



Thesis Defence: Evaluation of Gene Finding Tools When Applied to *Trichoderma* Genomes

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Outline

- ***Trichoderma* fungi.**
- Motivation.
- Research objectives.
- Workflow overview.
- Results highlights.
- Conclusions and future work.



What is *Trichoderma*?

- Filamentous fungi common in soil; key role in nutrient cycling.
- Used in biocontrol and as biofertilizers.
- Produce plant cell wall degrading enzymes and **secondary metabolites**.
- Future studies reveal their biology and potential applications.

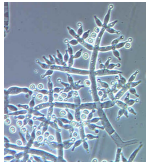


Figure: *T. harzianum* ¹



Figure: *Trichoderma* colony ²

¹Source: <https://en.wikipedia.org/wiki/Trichoderma>

²Source: <https://biocontrol.entomology.cornell.edu/pathogens/trichoderma.php>

Novel *Trichoderma* Genomes

- *Trichoderma* species are diverse, with many species not yet fully characterized.
- DC1 and Tsth20 both improve salt and drought tolerance.
- Tsth20 shows potential as a bioremediation agent.
- Recent advances in sequencing technology have made it possible to generate high-quality genomes for these species.
- Joint effort by Brendan Ashby, the Kaminskyj lab, and the Global Institute for Food Security.



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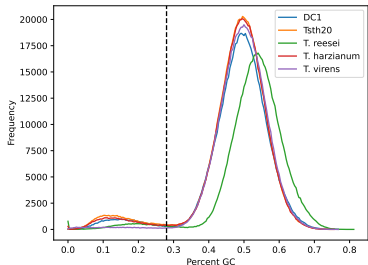
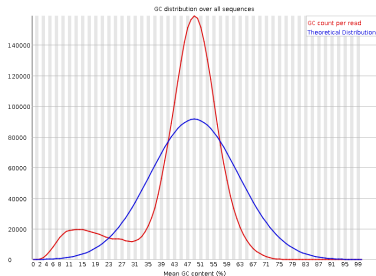
Motivation

- To understand *Trichoderma* biology, we must identify genes within their genomes.
- Gene prediction tools vary significantly in implementation and performance.
- Novel genomes with novel biology require gene annotation to connect phenotypes to genotypes.
- New high-quality *Trichoderma* genomes (DC1 and Tsth20) enable this comparative evaluation of gene finding tools.



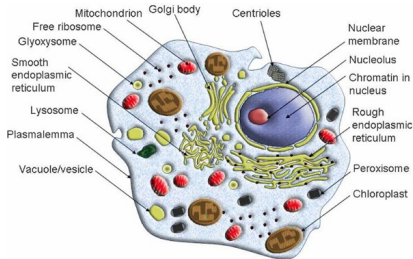
Gene Finding is Still Challenging

- We've been finding genes for decades. What's going on?
- Exons and introns as well as start and stop positions make gene finding tricky.
- Inherent properties of genomes also complicate gene finding.
- Stop codons in AT-rich regions are highly prevalent.

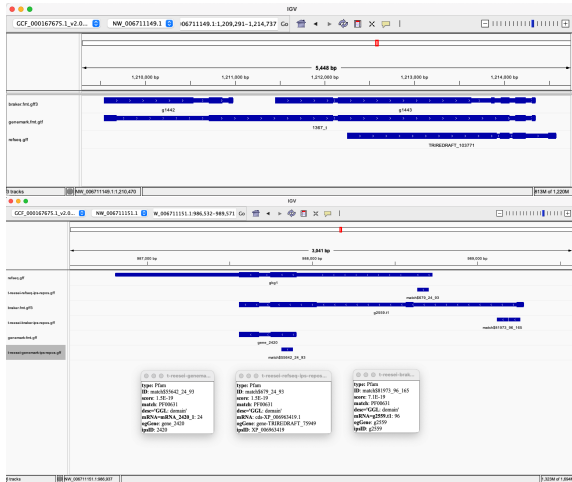


The Details Matter

- Minor changes in start/stop positions can have major impacts on predicted protein sequences.
- Ex. The subcellular localization process relies heavily on N-terminal sequences.
- Links back to cell wall degrading enzymes and secondary metabolites.



