willoughby
conservation and genomics lab

Integrative bioinformatic and molecular approaches to elucidate the genomics of *Lecanosticta acicola* and its impacts on Brown Spot Needle Blight

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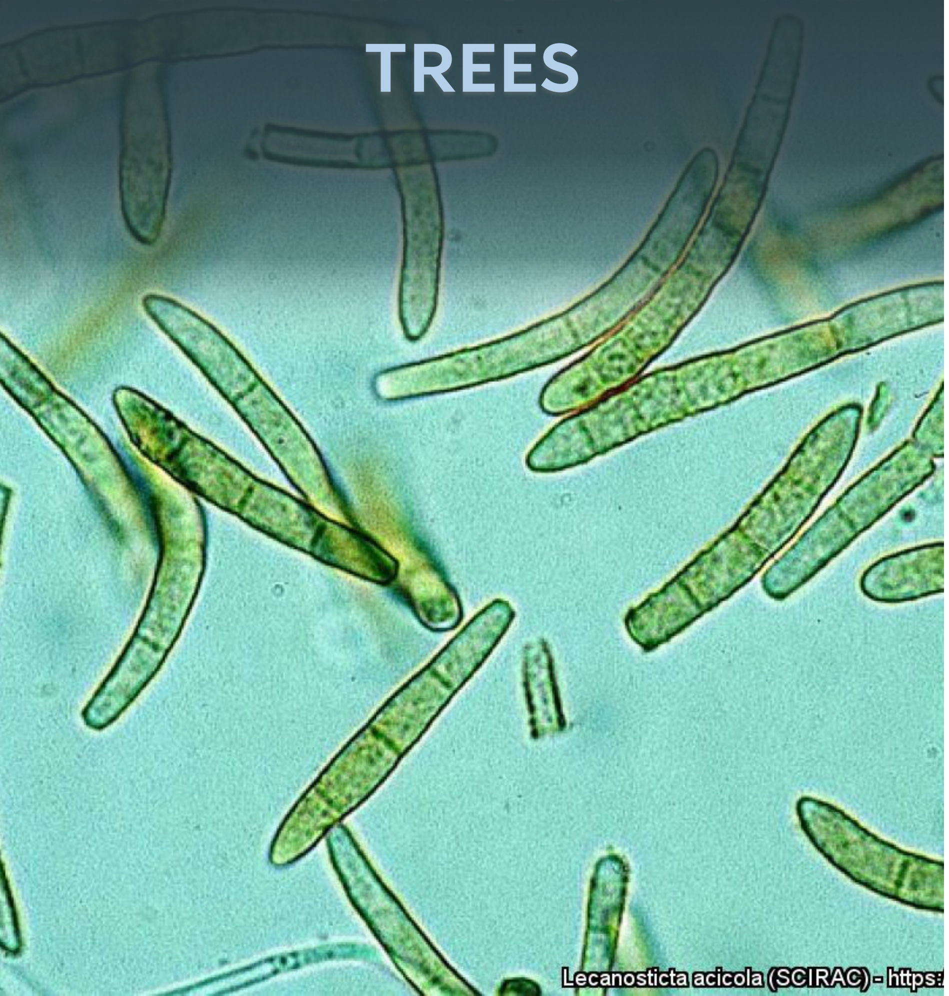
Temitope R Folorunso
Lori G Eckhardt, Ph.D.
Janna R Willoughby, Ph.D.

