# Introduction to Snakemake

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# Lesson 1

# Lesson 1

1. Why Snakemake?
2. Introducing Workflows
3. Workflow Syntax
4. Running Snakemake
5. Assignment 1

Preparation for Assignment 1:

```
sbatch -c 1 --mem 4G -J conda --wrap \
"conda create -n prokka -c conda-forge -c bioconda prokka"

sbatch -c 1 --mem 4G -J conda --wrap \
"conda create -n pirate -c bioconda -c conda-forge pirate"
```

# Why Snakemake?

**Automation**

Reproducibility

Others' Snakefiles

Front-loading your effort.

Modest investment at the beginning of a project yields a hands-off tool for performing routine analyses.

Snakemake will only run what it needs to - will only evaluate what changes.

# Why Snakemake?

Automation

**Reproducibility**

Others' Snakefiles

Guarantee that the same inputs will give the same outputs.

Altering an input will make Snakemake re-evalutate any outputs that depend upon it.

Built-in version tracking.

# Why Snakemake?

Automation

Reproducibility

**Others' Snakefiles**

Understand and modify the tools others have created for you.

# Baking Cookies

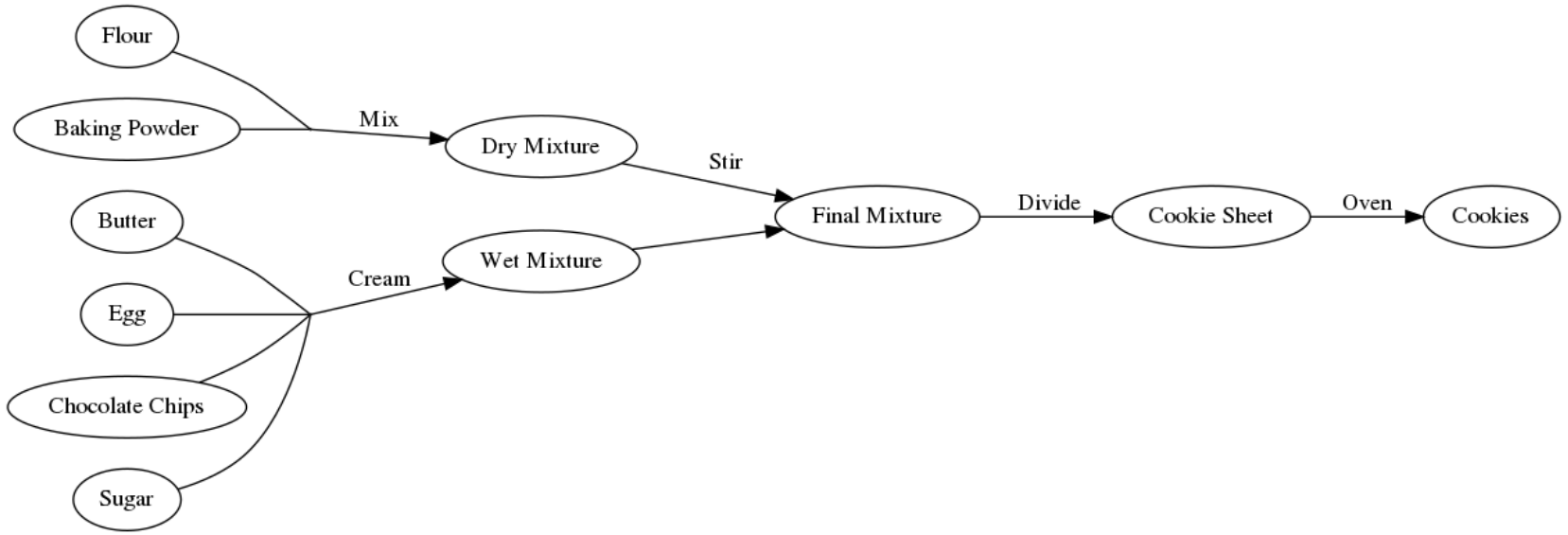
Procsilas Moscas - Wikimedia Commons

# Baking Cookies by Following a "Script"

1. *Mix* **flour** & **baking powder** in a bowl
2. *Cream* **butter**, **egg**, **chocolate chips**, and **sugar** in another bowl
3. *Stir* the **two bowls** together
4. *Divide* **dough blobs** onto a cookie sheet
5. Bake in an *oven* to get **cookies**