Variability in Gene Predictions



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Research Objectives

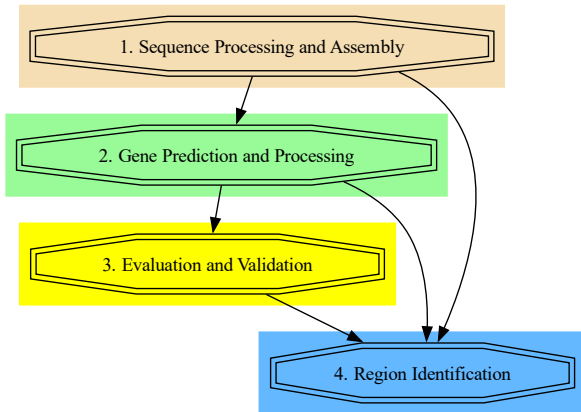
- Assemble and evaluate novel assemblies of DC1 and Tsth20.
- Apply gene finders Braker2 and GeneMark in DC1, Tsth20, and three other RefSeq assemblies.
- Compare gene finding tools based on relevant criteria, including:
 - Number of genes and isoforms predicted.
 - Presence of conserved single-copy orthologs (BUSCOs).
 - Presence of functional domains and closely related protein sequences.
 - Presence of genes in AT-rich sequence.
 - Agreement of gene finders on start and stop positions of a gene.



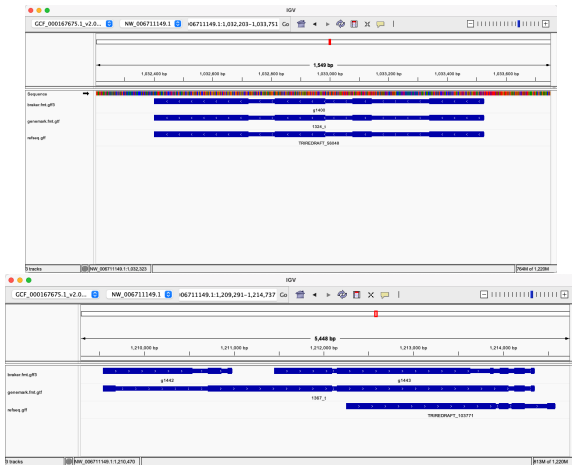
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Workflow Overview



Region Identification



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Assemblies of DC1 and Tsth20

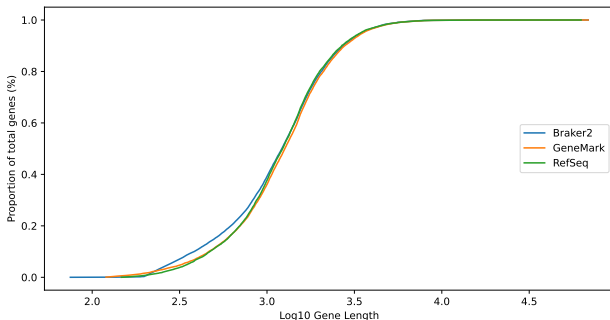
- Both assemblies are of high quality.
- Few contigs, which is good for gene finding.
- BUSCO analysis sees similar benefits.
- RefSeq assemblies are highly fragmented in comparison.

Strain	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
<i>T. harzianum</i>	532	40.98 Mb	4.08 Mb	47.61	2.41 Mb	7
<i>T. virens</i>	93	39.02 Mb	3.45 Mb	49.25	1.83 Mb	8
<i>T. reesei</i>	77	33.39 Mb	3.75 Mb	52.82	1.21 Mb	9



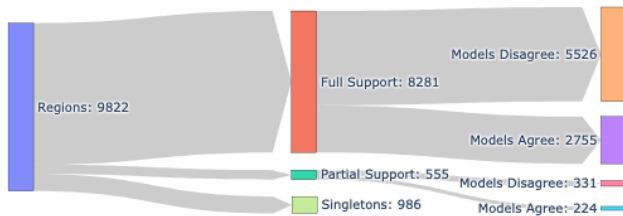
Small but Significant Details

- Before diving too deep, how can we objectively confirm that gene finding tools are systematically different?
- Gene finders may be biased to predict certain gene lengths.
- Kolmogorov-Smirnov (KS) tests were performed on gene length distributions from preliminary gene predictions.