Aug 13, 2024

THREAT TO THE ECONOMIC AND ECOLOGICAL SIGNIFICANCE OF PINE TREES



https://gd.eppo.int/taxon/SCIRAC/photos

BROWN SPOT NEEDLE BLIGHT


ONTARIO

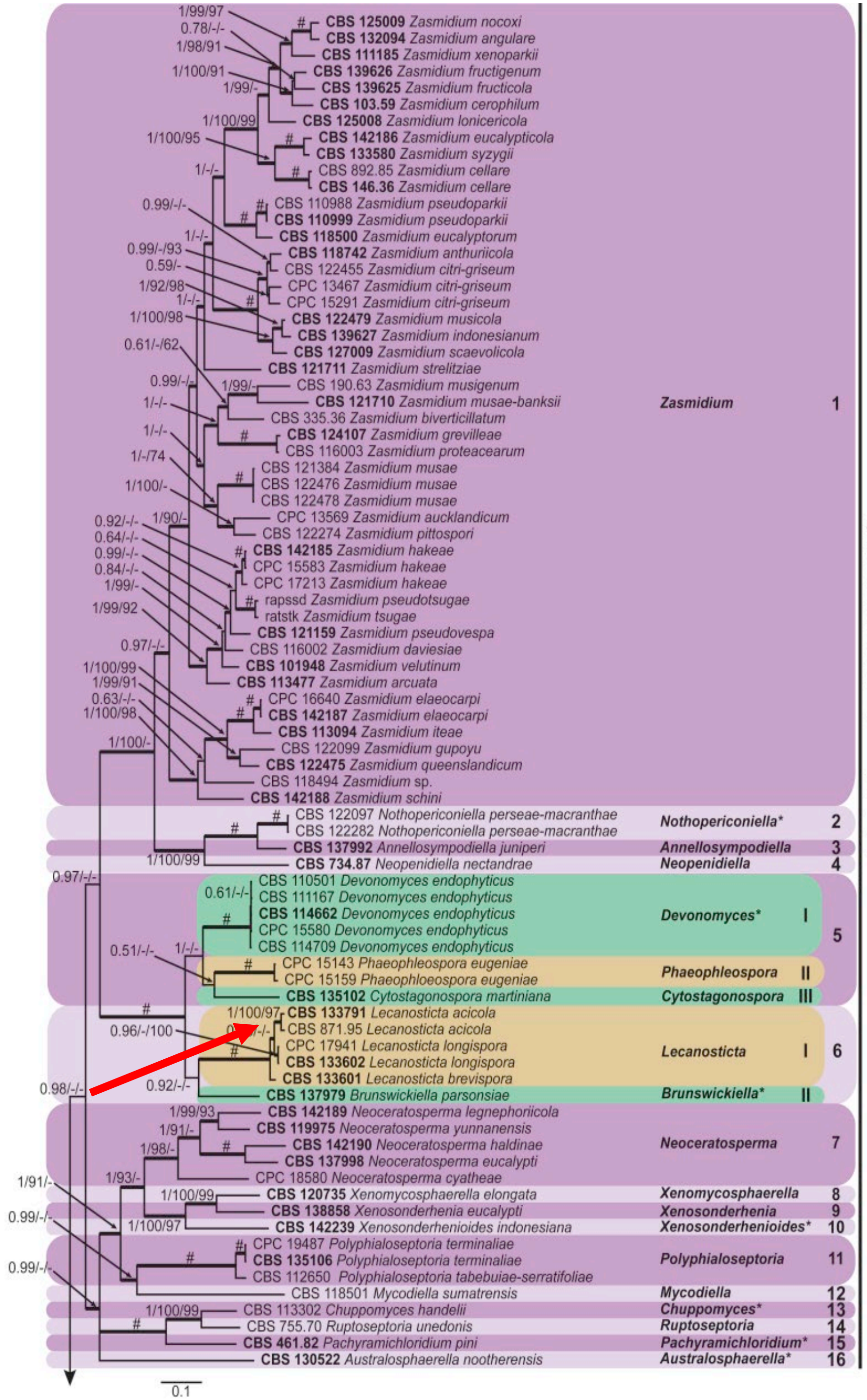

ALABAMA

ARKANSAS

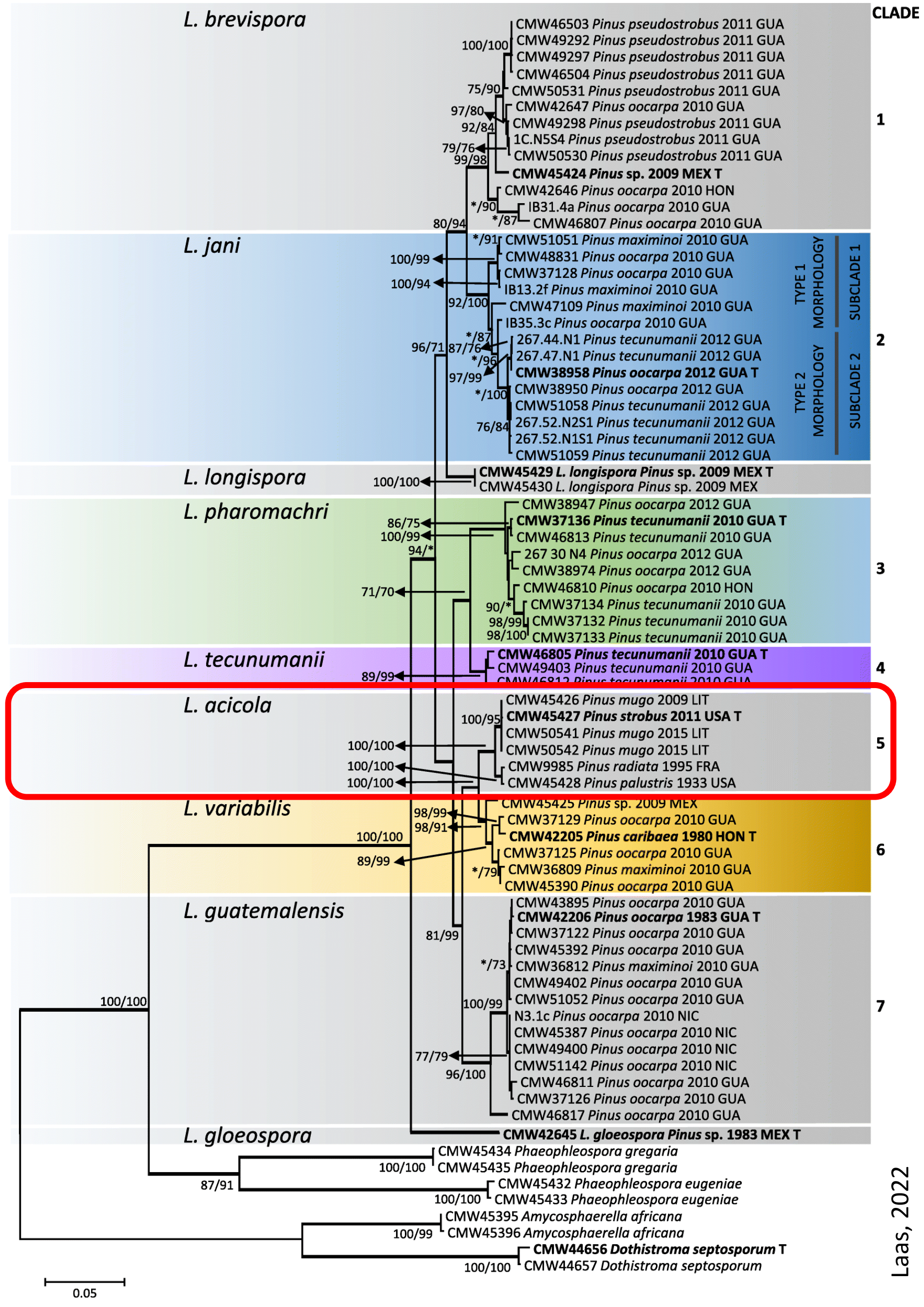

FLORIDA


ORIGINS, EVOLUTION & TAXONOMY

- Central America
    - Genus
- North America
    - Species



Videira et al., 2017

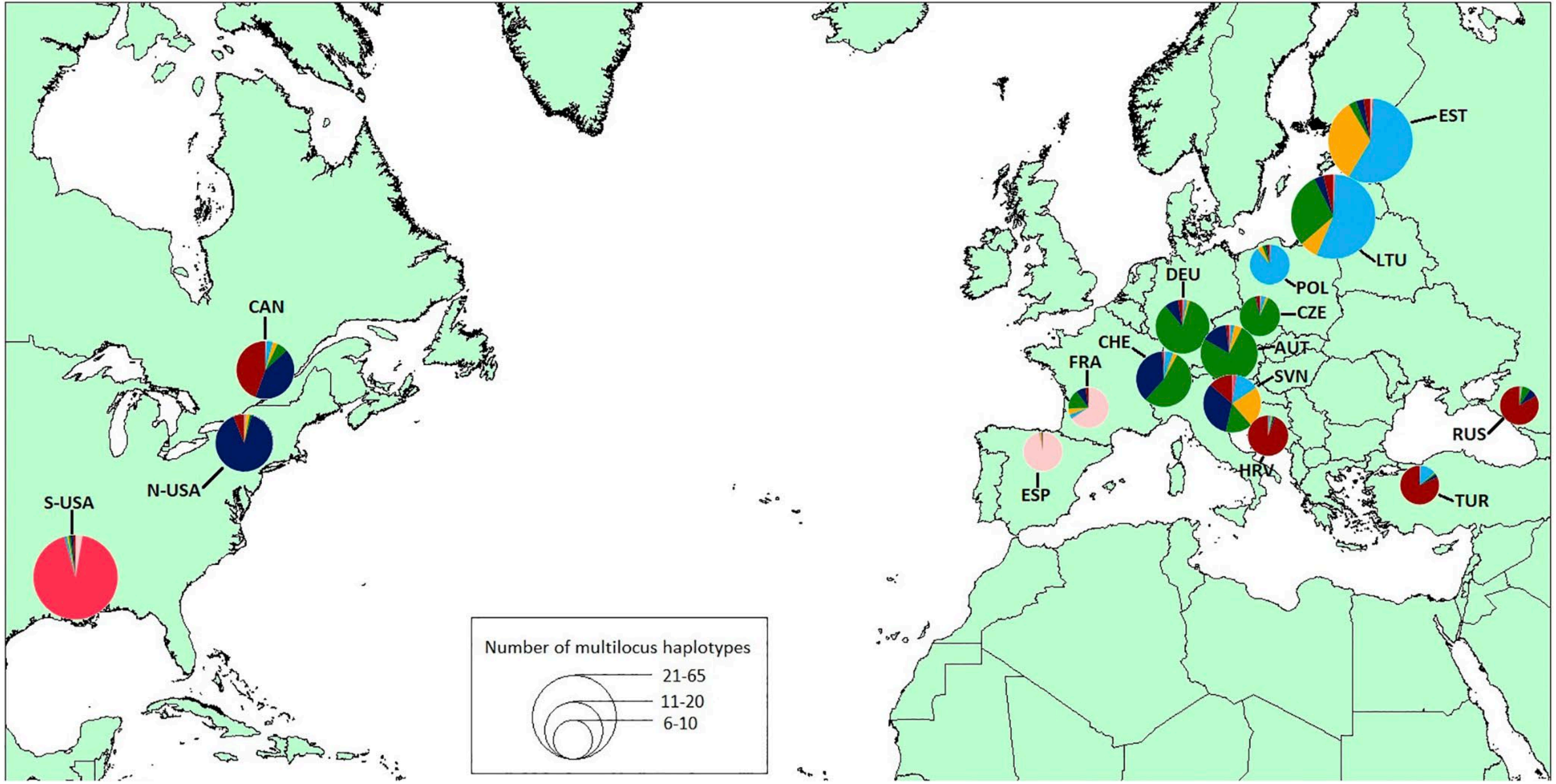


Laas, 2022

SPREAD AND DIVERSITY

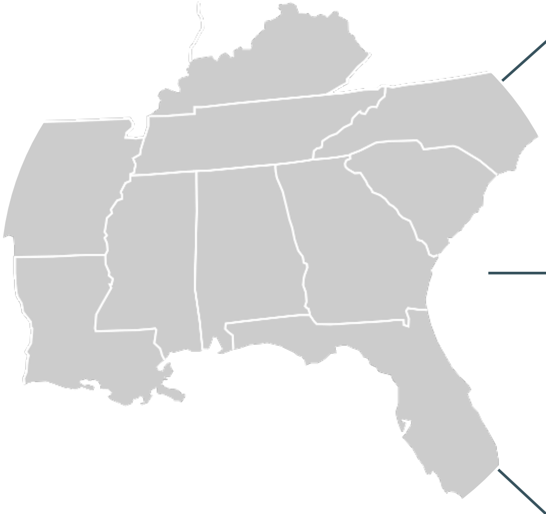
Southern USA is remarkably distinct

Tubby, et al. (2023)



Laas, M., et al. (2023)

RESEARCH GAPS



Regional genomic data

- Limited genomic studies in the Southeastern U.S.

Host specificity

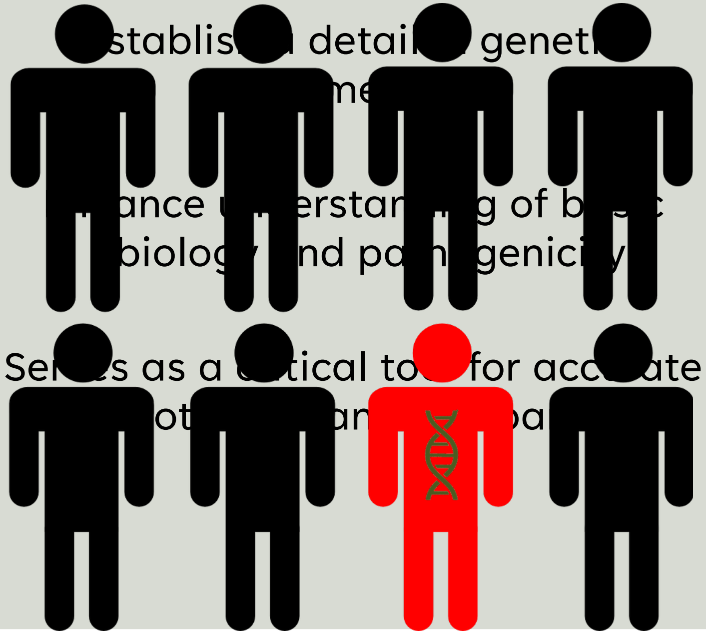
- Insufficient understanding on host specificity and the pathogen’s interaction with different pine species.

Disease management

- Need for targeted disease management strategies based on genomic insights.

APPROACHES

Reference genome



stablis detai geneti
me

ance u erstan g of b c
biolog nd pa genicity

Se s as a tical to for acc ate
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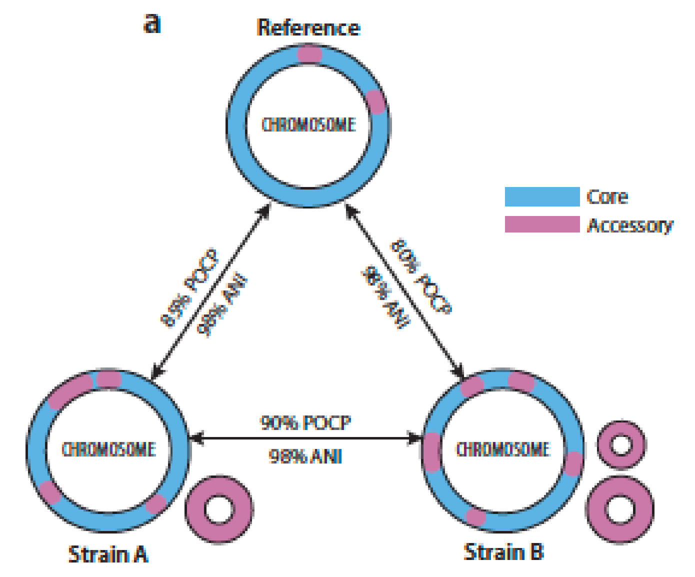
Pan-genome



Combined approach

Complete picture of the pathogen’s genetics
Integrate foundational insights with ecological implications
Enhance understanding of evolutionary pressures on *L. acicola*

Objectives

Genome Assembly and Analysis	Produce new genome assemblies of *Lecanosticta acicola* using long-read and short-read sequencing and perform comparative genomic analyses with other fungal pathogens.
Phylogenetic and Evolutionary Insights	Conduct accurate phylogenetic analysis to refine the evolutionary placement of *L. acicola* and identify genomic features specific to the southeastern U.S.
Genomic Diversity and Disease Targets	Explore genomic diversity, host specificity, and identify potential genetic targets associated with disease in *L. acicola*.

