Introduction to Snakemake

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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 --wrap \
"conda create -n smk-lesson-1 -c conda-forge -c bioconda prokka 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.

Why Snakemake?

Automation

Reproducibility

Others' Snakefiles

Guarantee that the same inputs will give the same outputs.

Altering any input will make Snakemake re-evalutate the outputs.

Built-in version tracking.

Why Snakemake?

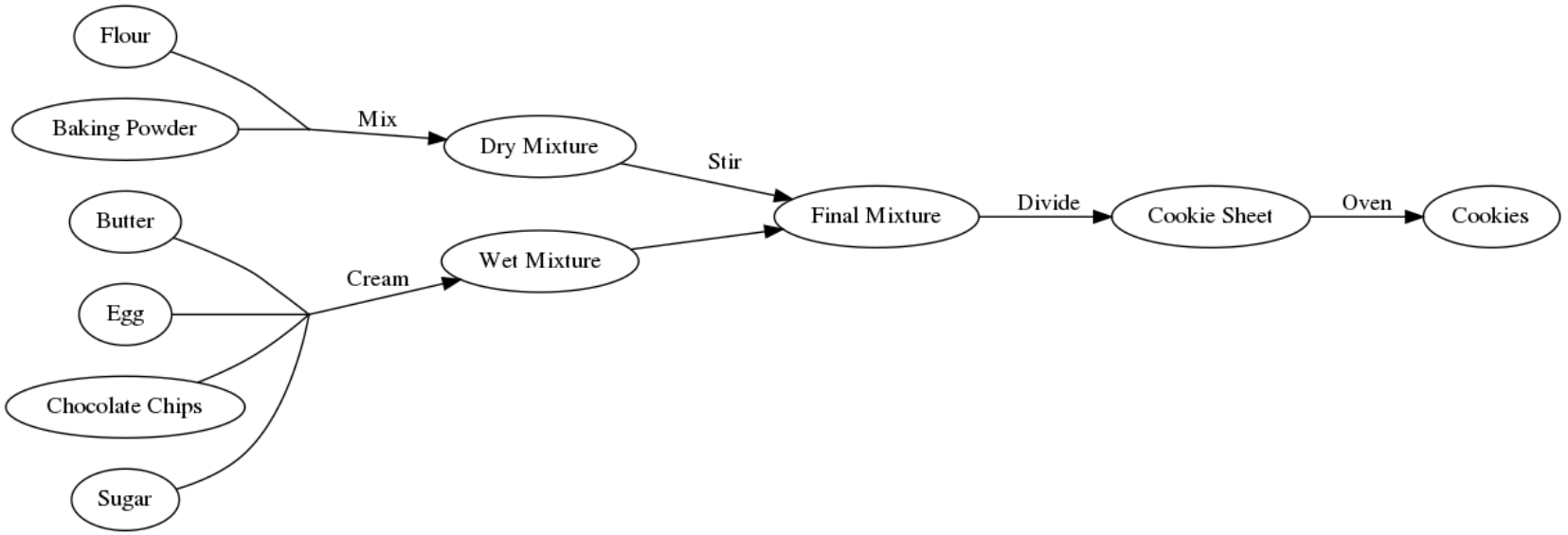
Automation

Reproducibility

Others' Snakefiles

Understand and modify the tools others have created for you.

