# Evaluation of Gene Finding Tools on *Trichoderma* Genomes - Update

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**Connor Burbridge**

University of Saskatchewan

# Outline

- Progress update with results
- Progress assessment
- Timeline for completion
- Discussion of next steps

# Background

- *Trichoderma* species are important for agriculture and biotechnology.
- Gene finding is a critical step in understanding *Trichoderma*
- Various tools exist for gene prediction, but their implementations and performance can vary significantly.
- Few studies have compared these tools in fungi, and even fewer in *Trichoderma*.
- In addition, high-quality reference genomes are not available for all species, which makes comparisons difficult.

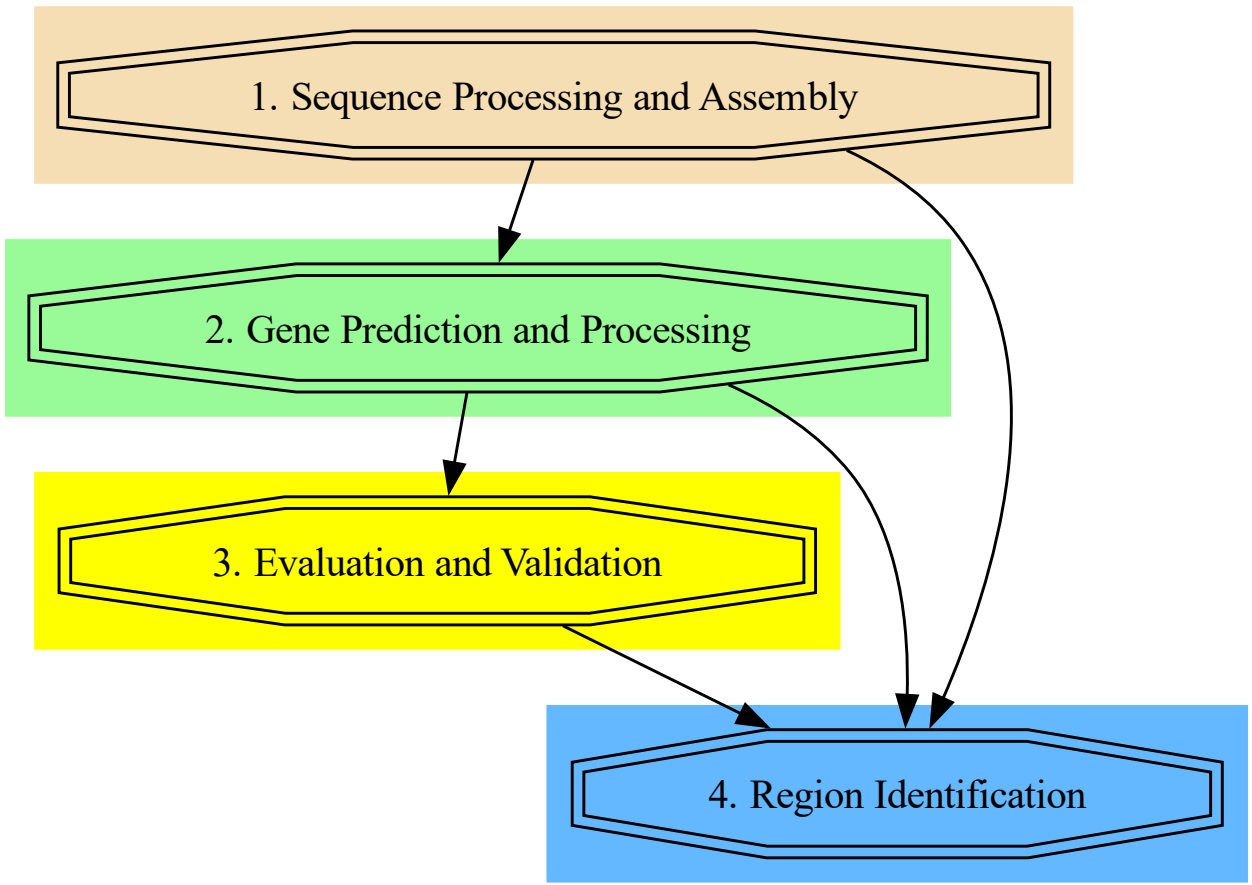
# Why *Trichoderma*?