- We can follow a linear recipe start-to-finish
- Imagine a robot following these steps
  - Follows instructions, but can only run in sequence
  - If the eggs were spoiled, it would get new eggs and start from the very beginning

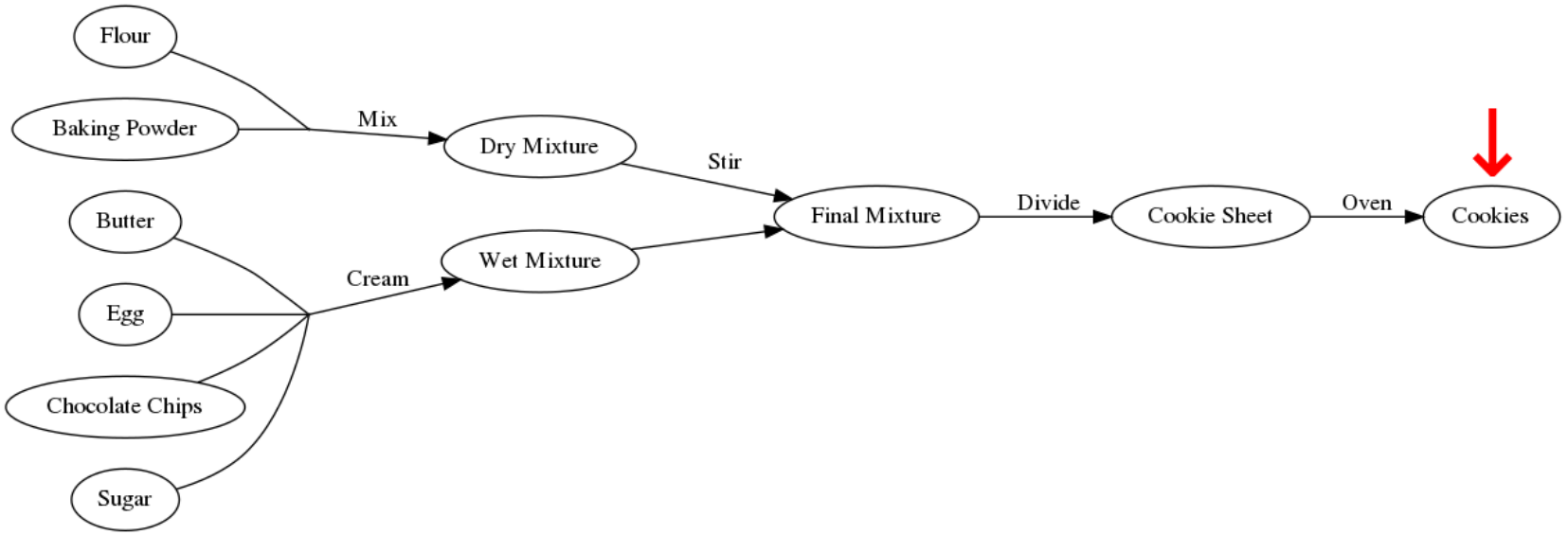
# Baking with Graphs



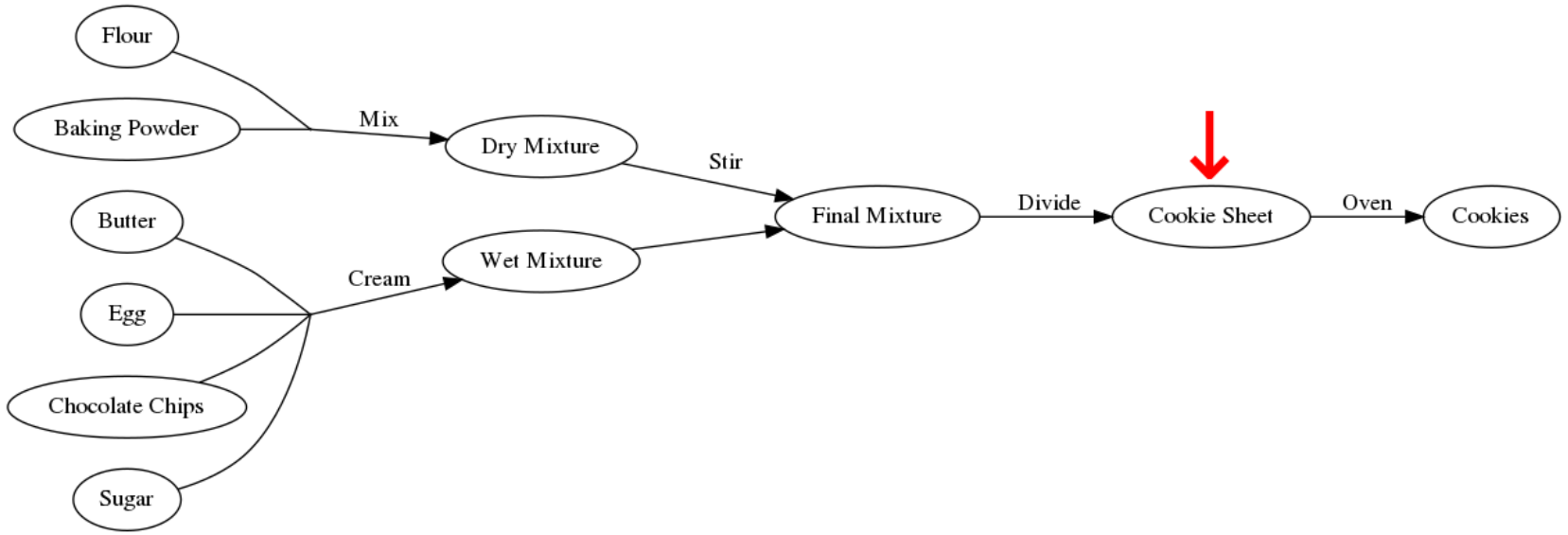
# Thinking Backwards

- Snakemake lessons/figures out how to achieve the desired result:
  - starts at the final product
  - works backwards until it finds what it needs
- A **collection of relationships** not a **sequence of instructions**
- You tell it how to convert each input to each output

