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Genomic tools for *Cannabis sativa* and *Psilocybe cubensis* propagation



Use Powerful Genomic Data to
Advance Your Operation

Kevin McKernan
Chief Scientific Officer
Medicinal Genomics

THE EMERALD CONFERENCE

Transparent and Public Data Resources

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bioRxiv is receiving many new papers on coronavirus SARS-CoV-2. A reminder: these are preliminary reports that have not undergone peer review. They should not be regarded as conclusive, used to guide clinical practice, or reported in news media.

New Results

Comments (2)

Sequence and annotation of 42 cannabis genomes reveals extensive copy number variation in cannabinoid synthesis and pathogen resistance genes

Kevin J. McKernan, Yvonne Helbert, Liam T. Kane, Heather Ebling, Lei Zhang, Biao Liu, Zachary Eaton, Stephen McLaughlin, Sarah Kingan, Primo Baybayan, Gregory Concepcion, Mark Jordan, Alberto Riva, William Barbazuk, Timothy Harkins



42 Cannabis genome public
Largest Cannabis DNA sequence
repository. 10X larger than all
previous submissions

RESEARCH ARTICLE

Check for updates

REVISIED **Cannabis** microbiome sequencing reveals several mycotoxigenic fungi native to dispensary grade *Cannabis* flowers [version 2; peer review: 2 approved]

Kevin McKernan, Jessica Spangler, Lei Zhang, Vasisht Tadigotla, Yvonne Helbert, Theodore Foss, Douglas R. Smith

Author details

This article is included in the **Agriculture, Food and Nutrition** gateway.

Abstract

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In 2016 we published the first
Cannabis Flower Microbiomes
studies

RESEARCH ARTICLE

Check for updates

Metagenomic analysis of medicinal *Cannabis* samples; pathogenic bacteria, toxigenic fungi, and beneficial microbes grow in culture-based yeast and mold tests [version 1; peer review: 3 approved, 1 approved with reservations]

Kevin McKernan, Jessica Spangler, Yvonne Helbert, Ryan C. Lynch, Adrian Devitt-Lee, Lei Zhang, Wendell Orphe, Jason Warner, Theodore Foss, Christopher J. Hudalla, Matthew Silva, Douglas R. Smith

Author details

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Published TYM qPCR primers that
navigate cannabis ITS problems

F1000Research Search

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Home » Browse » A draft reference assembly of the *Psilocybe cubensis* genome

DATA NOTE

Check for updates

REVISED A draft reference assembly of the *Psilocybe cubensis* genome [version 2; peer review: 2 approved]

Previously titled: A draft sequence reference of the *Psilocybe cubensis* genome

Kevin McKernan¹, Liam T. Kane¹, Seth Crawford², Chen-Shan Chin³, Aaron Trippe², Stephen McLaughlin³

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Most complete *Psilocybe cubensis* genome reference

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RESEARCH ARTICLE

Check for updates

REVISED A whole genome atlas of 81 *Psilocybe* genomes as a resource for psilocybin production. [version 2; peer

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81 additional *Psilocybe* genomes

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BROWSE GATEWAYS & COLLECTIONS HOW TO PUBLISH ABOUT BLOG

Home » Browse » Whole genome sequencing of colonies derived from cannabis flowers...

METHOD ARTICLE

Check for updates

REVISED Whole genome sequencing of colonies derived from cannabis flowers and the impact of media selection on benchmarking total yeast and mold detection tools [version 2; peer review: 2 approved]

Kevin McKernan¹, Yvonne Helbert, Liam Kane¹, Nathan Houde, Lei Zhang, Stephen McLaughlin

Author details

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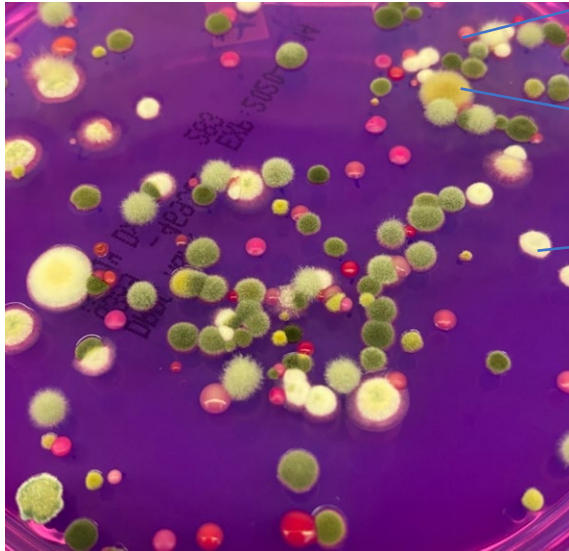
Comparison of TYM qPCR to PDA, PDA with CAMP, DRBC via Whole Genome sequencing of 45 genomes

DRBC 5 day plating

>100 inclusion organisms on TYM

>40 exclusion organisms

Recalibrate on new media



Enumeration Tests in Validation



> PeerJ. 2021 May 13;9:e11446. doi: 10.7717/peerj.11446. eCollection 2021.

Cannabinoids from inflorescences fractions of *Trema orientalis* (L.) Blume (Cannabaceae) against human pathogenic bacteria

Tiwtawat Napiroon¹, Keerati Tanruean², Pisit Poolprasert², Markus Bacher³, Henrik Balslev⁴, Manop Poopath⁵, Wichai Santimaleeworagun⁶

Affiliations + expand

PMID: 34035994 PMCID: PMC8126263 DOI: 10.7717/peerj.11446

[Free PMC article](#)

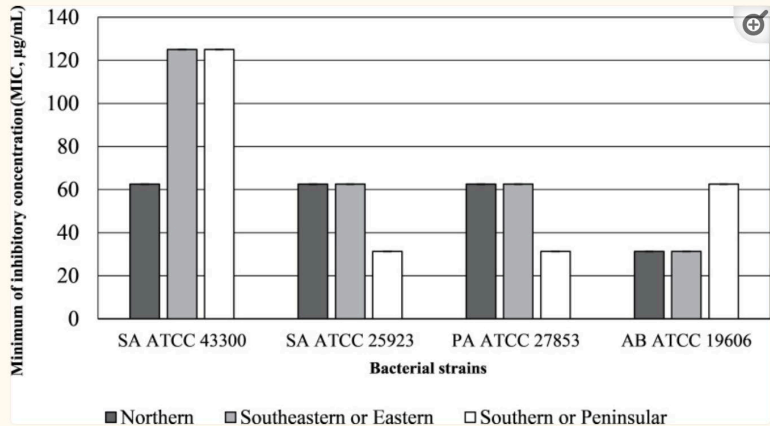
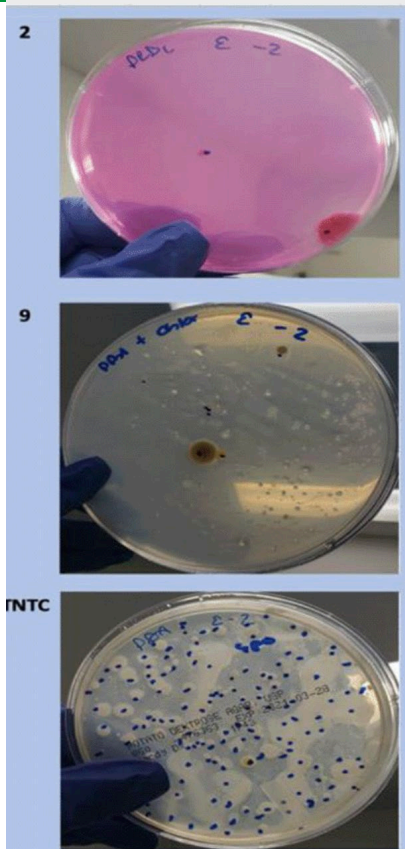


