Creating supermatrices and phylogenetic datasets with SuperCRUNCH

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Phylogenetic Data Workflows

Handful of tools available for assembling phylogenetic data

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Each has great features!

PhyLoTa	(Sanderson et al. 2008)
PHLAWD	(Smith et al. 2009)
phyloGenerator	(Pearse & Purvis 2013)
SUMAC	(Freyman, 2015)
SUPERSMART	(Antonelli et al. 2017)
PhylotaR	(Bennett et al. 2018)
PyPHLAWD	(Smith & Walker 2018)

Phylogenetic Data Workflows

Common limitations for my work needs

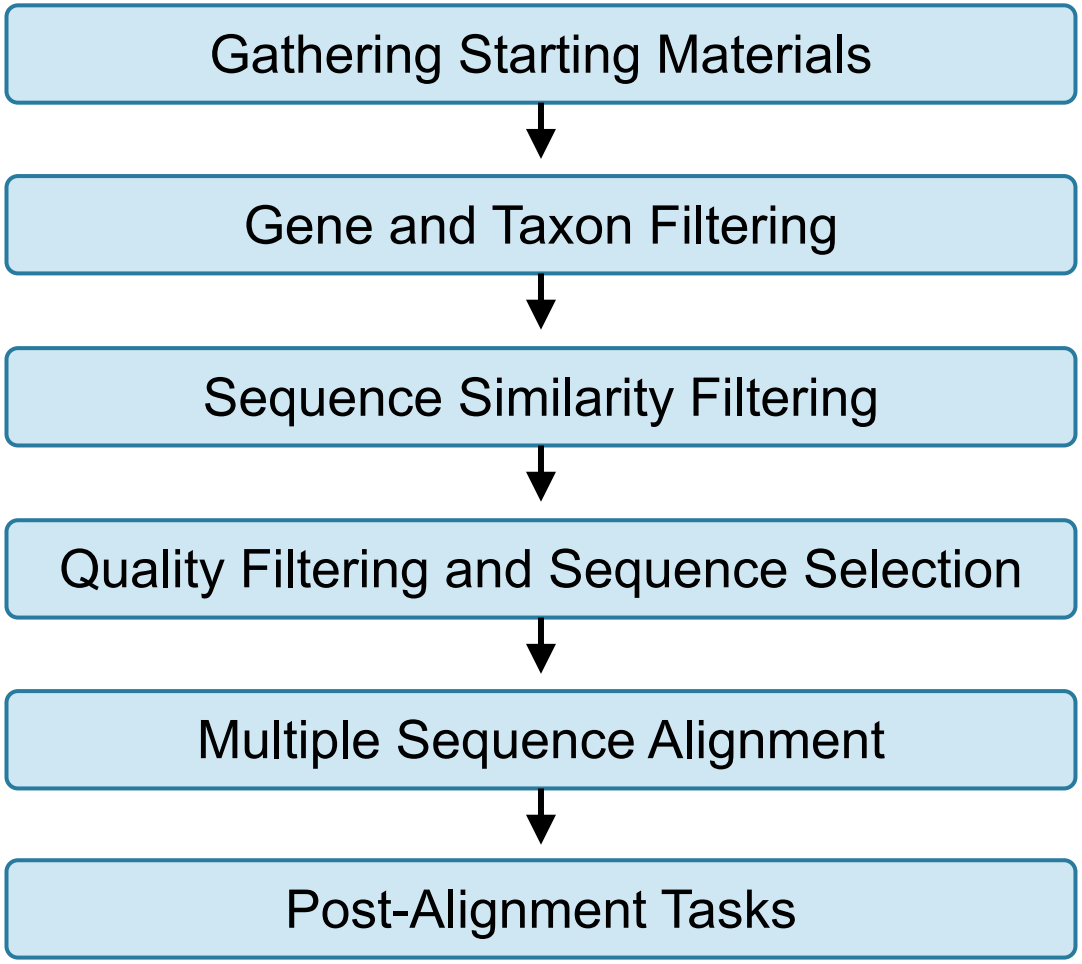
- Sequences fetched using GenBank release database
- Reliance on NCBI Taxonomy for identifying sequences
- Automated clustering of whole sequence sets
- Black-box methods for sequence filtering and selection

SuperCRUNCH

FOR PHYLOGENETIC DATA

- Bioinformatic toolkit for phylogenetic data
- Allows ANY fasta-formatted sequence data to be used
- Targeted searches based on list of taxa and genes
- Transparent methods for orthology-filtering and sequence selection, including detailed output files
- Can be used to assemble traditional supermatrices as well as population-level datasets
- Can process large sequence capture datasets (UCE's)

SuperCRUNCH Workflow



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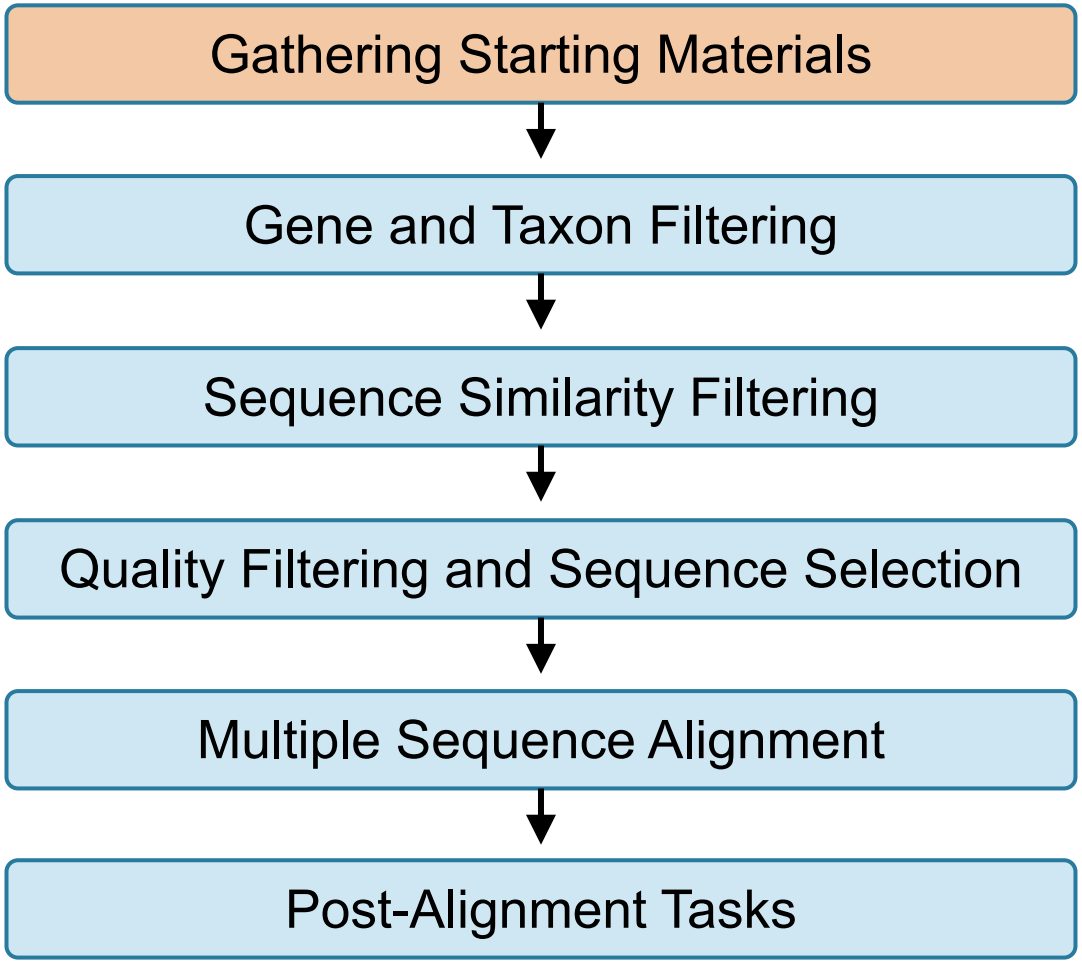