- describe trichoderma here*********
- *Trichoderma* species are ubiquitous in soil and play a significant role in nutrient cycling.
- They are also used in biocontrol and as biofertilizers.
- Known for their production of plant cell wall degrading enzymes, and secondary metabolites.
- Further genomic studies can help in understanding their biology and potential applications.

# Novel *Trichoderma* Genomes

- *Trichoderma* species are diverse, with many species not yet fully characterized.
- Recent advances in sequencing technology have made it possible to generate high-quality genomes for these species.
- Two novel genomes of *Trichoderma* species have been sequenced at the Global Institute for food Security
- DC1 and Tsth20, shown to improve drought and salt tolerance when applied to crops.
- These genomes provide an opportunity to evaluate gene finding tools in a comparative context, and contribute to the understanding of *Trichoderma* biology.

# Workflow Overview



# Datasets and Tools

- **Datasets:**
  - Novel *Trichoderma* genomes: DC1 and Tsth20.
  - Reference genomes and annotations: *T. reesei, T. harzianum, T.virens*.
  - RNAseq training data from *T. reesei*.
  - Benchmarking Universal Single-Copy Orthologs (BUSCO) fungal database.
  - Protein sequence queries for tblastn from *T. atroviride, Fusarium graminearum, and Saccharomyces cerevisiae*.
- **Tools:**
  - Sequence processing: FastQC, Trimmomatic, Hisat2.
  - Genome assembly: NextDenovo and NextPolish.
  - Gene finding tools: Braker2 and GeneMark-ES.
  - Evaluation tools: BUSCO, tblastn, InterProScan, and custom scripts.

# Assembly Results

| Name | Total Contigs | Total Length | Largest Contig | GC% | N50 | L50 |
|---|---|---|---|---|---|---|
| DC1 | 8 | 38.6 Mb | 11.49 Mb | 47.97 | 5.69 Mb | 3 |
| Tsth20 | 7 | 41.58 Mb | 8.02 Mb | 47.33 | 6.52 Mb | 3 |
| *T. harzianum* | 532 | 40.98 Mb | 4.08 Mb | 47.61 | 2.41 Mb | 7 |
| *T. virens* | 93 | 39.02 Mb | 3.45 Mb | 49.25 | 1.83 Mb | 8 |
| *T. reesei* | 77 | 33.39 Mb | 3.75 Mb | 52.82 | 1.21 Mb | 9 |

- DC1 and Tsth20 assemblies are of high quality with few contigs in comparison to other *Trichoderma* assemblies.
- Input sequences and assemblies show a bimodal distribution of GC content.
- AT-rich sequence content may be related to transposable elements and repeat-induced mutations, which may be of interest in secondary metabolite production.

# Gene Finding Results

| Assembly | Braker2 | | GeneMark | | RefSeq | |
|---|---|---|---|---|---|---|
| | Genes | CDS | Genes | CDS | Genes | CDS |
| DC1 | 8546 | 8637 | 11353 | 11353 | N/A | N/A |
| Tsth20 | 8784 | 8858 | 12362 | 12362 | N/A | N/A |
| *T. reesei* | 9659 | 10175 | 9196 | 9196 | 9109 | 9118 |
| *T. harzianum* | 8314 | 8385 | 12164 | 12164 | 14269 | 14090 |
| *T. virens* | 7801 | 7863 | 11866 | 11866 | 12405 | 12406 |

- Braker2 predicts more genes and coding sequences than GeneMark and RefSeq in *T. ressei*, but fewer in other assemblies.
- GeneMark and RefSeq predictions are similar, except in *T. harzianum*, where RefSeq predicts 17% more genes.
- GeneMark only predicts one coding sequence per gene, while Braker2 and RefSeq predict multiple coding sequences.