Regions of Gene Predictions

- Agreement of gene finders assessed by identifying regions of gene predictions.
- Regions classified based on agreement of start and stop positions.
- Transitive closure used to group overlapping predictions.



AT-rich Sequence Analysis

- GC content varies across genomes.
- Gene finders struggle in AT-rich ($>72\%$ AT content) regions.

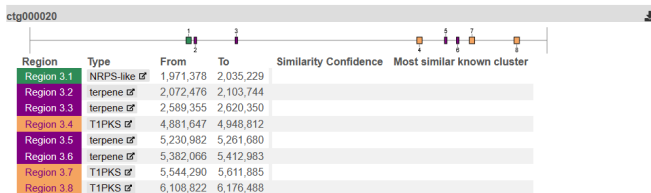
Genome	Total Length (bp)	Low GC Segments	Low GC Length (bp)	Proportion (%)
DC1	38,616,239	610	2,064,202	5.35
Tsth20	41,588,851	245	891,216	2.14
<i>T. reesei</i>	33,395,713	689	1,269,347	3.80
<i>T. harzianum</i>	40,980,648	1,311	2,527,773	6.17
<i>T. virens</i>	39,022,666	96	162,356	0.42

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



Secondary Metabolite Gene Clusters

- antiSMASH used to identify candidate secondary metabolite gene clusters.
- Tsth20 contains multiple candidate clusters.
- 35 candidates from Braker2 predictions and 58 from GeneMark.
- Limited exploration of predicted clusters performed.



Ranking Gene Finders

Category	Braker2	GeneMark	RefSeq
Availability	2	2	N/A
Ease of install	1	2	N/A
Ease of use	2	2	N/A
Number of genes predicted	1	3	3
Number of isoforms predicted	3	0	2
BUSCO Performance	3	2	1
Performance in AT-rich sequence	2	1	3
Predictions with InterProScan support	2	3	1
Cumulative Rank (Considering Availability)	16	15	N/A
Cumulative Rank (Ignoring Availability)	11	9	10



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Key Findings

- Overall performance of gene finders are similar, but vary by specific criteria.
- Braker2 ranks highest, particularly when relevant training data is available.
- Candidate secondary metabolite gene clusters were found in Tsth20 assembly.
- DC1 and Tsth20 assemblies are of high quality, providing a solid foundation for future genomic studies.
- Explored a reference-agnostic approach to gene prediction comparisons.



Future Work

- A few of many potential avenues...
 - Explore additional gene finding tools.
 - Adjust parameters of existing tools.
 - Experiment with different types of training data for Braker2.
 - Extend analysis of identified regions of gene predictions.
 - Explore secondary metabolite gene clusters in more detail.
 - Expand the number of *Trichoderma* genomes analyzed.



Acknowledgements

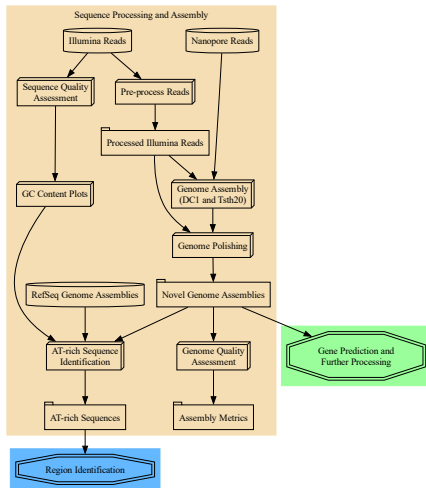
- My supervisors for their committed support through COVID and other challenges.
- Brendan Ashby and the Kaminskyj lab for providing the data for this project.
- Global Institute for Food Security for providing a portion of my funding.
- My committee members for their feedback and support.