Gathering Starting Materials

Sequence Data

Taxon List

Gene List

Gathering Starting Materials

Sequence Data | Taxon List | Gene List

NCBI/GenBank sequences and/or Locally generated sequences

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Fasta format:

```
>KP820543.1 Callisaurus draconoides voucher R45 aryl hydrocarbon
receptor (anr) gene, partial cds
CACAATGAGAAAGCCTTGATAAACCGTGATCGGACTTTGCCACTCGTTGAAGAAATAGATGAGAGCT…

>KP820544.1 Urosaurus ornatus voucher UWBM7587 aryl hydrocarbon
receptor (anr) gene, partial cds
TAAAATCTCCTTTGAAAGGAACCTTTTTGTGGACACCAGGGATGAATTAGGTAATGTAATGGCCAGA…
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Gathering Starting Materials

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Fasta format: > **Unique Identifier** **Taxon Label** **Description**

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Gathering Starting Materials

Sequence Data | Taxon List | Gene List

Text file with species and subspecies labels

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Callisaurus draconoides draconoides
Callisaurus draconoides inusitanus
Cophosaurus texanus
Holbrookia maculata
Uma exsul
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List Sources

- Taxonomic Databases
- NCBI Taxonomy Browser
- Directly from sequences (via SuperCRUNCH)

Gathering Starting Materials

Sequence Data | Taxon List | Gene List

Tab-delimited text file with search terms

- Search terms include a gene abbreviation and description
- Can include any number and combination of genes (mtDNA, nuclear, seq-cap)
- Tetrapod 5k UCE set and other example files on github

Gathering Starting Materials

Sequence Data | Taxon List | **Gene List**

Tab-delimited text file with search terms

Label	Abbreviation	Description
CMOS	CMOS;C-MOS	oocyte maturation factor
CXCR4	CXCR4	chemokine C-X-C motif receptor 4;C-X-C …
DLL1	DLL1;DLL	distal-less
DNAH3	DNAH3	dynein axonemal heavy chain 3;dynein ax…
ECEL1	ECEL1;ECEL	endothelin converting;endothelin conver…
ENC1	ENC1	ectodermal neural cortex 1
EXPH5	EXPH5	exophilin;exophilin 5;exophilin-5;exoph…
FSHR	FSHR	follicle stimulating hormone;follicle-s…

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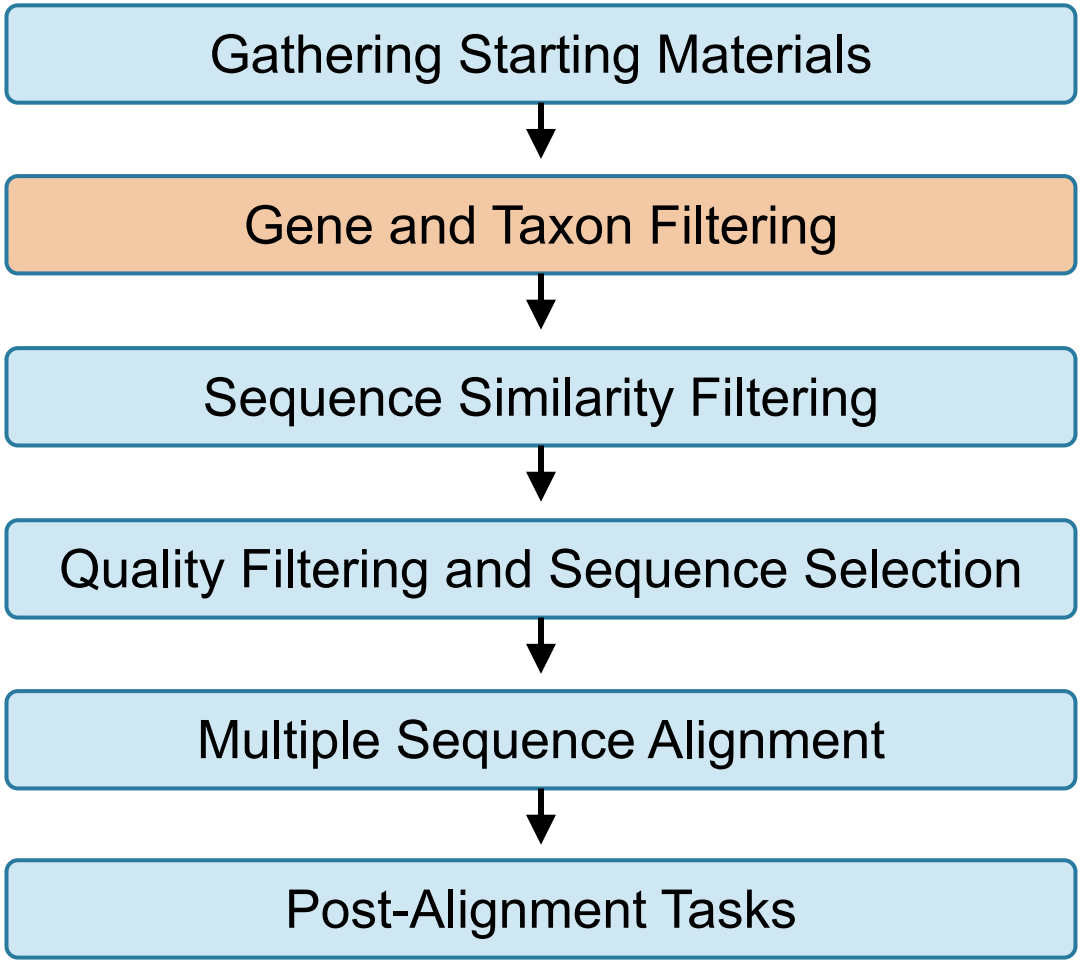
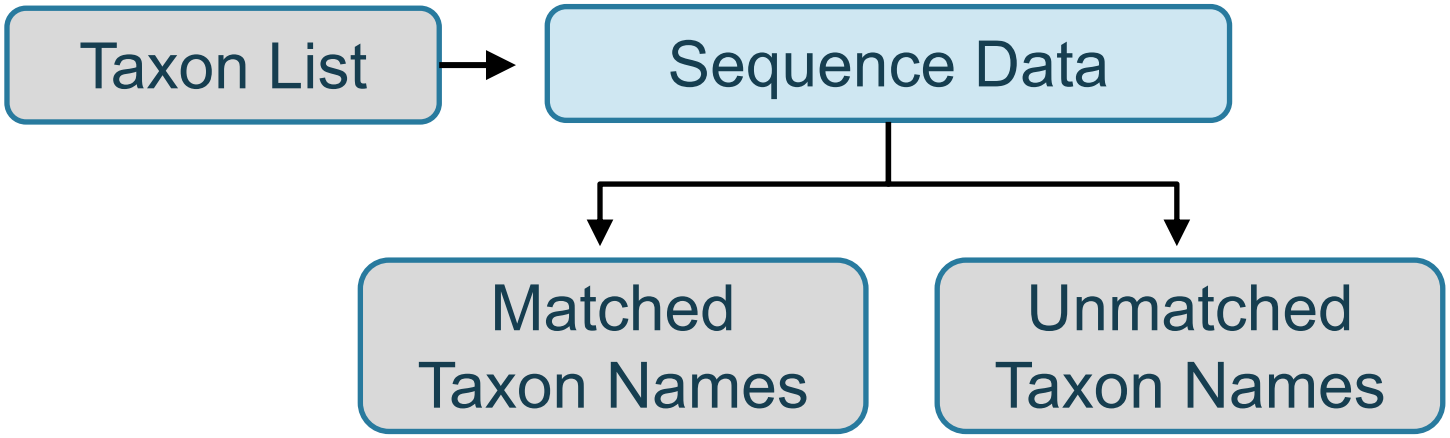


Gene and Taxon Filtering

Optional assessment and cleaning step



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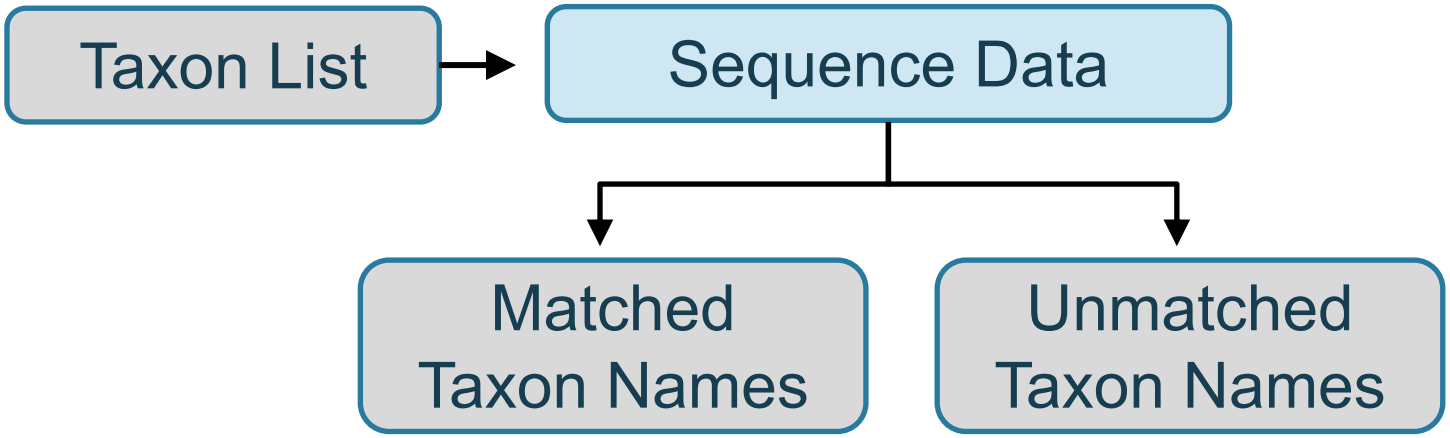

Bad Records