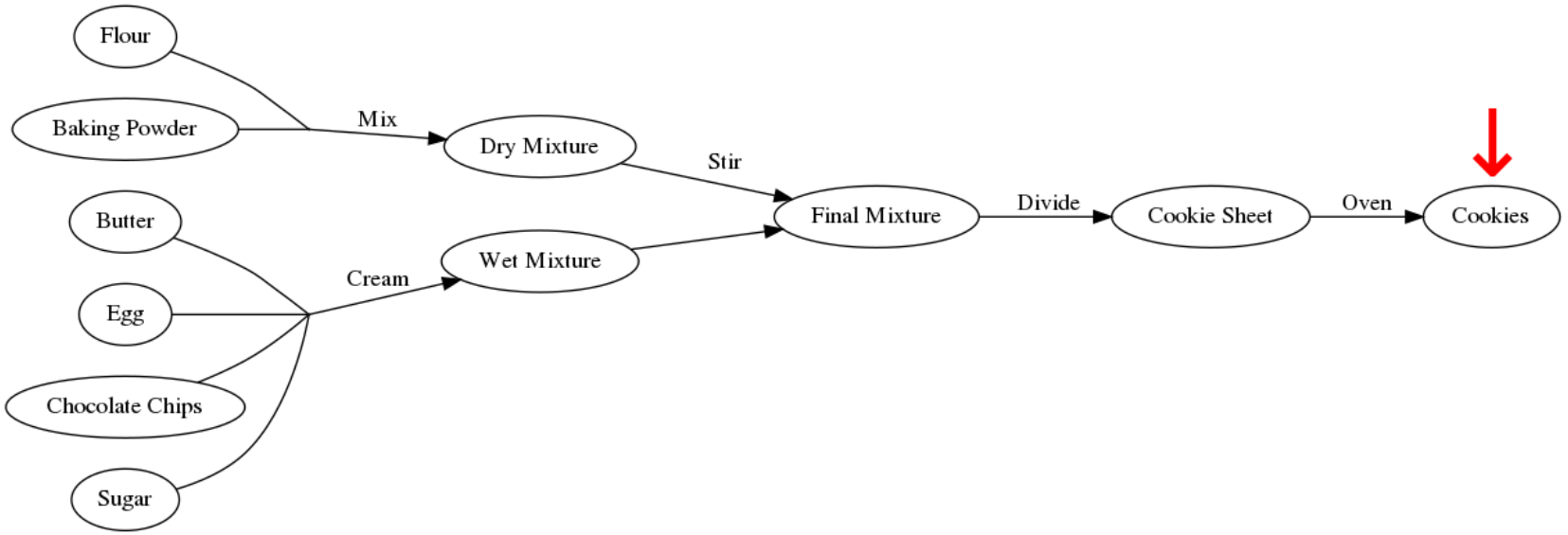
Baking with Graphs



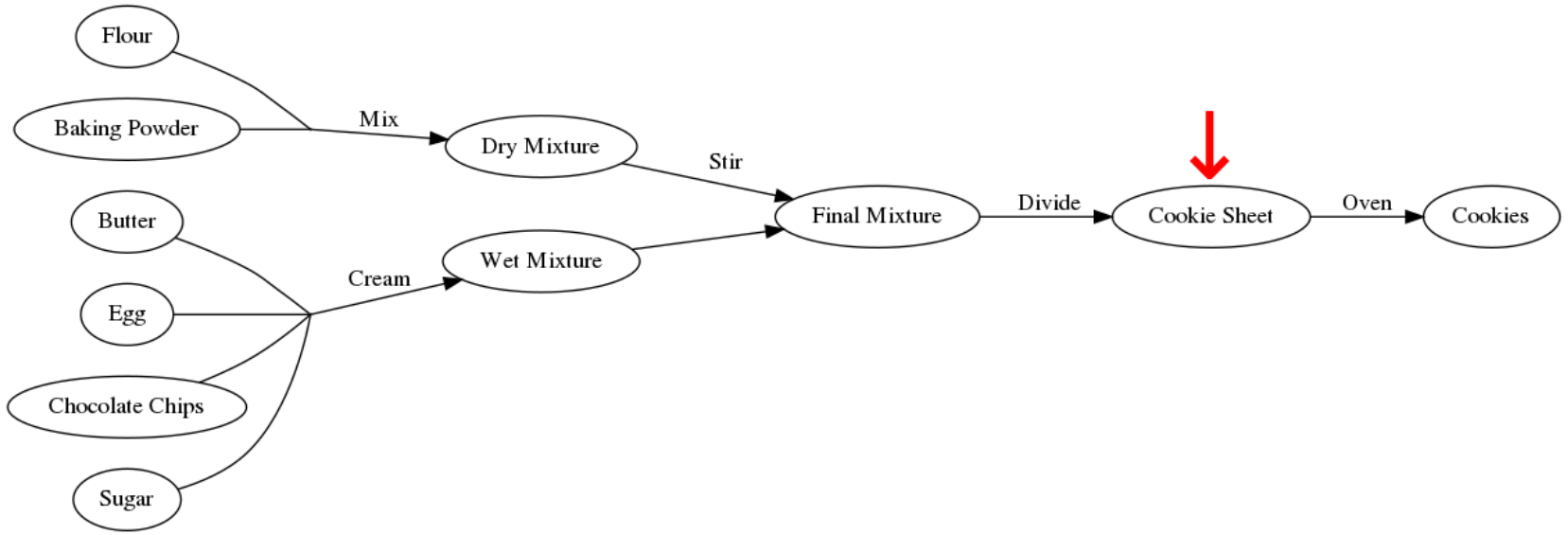
Thinking Backwards

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

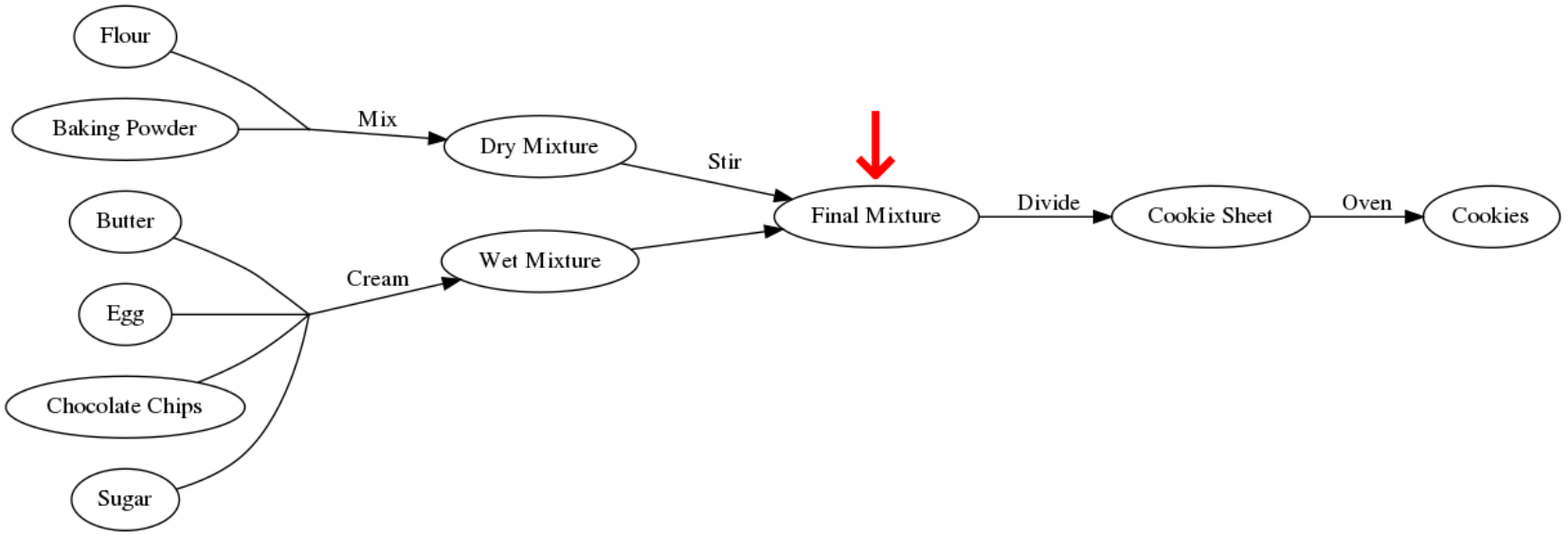
Thinking Backwards



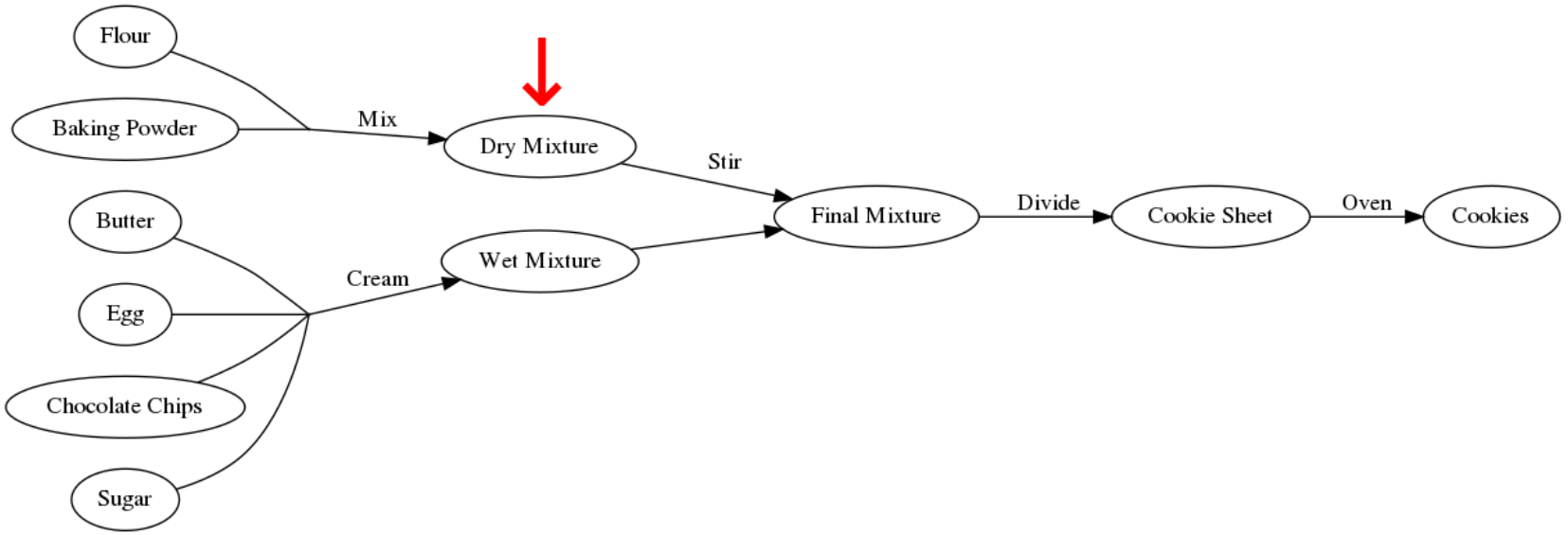
Thinking Backwards



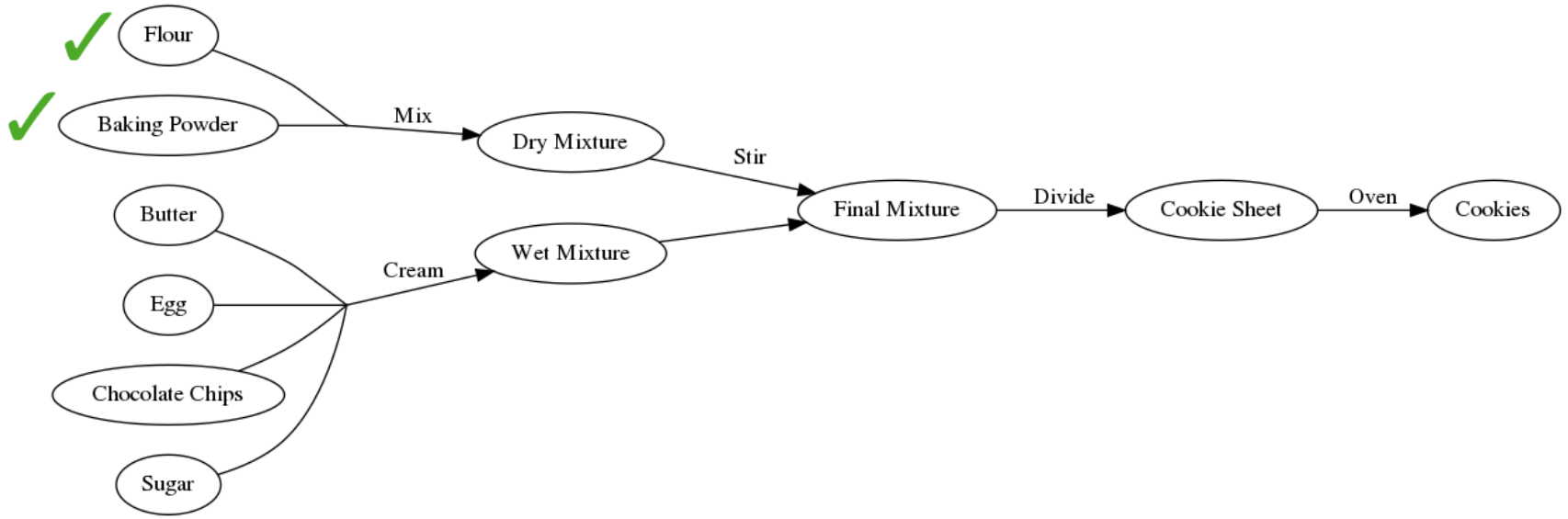
Thinking Backwards



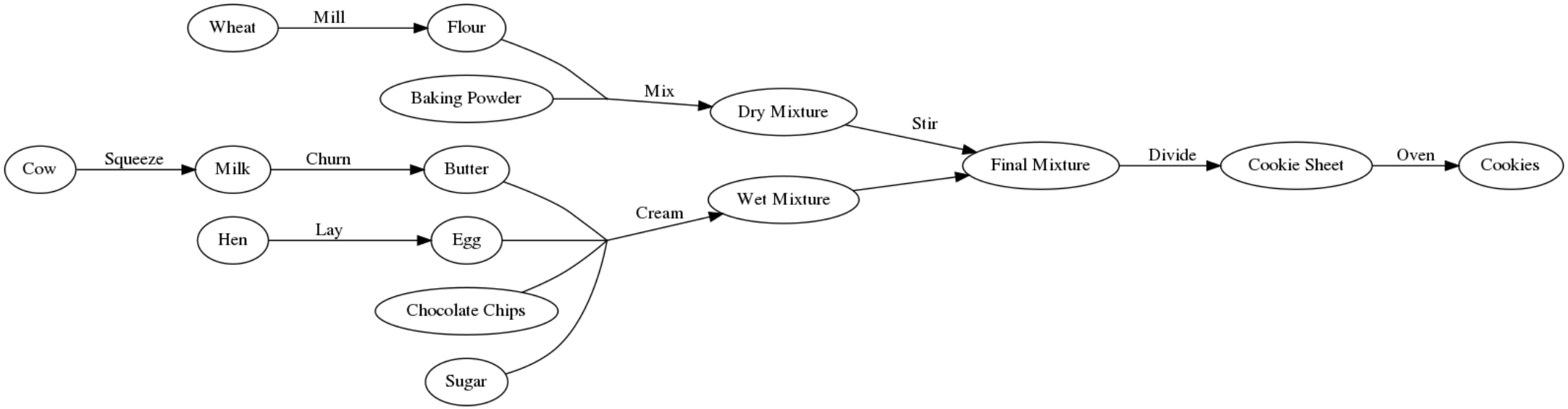
Thinking Backwards



Thinking Backwards



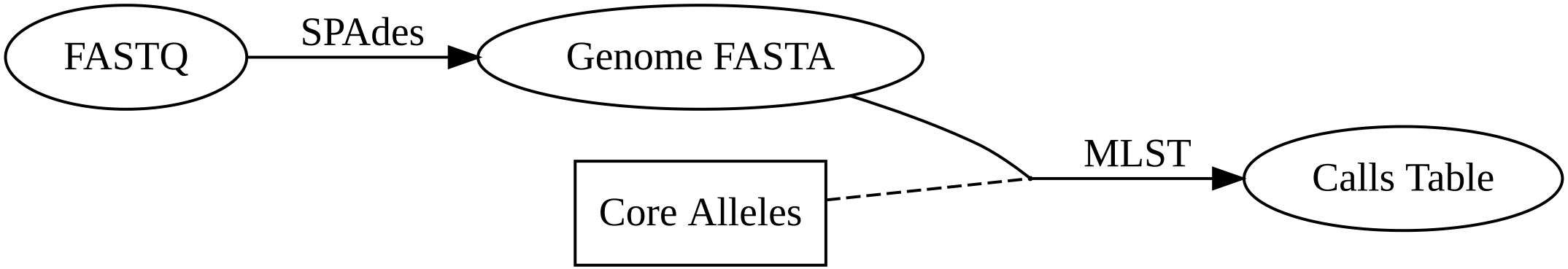
Thinking Backwards



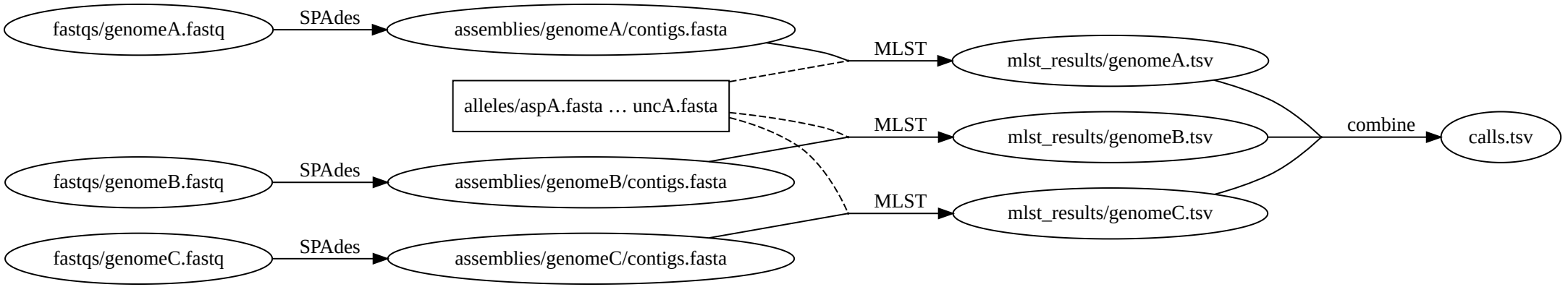
Rules for Baking

```
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}"
```

Baking → Bioinformatics



Multiple Samples



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 will be applied in parallel to each file matching the rule