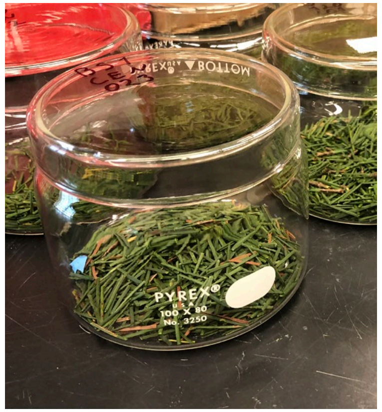
SAMPLE DISEASED TREES

LOBLOLLY

LONGLEAF

SLASH

SAMPLE SURFACE STERILIZATION



Bleach → Water → Ethanol → Water

FUNGUS CULTIVATION

PLATING

- PNA – Pine needle agar
- MEA 1% – Malt extract agar
- CMA – Corn meal agar
- APDA – Acid potato dextrose agar
- PNA + ME – Pine needle agar with 0.5% malt extract
- MEA 2% – Malt extract agar

FUNGUS ISOLATION

NEEDLE PLATING

Needles with spots

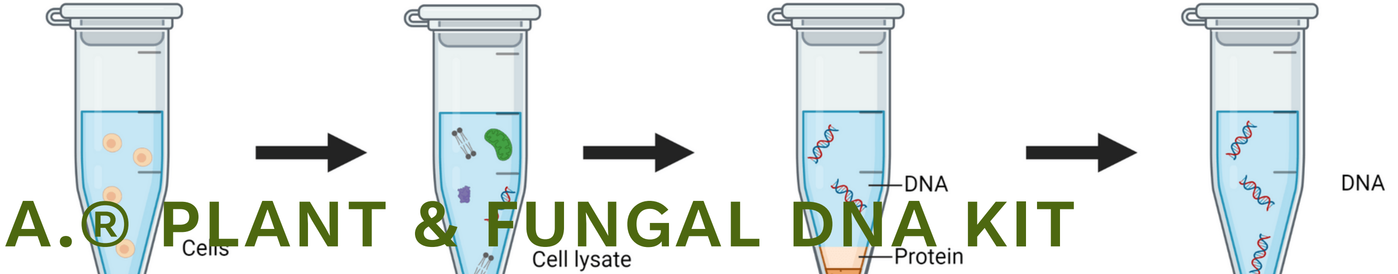
SUBCULTURING

Suggestive morphology

Replate into same media

INITIAL DNA EXTRACTION

PHENOL:CHLOROFORM PROTOCOL



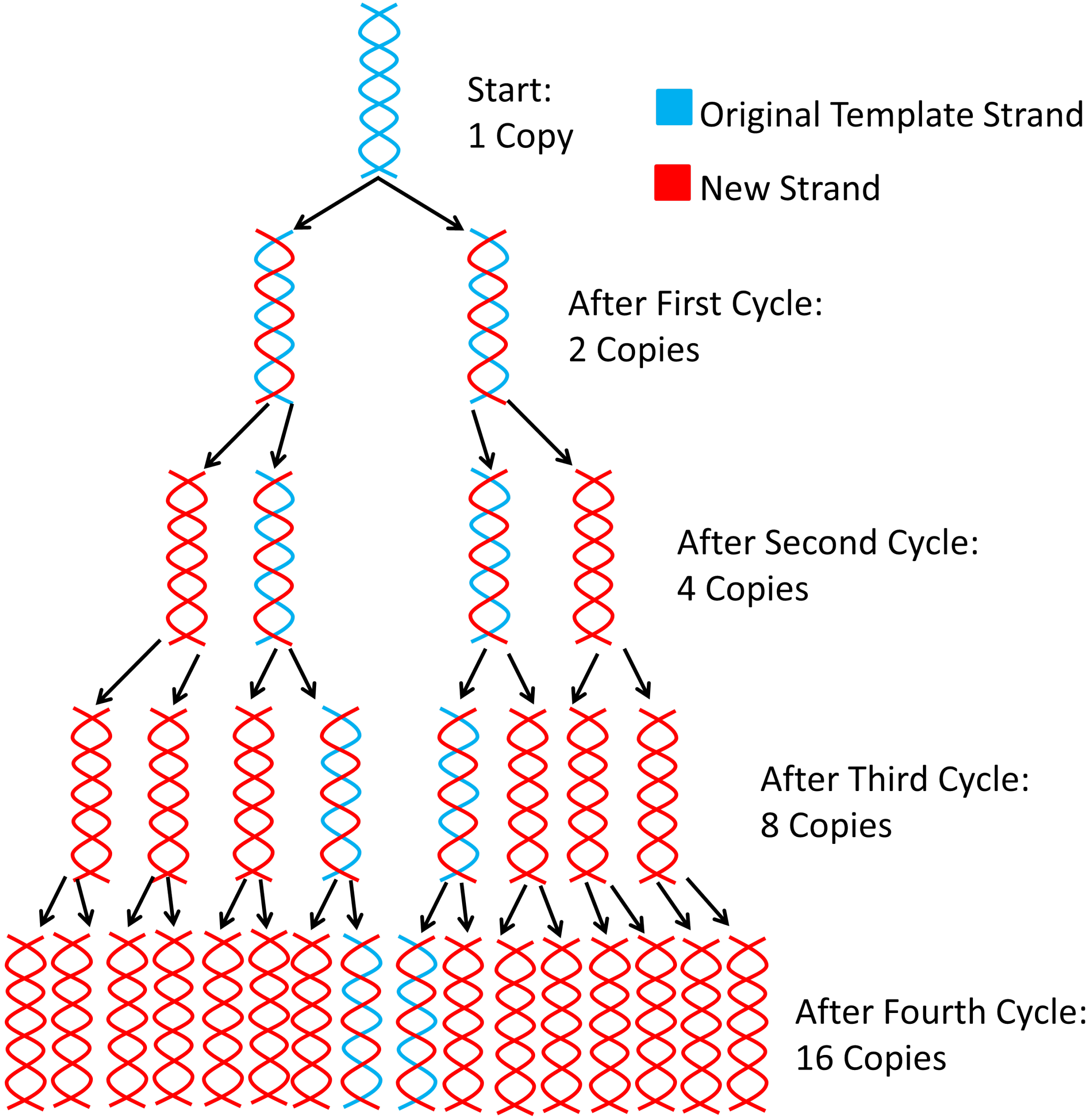
Add phenol-chloroform
Vortex and centrifuge

