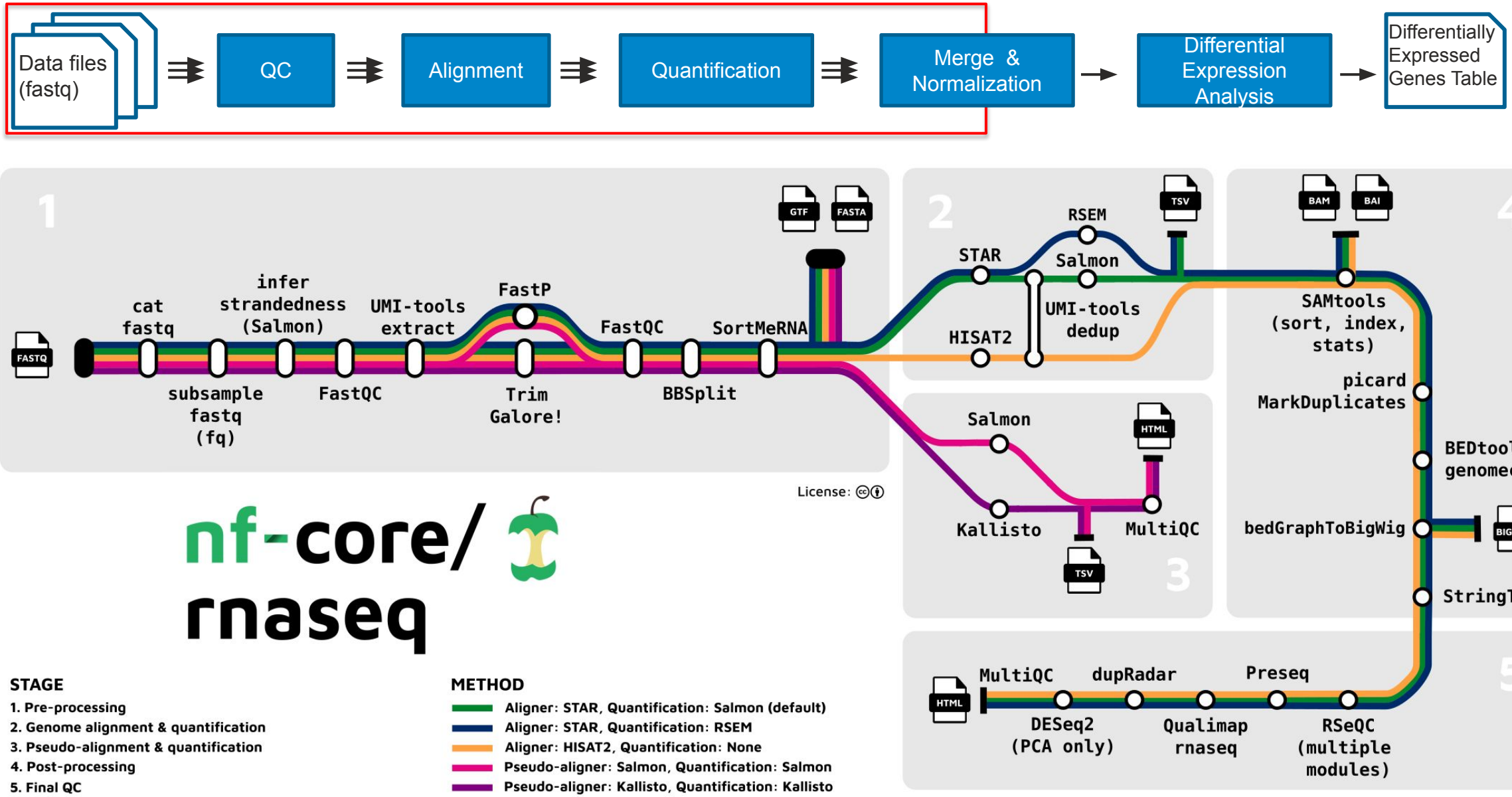
Alternative approaches to Quantification





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After expression is assessed in each sample, they are merged into a “count matrix”

gene_name	AL_TO_rep01	AL_TO_rep02	AL_TO_rep03	DR_TO_rep01	DR_TO_rep02	DR_TO_rep03
aap-1	753	747	743	940	947	982
aat-1	27	24	14	15	28	14
aat-2	30	33	24	60	65	68
aat-3	134	137	127	78	67	93
aat-4	23	45	35	22	30	27
aat-5	38	33	29	123	84	105
aat-6	40	39	28	41	46	55
aat-7	1	1	0	2	4	6
aat-8	1	1	2	14	3	10
aat-9	362	399	374	370	328	370

After the NF-core: working with your output

- NF-core pipelines generally focus on the standard common analysis step
- Many summary output files are available
- Output tables can become input to other tools
  - RNA-seq analysis with Sequin
  - https://sequin.ncats.io/app/