- In `shell` block, you can access these when preceded by `wildcards`
  - *e.g.* `{wildcards.sample}`

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 --cpus {threads} -o annotations/{wildcards.sample} {input}"
```

Caveats and Assumptions

Directory Structure

- Workflows tightly coupled to any directory structure described within
- Will implicitly create any directories it needs
  - No need for `mkdir`

Independent Jobs

- Failure of any job will abort all other jobs
  - 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

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
  - *Don't run it on the head node!*
- Two parts:
  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 command to pass to **Slurm**
- May access Snakemake special variables like `{threads}`
  - **More on this later**

```
--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

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 smk-lesson-1
```

Lesson 2

Lesson 2

1. Assignment 1 Answers
2. Conda Integration
3. Params and Threads
4. Mixing in Python (and R and Julia)
5. Assignment 2

Assignment 1

Conda Integration

- Snakemake can manage conda directly
- No need to manually build or activate conda environments

Conda directive

```
rule annotate_genome:
    input: "genomes/{sample}.fasta"
    output: "annotations/{sample}/{sample}.gff"
    conda: "envs/prokka.yaml"
    shell: "prokka --cpus {threads} -o annotations/{wildcards.sample} {input}"
```

Conda YAML files

- Placed **relative to the Snakefile**, *not* the project directory

```
# annotate.smk
rule annotate_genome:
    input: "genomes/{sample}.fasta"
    output: "annotations/{sample}/{sample}.gff"
    conda: "envs/prokka.yaml"
    shell: "prokka --cpus {threads} -o annotations/{wildcards.sample} {input}"
```

The above will look for the following directory structure:

```
snakefiles/
├── annotate.smk
└── envs
    └── prokka.yaml
```

Conda YAML files

This YAML file ...

```
name: prokka
channels:
    - conda-forge
    - bioconda
    - defaults
dependencies:
    - prokka
```

... is equivalent to this conda command:

```
conda create -n prokka -c conda-forge -c bioconda -c defaults prokka
```

Using Conda Directives with Snakemake

- Must explicitly tell Snakemake to use Conda

```
snakemake --use-conda <…>
```

- Automatic installation and activation

Config

Configuration is possible through `config`

- Python `dict` available within the Snakefile
- Available through two methods
  - `--config` passes arguments directly via command line
  - `--configfile` points to a YAML file that provides values

`--config "key=value"` is equivalent to `--configfile config.yaml` where...

```
# config.yaml
key: "value"
```

Configuration via:

`--config` flag:

- ↓ effort
- ↑ flexible
- ↓ reproducible

YAML file:

- ↑ effort
- ↓ flexible
- ↑ reproducible

Params

- Non-file parameters may be provided in the `params` directive

Abusing Params to Fine-tune Resources

```
snakemake <…> --cluster 'sbatch -c {threads} --mem {params.mem} --time {params.time} '
```