```
A.porcus mitochondrial
Calumma sp.
Jp 2016507218-a/5
Unverified calotes
```

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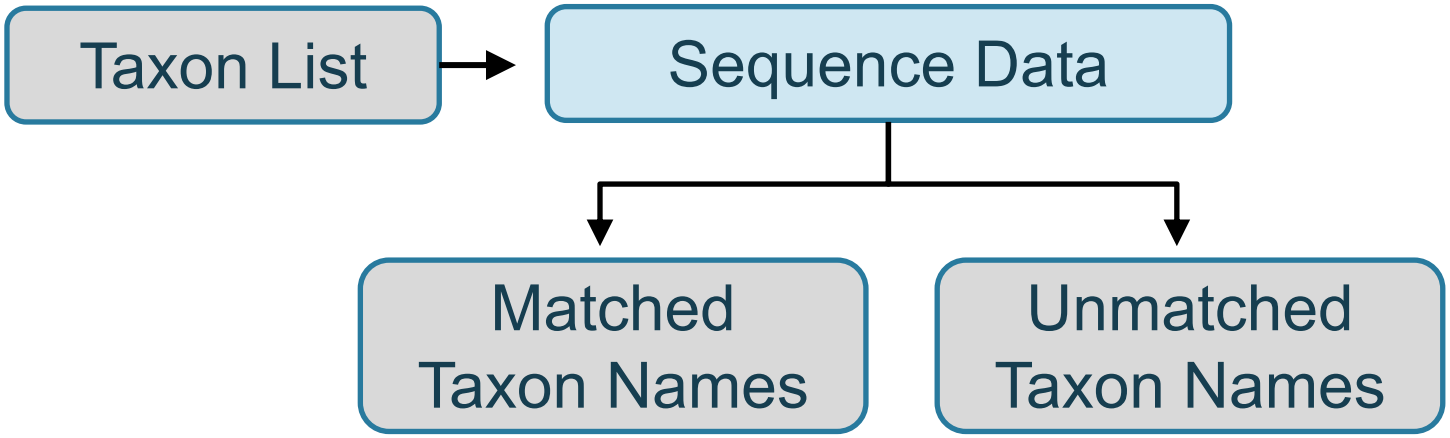

Good records, but need to be corrected

Uromastix acanthinura	→	Uromastyx acanthinura	Spelling errors
Causus defilippi	→	Causus defilippii	
Vipera raddei	→	Montivipera raddei	Change in taxonomy

Gene and Taxon Filtering

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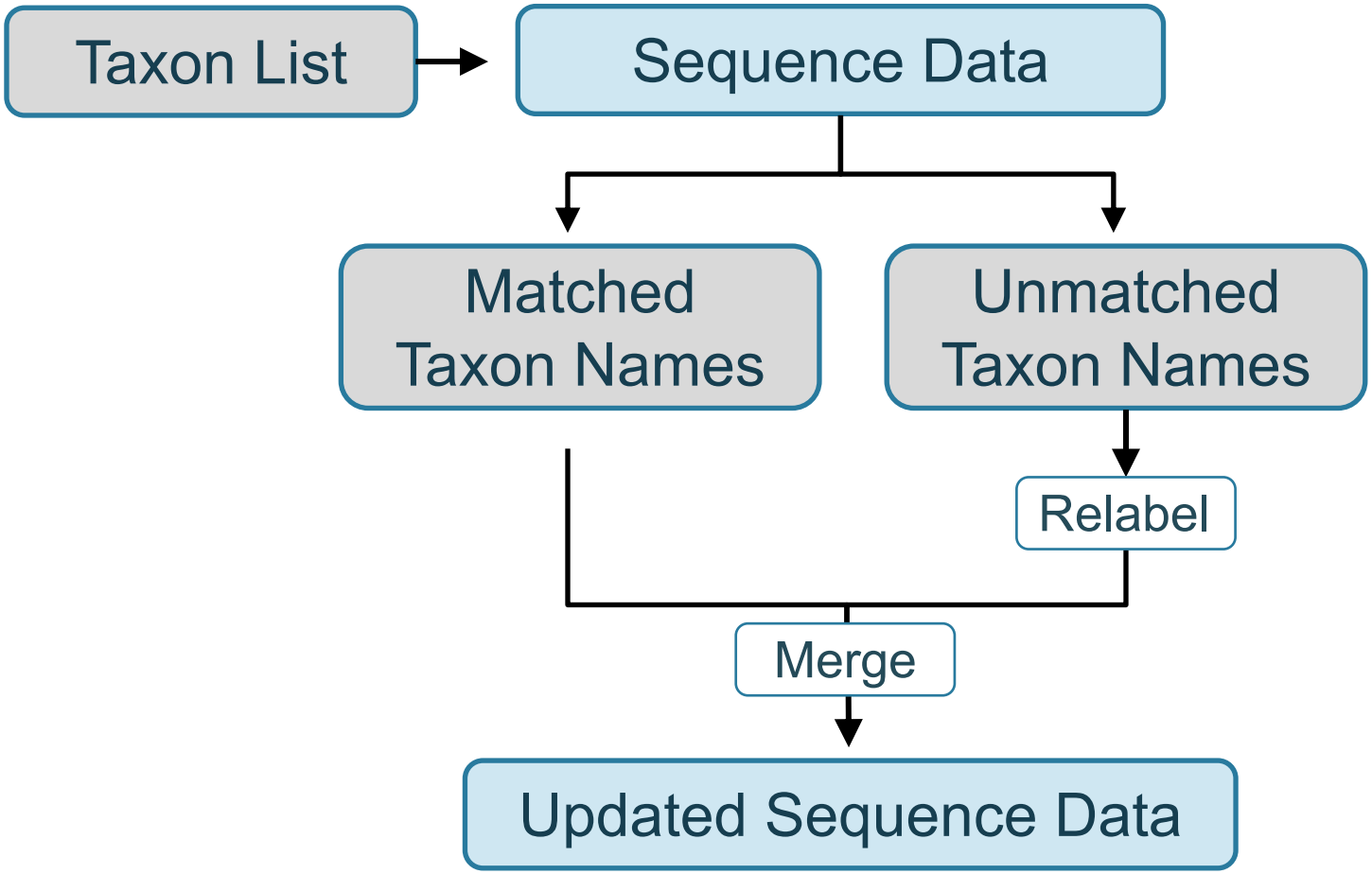

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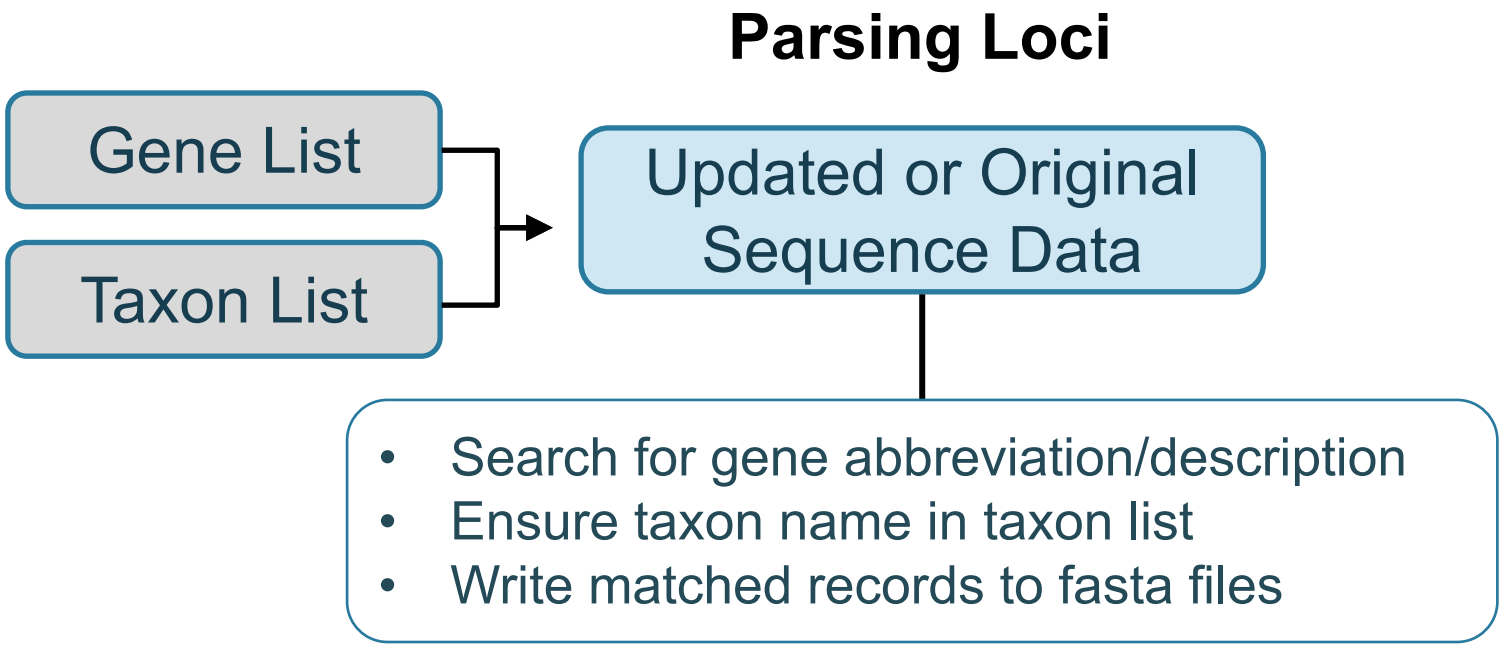

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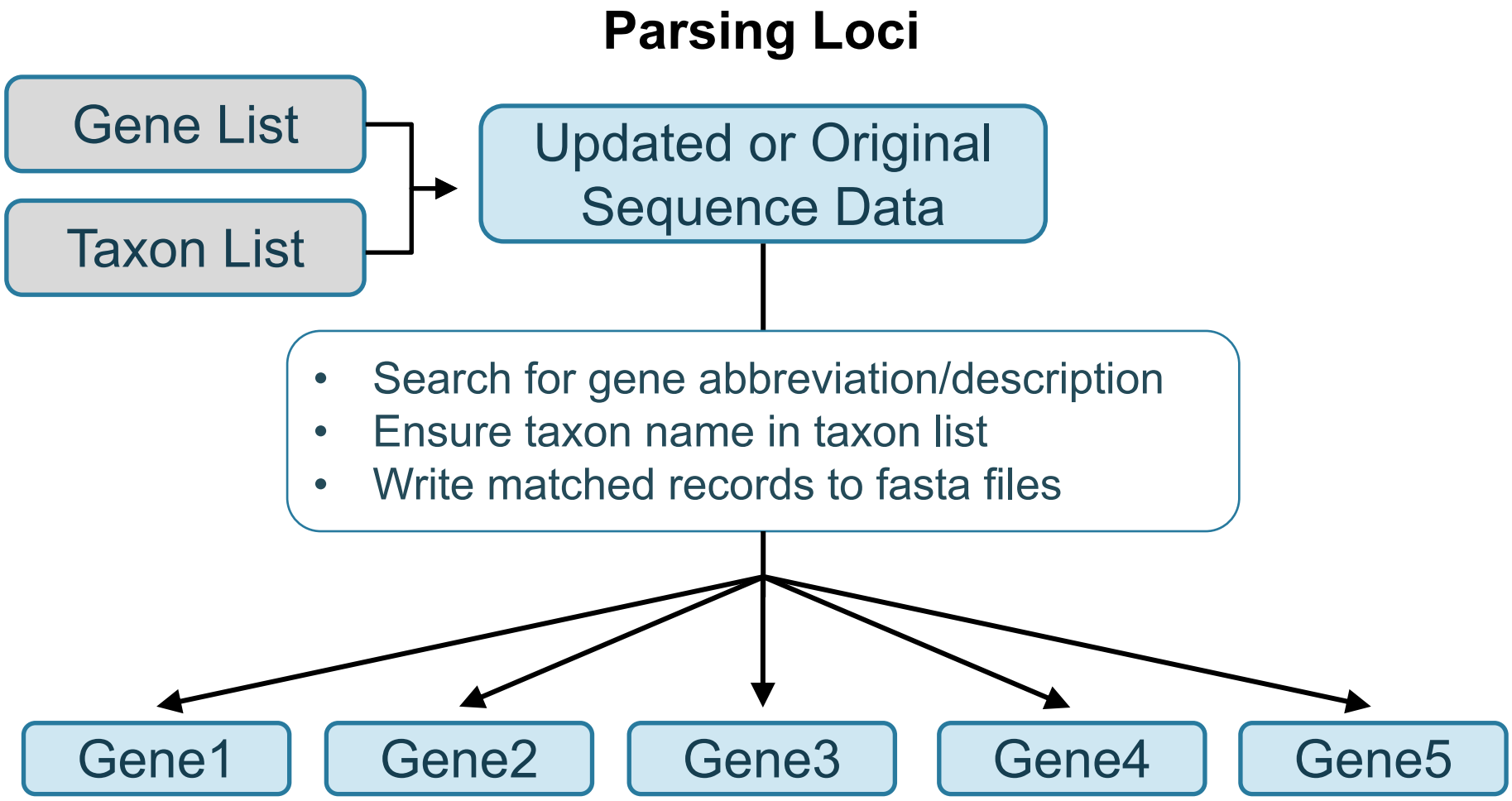
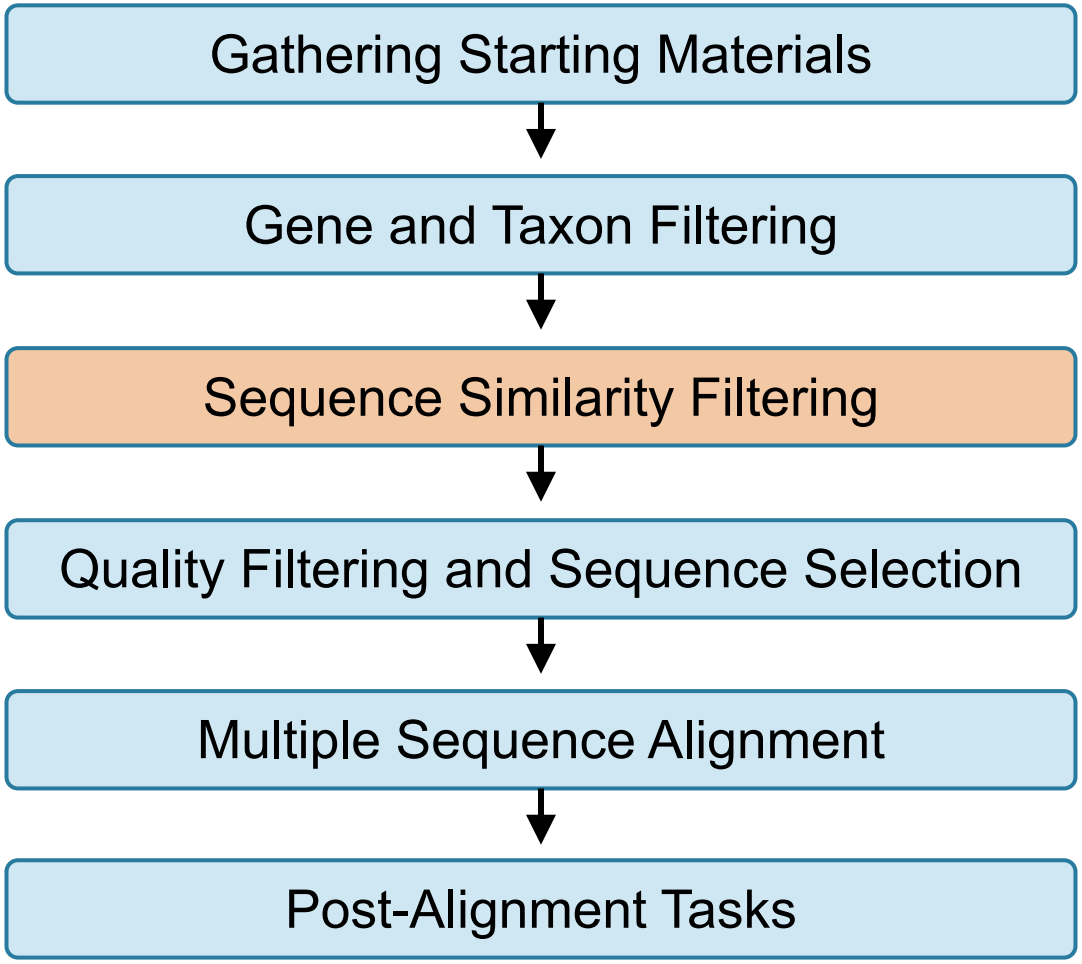


SuperCRUNCH Workflow



Sequence Similarity Filtering

Use BLASTn to identify and extract target sequence

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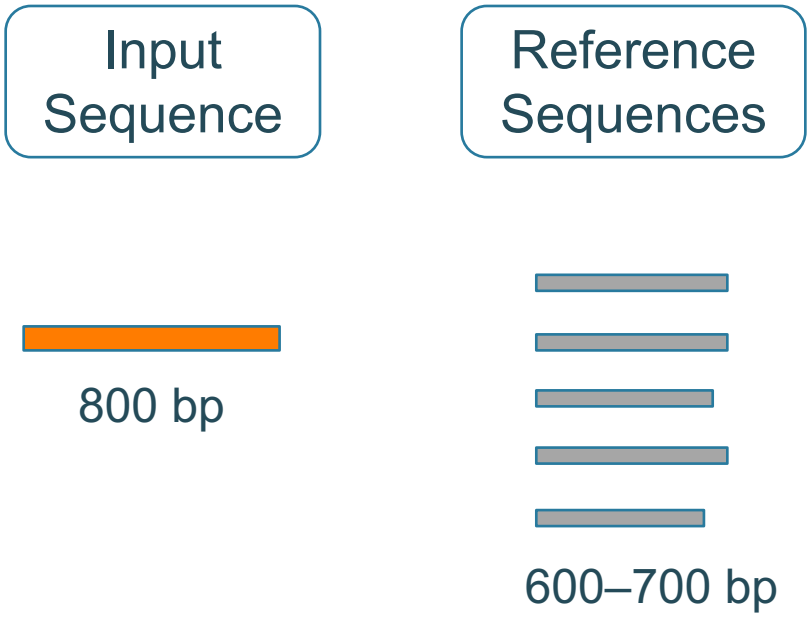

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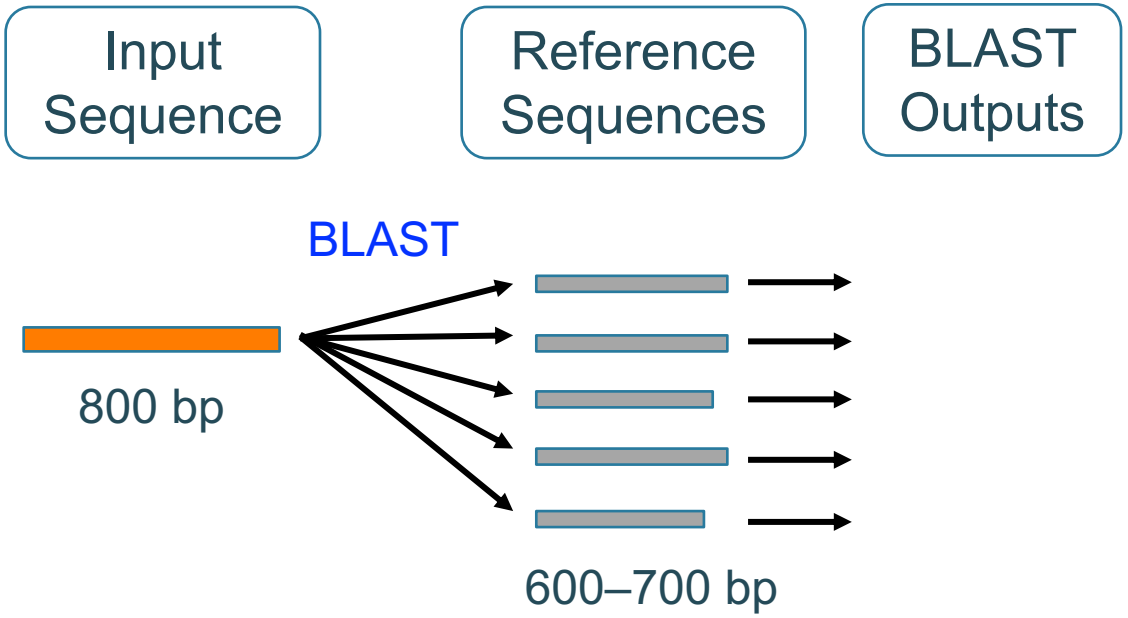