Figure 3

Minimum inhibitory concentration (MIC, µg/mL) of inflorescence fraction (S3).



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OSF PREPRINTS

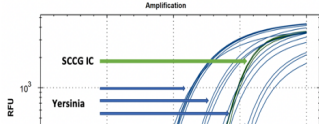
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Pathogenic Enterobacteriaceae require multiple culture temperatures for detection in Cannabis sativa L.

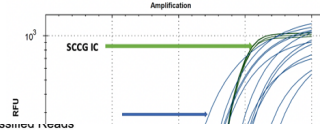
AUTHORS
Kevin McKernan, Vonne Helbert, Liam T. Kane, Lei Zhang, Nathan Houde, Anne Bennett, Juliana Silva, Heather Ebling, Stephen McLaughlin

AUTHOR ASSERTIONS
Conflict of Interest: Yes Public Data: Available Preregistration: Not applicable

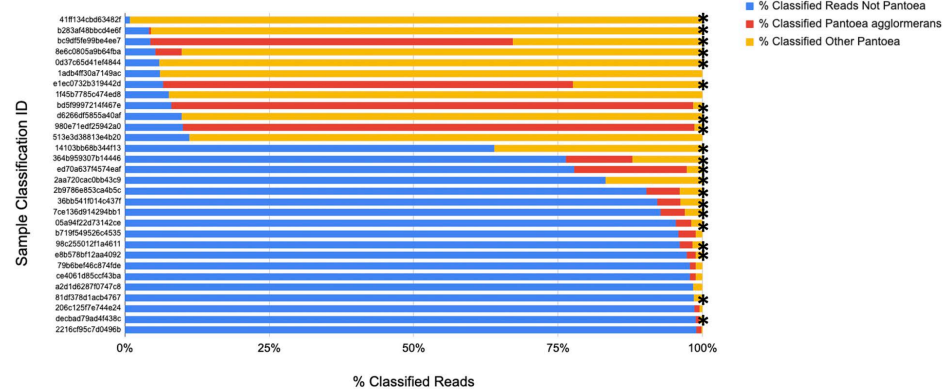
Yersinia enterocolitica Entero (blue) qPCR dilution series with SCCG (green) internal control



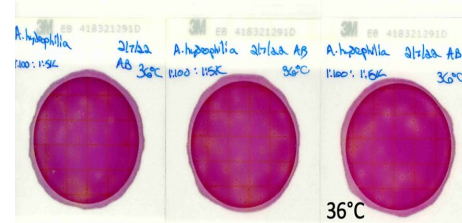
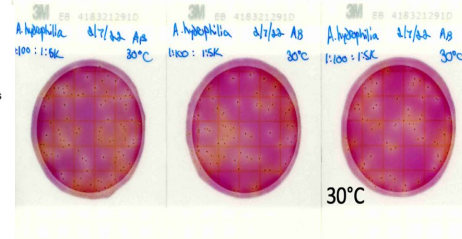
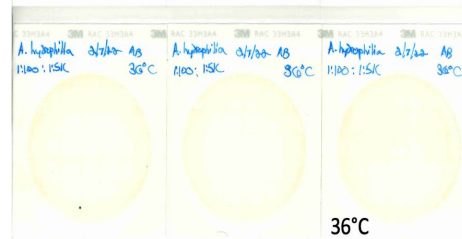
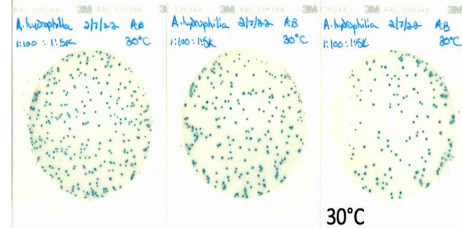
Aeromonas hydrophila Entero (blue) qPCR dilution series with SCCG (green) internal control



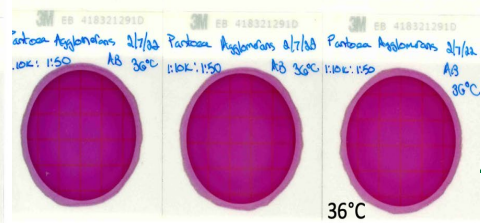
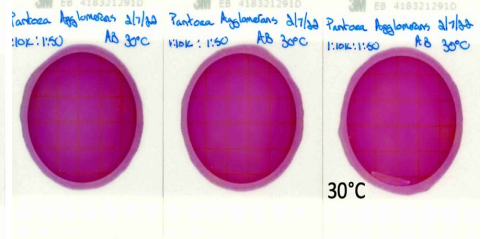
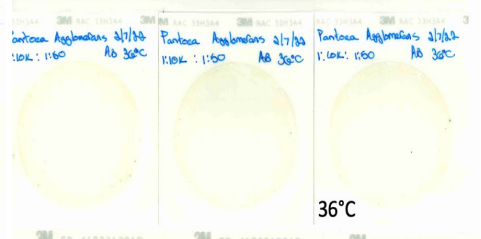
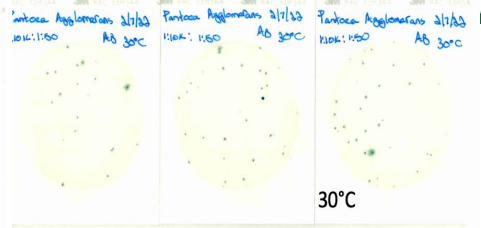
%Reads Classified as Pantoea in Samples >1% Pantoea



Aeromonas hydrophila ATCC 7966 @30°C vs 36°C
RAC plates (top) and EB plates (bottom)



Pantoea agglomerans: 30°C vs 36°C
RAC plates (top) and EB plates (bottom)





bioRxiv is receiving many new papers on coronavirus SARS-CoV-2. A reminder: these are preliminary and should not be regarded as conclusive, guide clinical practice/health-related behavior, or be reported in news media.