# Examining Coding Sequence Lengths

| Genome | Tool #1 | Tool #2 | *P*-value |
|---|---|---|---|
| DC1 | Braker2 | GeneMark | 0.999 |
| Tsth20 | Braker2 | GeneMark | 0.965 |
| *T. reesei* | Braker2 | GeneMark | $9.481 * 10^{-07}$ |
| *T. reesei* | GeneMark | RefSeq | 0.002 |
| *T. reesei* | Braker2 | RefSeq | $1.340 * 10^{-07}$ |
| *T. harzianum* | Braker2 | GeneMark | 0.863 |
| *T. harzianum* | GeneMark | RefSeq | $4.313 * 10^{-52}$ |
| *T. harzianum* | Braker2 | RefSeq | $4.674 * 10^{-55}$ |
| *T. virens* | Braker2 | GeneMark | 0.635 |
| *T. virens* | GeneMark | RefSeq | $7.352 * 10^{-12}$ |
| *T. virens* | Braker2 | RefSeq | $1.794 * 10^{-09}$ |

# BUSCO Results

| Strain | Complete | Single | Duplicated | Fragmented | Missing |
|---|---|---|---|---|---|
| DC1 | 99.5 | 80.2 | 19.3 | 0.1 | 0.4 |
| Tsth20 | 99.9 | 81.7 | 18.2 | 0.0 | 0.1 |
| *T. harzianum* | 99.7 | 80.2 | 19.5 | 0.0 | 0.3 |
| *T. virens* | 99.8 | 79.0 | 20.8 | 0.1 | 0.1 |
| *T. reesei* | 99.9 | 85.5 | 14.4 | 0.1 | 0.0 |

**(a)** Braker2

| Strain | Complete | Single | Duplicated | Fragmented | Missing |
|---|---|---|---|---|---|
| DC1 | 99.2 | 98.8 | 0.4 | 0.3 | 0.5 |
| Tsth20 | 99.8 | 99.1 | 0.7 | 0.0 | 0.2 |
| *T. harzianum* | 99.6 | 98.9 | 0.7 | 0.0 | 0.4 |
| *T. virens* | 99.7 | 99.2 | 0.5 | 0.1 | 0.2 |
| *T. reesei* | 99.6 | 99.5 | 0.1 | 0.0 | 0.4 |

**(b)** GeneMark

| Strain | Complete | Single | Duplicated | Fragmented | Missing |
|---|---|---|---|---|---|
| *T. harzianum* | 99.9 | 99.2 | 0.7 | 0.0 | 0.1 |
| *T. virens* | 99.5 | 98.8 | 0.7 | 0.3 | 0.2 |
| *T. reesei* | 99.8 | 99.5 | 0.3 | 0.0 | 0.2 |

**(c)** RefSeq

# Agreement of Gene Predictions

- In DC1 and Tsth20, BRaker2 and GeneMark tend to agree on theh start and stop positions of genes when they predict the same gene. Both gene finders have a large portion of singleton predictions.