To interpret our count matrix, we need an Experimental Design File

- At minimum, the Design File must contain
  - Identifiers for each sample (ideally matched to a data filename)
  - Assignment of all experimental parameters under consideration to each sample
- Ideally- ANY feature/variable that might vary between samples

sample	treatment	rep
AL_TO_rep01	AL	rep01
AL_TO_rep02	AL	rep02
AL_TO_rep03	AL	rep03
DR_TO_rep01	DR	rep01
DR_TO_rep02	DR	rep02
DR_TO_rep03	DR	rep03

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In the end, a table of DE Gene Scores (*e.g.*, with DESeq2)

id	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
aagr-1	269.129364535602	-1.7442675672456	0.117789943380256	-14.8082893767481	1.29494023689411E-49	4.77497892947039E-48
aagr-3	2008.77205021688	-0.150425067741619	0.0418534931952695	-3.59408632965959	0.0003255318965062	0.00115773135585242
aak-2	243.639422569596	0.278051661358966	0.118395785760709	2.34849289248301	0.0188495589454439	0.0458599158655762
aakb-2	415.838439463941	0.561118701249279	0.100734483891487	5.57027424544835	2.54338675055636E-08	1.56247902940004E-07
aakg-1	365.852541550914	0.50046549824763	0.0971820567244253	5.149772654707	2.6080239032197E-07	1.40999077665866E-06
aakg-3	14.7626365586319	1.32753612196484	0.538581116196545	2.46487684406741	0.0137060352076937	0.034635107180592
aakg-4	72.0407425048923	1.73861272918138	0.251164464315594	6.92220825871598	4.44656882831695E-12	3.78784449623833E-11
aakg-5	736.490245516047	-0.171877365357521	0.063262738817093	-2.71688150989571	0.00659001957329092	0.018076559672254
aap-1	846.749244306947	0.216032066870877	0.0694242604499855	3.11176619629258	0.00185971722699245	0.00572240163660642
aars-2	2065.39673387659	0.132015428540962	0.0446828104046726	2.9545014591821	0.0031317467246952	0.00916240085776832
aat-2	45.7630124225589	1.01639572482027	0.300017225171763	3.38779123178136	0.000704578716201275	0.00236338649524766

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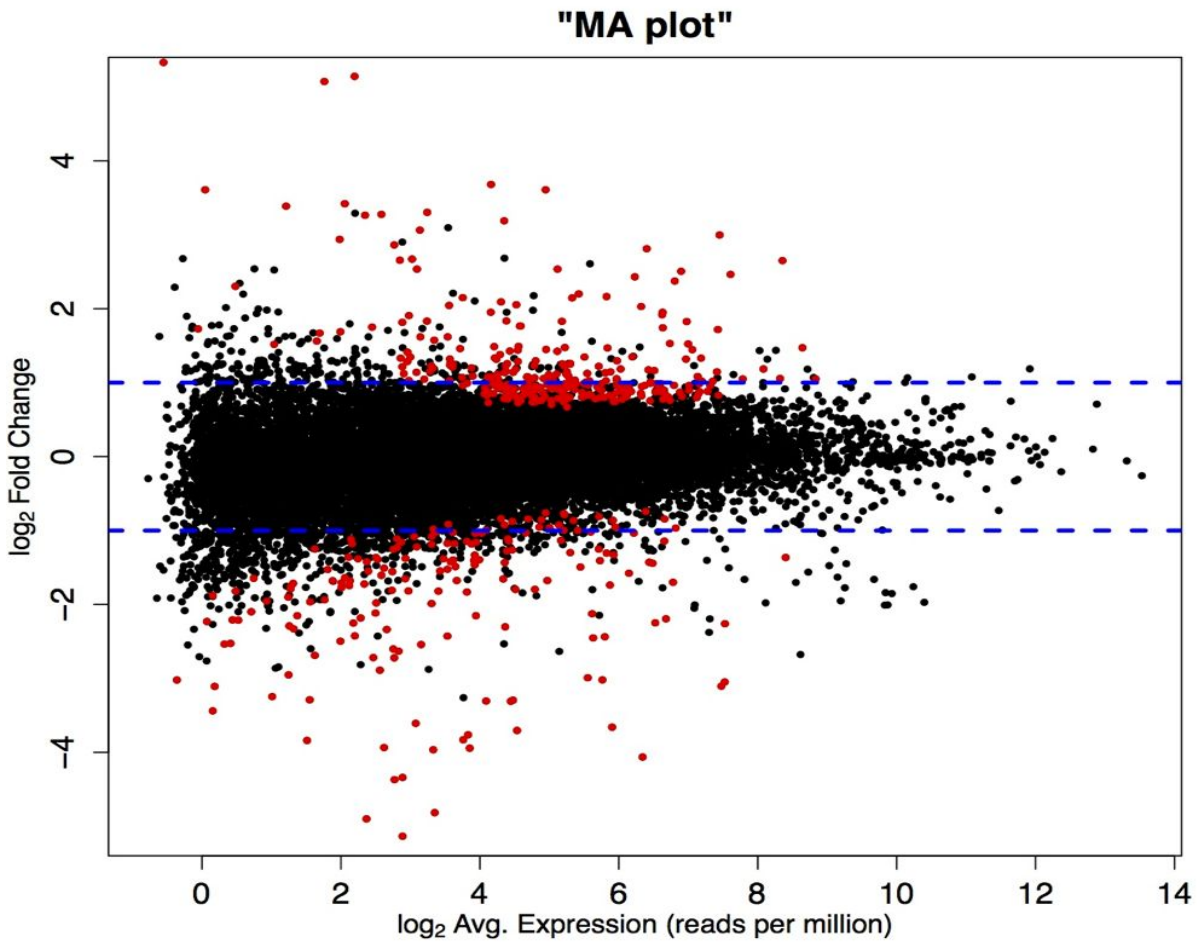
The end result for all genes (in visual form)