New Results

[Comments \(2\)](#)

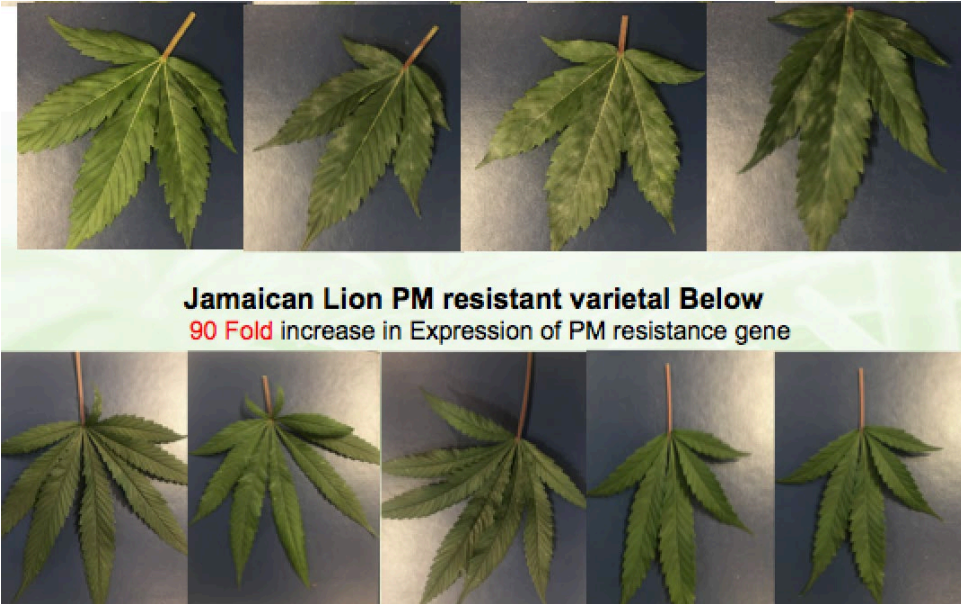
Sequence and annotation of 42 cannabis genomes reveals extensive copy number variation in cannabinoid synthesis and pathogen resistance genes

[ID](#) Kevin J. McKernan, Yvonne Helbert, Liam T. Kane, Heather Ebling, Lei Zhang, Biao Liu, Zachary Eaton, Stephen McLaughlin, [ID](#) Sarah Kingan, Primo Baybayan, [ID](#) Gregory Concepcion, Mark Jordan, Alberto Riva, William Barbazuk, [ID](#) Timothy Harkins

doi: <https://doi.org/10.1101/2020.01.03.894428>

This article is a preprint and has not been certified by peer review [what does this mean?].





PM
susceptible

PM
Resistant

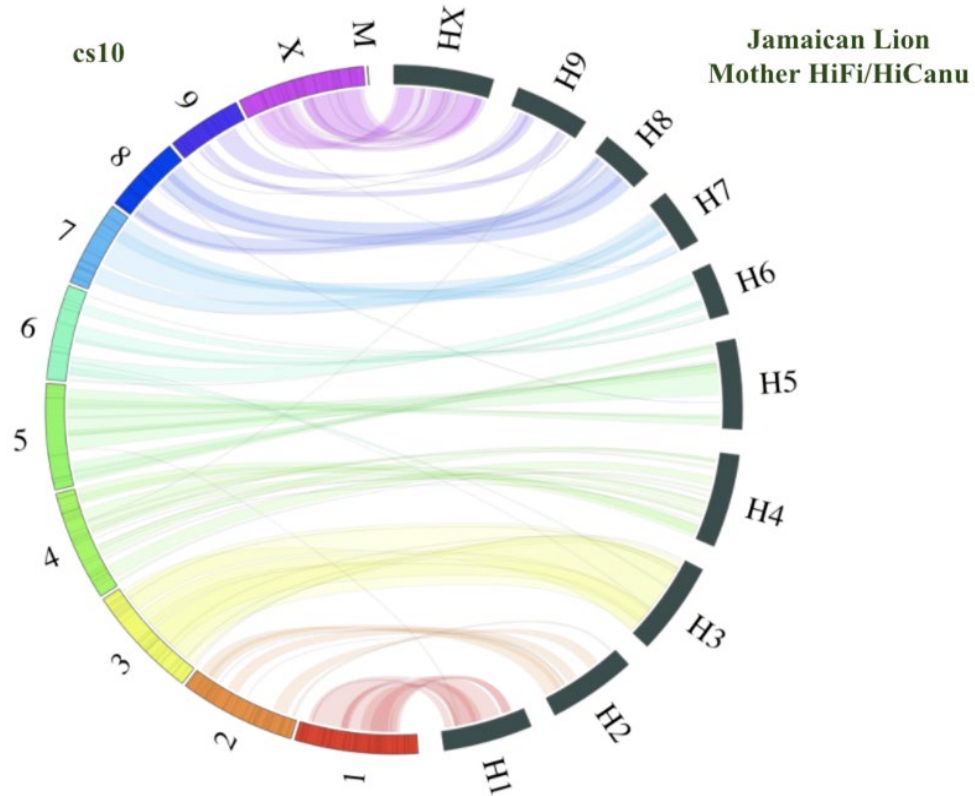
THE EMERALD HiC + HiFi on a Cannabis Trio CONFERENCE

Table 1A. Pacific Biosciences coverage and sequencing statistics of three Jamaican Lion cannabis genomes. Genomes were sequenced with continuous long read mode (CLR). F1 was female. BUSCO: benchmarking universal single-copy orthologs. Software and database versions: BUSCO.py 3.0.2, Augustus 3.3.21, [Hmmer](#) 3.2.1, Blast 2.7.1, eudicotyledons_odb10(Simao et al. 2015).