Transfer and retain aqueous phase DNA

verview-of-dna-extraction-methods

E.Z.N.A.® PLANT & FUNGAL DNA KIT

PCR CONFIRMATION



Start:
1 Copy

🟦 Original Template Strand

🟥 New Strand

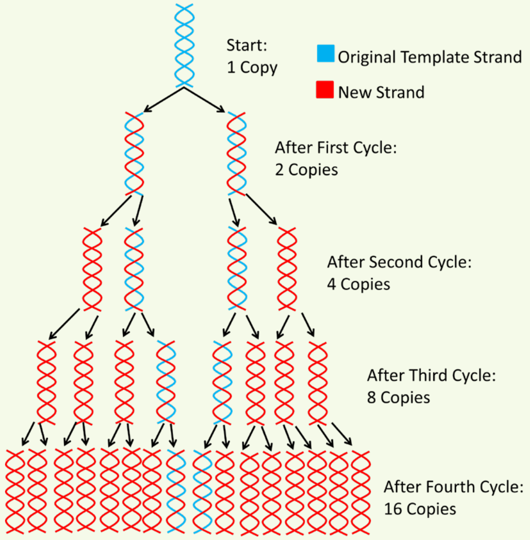
After First Cycle:
2 Copies

After Second Cycle:
4 Copies

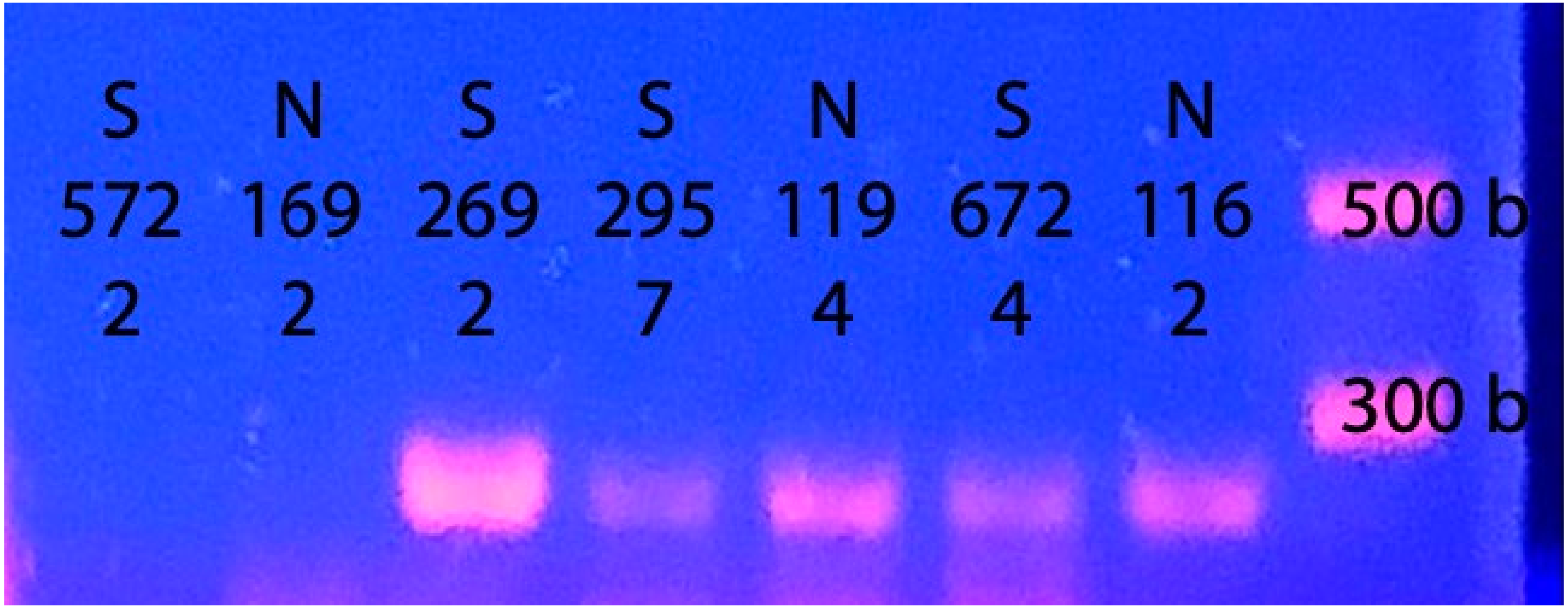
After Third Cycle:
8 Copies

After Fourth Cycle:
16 Copies

PCR CONFIRMATION



Latef Primers (Ioos, 2011) - *TEF*1 Region



✗ ✗ ✓ ✓ ✓ ✓ ✓

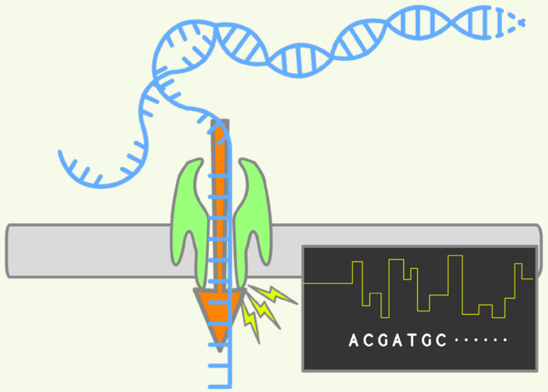
FUNGUS CULTIVATION

DNA EXTRACTION

3% MALT EXTRACT LIQUID

CTAB – PHENOL:CHLOROFORM PROTOCOL

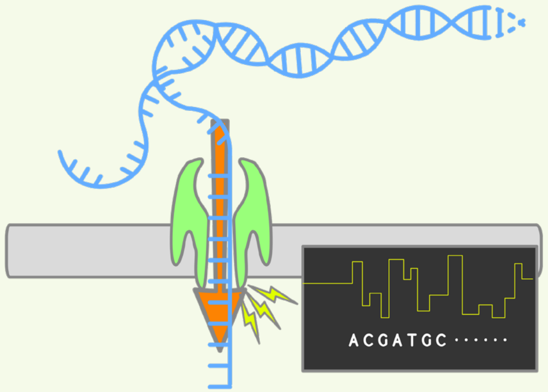
DNA SEQUENCING

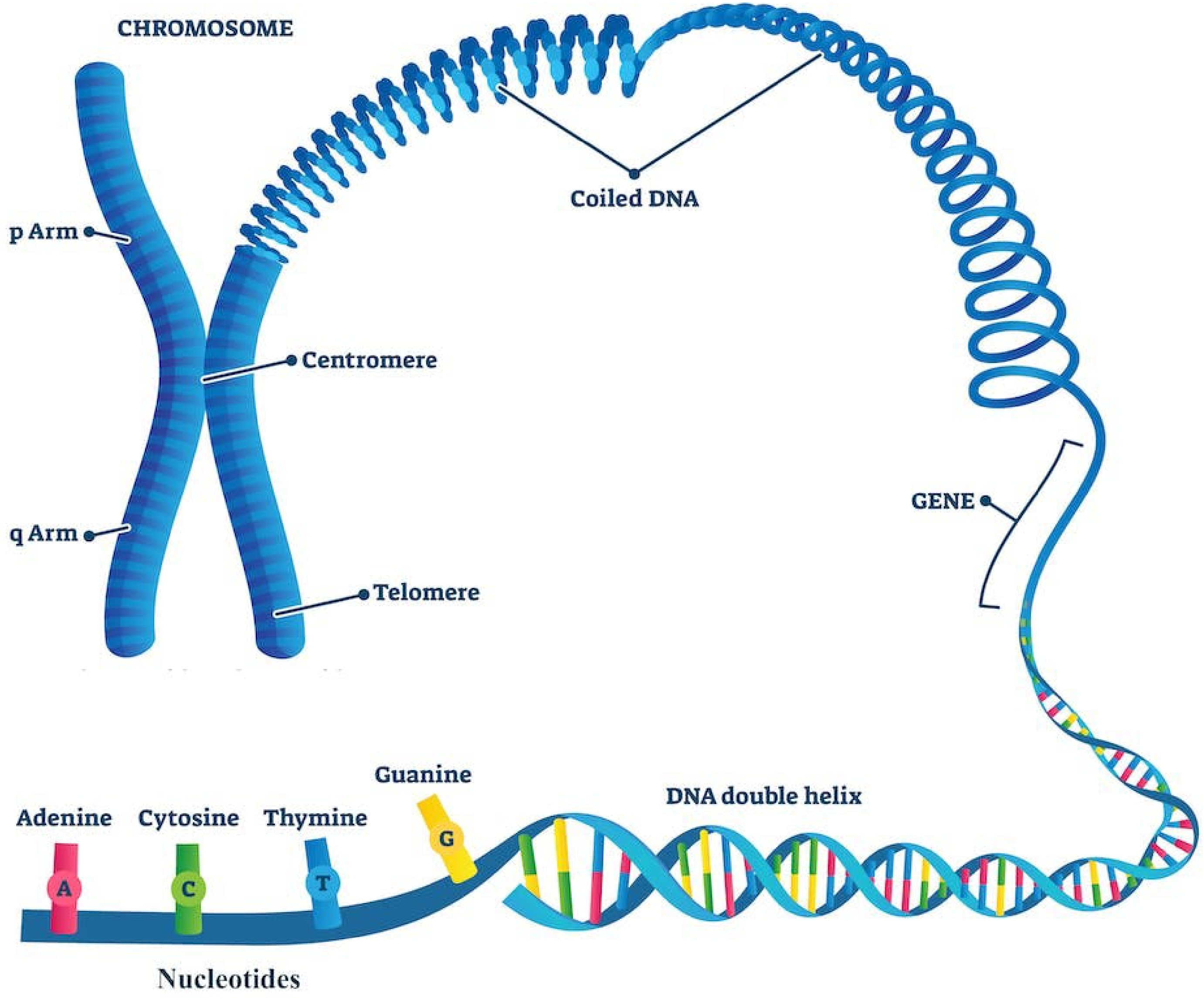


OXFORD NANOPORE

MINION MK1C

DNA SEQUENCING





Source: https://theconversation.com/the-human-genome-project-pieced-together-only-92-of-the-dna-now-scientists-have-finally-filled-in-the-remaining-8-176138

DATA ANALYSIS

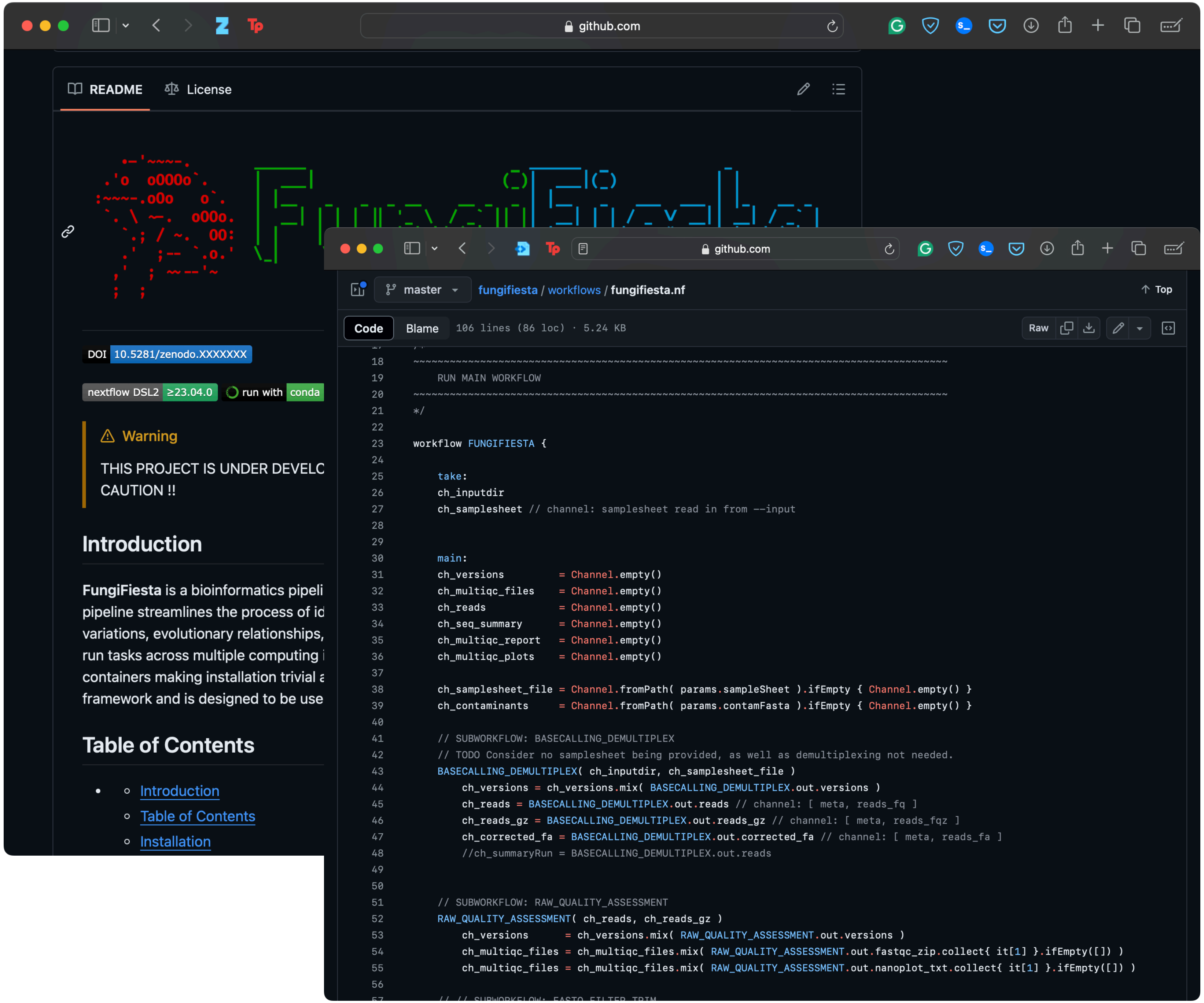
WHAT IS A PIPELINE?