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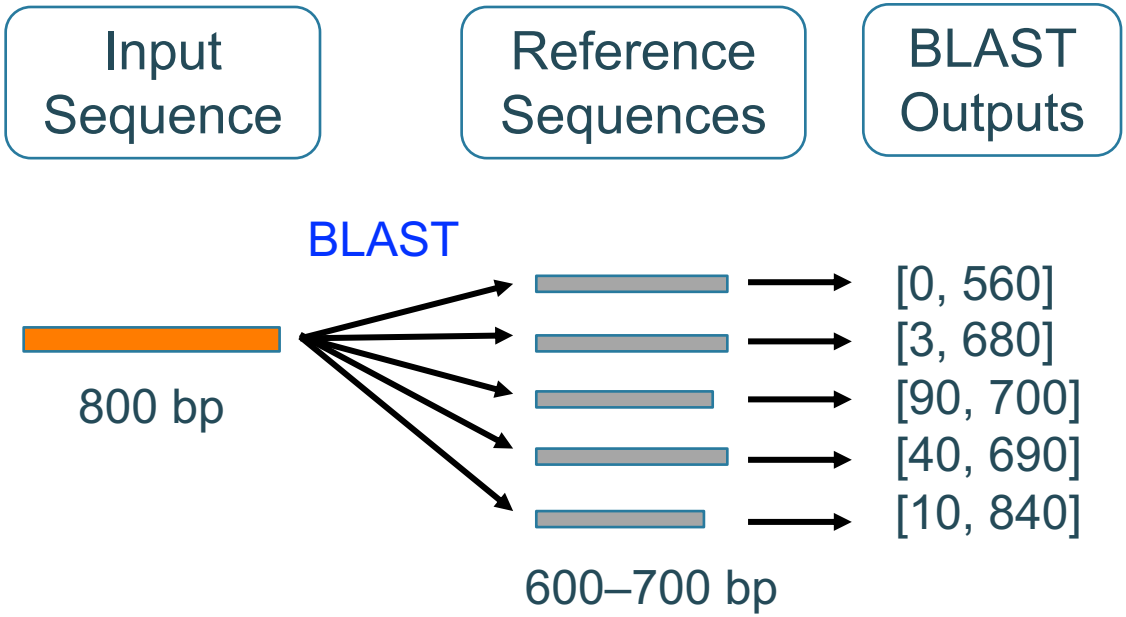

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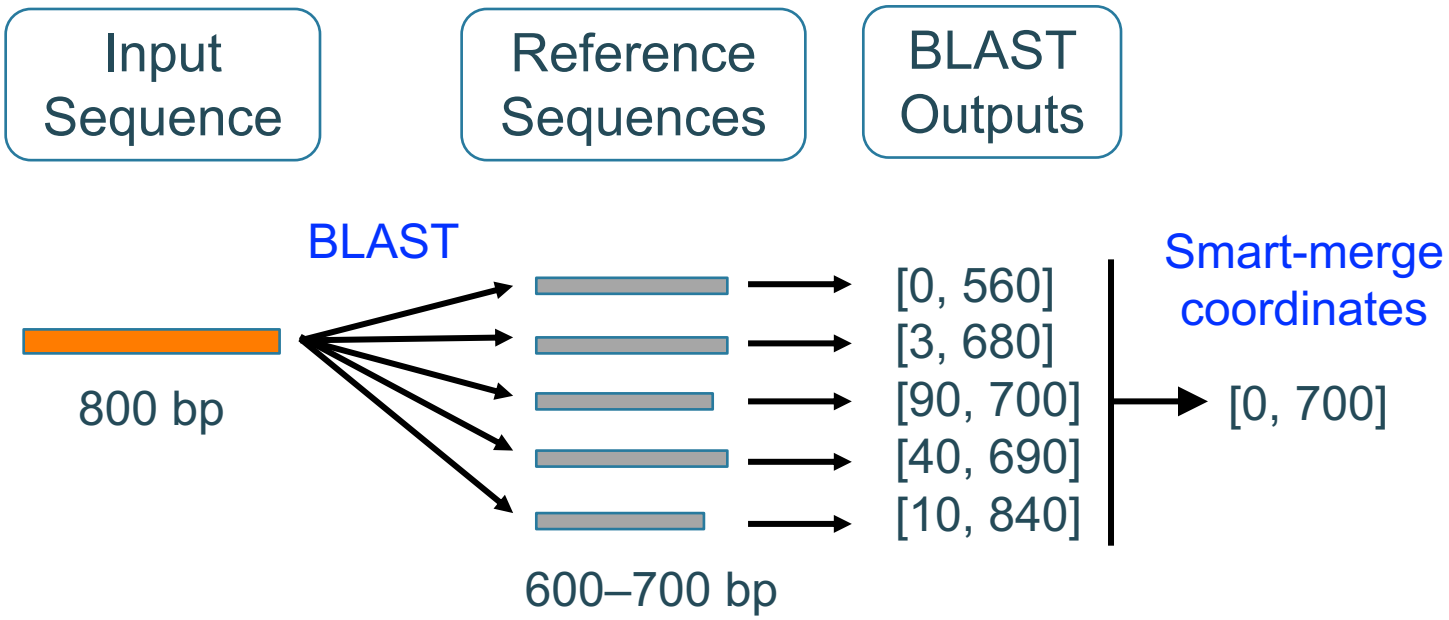

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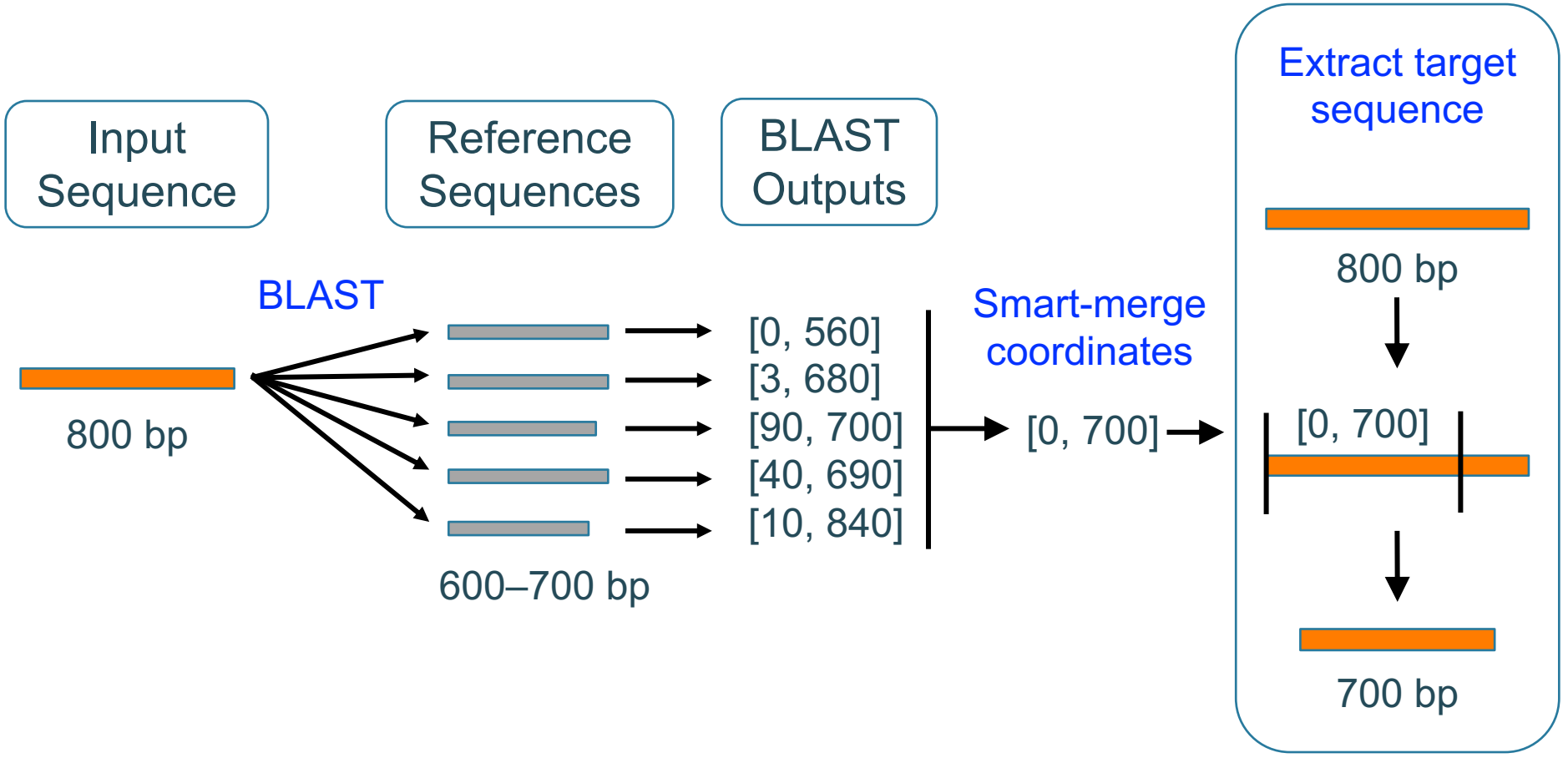

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‘Simple’ Records

‘Complex’ Records

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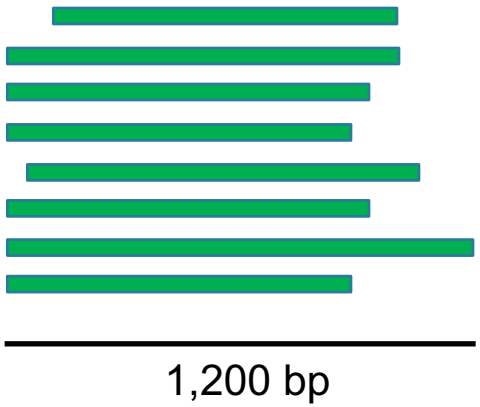

- Single gene
- Same region obtained
- Minor length variation

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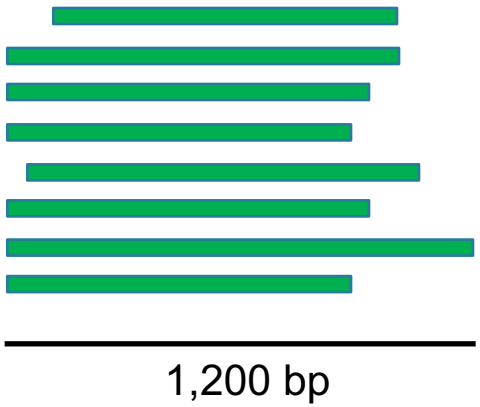

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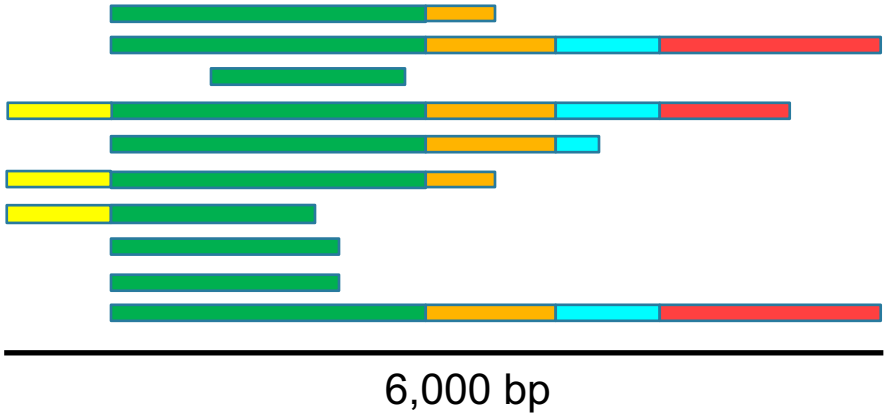

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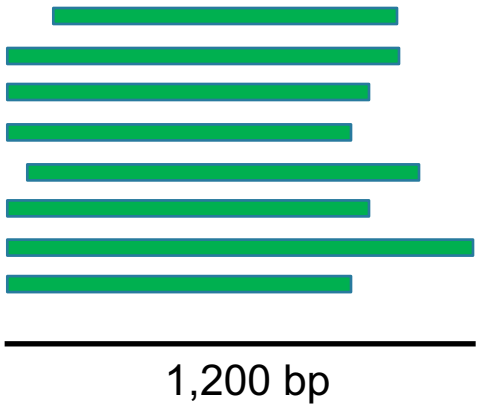

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Automatic selection of reference sequences