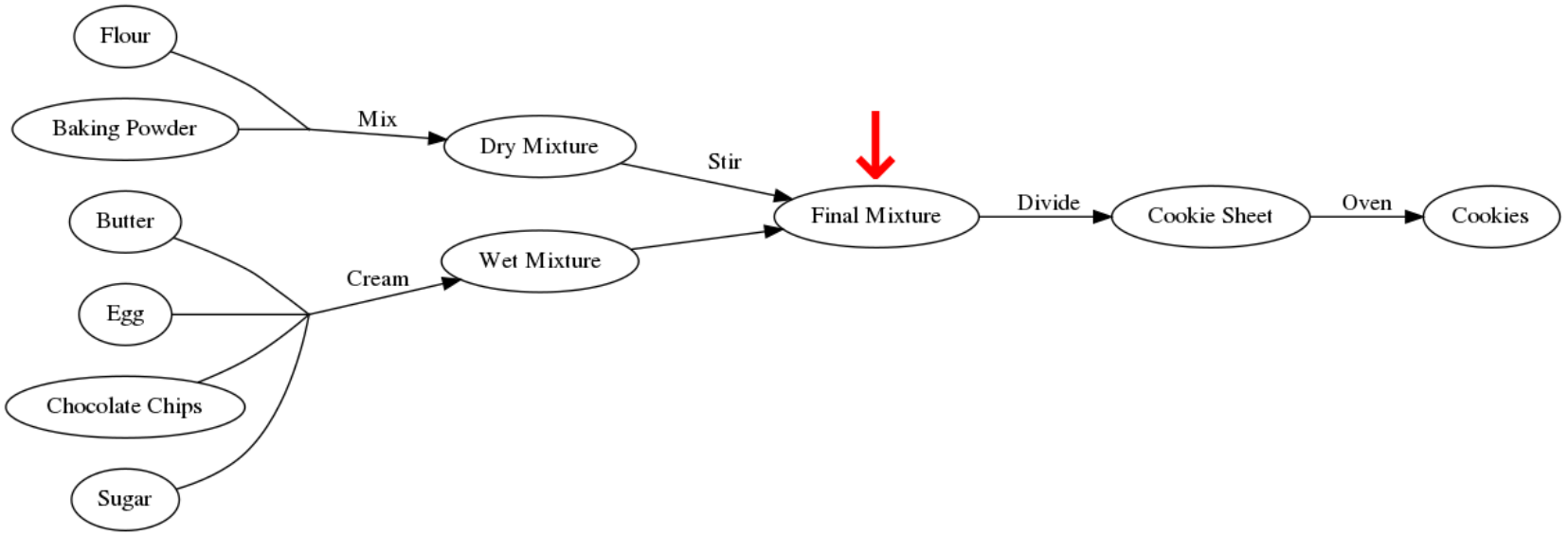
# Thinking Backwards



# Thinking Backwards



# Thinking Backwards



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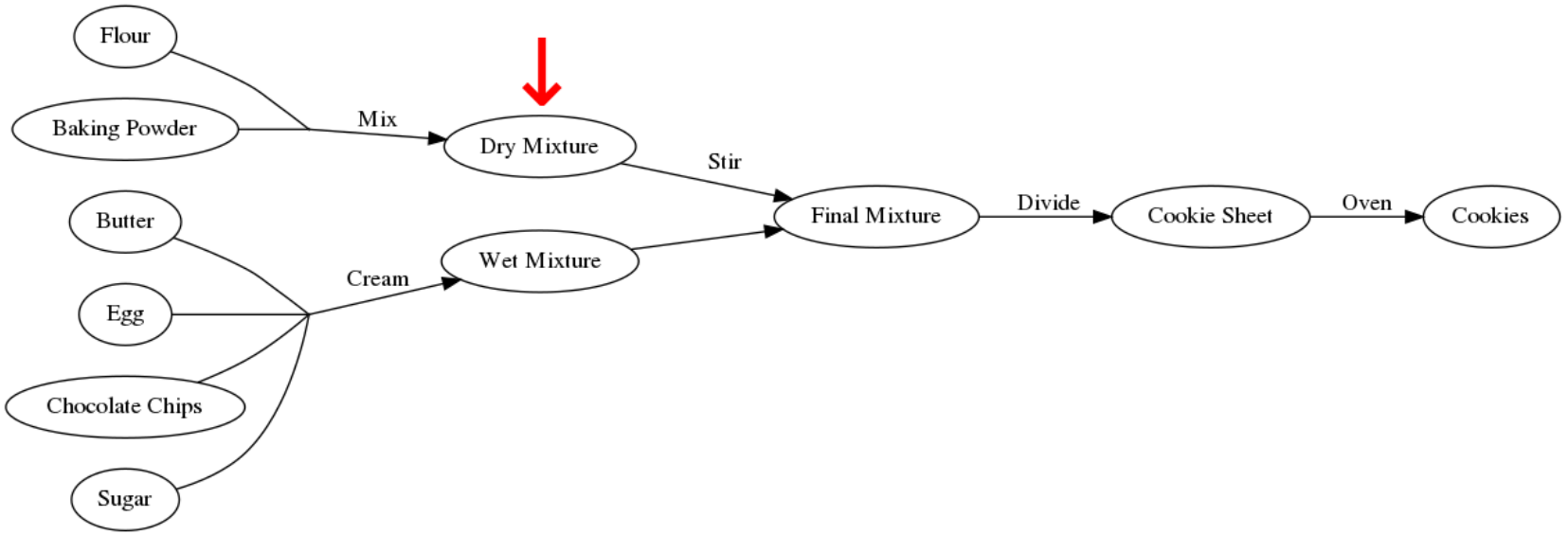