Coverage	Mother	Father	Daughter (JL5)
N50	3,283,100	1,668,042	3,491,975
Contig number (> 5 Kb)	481	1264	658
Genome size (bp)	875,793,298	1,009,156,132	999,122,115
Complete BUSCOs (%)	96.1	97.0	97.3
Single-copy BUSCOs (%)	83.5	63.3	63.5
Duplicated BUSCOs (%)	12.6	33.7	33.8
Sequencing statistics			
Unique molecular yield (Gb)	125	150	84.8
N50 RL (Kb)	34.6	35.6	50
N50 Subread (Kb)	20	24	19

The 33X coverage HiFi assembly delivered a higher
N50 (5.2Mb) assembly
BUSCO Primary (93.7%C, 82.8% single, 10.9% duplicate)
BUSCO Primary + Alts (**97.4%**, 41.2% single, 56.2% duplicate)

THE EMERALD HiC + HiFi CONFERENCE

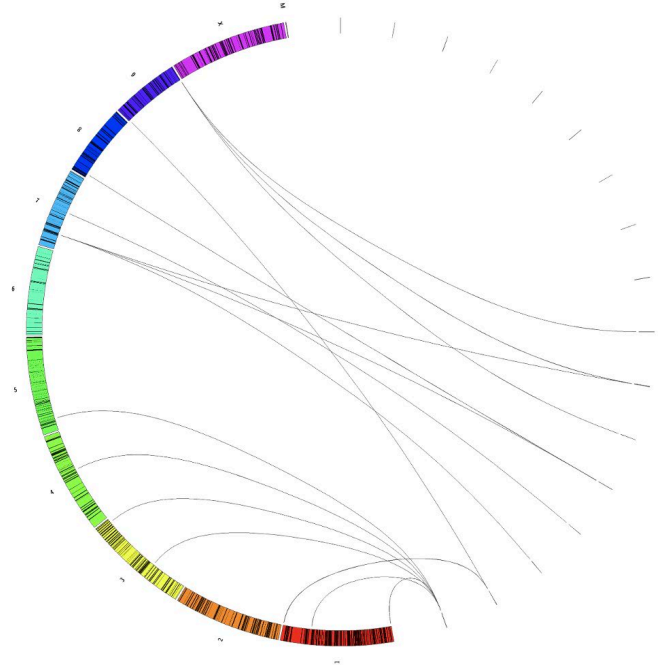


THE EMERALD HiCanu Telomeres CONFERENCE

Supplementary Table 1. Contigs in the Jamaican Lion HiFi-HiCanu assembly with terminal Telomeric sequences and their respective lengths.

Contig	Telomere Len (Kb)	Contig Length
tig00005375	25.7	250,103
tig00005432	2.9	230,364
tig00005460	23.8	142,906
tig00005486	7.7	167,894
tig00005511	1.8	170,382
tig00005736	16.6	102,145
tig00005782	35.2	85,691
tig00005821	26	67,613
tig00005878	26.9	107,231
tig00005912	22.4	27,710
tig00005913	17.8	27,527
tig00005915	23.1	44,441
tig00006147	13.2	32,702
tig00006532	5.3	35,243
tig00007704	18.9	26,164
tig00043130	4.2	401,659
tig00043131	42.9	45,132

Supplemental Figure 18. Jamaican Lion HiCanu telomeric contigs (right) mapped to the Cs10 assembly (Left) with minimap and displayed with Circos.

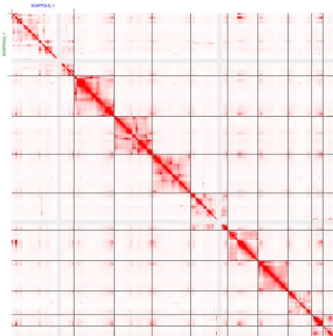


63% AT rich

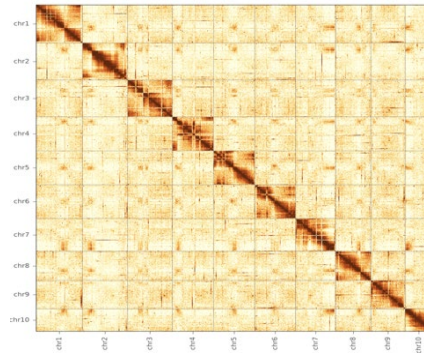
63% Long Terminal Repeats (LTR)

Lots of Simple Sequence Repeats (SSRs)

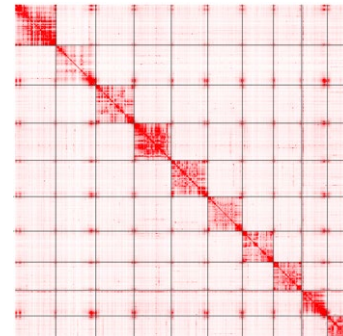
JL scaffolds organized according to BLAST to Cs10 Chromosomes