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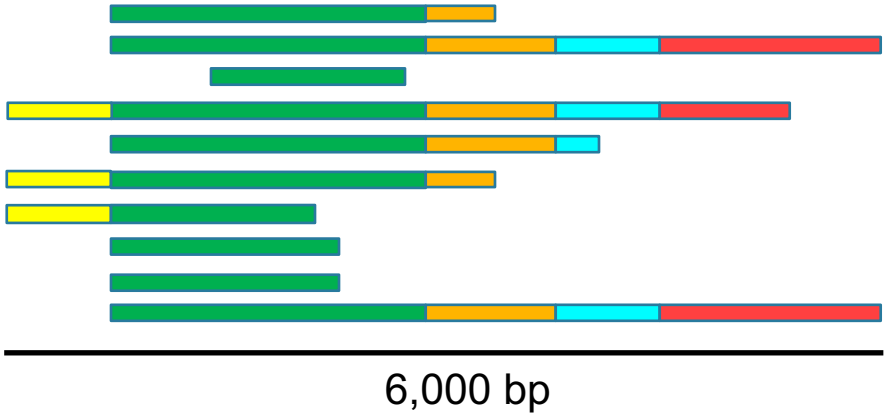

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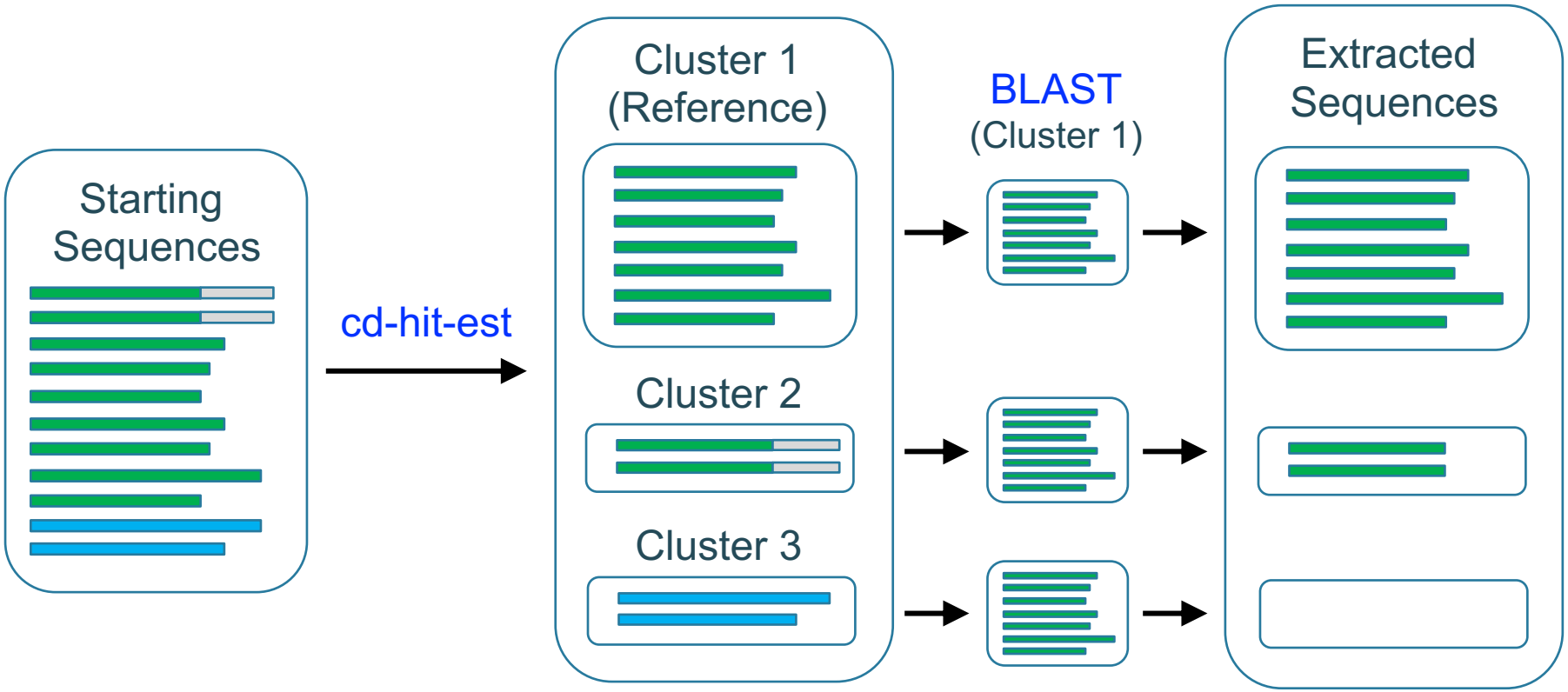
Requires a user-supplied reference sequences

Sequence Similarity Filtering

‘Simple’ Records

Automatic selection of reference sequences



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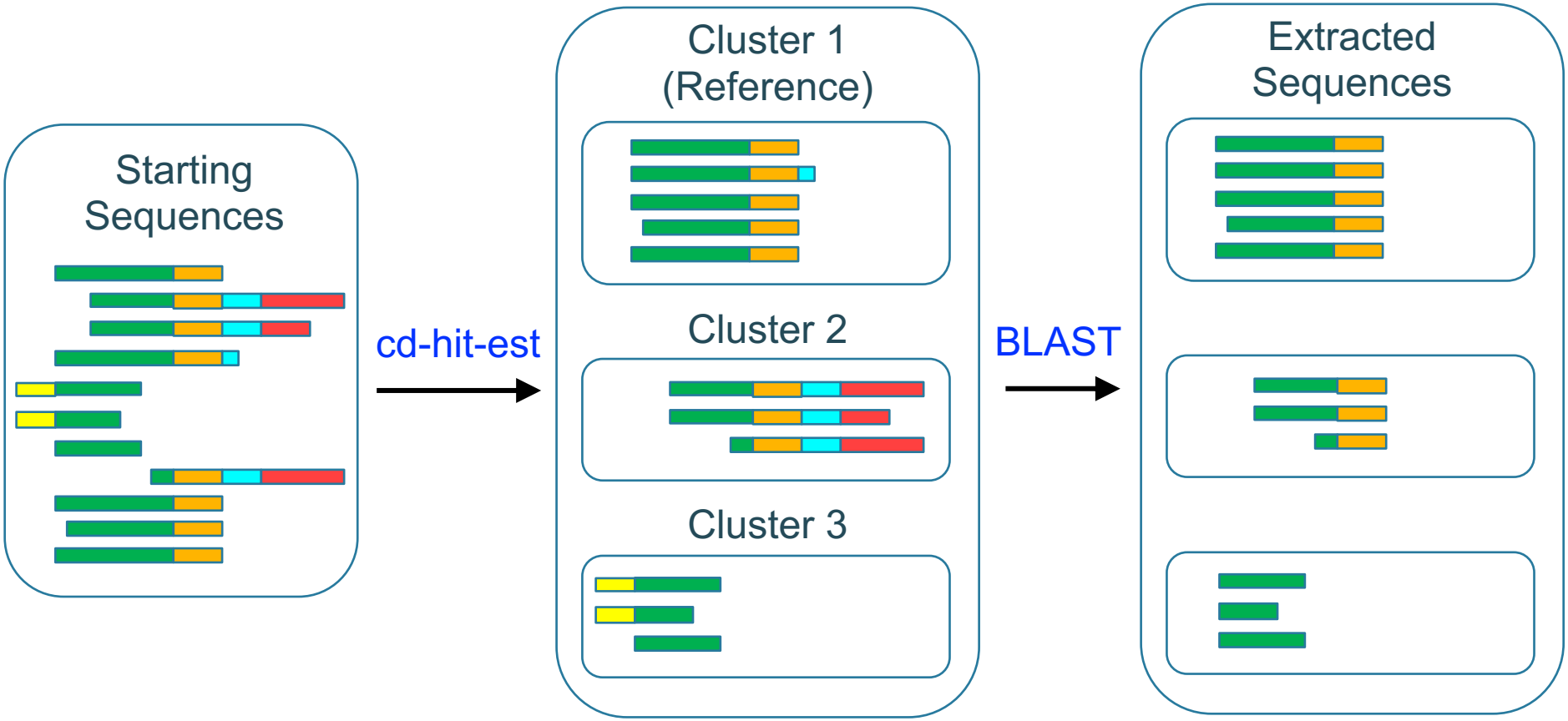
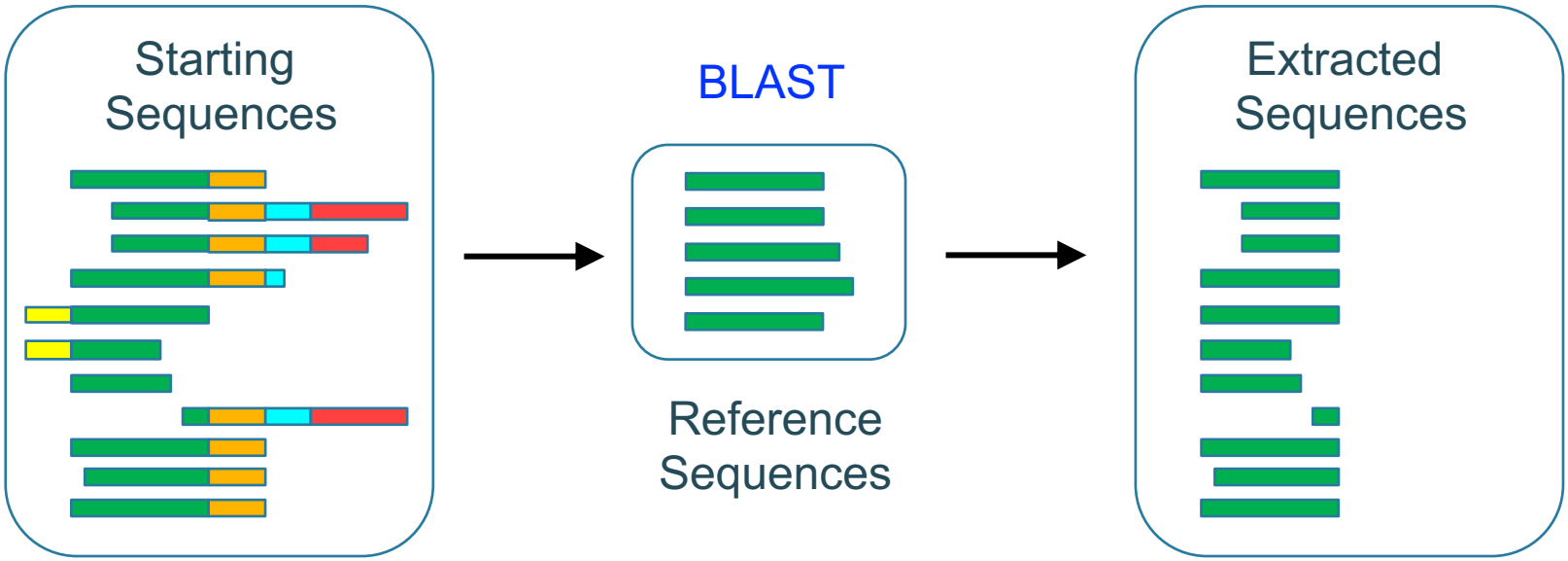


Sequence Similarity Filtering

‘Complex’ Records

Supply validated reference sequences



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  - blastn, megablast, dc-megablast

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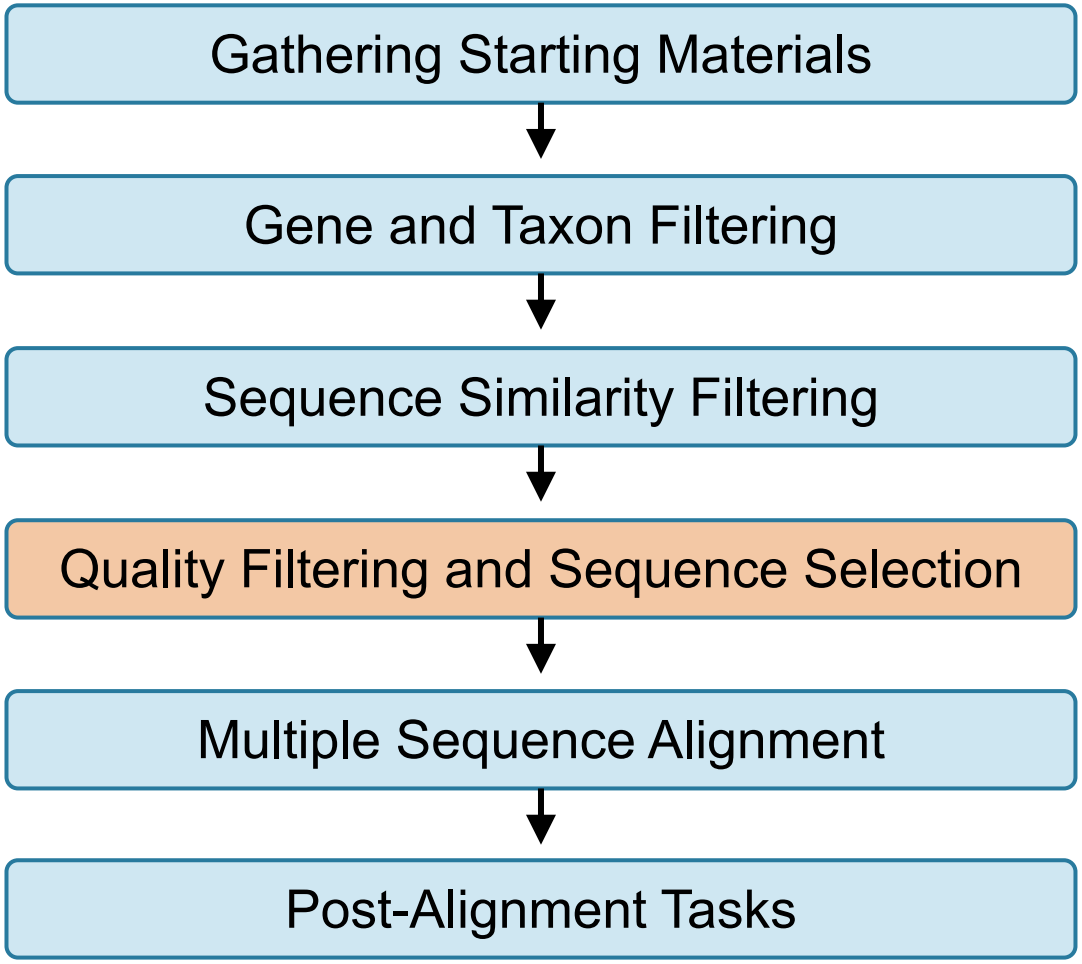
'Simple' Records

Record	Original Length	Extracted Length	Coordinates Used
GU432436.1	772	772	[0, 771]
GU432437.1	753	753	[0, 752]
GU432438.1	773	773	[0, 772]
GU432439.1	749	749	[0, 748]
JF818222.1	719	717	[2, 718]

'Complex' Records

Record	Original Length	Extracted Length	Coordinates Used