DATA ANALYSIS

GitHub

AUHPC

> hpc.auburn.edu



DOI 10.5281/zenodo.XXXXXXX

nextflow DSL2 ≥23.04.0 run with conda

⚠ Warning

THIS PROJECT IS UNDER DEVELC
CAUTION !!

Introduction

FungiFiesta is a bioinformatics pipeli
pipeline streamlines the process of id
variations, evolutionary relationships,
run tasks across multiple computing i
containers making installation trivial a
framework and is designed to be use

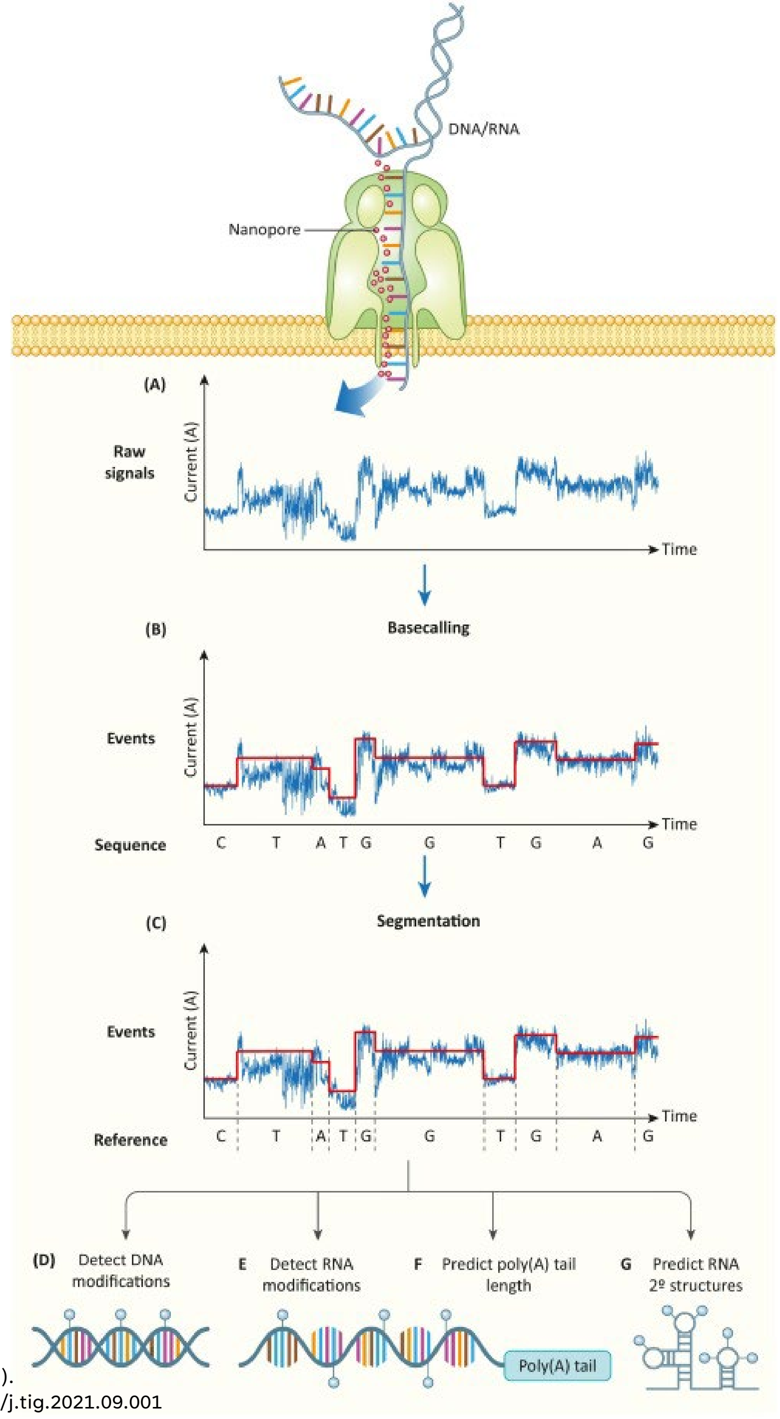
Table of Contents

- Introduction
- Table of Contents
- Installation

```
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    RUN MAIN WORKFLOW
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
*/

workflow FUNGIFIESTA {

    take:
    ch_inputdir
    ch_samplesheet // channel: samplesheet read in from --input


    main:
    ch_versions          = Channel.empty()
    ch_multiqc_files     = Channel.empty()
    ch_reads             = Channel.empty()
    ch_seq_summary       = Channel.empty()
    ch_multiqc_report    = Channel.empty()
    ch_multiqc_plots     = Channel.empty()

    ch_samplesheet_file = Channel.fromPath( params.sampleSheet ).ifEmpty { Channel.empty() }
    ch_contaminants     = Channel.fromPath( params.contamFasta ).ifEmpty { Channel.empty() }

    // SUBWORKFLOW: BASECALLING_DEMULTIPLEX
    // TODO Consider no samplesheet being provided, as well as demultiplexing not needed.
    BASECALLING_DEMULTIPLEX( ch_inputdir, ch_samplesheet_file )
        ch_versions = ch_versions.mix( BASECALLING_DEMULTIPLEX.out.versions )
        ch_reads = BASECALLING_DEMULTIPLEX.out.reads // channel: [ meta, reads_fq ]
        ch_reads_gz = BASECALLING_DEMULTIPLEX.out.reads_gz // channel: [ meta, reads_fqz ]
        ch_corrected_fa = BASECALLING_DEMULTIPLEX.out.corrected_fa // channel: [ meta, reads_fa ]
        //ch_summaryRun = BASECALLING_DEMULTIPLEX.out.reads


    // SUBWORKFLOW: RAW_QUALITY_ASSESSMENT
    RAW_QUALITY_ASSESSMENT( ch_reads, ch_reads_gz )
        ch_versions      = ch_versions.mix( RAW_QUALITY_ASSESSMENT.out.versions )
        ch_multiqc_files = ch_multiqc_files.mix( RAW_QUALITY_ASSESSMENT.out.fastqc_zip.collect{ it[1] }.ifEmpty([]) )
        ch_multiqc_files = ch_multiqc_files.mix( RAW_QUALITY_ASSESSMENT.out.nanoplot_txt.collect{ it[1] }.ifEmpty([]) )
```

BIOINFORMATICS

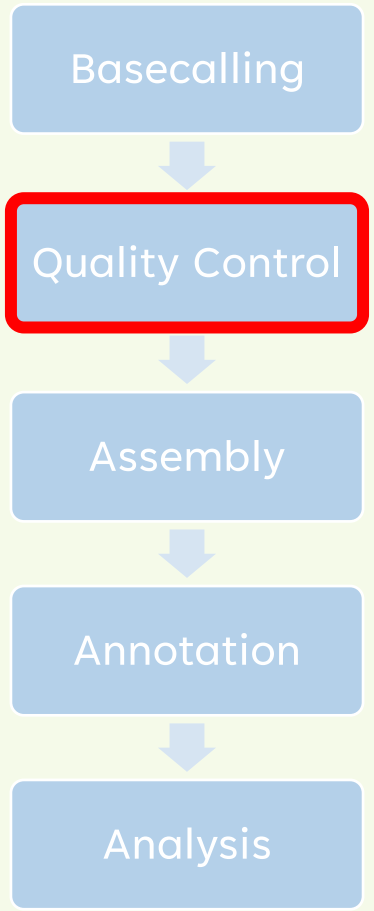
- Basecalling
- Quality Control
- Assembly
- Annotation
- Analysis