Increased by treatment

Decreased by treatment



Increased average expression across all samples

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Summary and concluding thoughts

- Workflows allow for systematic and reproducible execution of complex, multi-step analysis of genome-scale data
- Community-supported workflows let you
  - Carry out best-in-practice analysis plans
  - Reduce effort and potential error
  - Keep track of analysis steps and output for subsequent downstream analysis and reporting/publication
- The learning curve is still not trivial
  - We can help

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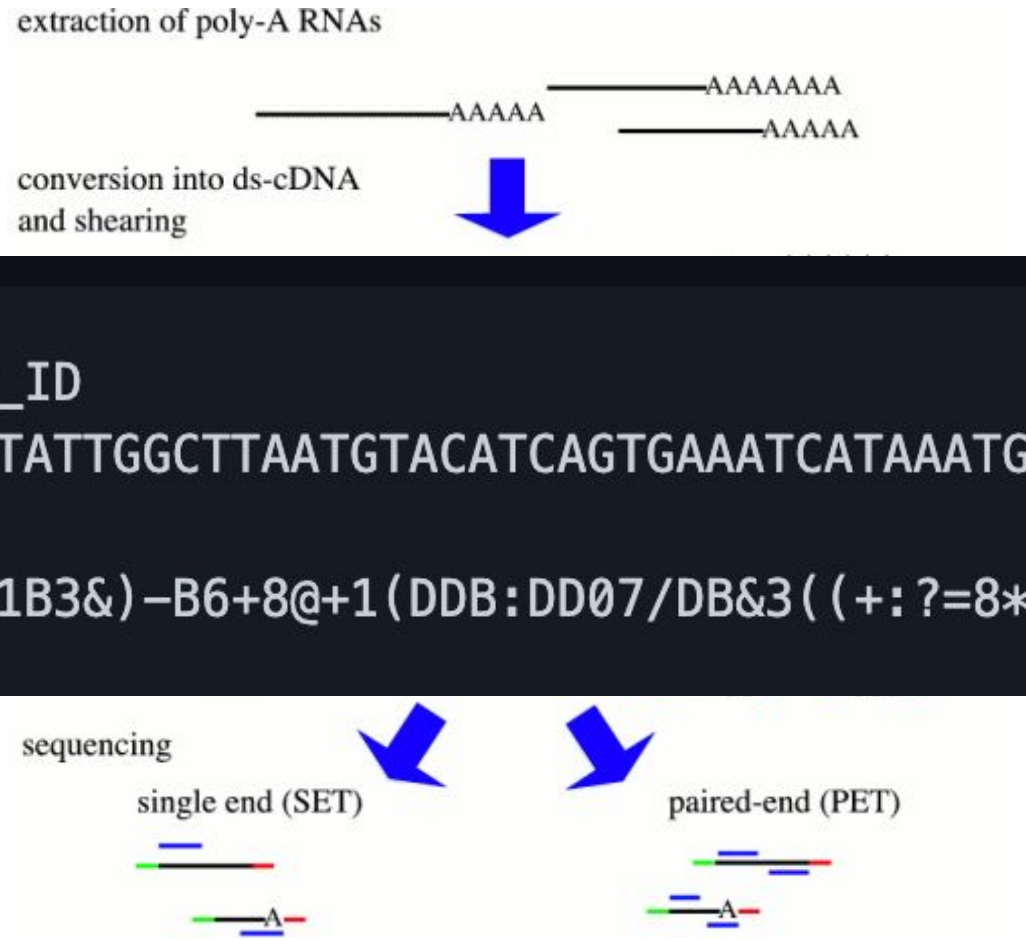
A typical RNA Seq experiment (and why we need QC)



http://cmb.molgen.mpg.de/2ndGenerationSequencing/Solas/RNA-seq.html

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Computational normalization is critical for transcriptome analysis

- Three standard approaches to computational normalization
  - Internal normalization (Quantile, VST, FPKM, TPM, etc)
    - Assume all samples are roughly the “same,” and force equal distributions
    - Insensitive to global changes
  - Internal standard normalization
    - Identify a relatively small number of “unchanging” targets and scale all values so that these values are equal in all samples
  - External standard normalization
    - Add a known control (“Spike-in”) and then scale values such that the values for the controls are the same

Community supported workflows: NextFlow/NF-core

- https://nf-co.re/
- Nf-core Pipelines are
  - (Mostly) focused on specific data type
  - Supported by teams of volunteers
  - A systematic way to get systematic execution, logging, and organized output
  - Generally “best-practice” accepted steps