AM055651.1	510	510	[0, 509]
AM055653.1	508	508	[0, 507]
JF317635.1	17,388	1,575	[1,060, 2,634]
JF317636.1	17,344	1,574	[1,061, 2,634]
AF440085.1	3,700	1,473	[1,014, 2,486]
AF440019.1	3,693	1,470	[1,010, 2,479]
AF338325.1	1,071	41	[1,030, 1,070]
AF338329.1	1,085	84	[1,001, 1,084]

SuperCRUNCH Workflow



Quality Filtering and Sequence Selection

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Example from Iguania dataset:

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426,941,590,086,091,766,797,665,819,027,414,303,769,493,004,175,161,067,612,739,804,543,200,225,182,323,549,827,313,258,790,810,403,584,768,416,770,835,213,996,198,117,363,132,678,180,852,868,614,265,036,
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000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,
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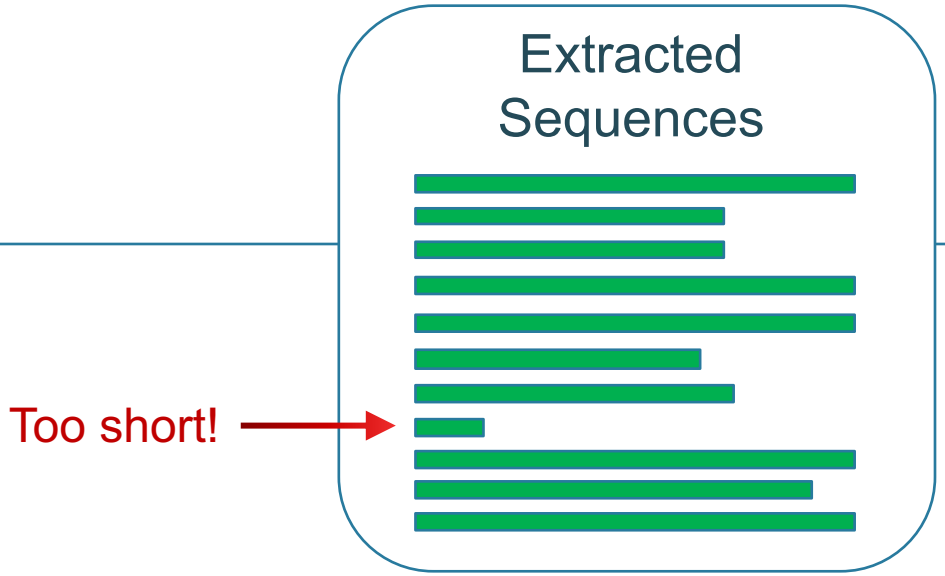
000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000

Combinations: 4.27×10^{3155}