Gao et al JL-China

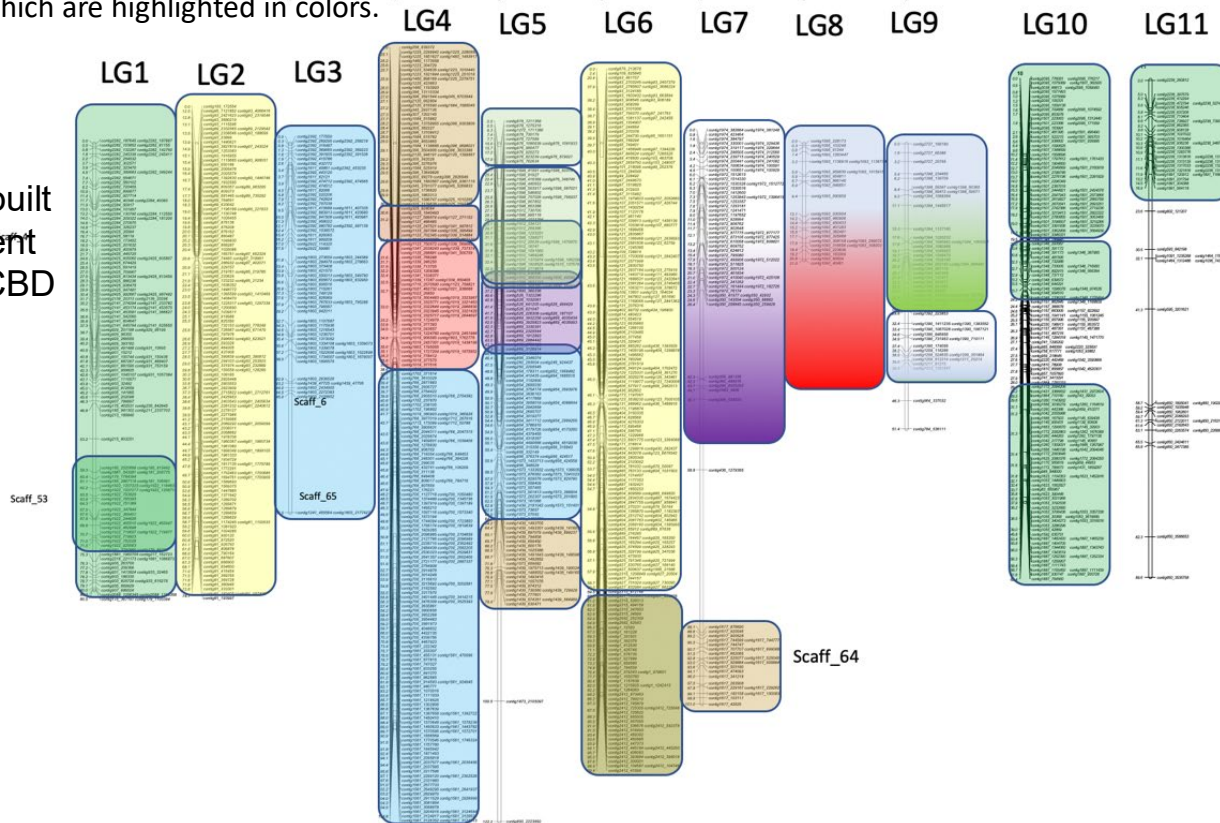


JL Hi-C mapped to Cs10 reference



Genetic Map Concordance with HiC 85K SNP Chip - CannSNP90

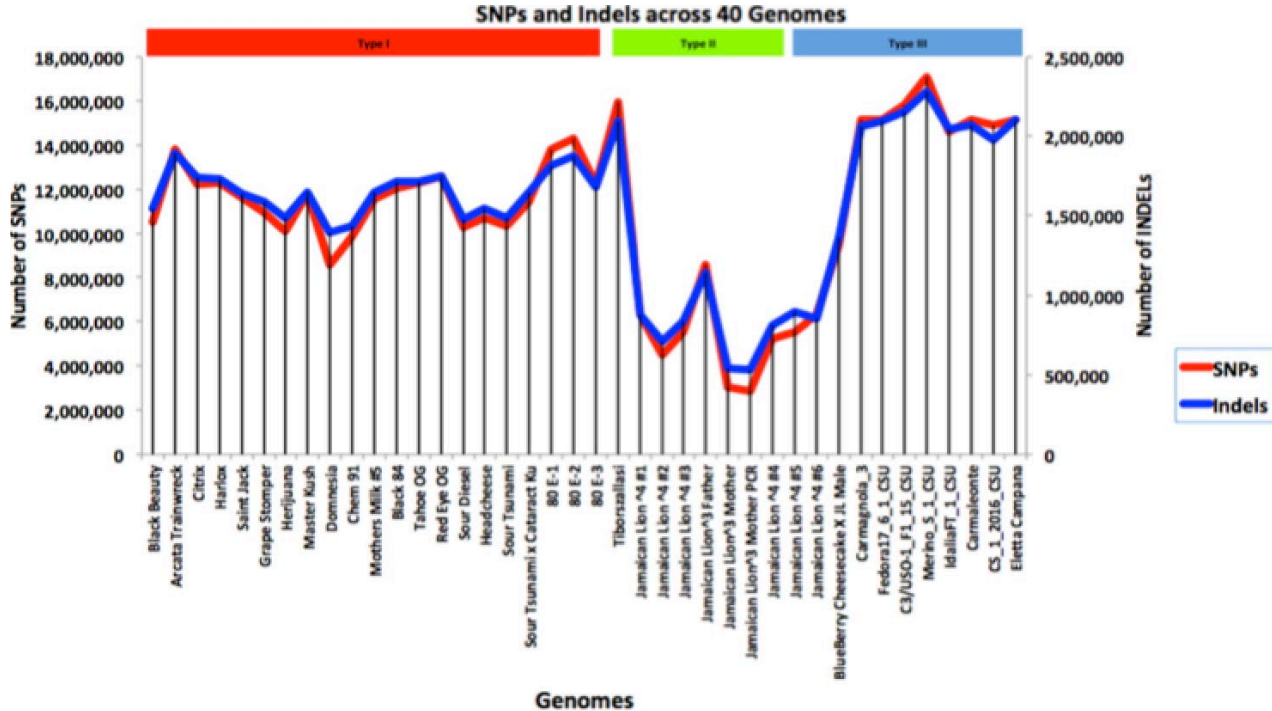
The genetic map and physical map was compared and most of the marker position was confirmed which are highlighted in colors.



Genetic Map was built with F2 from different cultivars: Oregon CBD

Genetic Map from Jamaican Lion F1 underway

58,262,496 Unique SNPs across 40 Genomes.

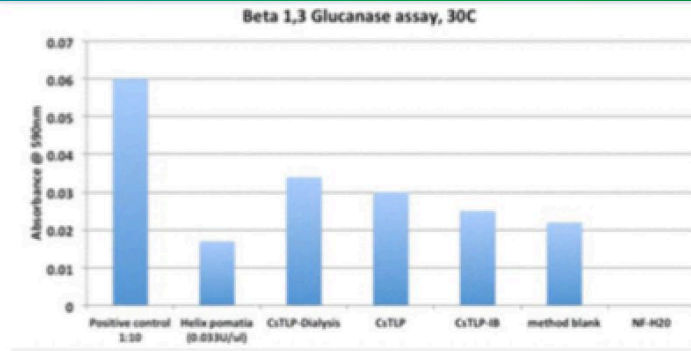
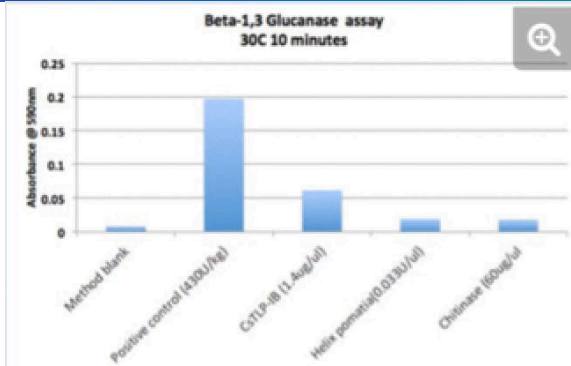


Type I = 10-14 SNPs
Type III = 12-17M SNPs

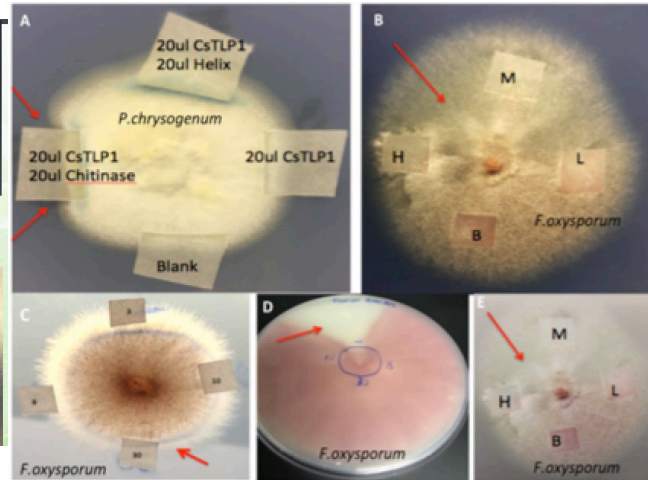
Mapped with DRAGEN and ParaBricks

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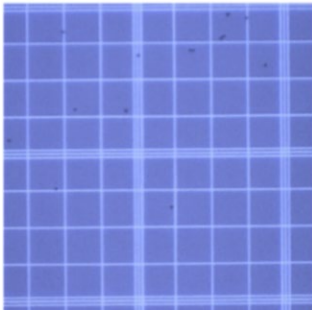
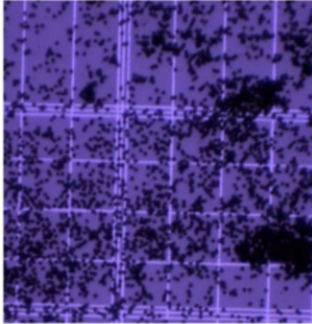
Functional Validation of Cloned Cannabis Genes