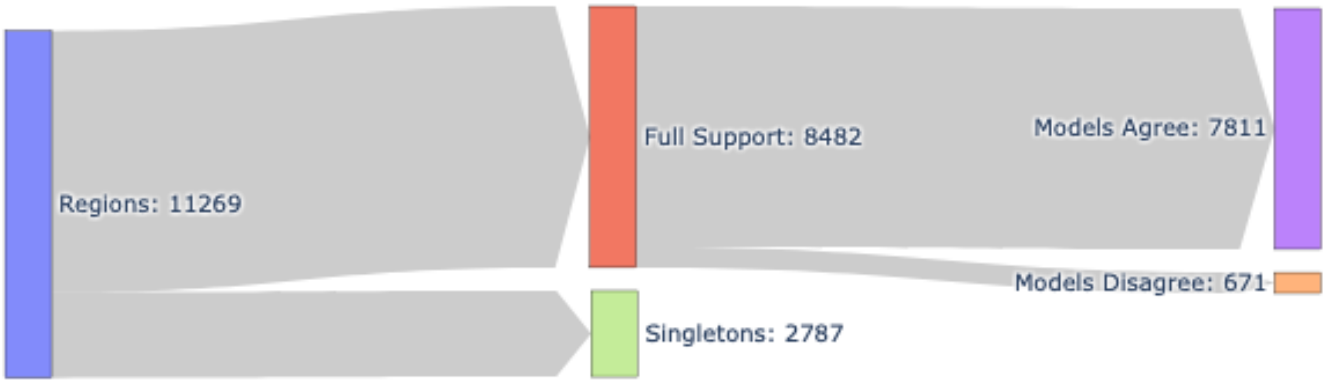



Figure: DC1

- In *T. reesei*, Braker2, GeneMark and RefSeq tend to disagree more on the start and stop positions of genes when they predict the same gene.
- Disagreement is more pronounced in *T. harzianum* and *T. virens*, where genes with partial support from more than one gene finder are common.

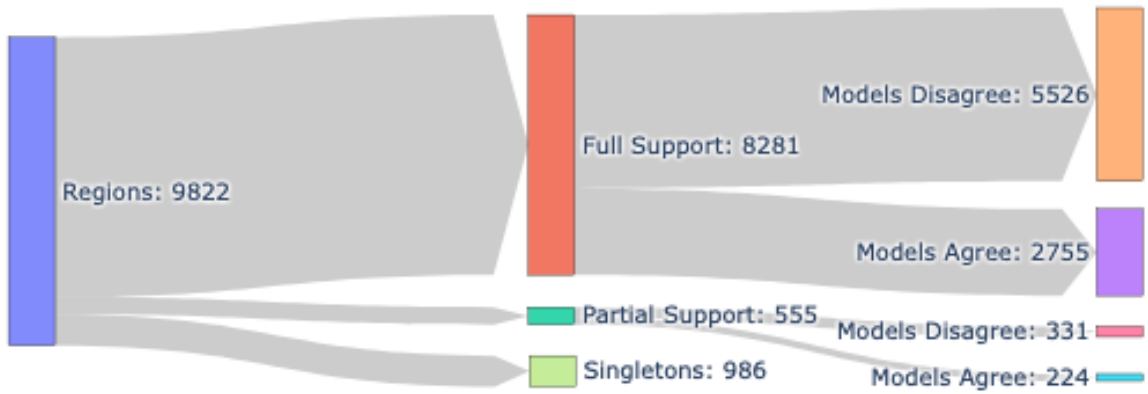



Figure: *T. reesei*

# InterProScan Results

- I need to look into this. Braker2 numbers strange

| Assembly | Braker2 | GeneMark | RefSeq |
|---|---|---|---|
| DC1 | $\frac{10676}{14479}$ | $\frac{8416}{11354}$ | N/A |
| Tsth20 | $\frac{11389}{15546}$ | $\frac{9168}{12373}$ | N/A |
| *T. reesei* | $\frac{8471}{11704}$ | $\frac{6990}{9196}$ | $\frac{6964}{9111}$ |
| *T. harzianum* | $\frac{11370}{15408}$ | $\frac{9061}{12164}$ | $\frac{9293}{14065}$ |
| *T. virens* | $\frac{11249}{15062}$ | $\frac{8871}{11866}$ | $\frac{9062}{12383}$ |

# BLAST Results

| Reference | Ref. Proteins | DC1 | Tsth20 | *T. reesei* | *T. harzianum* | *T. virens* |
|---|---|---|---|---|---|---|
| *Trichoderma atroviride* | 11807 | 11552 | 11080 | 10601 | 11081 | 11078 |
| *Fusarium granminarium* | 13312 | 10327 | 10429 | 10064 | 10434 | 10490 |
| *Saccharomyces cerevisiae* | 6014 | 3537 | 3517 | 3445 | 3509 | 3500 |