```
rule annotate_genome:
    input: "genomes/{sample}.fasta"
    output: "annotations/{sample}/{sample}.gff"
    threads: 8
    params:
        time="45:00",
        mem="16G"
    shell: "prokka --cpus {threads} -o annotations/{wildcards.sample} {input}"

rule symlink_gff:
    input: "annotations/{sample}/{sample}.gff"
    output: "gffs/{sample}.gff"
    threads: 1
    params:
        time="01:00",
        mem="100M"
    shell: "ln -sr {input} {output}"
```

Config vs Params

- Params are fairly "fixed"
  - Used primarily to simplify `shell` block
- Config for run-specific information
  - *e.g.* providing a particular host database to `kat` or training file to `chewBBACA`

Mixing in Python

- Python may be mixed in arbitrarily into Snakemake
  - *i.e.* All Python is valid Snakemake
- Two main ways of using Python in Snakemake
  - `run` blocks
  - Python used directly in the Snakemake file

Python → Snakemake, get it?

Run blocks

- `run` blocks can be used in place of `shell` blocks
- Write Python inside the `run` block, rather than Bash in a `shell` block
- May access snakemake values like `input` and `output`

```
rule transpose_table:
    input: "data/results_table.csv"
    output: "data/results_table_transposed.csv"
    run:
        import pandas as pd
        original = pd.read_csv(input[0], header=0)
        transposed = original.transpose()
        transposed.to_csv(output[0], header=False)
```

Directly Using Python in Snakemake

- You can directly use Python in Snakemake
- Particularly useful for handling cases where a rule generates variable output
  - *e.g.* The number of gene FASTAs generated by a pangenome analysis
- Can provide a Python function to `input` instead of a file pattern

- `select_high_quality_genomes` takes a list of FASTAs, then symlinks high-quality ones into `./good_genomes/` and writes a report called `quality_report.txt`
- We don't know in advance which genomes will pass QC, so we need an input function

```
rule quality_filter_genomes:
    input: expand("genomes/{sample}.fasta", sample=samples)
    output: "quality_report.txt"
    shell: "select_high_quality_genomes {input} > {output}"

# input functions need to take parameter `wildcards`
def collect_good_genome_sample_names(wildcards):
    good_genomes = Path("good_genomes/").glob("*.fasta")
    return list(good_genomes)

# use the report as a dummy input to make sure quality_filter_genomes executes
rule run_abricate:
    input: report="quality_report.txt", fastas=collect_good_genome_sample_names
    output: "amr_results.tsv"
    shell: "abricate {input.fastas} > {output}"
```

Assignment 2

- Build upon Assignment 1

1. Create conda YAMLs for `prokka` and `pirate`
2. Give appropriate resources to each rule with `params`
3. Write a rule with a `run` block that reads `PIRATE.gene_families.tsv`, finds loci present in 100% of genomes, and writes their names to a text file
    - columns of interest: `gene_family` & `number_genomes`
4. A rule that:
    - Uses an input function reads selected genes from the text file in **Part 3**
    - Symlinks these into a directory called `loci`
        - Either `shell` or `run` at your preference

Assignment 2 Hints

`pandas` for easily reading tabular files

```
import pandas as pd
data_table = pd.read_csv(input[0], sep = "\t")
# select rows from columnA where columnC is greater than 42
selected_rows = data_table["columnC"] > 42
selected_columnA = data_table["columnA"].loc[selected_rows]
```

Creating symlinks from a list of file basenames

```
# list_of_names = ["larry", "moe", "curly"]
import os
for name in list_of_names:
    src = f"originals/{name}.txt"
    dst = f"filtered/{name}.txt"
    os.symlink(src, dst)
```

Assignment 2 Hints

Reading a text file into a list with Python

- Consider combining functions like this with `expand()`
  - `expand("path/to/{sample}.txt", sample=read_list_to_list())`

```
def read_lines_to_list(path: str):
    lines = []
    with open(path, "r") as f:
        for line in f:
            trimmed_line = line.strip()
            lines.append(trimmed_line)
    return lines
```

Reading a text file, but using at as an input function

```
def aggregate_files(path: str):
    lines = []
    with open(path, "r") as f:
        for line in f:
            trimmed_line = line.strip()
            lines.append(trimmed_line)
    lines_with_paths = [f"path/to/{sample}.txt" for sample in lines]
    return lines_with_paths
```

```
rule do_something:
    input: aggregate_files
    output: "somefile.txt"
    shell: "my_program {input} -o {output}"
```