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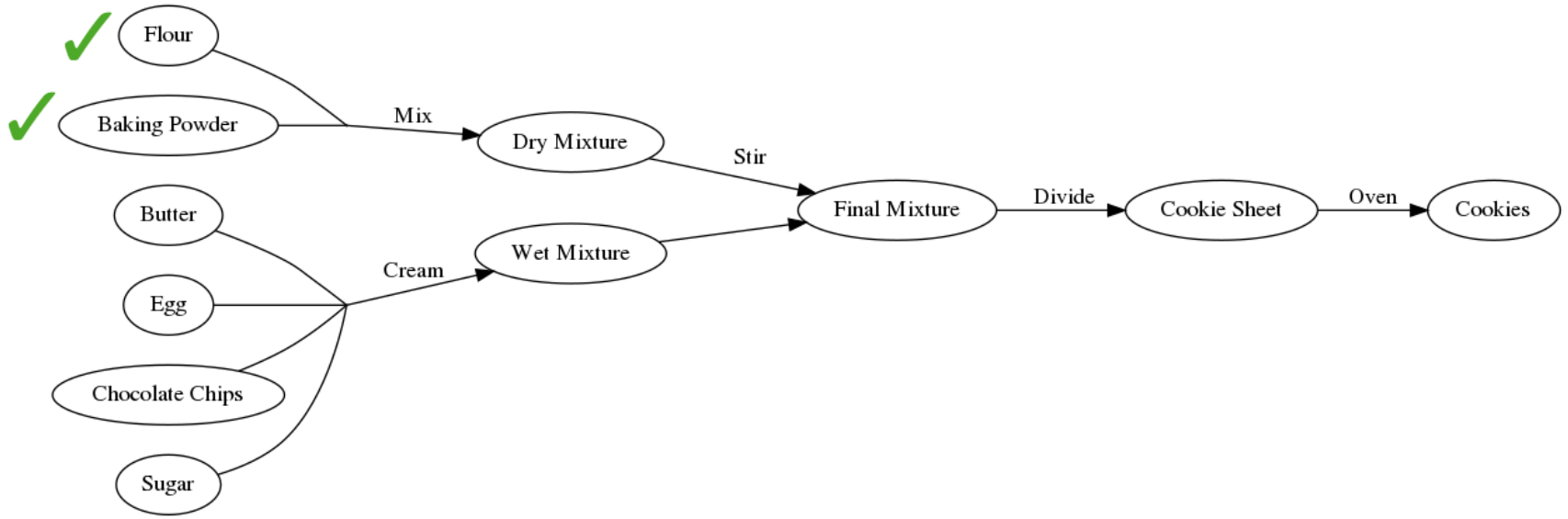
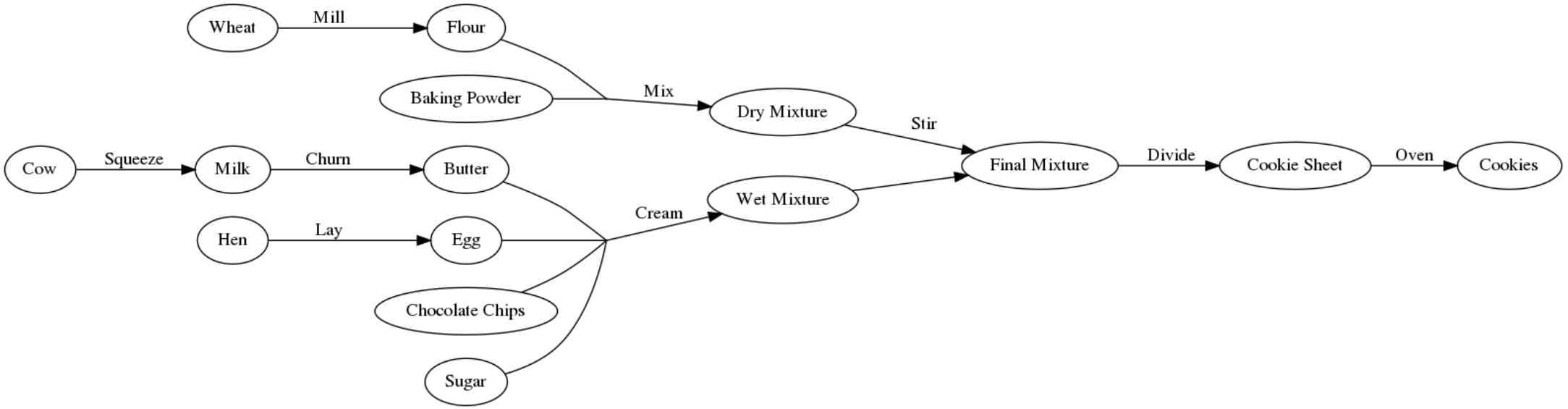