- The *T. atroviride* and *Fusarium* datasets are well respresented in the tblastn searches, while the *S. cerevisiae* dataset is less well represented.

| Subject | Query | Braker2 | GeneMark | RefSeq |
|---|---|---|---|---|
| DC1 | *T. atroviride* | 5902 | 4679 | N/A |
| DC1 | *F. graminarium* | 4955 | 4114 | N/A |
| DC1 | *S. cerevisiae* | 2105 | 1850 | N/A |
| *T. reesei* | *T. atroviride* | 5072 | 5174 | 4989 |
| *T. reesei* | *F. graminarium* | 4577 | 4685 | 4529 |
| *T. reesei* | *S. cerevisiae* | 2055 | 2114 | 2022 |
| *T. harzianum* | *T. atroviride* | 6363 | 4611 | 6835 |
| *T. harzianum* | *F. graminarium* | 5659 | 4198 | 5982 |
| *T. harzianum* | *S. cerevisiae* | 2424 | 1963 | 2560 |

- Regions with Braker2 and RefSeq gene predictions consistently have more tblastn hits than GeneMark, with the exception of *T. reesei*.

# Gene Predictions in AT-rich Sequence

| Assembly | Braker2 | GeneMark | RefSeq |
|---|---|---|---|
| DC1 | 31 | 11 | N/A |
| Tsth20 | 11 | 2 | N/A |
| *T. reesei* | 39 | 48 | 107 |
| *T. harzianum* | 81 | 30 | 154 |
| *T.virens* | 21 | 8 | 20 |

- Very few genes are predicted in AT-rich regions of DC1, Tsth20 and *T. virens* assemblies in comparison to the *T. reesei* and *T. harzianum* assemblies.
- Possibly due to higher quality assemblies? Although *T. virens* is still fragmented.
- A two-sided binomial test confirms that gene finders do not predict the same proportion of genes in AT-rich sequence as they do in normal genomic sequence.

# Conclusions

- In terms of gene finding performance based on several criteria, RefSeq generally performs the best, while GeneMark performs the worst.
- Braker2 performs well in *T. reesei*, but not as well in the other assemblies.
- Be carfeul with the training data you select for Braker2, as it can have a significant impact on the results.
- RefSeq is not always available, so Braker2 should be used if appropriate training is available, but GeneMark can be used as a fallback.

| Category | Braker2 | GeneMark | RefSeq |
|---|---|---|---|
| Availability | 3 | 3 | 0 |
| Ease of install | 1 | 2 | 0 |
| Ease of use | 3 | 3 | 0 |
| # of genes predicted | 0 | 3 | 3 |
| # of transcripts predicted | 3 | 0 | 2 |
| Predicts shortest genes | 2 | 1 | 0 |
| Predicts more shorter genes | 1 | 0 | 3 |
| BUSCO Performance | 2 | 1 | 3 |
| Performance in AT-rich sequence | 2 | 1 | 3 |
| Predictions with InterProScan support | 3 | 3 | 3 |
| Final Score (Publicly Available) | 20 | 17 | N/A |
| Final Score (Ignoring Availability) | 13 | 9 | 17 |

# Future Work

- Extend the analysis to include more *Trichoderma* species, more gene finding tools, and more datasets.
- Investigate further methods for validation of gene predictions.
- Explore the landscape of secondary metabolite gene clusters in *Trichoderma* genomes further.
- Continue to analyze the newly assembled genomes of DC1 and Tsth20.

# Timeline for Completion

- Initial draft of thesis complete - end of August 2025
- Revisions and changes - Sept./Oct. 2025
- Submit thesis to committee - Early November 2025
- Schedule defence for December 2025 or January 2026
- Time for extra revisions post-defence - January/early February 2026
- Completion - End of February 2026

# Current Status

- Introduction - 0%
- Background - 80%
- Methods - 85%
- Research Questions - 75%
- Results and Discussion - 80%
- Conclusions and Future Work - 70%
- What else needs to be done?