Trends in Genetics

Wan, Y. K., et al. (2022).
https://doi.org/10.1016/j.tig.2021.09.001

BIOINFORMATICS


Basecalling

Quality Control

Assembly

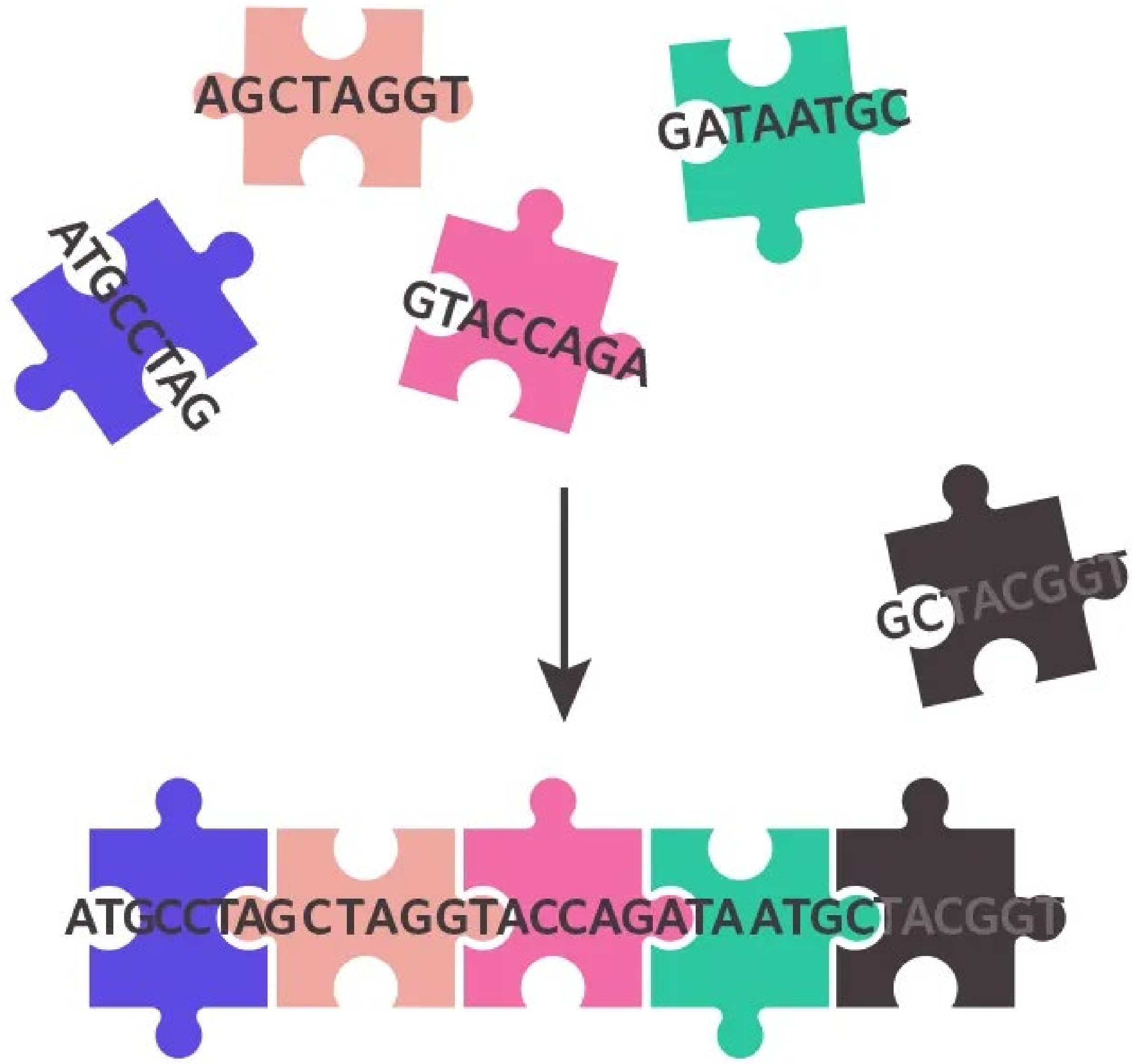
Annotation

Analysis

Removal of low-quality data

BIOINFORMATICS

- Basecalling
- Quality Control
- **Assembly**
- Annotation
- Analysis

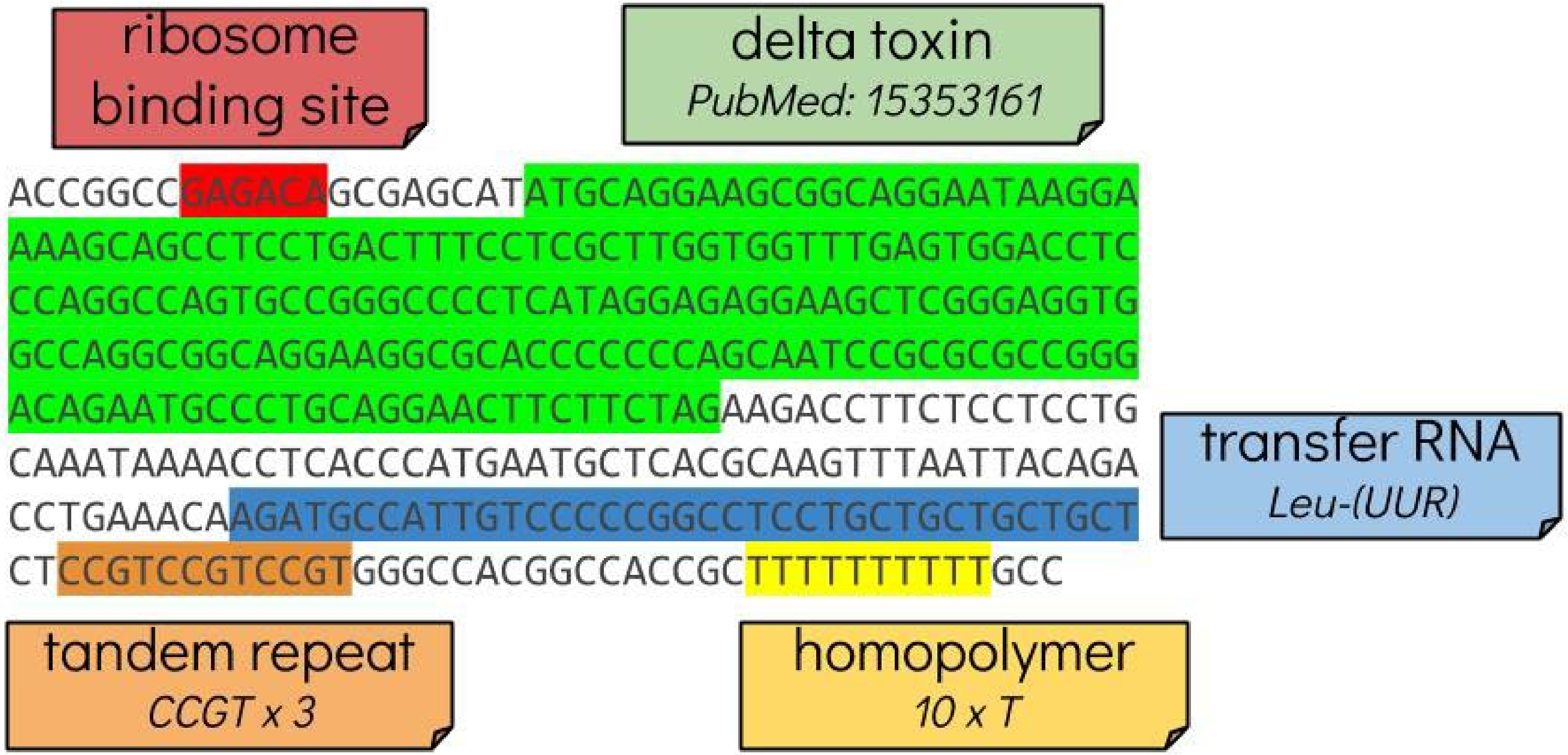


Source: https://www.the-scientist.com/infographics/infographic-the-sequencing-and-assembly-of-the-human-genome-70427

BIOINFORMATICS

- Basecalling
- Quality Control
- Assembly
- Annotation
- Analysis

Adding biological info to sequences



Source: https://training.galaxyproject.org/training-material/topics/genome-annotation/tutorials/annotation-with-prokka/slides-plain.html

BIOINFORMATICS

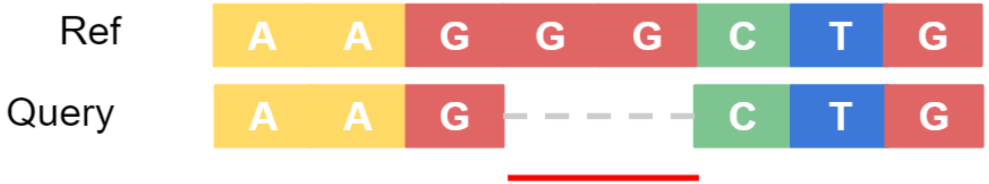
- Basecalling
- Quality Control
- Assembly
- Annotation
- **Analysis**

Sequence Variants

SNV (**S**ingle **N**ucleotide **V**ariant)

Ref	A	A	G	G	G	C	T	G
Query	A	A	G	G	A	C	T	G

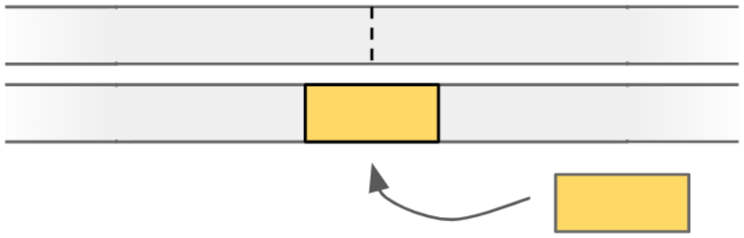
INDEL (**In**sertion or **Del**etion)



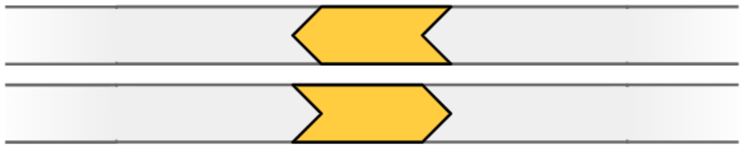
Ref	A	A	G	G	G	C	T	G
Query	A	A	G	-	-	C	T	G

Structural Variants

Insertion



Inversion



https://www.melbournebioinformatics.org.au/tutorials/tutorials/longread_sv_calling/longread_sv_calling/