Jamaican Lion PM resistant varietal Below
90 Fold increase in Expression of PM resistance gene



Declump and
count 15 Spores



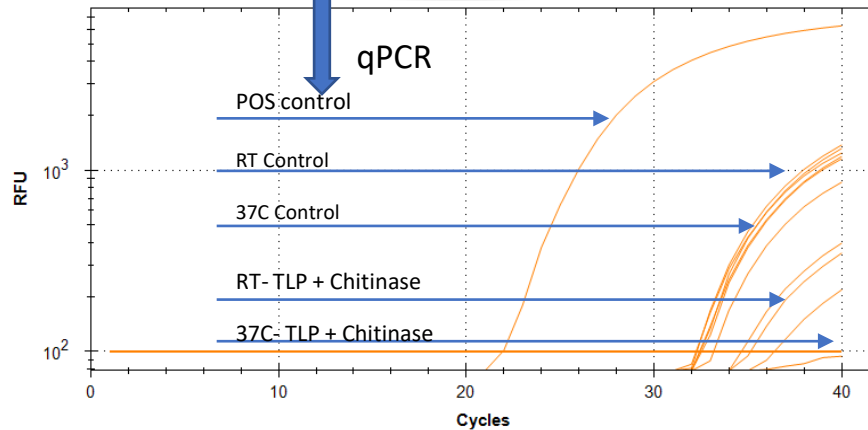
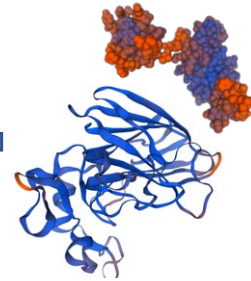
AOAC EUA TYM