# Thinking Backwards



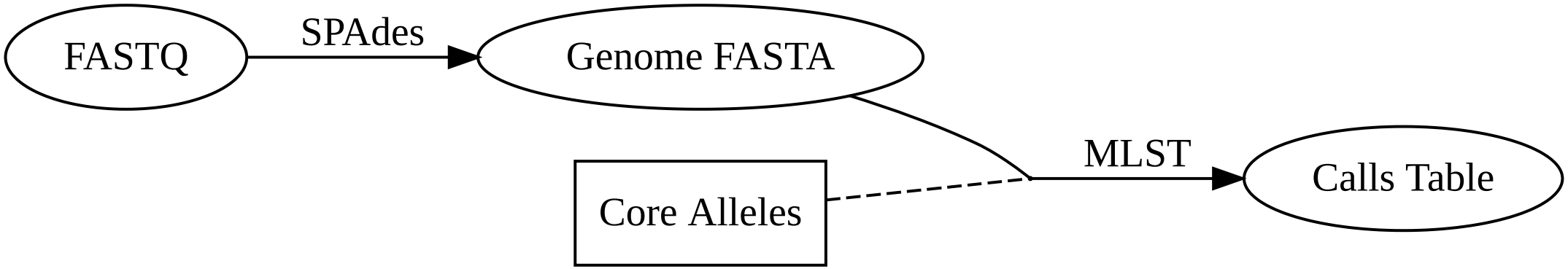
# Rules for Baking

```
rule all:
    input: "cookies"

rule bake_cookies:
    input: "pan/dough.blobs"
    output: "cookies"
    shell: "oven -i {input} -o {output} --temp 350 --time 15"

rule apportion_dough_blobs:
    input: "bowls/final.mix"
    output: "pan/dough.blobs"
    shell: "scoop -n 24 {input} > {output}"

rule combine_bowls:
    input: wet="bowls/wet.mix", dry="bowls/dry.mix"
    output: "bowls/final.mix"
    shell: "mixer {input.wet} {input.dry} > {output}"
```