Quality Filtering and Sequence Selection

- What additional filters should be applied to sequences?
  - Minimum length (defined by user)
- If multiple sequences are available for a species, how do we choose?



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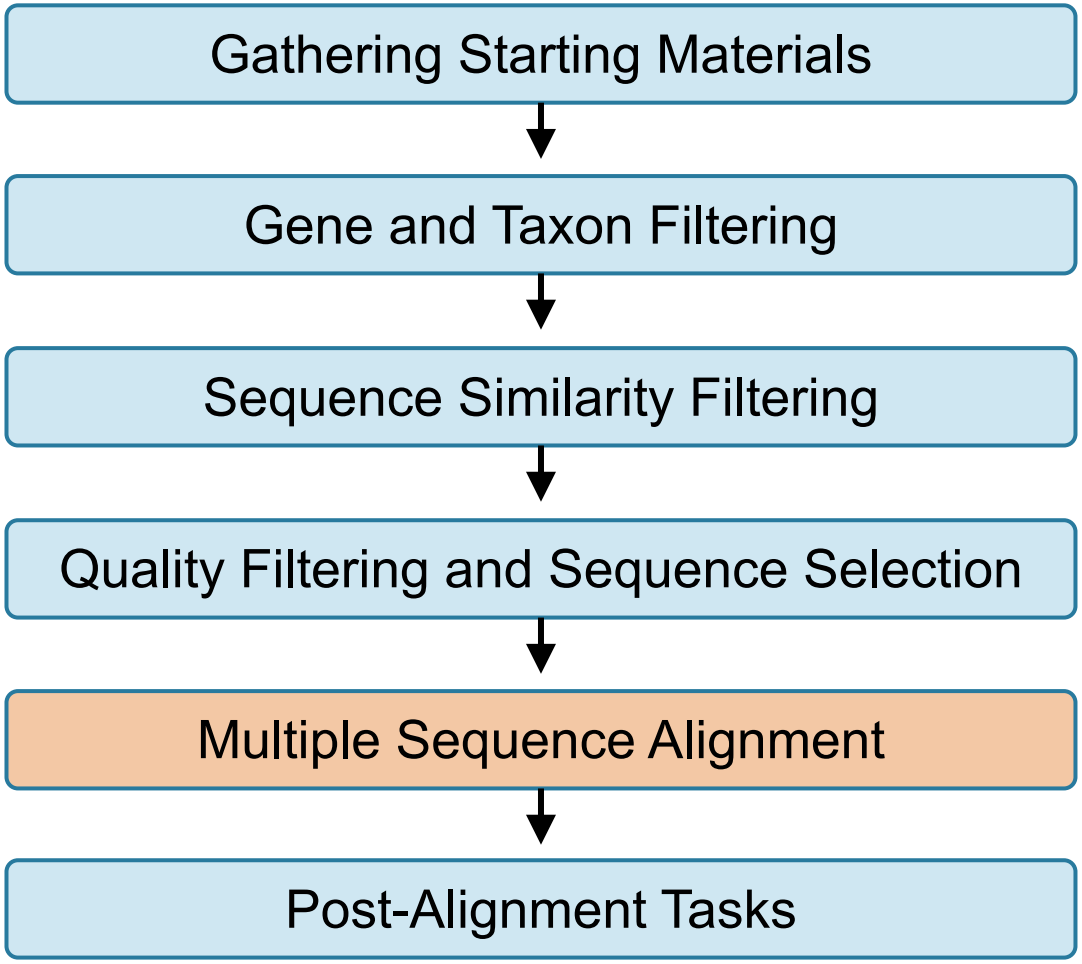
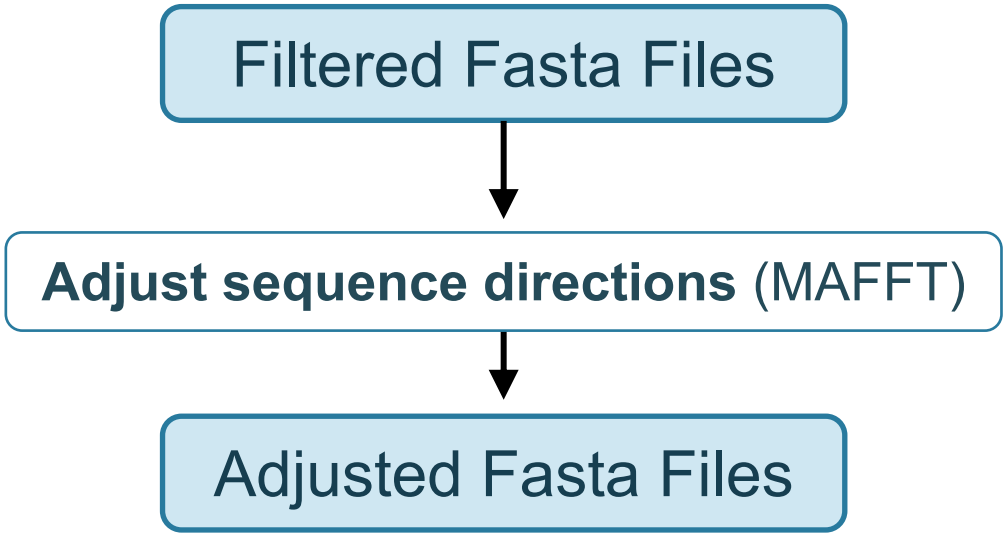
Species-level Data — **One sequence per species per gene**

Population-level Data — **All sequences per species per gene**

SuperCRUNCH Workflow

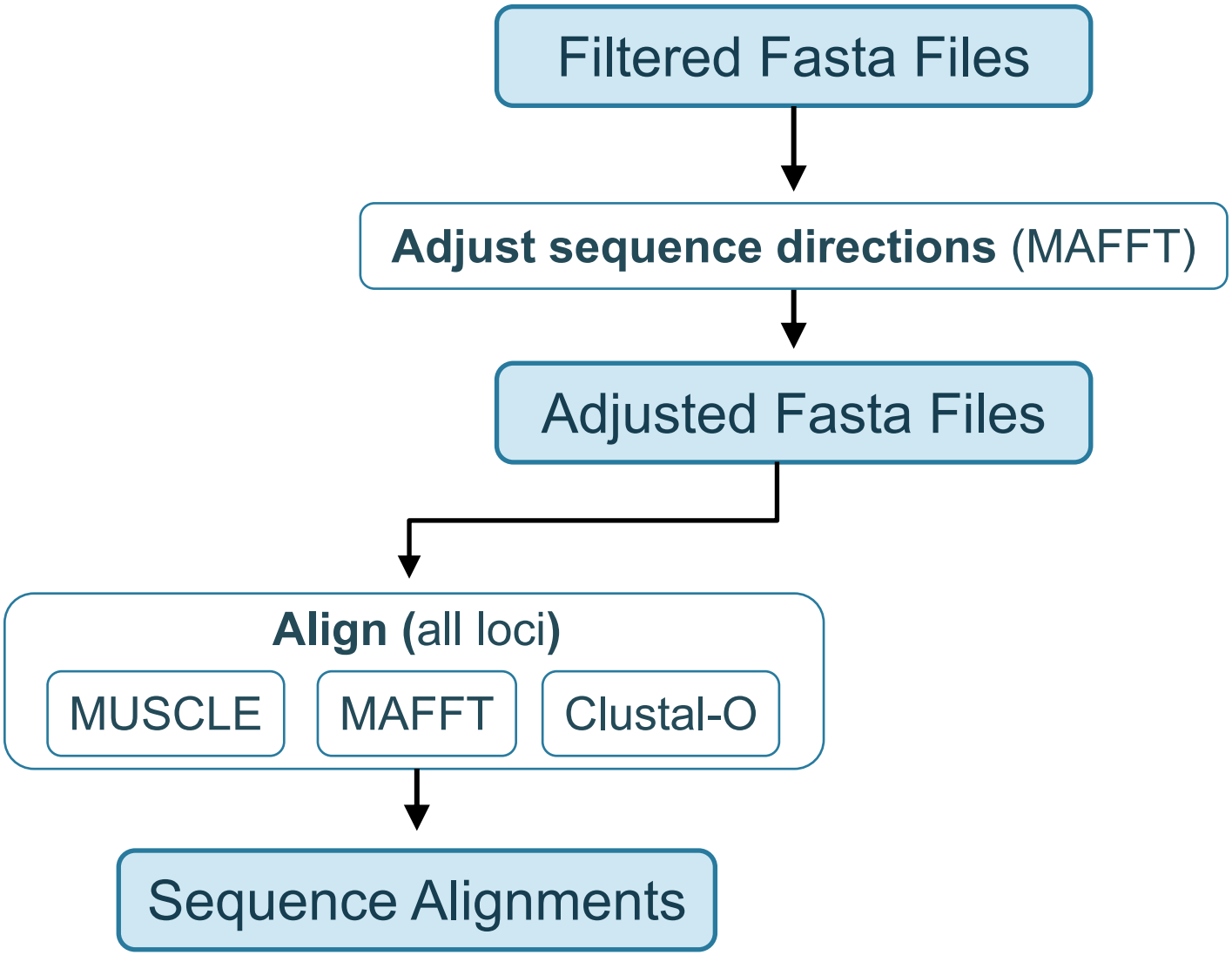


Multiple Sequence Alignment

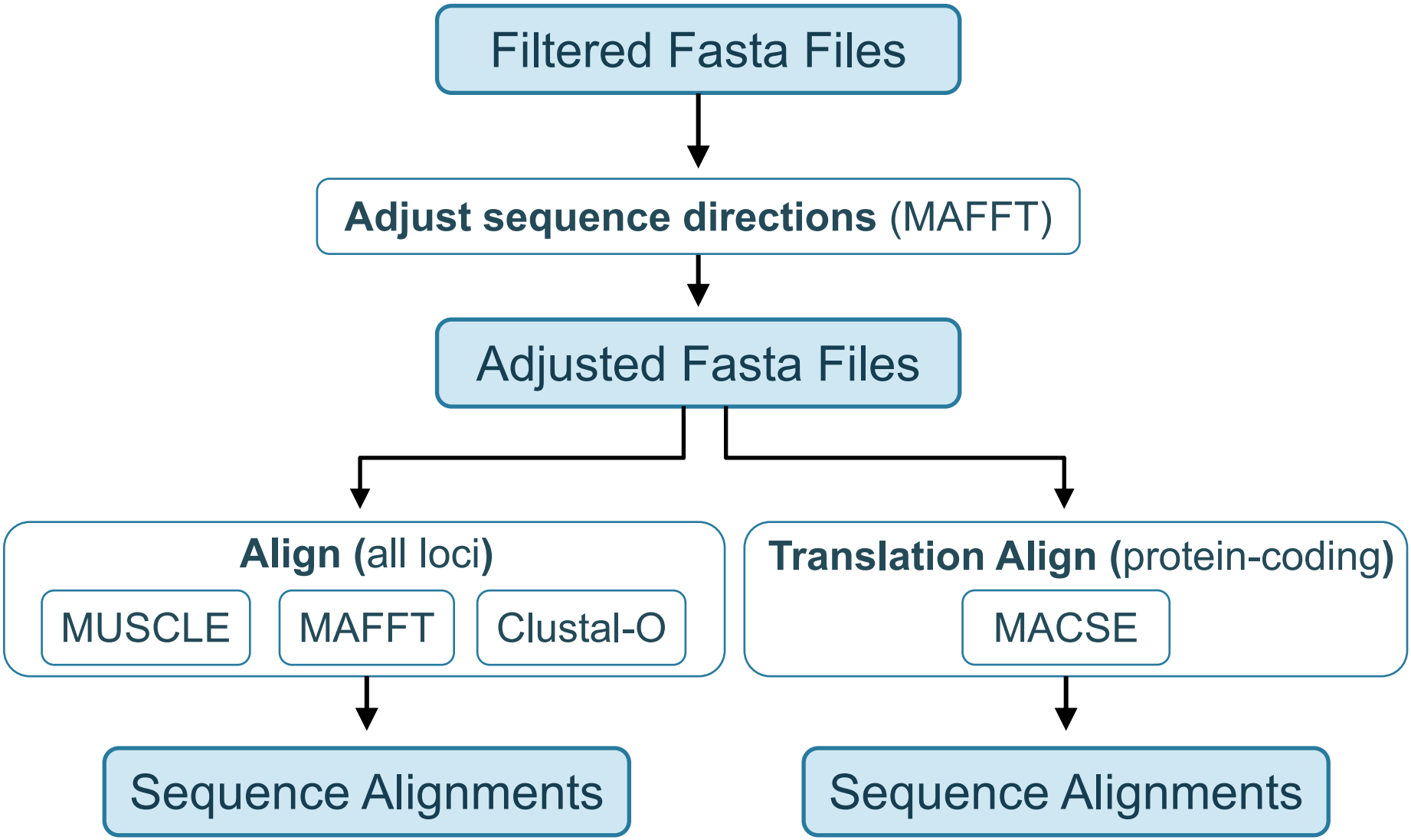


Locus	Correct Direction	Direction Adjusted
UCE-1003	66	5
UCE-1005	62	5
UCE-1012	57	11
UCE-1013	15	4

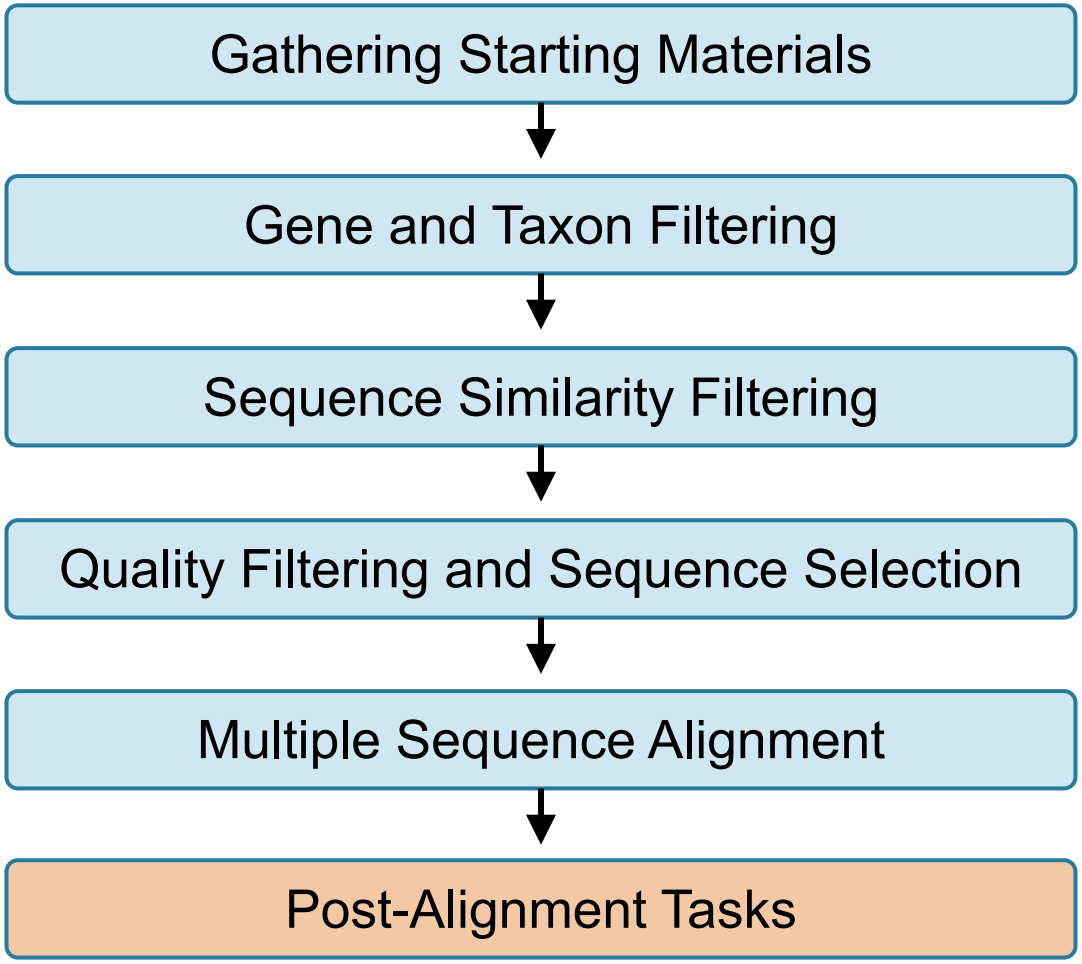
Multiple Sequence Alignment



Multiple Sequence Alignment



SuperCRUNCH Workflow



Post-Alignment Tasks

- Relabel sequence records

Post-Alignment Tasks

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Original Labels

