The Nextflow Automation Blueprint

Phase 1: Environment Setup

Before writing code, you need the engine and the garage.

1. **Install Nextflow:** The "engine" that runs the script.
2. **Install Docker:** The "garage" that holds all the tools (FastQC, BWA, etc.) so you don't have to install them on your actual computer.
3. **Organize Folders:** Create a project root with a modules/ folder and a data/ folder.

The Role of Containerization in Bioinformatics

Abstract Analogy: The "Shipping Container" Model

Docker utilizes a **containerization** strategy analogous to global intermodal shipping. Just as a standard shipping container encapsulates diverse goods—ensuring they can be transported via ship, rail, or truck without altering the internal contents—a Docker container packages bioinformatics software with its entire runtime environment. This ensures that the application remains functional and consistent regardless of the host operating system.

The Problem: Dependency Hell and Software Conflicts

In complex genomic pipelines, different tools often require mutually exclusive environments. For example:

- **Tool A (e.g., BWA):** May rely on legacy libraries or specific versions of Python (e.g., 2.7).
- **Tool B (e.g., MultiQC):** May require modern dependencies (e.g., Python 3.10+).

Installing these directly on a single host machine creates **dependency conflicts**, where updating a library for one tool inadvertently breaks another. Docker resolves this by providing **process isolation**; each tool resides in its own discrete environment, preventing cross-tool interference.

Core Definitions: Images vs. Containers

Term	Technical Definition	Practical Analogy
Docker Image	A read-only, executable package that includes everything needed to run an application—code, runtime, libraries, and environment variables.	The **Blueprint**: A static template used to build the environment.
Docker Container	A runtime instance of an image. It is a lightweight, standalone, and executable software package that runs in isolation on the host OS.	The **Live Instance**: The active "worker" that executes the task and is decommissioned upon completion.

Conclusion: Why Containerization is Mandatory

For high-throughput sequencing pipelines, Docker is utilized to achieve:

1. **Reproducibility:** Ensuring that the same code produces identical results across different computational infrastructures.
2. **Portability:** Enabling the seamless transition of workflows from local workstations to high-performance computing (HPC) clusters or cloud environments.
3. **Efficiency:** Eliminating the time-intensive process of manual tool installation and configuration.

Phase 2: Component Engineering (The Modules)

For every tool in your pipeline, you create a dedicated .nf file in the modules/ directory.

- **Identify the Tool:** (e.g., Samtools).
- **Find the Container:** Search Biocontainers for the Docker URL.
- **Define Inputs/Outputs:** Decide what file comes in (FASTQ) and what file goes out (BAM).
- **Write the Script:** The exact command you would type in a terminal.

Phase 3: The Brain (The Main Workflow)

Create the main.nf file to act as the conductor.

1. **Define Parameters:** Set default paths for inputs (params.fastq) and outputs (params.outdir).
2. **Import Modules:** Use the include statement to bring in your module files.
3. **Construct Channels:** Use Channel.fromPath to feed your files into the pipeline.
4. **Connect the Dots:** Pass the output of one process as the input to the next (e.g., TRIM(FASTQC.out)).

Phase 4: Configuration & Safety

1. **nextflow.config:** This file acts as the master control.
   Docker: docker.enabled = true
   Absolute Paths: You must define the exact location of software binaries here so the pipeline doesn't have to "guess" where they are.
   Example: params.bwa_bin = "/usr/bin/bwa"

2. **.gitignore:** The purpose of a .gitignore file is to act as a **filter**. It tells Git exactly which files or folders in your project should be **ignored** (not tracked or uploaded to GitHub).

```
.nextflow/          //  --internal cache files that are only useful on local machine
.nextflow.log*      //  --unnecessary log files
work/               //  --contain 100s of temporary folders not needed in GitHub
```

results/ // --contains outputs
data/ // --contains raw FASTQ files
reference/ // --contains raw FASTQ files

3. **environment.yml** - The Conda

Purpose and Importance

While Docker handles the "tools" (like BWA), the environment.yml file handles the infrastructure (Nextflow itself and Python/Java dependencies).

- Reproducibility: It ensures that every scientist who uses your repo has the exact same version of Nextflow and other base tools.
- Reviewer Requirement: It serves as a fallback for users who cannot run Docker.
- Environment Isolation: It prevents your pipeline's requirements from messing up other software installed on your computer.

How to Use It

1. Creation: Define the tools in a .yml file.
2. Installation: Run conda env create -f environment.yml.
3. Activation: Run conda activate nextflow-env.

Phase 5: Version Control & Publishing

1. **Git Init:** Initialize your repository.
2. **Commit:** Save a "snapshot" of your code.
3. **GitHub Push:** Upload your code (using a Token) so it's safe in the cloud.