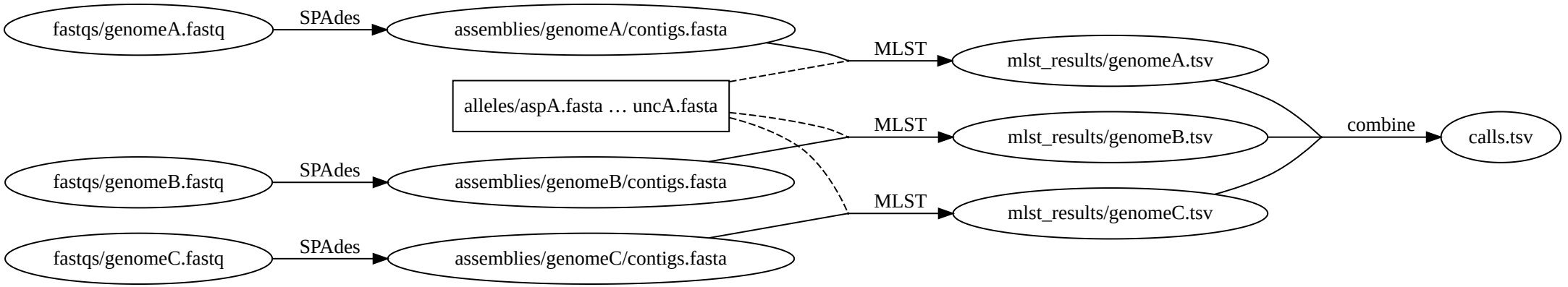
# Rules for Baking

- The `all` rule is the final target, and written first
- Each rule specifies its `input` and `output`
- The `shell` command defines how the `input` becomes the `output`
- Snakemake matches up `input` and `output` for all the rules until it can generate `all`

# Baking → Bioinformatics



# Multiple Samples



# Multiple Samples

- Recall that rules are only executed if their inputs update or outputs are missing
- Lets you run jobs without worrying you'll waste effort

## MLST Example from Above

1. If you have 1000 genomes and run the above MLST rule, it will calculate 1000 calls, and build the calls table
2. If you add 1 more genome, and rerun snakemake, only a single MLST will run, and the calls table is rebuilt

# Wildcards

- We can match every file with particular naming pattern with wildcards
- In a rule, wrap a variable name with curly braces
  - *e.g.* `{sample}`
- Rule is applied in parallel to each file matching the rule
- In `shell` block, you can access these when preceded by `wildcards`
  - *e.g.* `{wildcards.sample}`

# Expanding patterns

- The `expand()` function can be useful for taking a pattern and using it to get many files matching that pattern

```
# get sample names from starting fastas:
# genomes/foo.fasta, genome/bar.fasta, genome/baz.fasta
from pathlib import Path
samples = [p.stem for p in Path("genomes").glob("*.fasta")]
# samples = ["foo", "bar", "baz"]

rule all:
    input: "aggregated_results.txt"

rule process_single_genome:
    input: "genomes/{sample}.fasta"
    output: "results/{sample}.txt"
    shell: "frobnicate {input} > {output}"

rule takes_many_files:
    input: expand("results/{sample}.txt", sample=samples)
    output: "aggregated_results.txt"
```

# Rules for Multiple Samples

```
from pathlib import Path
sample_names = [fq.stem for fq in Path("fastqs").glob("*")]

rule all:
    input: "calls.tsv"

rule assemble:
    input:
        fwd="fastqs/{sample}/{sample}_1.fastq", rev="fastqs/{sample}/{sample}_2.fastq"
    output: "assemblies/{sample}/contigs.fasta"
    shell: "spades -1 {input.fwd} -2 {input.rev} -o assemblies/{wildcards.sample}"

rule get_sequence_types:
    input: "assemblies/{sample}/contigs.fasta"
    output: "mlst_results/{sample}.tsv"
    shell: "mlst --scheme campylobacter {input} > {output}"

rule combine_mlst_results:
    input: expand("mlst_results/{sample}.tsv", sample=sample_names)
    output: "calls.tsv"
    shell: "cat {input} > {output}"
```

# Threads

- Many (but not all!) bioinformatics tools use multiple CPU threads
- `threads` directive defaults to `1`
  - Accessible in the `shell` block, similar to `input` and `output`
    - `{threads}`

```
rule annotate_genome:
    input: "genomes/{sample}.fasta"
    output: "annotations/{sample}/{sample}.gff"
    threads: 8
    shell:
        "prokka --force --prefix {wildcards.sample} "
        "--cpus {threads} -o annotations/{wildcards.sample} {input}"
```

You can split the `shell` block over multiple lines. Leave a space at the end of each chunk!