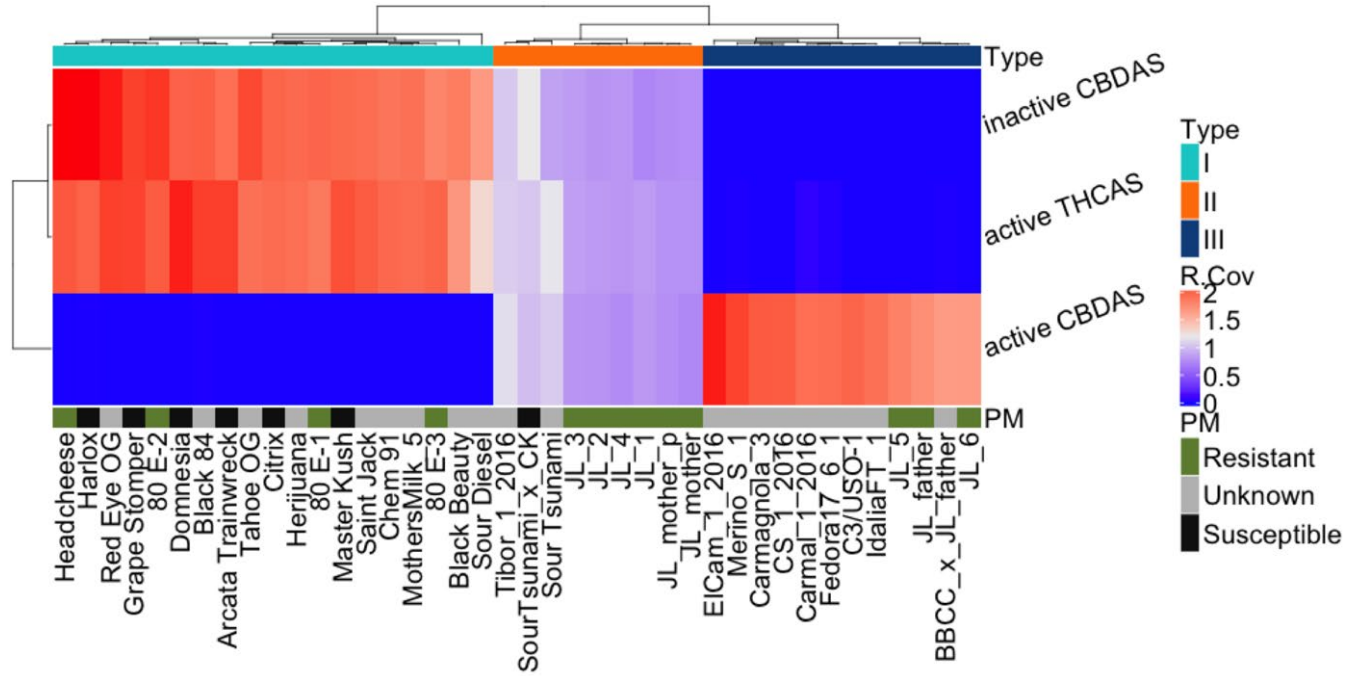
Grow at
RT and
37C for
22 hours



Amplification

TLP
&
Chitinase



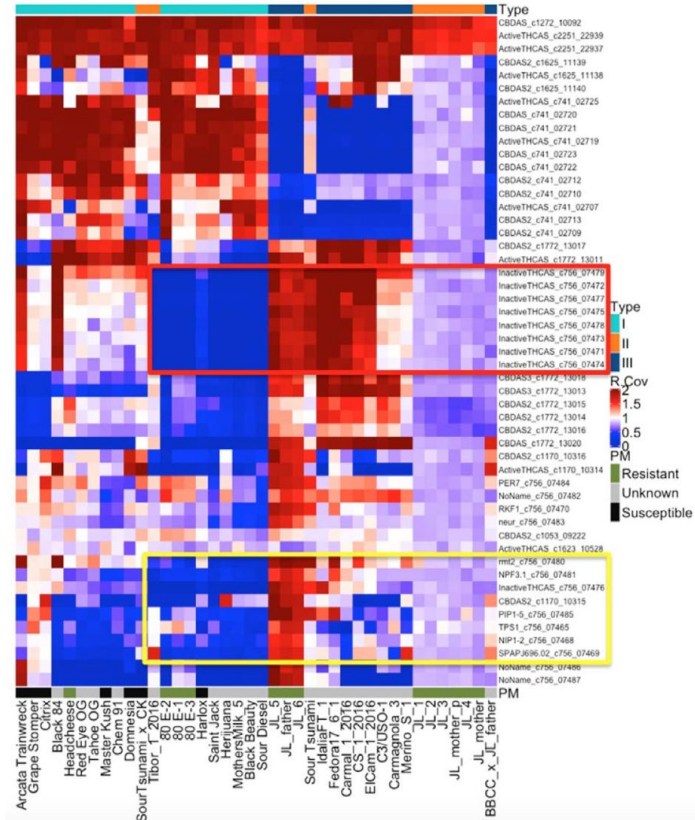


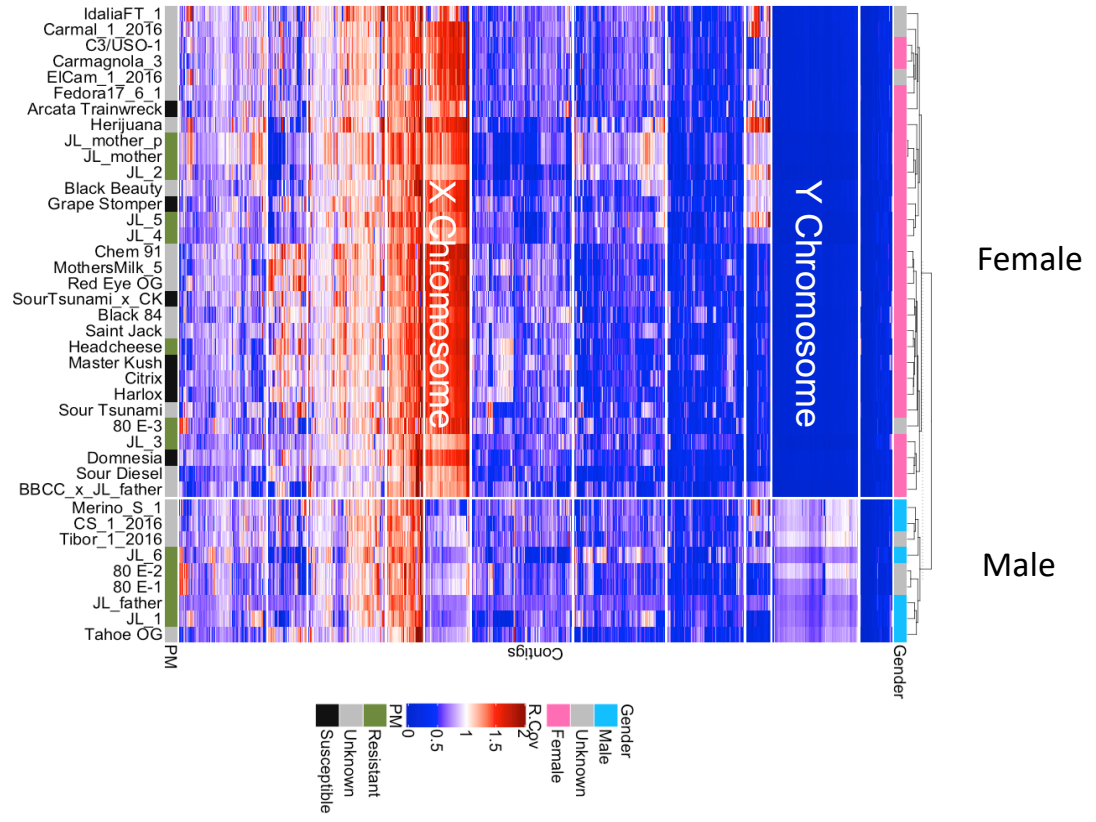
2Mb CBCA deletion Often co-deleted with Pathogen Response genes

Same
Contig




Gibberillin transporter –**NPF3**
Viral Defense –**RMT1**
PAMP-induced secreted peptides –**PIP1**
Aquaporin/H2O2 defense –**NIP1**





Blockchain based Genetic registry: The timestamps on your data are immutable

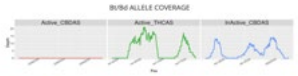


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THE SOURCE FOR CANNABIS KNOWLEDGE

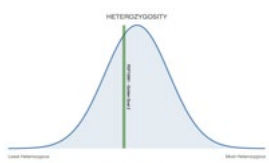
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GENETIC INFORMATION

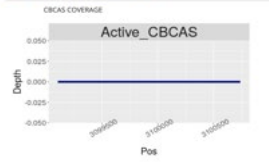
View this strain on the Phylotree
 Percent Heterozygosity: **0.96**
 Download VCF file: [Here](#)
 Download FastQ Files: [Read 1](#) [Read 2](#)
 Plant Type: **Type 1**



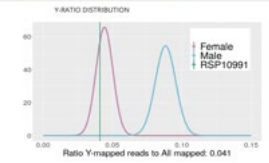
What does this visualization mean?



What does this visualization mean?



What does this visualization mean?



What does this visualization mean?

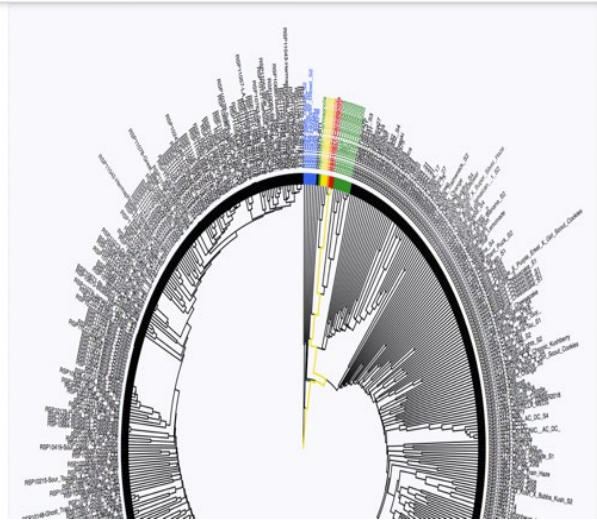
VARIANTS (THCAS, CBDAS, and CBCAS)

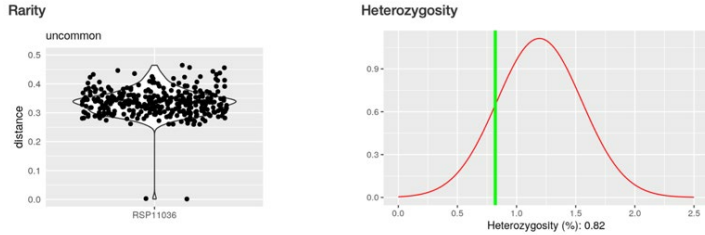
Show entries

Search:

Gene	HGVSc	HGVSp	Annotation	Annotation Impact	Contig	Contig Pos	Ref/Alt
THCAS	c.998C>G	p.Pro333Arg	missense_variant	MODERATE	contig741	4416830	G/C