Pipeline Execution Flowchart

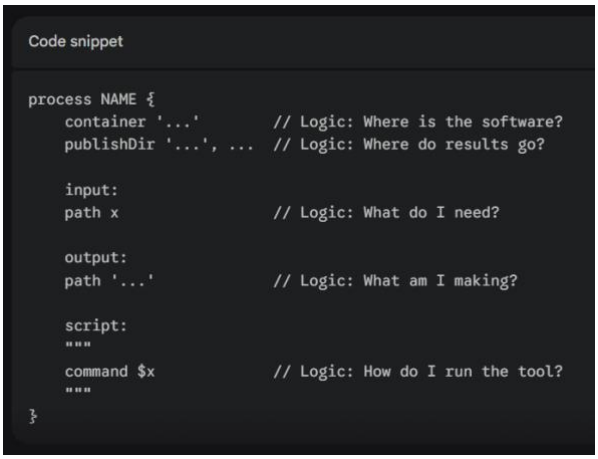
Step	Action	Input	Output
1	**Quality Control**	Raw FASTQ	HTML Report
2	**Trimming**	Raw FASTQ	Cleaned FASTQ
3	**Alignment**	Cleaned FASTQ + Ref	SAM File
4	**Processing**	SAM File	Sorted BAM + Index
5	**Variant Calling**	Sorted BAM + Ref	**VCF File**
6	**Reporting**	All QC Logs	**MultiQC Report**

The "From Scratch" Checklist

If you were to start a brand new project tomorrow, follow this order:

1. Initialize Git (git init).
2. Create .gitignore (Block work/ and data/).
3. Define environment.yml and activate it.
4. Set up nextflow.config with absolute paths for your system.
5. Write modules in modules/ using ${params.tool_bin} variables.
6. Construct the main logic in workflows/variant_calling.nf.
7. Call the workflow from main.nf.
8. Run with nextflow run main.nf.
9. Update README.md with setup instructions.
10. Push to GitHub.

Nextflow DSL2 Core Cheat Sheet



1. The Anatomy of a Process

Every step (module) follows this 5-part structure:

- **The container line (The Registry)**

Bioinformatics has a massive "library" of pre-made containers called **Biocontainers**, the container directive is used to link a process to a Docker image.

Where to find them: You go to biocontainers.pro and search for the tool (like fastqc or samtools).

The Pattern: Almost all of them follow this format: quay.io/biocontainers/TOOLNAME:VERSION--BUILD.

- **The publishDir line (Your Logic)**

This doesn't come from a website; **you invent it** based on how you want to organize your results.

The Logic: You ask yourself: "Where do I want the output of this specific step to go?"

The Pattern: It almost always looks like this: publishDir "${params.outdir}/FOLDER_NAME", mode: 'copy'

- **The command line**

Every step now uses the **Config-Binary Link**: Instead of writing fastqc $fastq, you write ${params.fastqc_bin} $fastq. This ensures the module pulls the path directly from your nextflow.config.

Section	Purpose	Example Syntax
Directive	Configuration	container 'URL', publishDir 'path', cpus 2
Input	What it needs	path fastq, val sample_id
Output	What it saves	path "*.bam", path "report.html"
When	Condition	when: params.run_this_step
Script	The command	""" ${params.fastqc_bin} $fastq """

2. Common Directives (The "Settings")

- **container**: Links to a Docker/Singularity image (look up on Biocontainers).
- **publishDir**: Moves files from the hidden work/ folder to a visible folder.
    - *Tip:* Always use mode: 'copy' so files don't disappear if you delete the work/ directory.
- **tag**: Adds a label to the logs (e.g., tag "$fastq").

3. Essential Channel Operators

Channels are the "pipes" that move data between processes.

- **Channel.fromPath('/path/*.fastq')**: How you get files into the pipeline.
- **.view()**: Prints the contents of a channel (great for debugging).
- **.mix()**: Combines two or more channels into one.
- **.collect()**: Waits for **all** items in a channel to finish before passing them as a single list (essential for **MultiQC**).

4. The Workflow Block (main.nf)

This is where you "glue" the modules together.
Do not put the workflow {} logic in main.nf.
main.nf should only contain the include statement and the trigger.

Code snippet

```
include { MY_PIPELINE }  from './worklfows/workflow.nf'

workflow {
   fastq_ch = Channel.fromPath(params.fastq, checkIfExists: true)
   ref_ch   = Channel.fromPath(params.ref, checkIfExists: true)

   MY_PIPELINE(fastq_ch, ref_ch)
}
```

5. Exam Troubleshooting Logic

If your pipeline fails, check these three things:

1. **Paths:** Did you provide the right path to the file?
2. **Wildcards:** Did you use * in the output: block to catch the files?
3. **Commas:** Did you forget a comma in the publishDir or input section?

6. Helpful Linux Commands for Nextflow

- nextflow run main.nf -resume: Runs the pipeline skipping finished steps.
- nextflow log: Shows a list of your previous runs.
- nextflow clean -f -q: Cleans up the heavy work/ directory to save space.