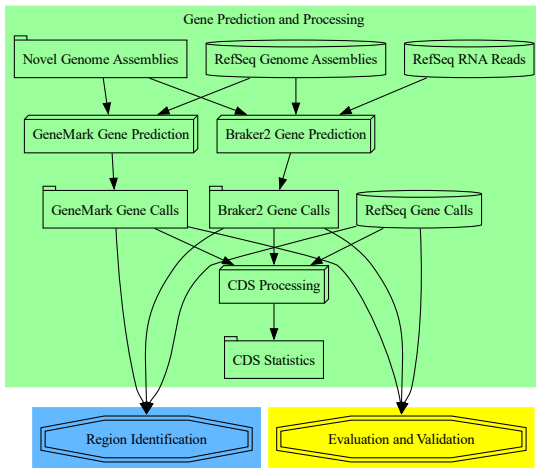
Questions and Discussion



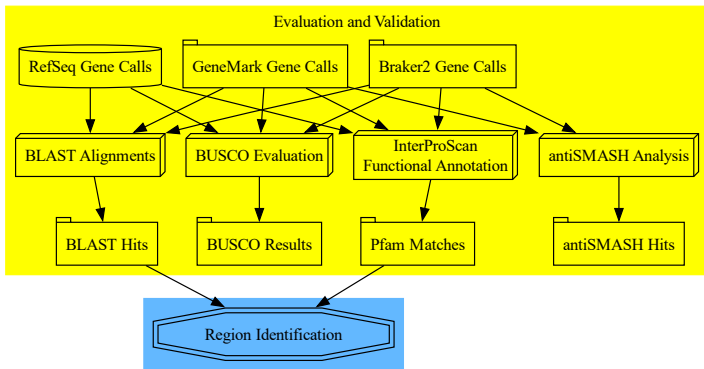
Workflow Details: Assembly



Workflow Details: Gene Finding

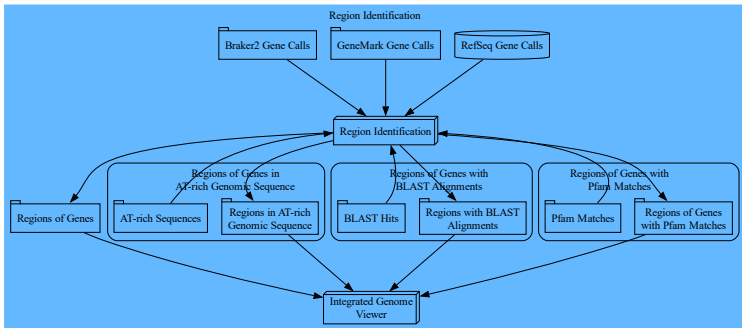


Workflow Details: Evaluation





Workflow Details: Regions



Datasets and Tools

- **Datasets:**

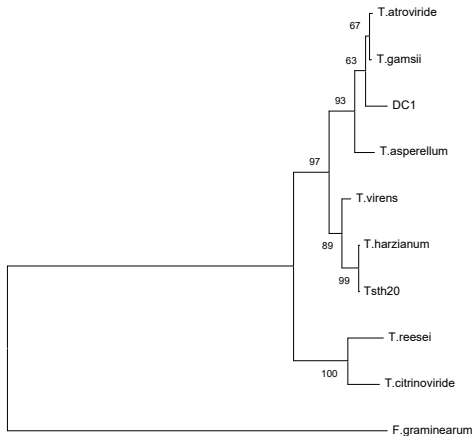
- Novel *Trichoderma* genomes: DC1 and Tsth20.
- Reference genomes and annotations: *T. reesei*, *T. harzianum*, *T. virens*.
- RNAseq training data from *T. reesei*.
- Benchmarking Universal Single-Copy Orthologs (BUSCO) fungal database.
- Protein sequence queries for tblastn from *T. atroviride*, *Fusarium 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.