# Caveats and Assumptions

## Directory Structure

- Snakefiles themselves can live anywhere
  - I keep mine in `~/snakefiles/`
- Workflows tightly coupled to its directory structure
  - The structure applies to the specified project directory
- Will implicitly create any directories it needs
  - No need for `mkdir`

# Directory Structure

This rule...

```
rule annotate_genome:
    input: "genomes/{sample}.fasta"
    output: "annotations/{sample}/{sample}.gff"
    threads: 8
    shell:
        "prokka --force --prefix {wildcards.sample} "
        "--cpus {threads} -o annotations/{wildcards.sample} {input}"
```

*requires* this structure:

```
analysis/
├── genomes/
│   ├── isolateA.fasta
│   ├── isolateB.fasta
│   └── isolateC.fasta
└── annotations/
    ├── isolateA.fasta
    ├── ...
```

# Caveats and Assumptions

## Independent Jobs

- Failure of *any* job will abort *all* other jobs
  - Default behaviour
- Override with `--keep-going`
  - Dependent jobs will still await all inputs
  - *e.g.* if stiring the dry cookie mixture fails, the wet mixture still gets made, but nothing goes in the oven
  - (I usually do this for routine work)

# Running Snakemake (Basic)

The **basic invocation** of Snakemake:

```
snakemake --jobs <number of parallel jobs> -s <path to your Snakefile> -d <work directory>
```

Example populated with real values:

```
snakemake --jobs 5 -s ~/snakefiles/assemble.smk -d ~/Projects/cj_population_study
```

# Running Snakemake on Waffles

- Snakemake can be run on HPCs like **Waffles**
    - *Must* be combined with Slurm if you're on Waffles
    - *Don't run it on the head node!*

## Two Parts for Cluster Submission

1. Tell Snakemake how to submit jobs with `--cluster`
2. Submit `snakemake` itself as a Slurm job

# Running Snakemake on Waffles

The `--cluster` argument:

- Create a template to pass to **Slurm**
- May access Snakemake special variables like `{threads}`
    - **More on this next lesson**

```
--cluster 'sbatch -c {threads} --mem 12G --partition NMLResearch '
```

# Running Snakemake on Waffles

Submitting the Snakemake job to Slurm:

```
sbatch -c 1 --mem 4G --wrap "snakemake --jobs 5 -s
~/snakefiles/assemble.smk -d ~/Projects/cj_population_study --cluster
'sbatch -c {threads} --mem 12G --partition NMLResearch '"
```

# Assignment 1

## Get The Test Data on Waffles

```
cp -r /Drives/W/Projects/CampyLab/snakemake-intro-data/ ~/snakemake-intro-data

# Or if you don't have access to Projects
cp -r /Drives/W/Temporary/snakemake-intro-data/ ~/snakemake-intro-data
```

## If You're Not Using Waffles

```
url="https://github.com/dorbarker/snakemake-intro/blob/main/data/snakemake-intro-data.zip"
fn="$HOME/snakemake-intro-data.zip"

curl -o $fn $url || wget -O $fn $url
unzip $fn
```

# Assignment 1

Write a Snakemake workflow that does the following:

1. Run Prokka on each genome
2. Symlink GFF annotations into `gffs/`
3. Build a pangenome with PIRATE

```
conda activate prokka
conda activate --stack pirate
```

Everything you need is in today's presentation:

https://github.com/dorbarker/snakemake-intro/blob/main/lessons/snakemake-intro-lesson-1.pdf

# Assignment 1 Hints

- Invoking PIRATE to generate nucleotide-based pangenome:
    - `PIRATE --input gffs/ --output pangenome/ --nucl --threads {threads}`
- PIRATE produces many files, but you can rely on `PIRATE.gene_families.tsv` being created
- You'll need to `expand()` your inputs to make sure all the GFF files are present