BIOINFORMATICS

Basecalling

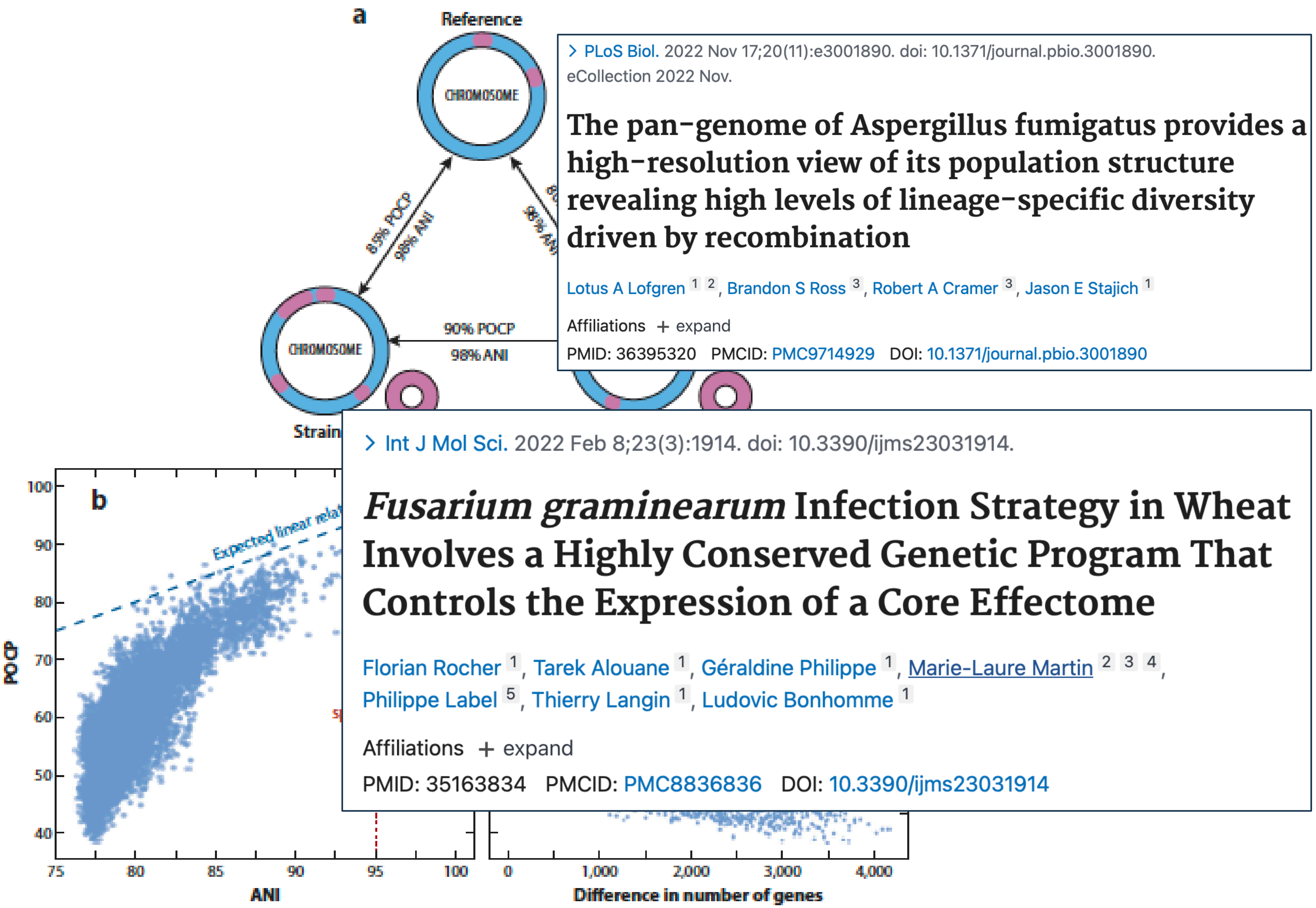
Quality Control

Assembly

Annotation

Analysis

Weisberg, A. J., et al. (2021)
https://doi.org/10.1146/annurev-phyto-020620-121736



> PLoS Biol. 2022 Nov 17;20(11):e3001890. doi: 10.1371/journal.pbio.3001890. eCollection 2022 Nov.

The pan-genome of Aspergillus fumigatus provides a high-resolution view of its population structure revealing high levels of lineage-specific diversity driven by recombination

Lotus A Lofgren [1] [2], Brandon S Ross [3], Robert A Cramer [3], Jason E Stajich [1]

Affiliations + expand

PMID: 36395320 PMCID: PMC9714929 DOI: 10.1371/journal.pbio.3001890

> Int J Mol Sci. 2022 Feb 8;23(3):1914. doi: 10.3390/ijms23031914.

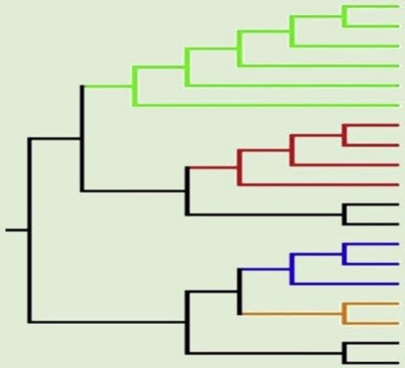
Fusarium graminearum Infection Strategy in Wheat Involves a Highly Conserved Genetic Program That Controls the Expression of a Core Effectome

Florian Rocher [1], Tarek Alouane [1], Géraldine Philippe [1], Marie-Laure Martin [2] [3] [4], Philippe Label [5], Thierry Langin [1], Ludovic Bonhomme [1]

Affiliations + expand

PMID: 35163834 PMCID: PMC8836836 DOI: 10.3390/ijms23031914

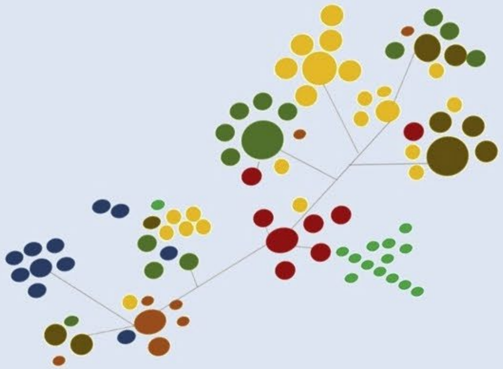
Phylogenomic



Estimate diversity and relationship between strains. Identify phylogenetic groups

Shows phylogenetic relationship among strains with its adapt to a host

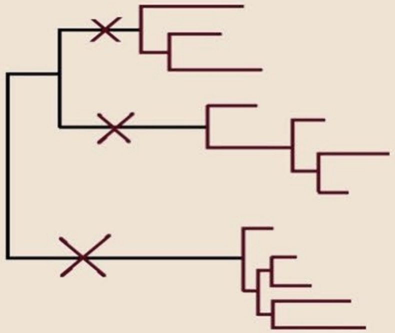
Epidemiology



Identify genotypic groups. Allow to interpret the origin and dissemination of strains

The classification of STs between strains correspond to adapt to a host and geographical location

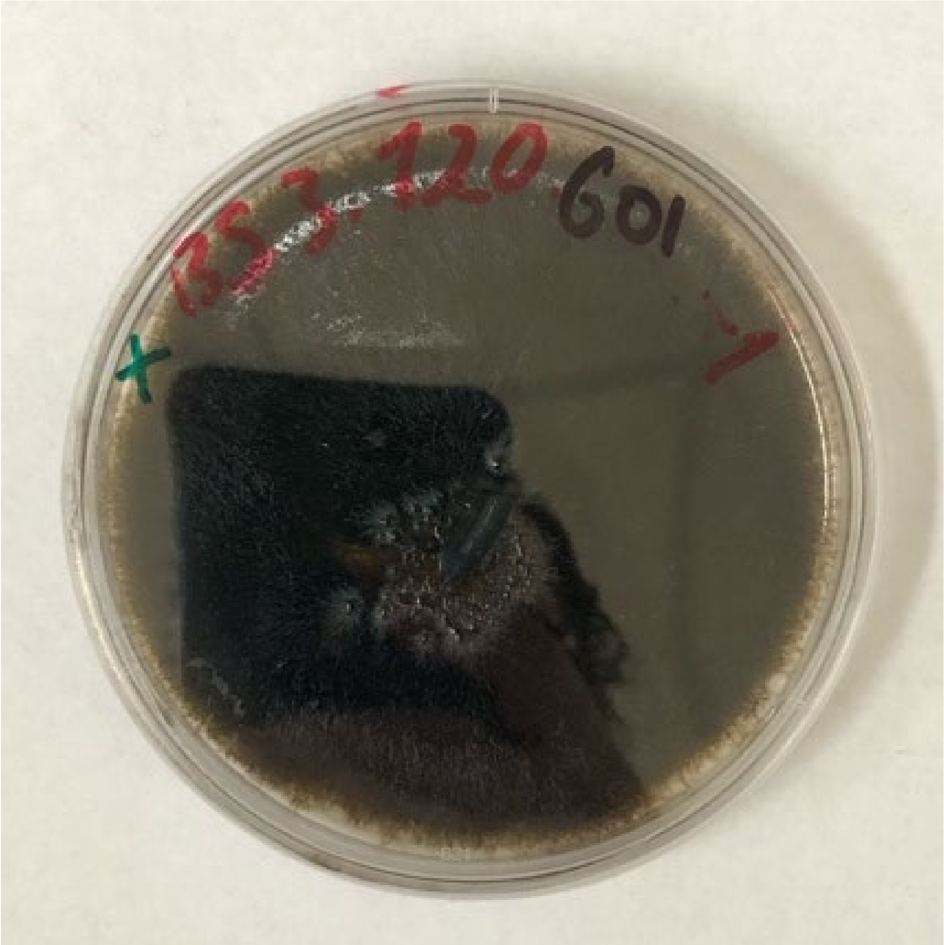
Positive selection



Identify genes and phylogenetic groups submit to positive selection

Shows pressure selection on proteins involve on interaction with immune system and host adaptation

Hurtado, R., Maturrano, L., Azevedo, V., and Aburjaile, F. (2020). Pathogenomics insights for understanding Pasteurella multocida adaptation. *International Journal of Medical Microbiology* 310, 151417. doi: 10.1016/j.ijmm.2020.151417.