Phylogenetics



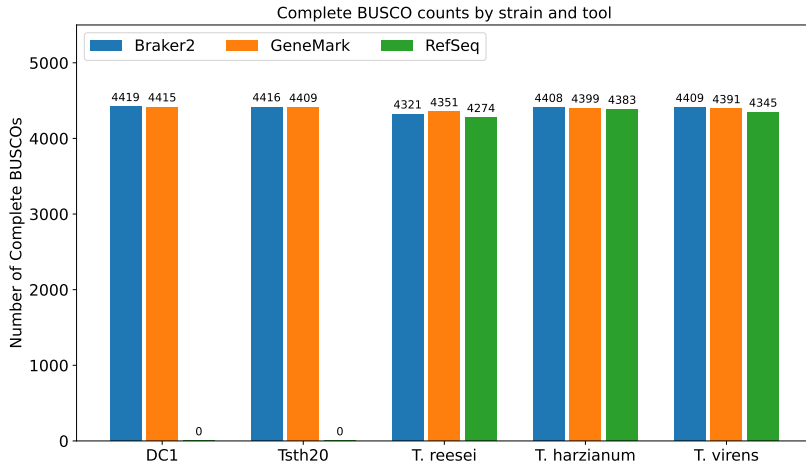
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Region Identification Algorithm

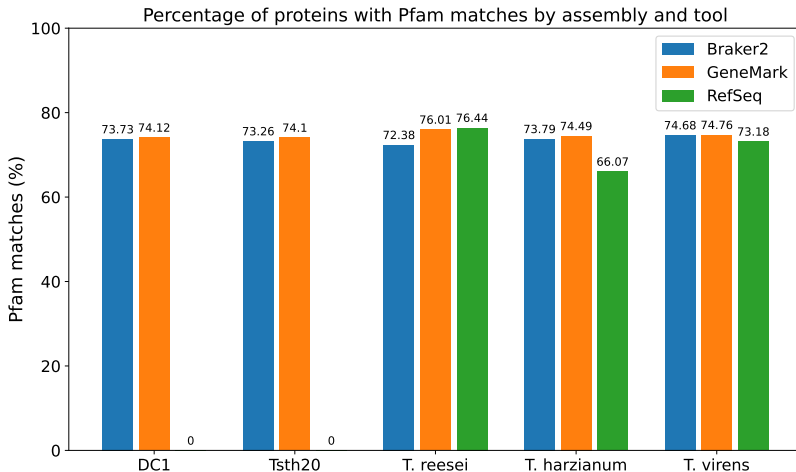
```
for <each feature in sortedFeatures> do
  if feature = firstFeature then
    currentRegion ← newRegion(feature)
    currentRegion.start ← feature.start
    currentRegion.end ← feature.end
  else if feature = lastFeature then
    regionList.append(currentRegion)
    return regionList
  else if feature overlaps currentRegion then
    currentRegion.updatePositions(currentRegion, feature)
    currentRegion.addFeature(feature)
  else
    regionList.append(currentRegion)
    currentRegion ← newRegion(feature)
    currentRegion.start ← feature.start
    currentRegion.end ← feature.end
  end if
end for
return regionList
```



BUSCO Results

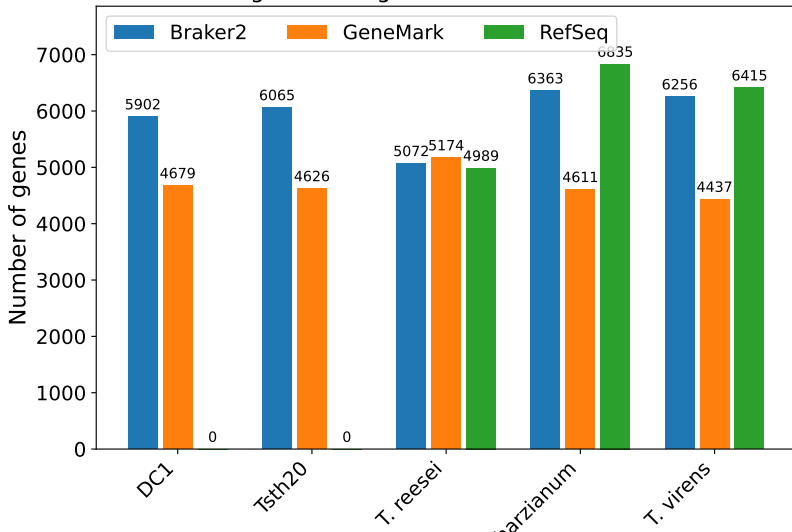


InterProScan Results



BLAST Results

Number of genes in regions with *T. atroviride* tblastn hits





AT-rich Sequence Localization