```
>JN881132.1 Daboia russelii activity-dependent neuroprotector...

>KU765220.1 Sceloporus undulatus voucher ADL182 activity-depe...
```

Post-Alignment Tasks

- Relabel sequence records

Original Labels

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Species

```
>Daboia_russelii

>Sceloporus_undulatus
```

Post-Alignment Tasks

- Relabel sequence records

Original Labels

```
>JN881132.1 Daboia russelii activity-dependent neuroprotector...

>KU765220.1 Sceloporus undulatus voucher ADL182 activity-depe...
```

Species

```
>Daboia_russelii

>Sceloporus_undulatus
```

Accession

```
>JN881132.1

>KU765220.1
```

Post-Alignment Tasks

- Relabel sequence records

Original Labels

```
>JN881132.1 Daboia russelii activity-dependent neuroprotector...

>KU765220.1 Sceloporus undulatus voucher ADL182 activity-depe...
```

Species

```
>Daboia_russelii

>Sceloporus_undulatus
```

Accession

```
>JN881132.1

>KU765220.1
```

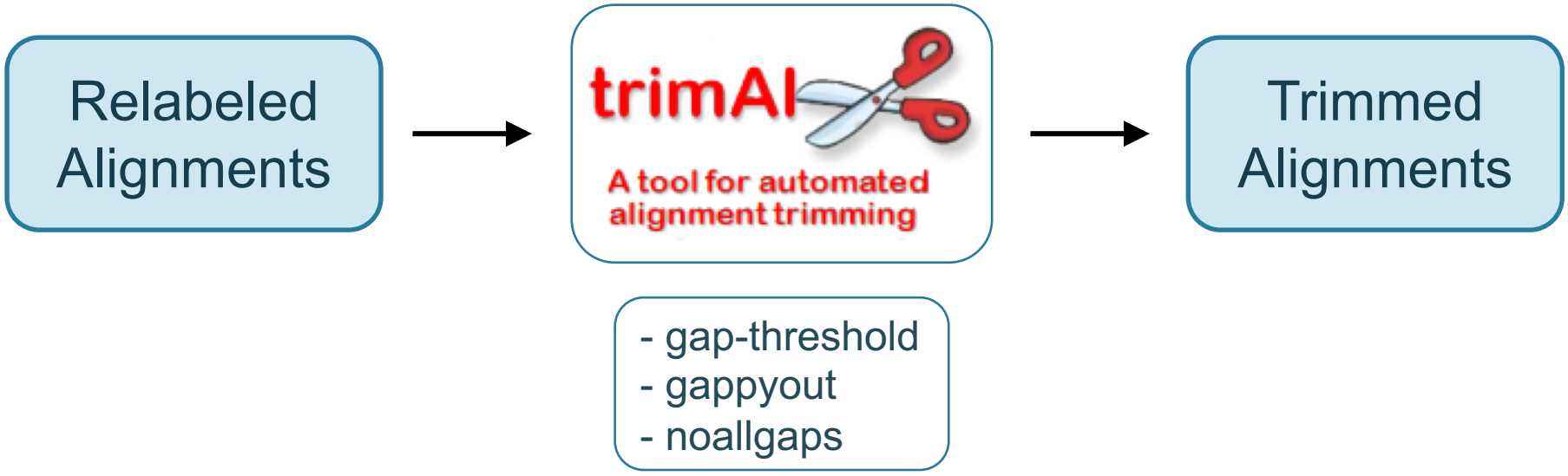
Species + Accession

```
>Daboia_russelii_JN881132.1

>Sceloporus_undulatus_KU765220.1
```

Post-Alignment Tasks

- Relabel sequence records
- Trim alignments



Post-Alignment Tasks

- Relabel sequence records
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- Convert to nexus, phylip format

Post-Alignment Tasks

- Relabel sequence records
- Trim alignments
- Convert to nexus, phylip format
- Concatenate alignments

Requires Species Labeling

```
>Daboia_russelii

>Sceloporus_undulatus
```

Missing Data Options

N - ?

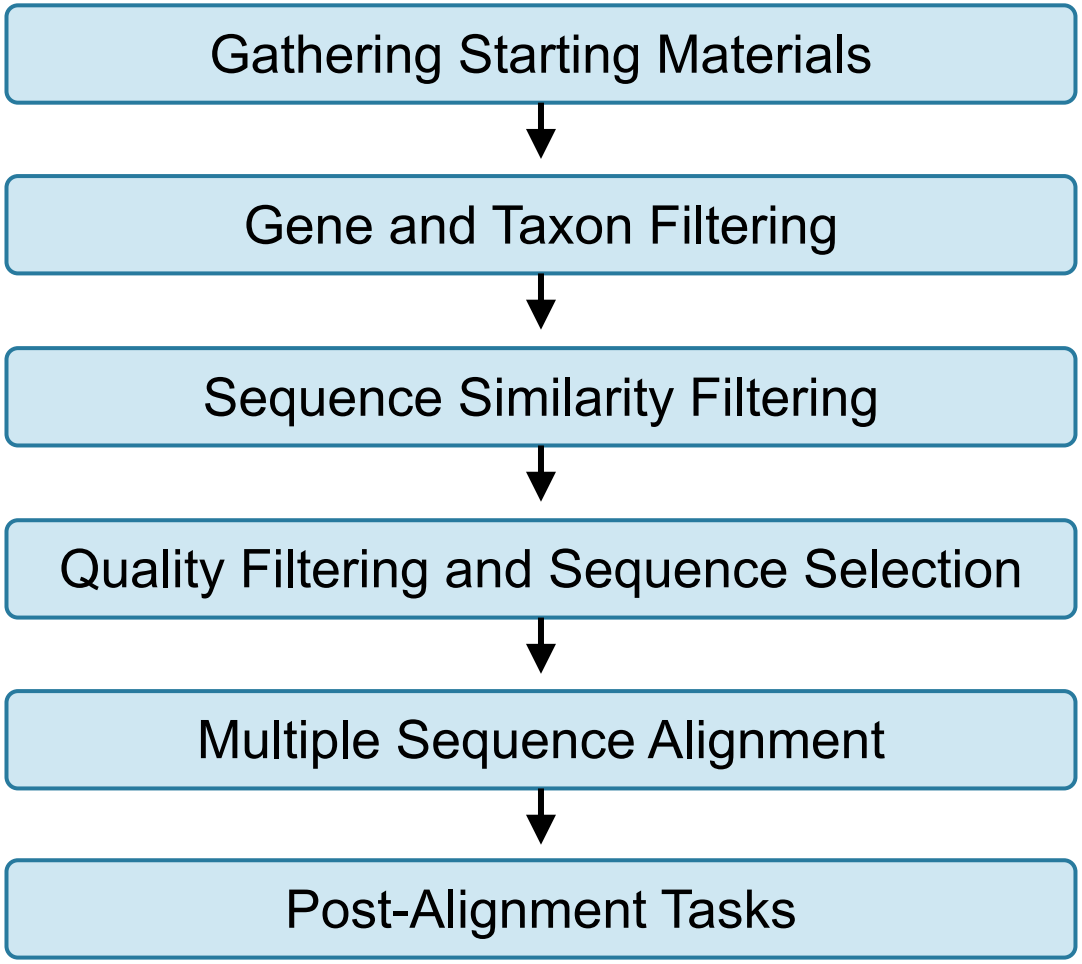
Outputs

Fasta or phylip

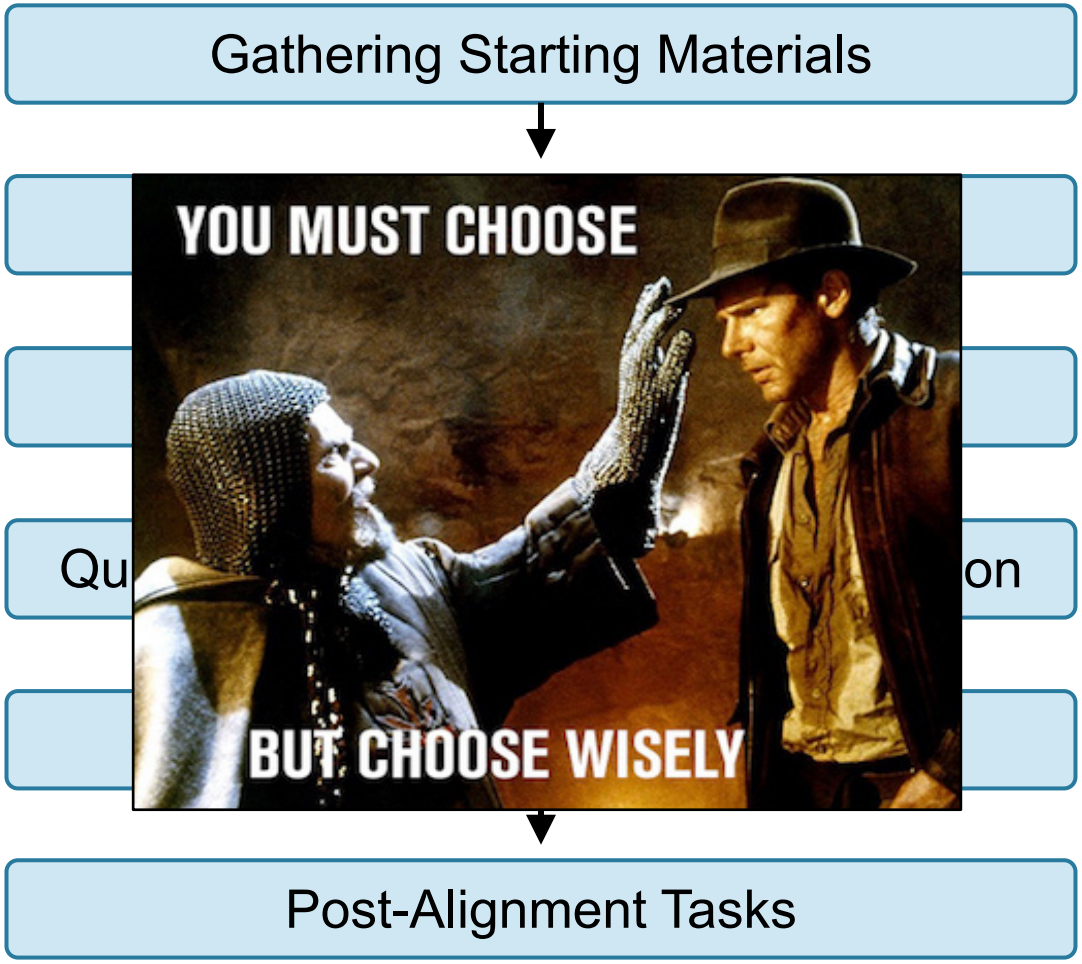
Partitions file

Loci per taxon

SuperCRUNCH Workflow



SuperCRUNCH Workflow



Benchmarking: Time

- Dataset dependent, but reasonable!
- Bottleneck is always sequence alignment

	Population level	Traditional Supermatrix	Genomic (UCE's)
File Size	52 MB	13.3 GB	205 MB
Sequences	82,557	8,785,378	338,942
Taxa	3	1,426	123
Genes	4	70	5,041
Total time	**~2.5 min**	**~4.5 hours**	**~10.5 hours**

Benchmarking: Other Programs

Comparison to PyPHLAWD

- PyPHLAWD uses GenBank release database and Taxonomy Browser to fetch starting sequences
- Default is automated clustering of all sequences (requires follow-up identification and processing)
- A ‘baited analysis’ can target specific loci, requires reference sequences

Benchmarking: Other Programs

Comparison to PyPHLAWD

- Compared supermatrix construction for Iguania, large clade of squamate reptiles (~1900 species)

- Targeted 68 loci
  - 61 nuclear – used automatic reference selection
  - 7 mtDNA loci – used validated reference sequences

Three analyses:

SuperCRUNCH NCBI direct-download	**SuperCRUNCH** PyPHLAWD database	**PyPHLAWD** PyPHLAWD database

Benchmarking: Other Programs

Comparison to PyPHLAWD

	Loci	Taxa	Sequences
SuperCRUNCH NCBI direct-download	66	1,426	13,419
SuperCRUNCH PyPHLAWD database	66	1,358	12,654
PyPHLAWD PyPHLAWD database	65	1,069	10,397

Benchmarking: Other Programs

- PyPHLAWD was comparable for nuclear loci
- PyPHLAWD failed to find 62% (2,125) of mtDNA seqs

Benchmarking: Other Programs

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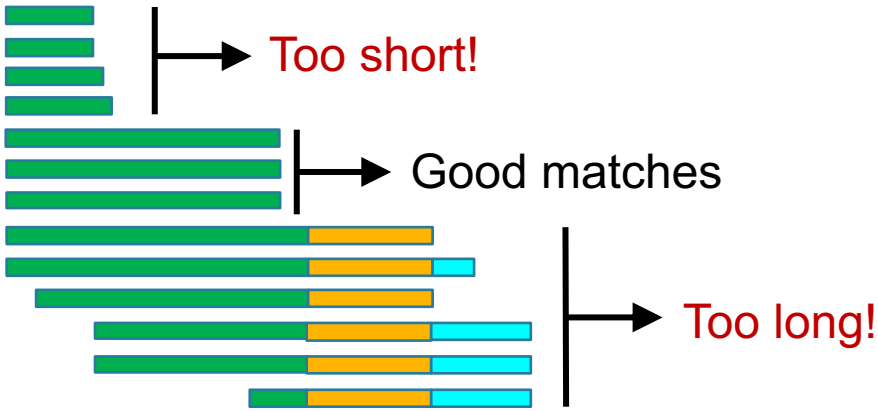
References

Sequences must be highly similar to bait to pass filter

Entire sequence is kept or discarded (no extraction)



Looking for Testers!

SuperCRUNCH

FOR PHYLOGENETIC DATA

Pre-Print	https://doi.org/10.1101/538728
Download	https://github.com/dportik/SuperCRUNCH
Documentation	https://github.com/dportik/SuperCRUNCH/wiki
Tutorials & Data	https://osf.io/bpt94/

Benchmarking

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File Size	52 MB	205 MB	13.3 GB
Sequences	82,557	338,942	8,785,378
Taxa	3	123	1,426
Genes	4	5,041	70
Gene and Taxon Filtering	5s	2hr 40min	34min
Sequence Similarity Filtering	8s	---	1hr 10min
Sequence Selection	1s	7min	7min
Sequence Alignment	2min	7hr 10min	2hr 40min
Post-Alignment Tasks	2s	21min	7s
Total cpu time	**~2.5 min**	**~10.5 hours**	**~4.5 hours**