PRELIMINARY RESULTS

BEST SOLID MEDIA

2% Malt Extract Agar

DNA EXTRACTION

E.Z.N.A.®

Plant & Fungal Kit

LIQUID MEDIA

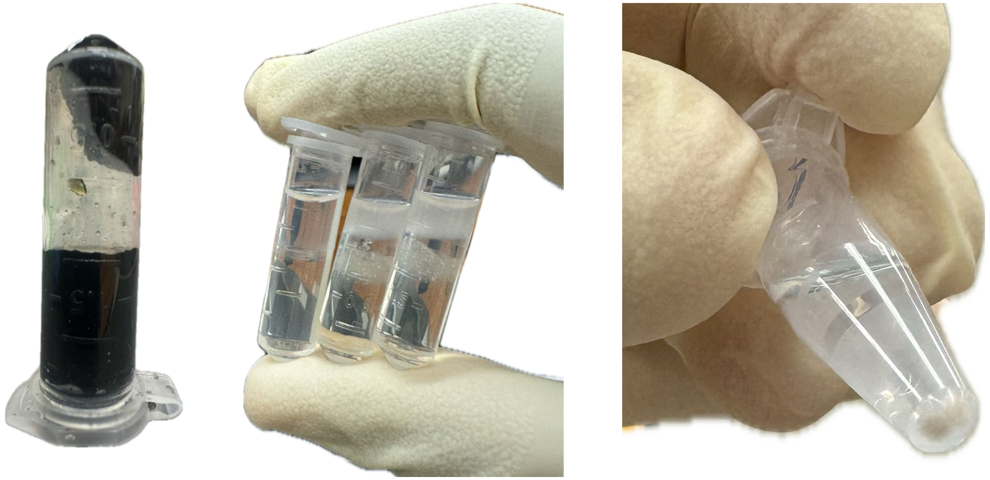
3% Malt extract can support growth

CONFIRMED ISOLATES

52 Total

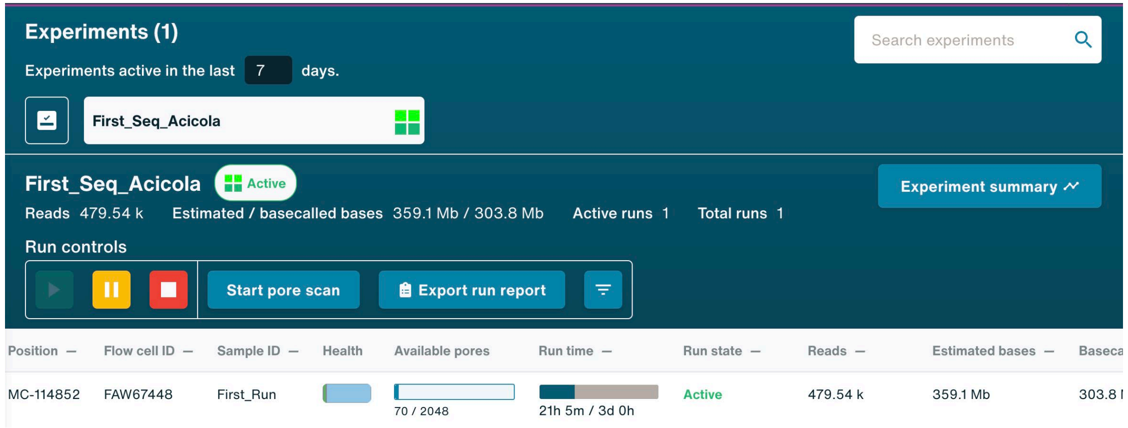
- 47 from loblolly
- 5 from long-leaf

PRELIMINARY RESULTS



Multiple isolates have been grown in liquid media
DNA extracted

Sequencing has begun
Methodologies being refined for scale-up.



Experiments (1)

Experiments active in the last 7 days.

First_Seq_Acicola

First_Seq_Acicola Active

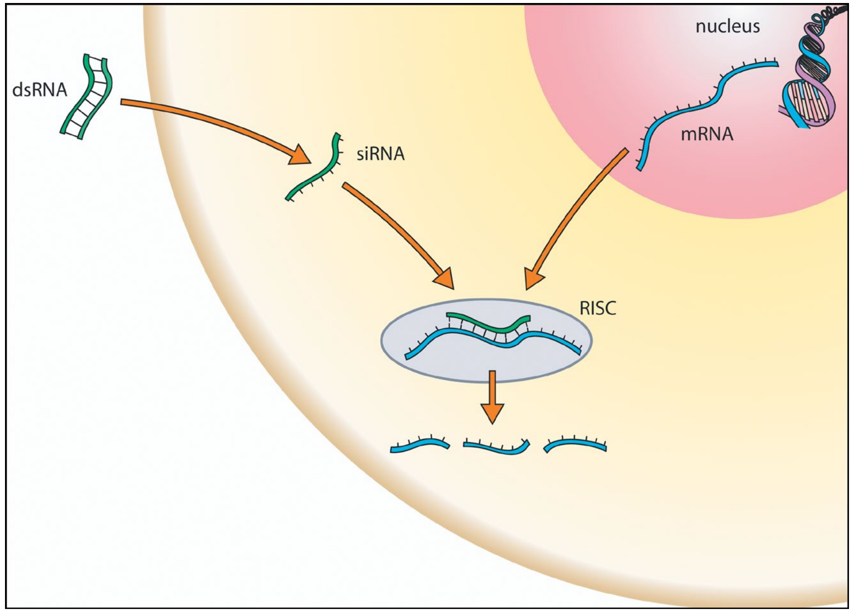
Reads 479.54 k Estimated / basecalled bases 359.1 Mb / 303.8 Mb Active runs 1 Total runs 1

Run controls

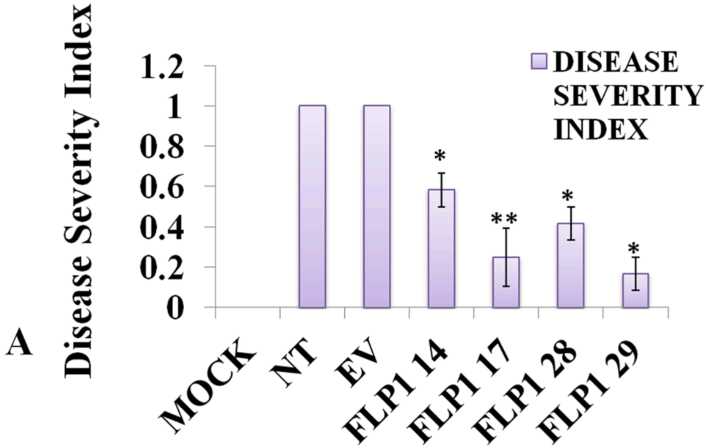
Position –	Flow cell ID –	Sample ID –	Health	Available pores	Run time –	Run state –	Reads –	Estimated bases –	Baseca
MC-114852	FAW67448	First_Run		70 / 2048	21h 5m / 3d 0h	Active	479.54 k	359.1 Mb	303.8

OUTCOMES - PAN-GENOME

Identification of Genomic Features	Discovery of common genomic features in L. acicola isolates responsible for infection mechanisms and virulence.
Identification of Genetic Disease-Associated Targets	Key genomic elements identified as potential targets for developing disease management strategies and resilient pine varieties.



Robinson, 2004



DSI of infected T_1 tomato progenies of FLP1-RNAi transgenics

Chauhan & Rajam, 2024

OUTCOMES - PHYLOGENETICS

Understanding Genomic Diversity and Host Specificity	Detailed understanding of the correlation between genomic diversity, host specificity, and geographic distribution.
Enhanced Understanding of Pathogen Evolution	Insights into the evolutionary pressures and adaptation mechanisms of *L. acicola*.

Disease Control and Pest Management

Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic Control

Ravi P. Singh, David P. Hodson, Yue Jin, Evans S. Lagudah, Michael A. Ayliffe, Sridhar Bhavani, Matthew N. Rouse, Zacharias A. Pretorius, Les J. Szabo, Julio Huerta-Espino, Bhoja R. Basnet, Caixia Lan, and Mogens S. Hovmøller

Affiliations

Published Online: 29 Jun 2015 | https://doi.org/10.1094/PHYTO-01-15-0030-FI

OUTCOMES

High-Quality Reference Genome	Comprehensive coverage, low error rates, and better resolution of complex genomic regions.
	Extensive annotation - Gene functions, regulatory sequences, non-coding RNAs.
	Biological functions and pathogenic mechanisms.
Phylogenetic Analysis	Evolutionary placement of L. acicola within the *Mycosphaerellaceae* family.
	Exploration of evolutionary trajectories, speciation events, and adaptations.
	Comparison with related species and other pine pathogens.

OUTCOMES

Comparative Genomics	Insights into genomic distinctions and similarities between *L. acicola* and other fungal species.
	Secretome, effectome, CAZymes, and transposable elements.
	Understanding pathogen-host interactions and plant cell wall degradation.
Genomic Plasticity & Evolution	Correlation of genomic differences with the evolution of *L. acicola*.
	Improved understanding of genomic plasticity and its implications for pathogenicity and adaptability.

FOREST SERVICE
U S
DEPARTMENT OF AGRICULTURE

ACKNOWLEDGMENTS

AUBURN
College of Forestry,
Wildlife and Environment

willoughby
conservation and genomics lab

Forest Health Dynamics Lab

Dr. Lori Eckhardt

Stallworth Land Company
Long Leaf Land & Timber Company
Osko Forest - Dr. Glenn Glover

REGIONS
Kris Bradley

THANK YOU!

QUESTIONS?

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CONSERVATION AND GENOMICS LAB

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