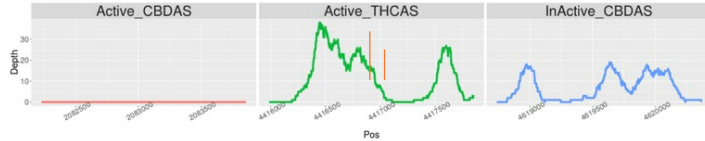
Showing 1 to 1 of 1 entries



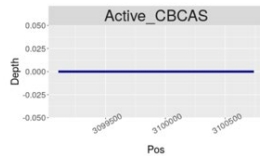


Bt/Bd ALLELE COVERAGE

Plant Type: Type I



CBCAS COVERAGE



Type IV

Variants in Select Genes

CBDAS, THCAS

Show 10 entries

Search:

Gene	HGVS.c	HGVS.p	Annotation	Annotation Impact	Contig	Contig Pos	Ref/Alt
1	THCAS	c.998C>G	p.Pro333Arg	missense_variant	MODERATE	contig741 4416830	G/C

Showing 1 to 1 of 1 entries

Previous **1** Next

CBDA in yeast makes ~20:1 CBDA:THCA

Type IV plant

1. No CBDAS gene.
2. Two Amino Acid Changing Mutations in THCAS
3. No CBCAS gene.
4. Zero THCA detected in 10%+ CBGA cultivar

NEAREST GENETIC RELATIVES IN PHYLOS DATASET:

Nearest Phylos Strain	Number of Overlapping SNPs	Concordance
SRR4448183	78	75

NEAREST GENETIC RELATIVES IN LYNCH DATASET:

Nearest Lynch Strain	Number of Overlapping SNPs	Concordance
SRR3495217	7	7

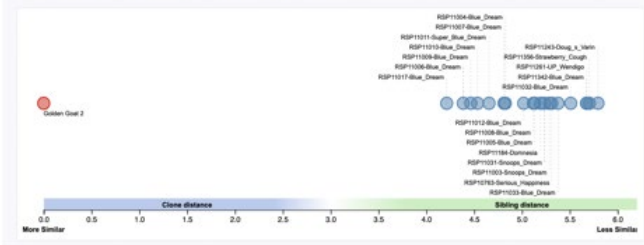
BLOCKCHAIN REGISTRATION INFORMATION:

Transaction ID: [fc01b0724f1cf53add58c49494dbcb7d901e28f2c945706d52714e87d82b73c9](#)
 Stamping Certificate: [PDF](#)
 SHASUM Hash: [b21a1f370d90ff4a0423cffeaa12b6e4091f0a46a1e0674b25823fff7197f1f](#)



Comparison to other public datasets and blockchain time stamps

NEAREST GENETIC RELATIVES TO Golden Goat 2:

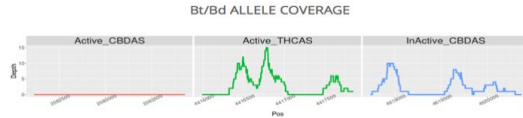


#	Relative	Genetic Distance
1	RSP11017-Blue Dream	4.21
2	RSP11006-Blue Dream	4.39
3	RSP11009-Blue Dream	4.46
4	RSP11010-Blue Dream	4.53
5	RSP11011-Super Blue Dream	4.66
6	RSP11007-Blue Dream	4.81
7	RSP11004-Blue Dream	4.83
8	RSP11012-Blue Dream	5.02
9	RSP11008-Blue Dream	5.12
10	RSP11005-Blue Dream	5.13

Relationships

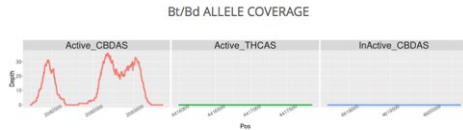
Sequencing Coverage

Active CBDAS Active THCAS Inactive CBDAS
Plant Type: *Type I*



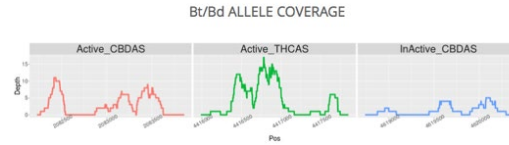
Type I
No Coverage on
CBDAS

Plant Type: *Type III*

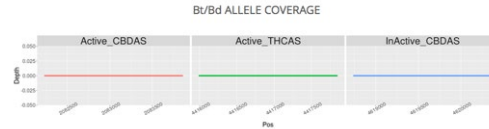


Type III
Good Coverage on
CBDAS
No Coverage on
THCA

Plant Type: *Type II*



Type II
Good Coverage on
CBDAS
Plant Type: *Type IV*



Type IV
No Coverage on THCAS
No Coverage on CBDAS.

January 25, 2022

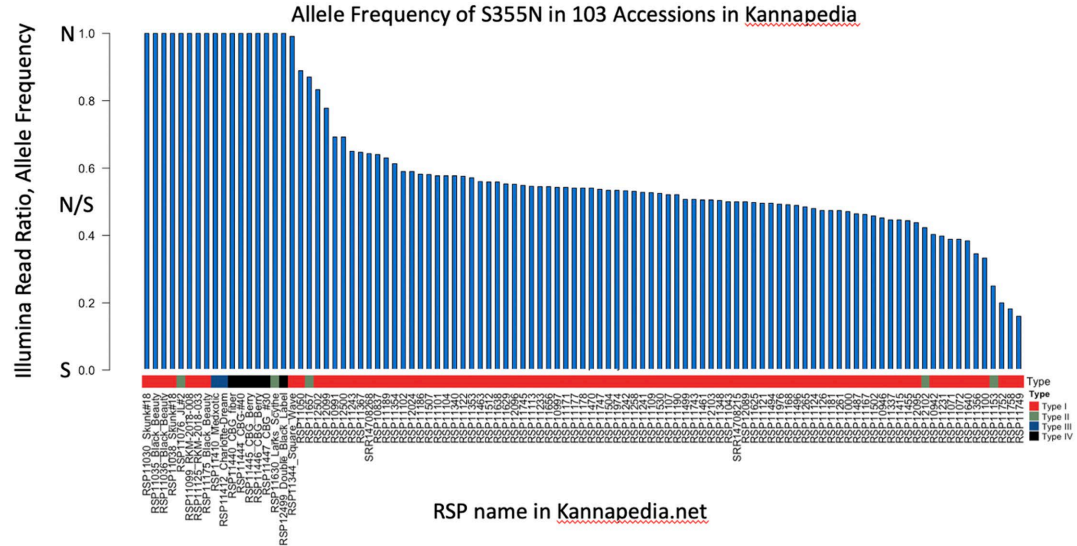
Preprint Open Access

Missense mutations in THCAS are associated with Cannabigerolic Acid expression in Cannabis sativa L.

Kevin McKernan; Lei Zhang; Yvonne Helbert; Liam T Kane; McLaughlin, Stephen

The chemical expression of THCA and CBDA has been previously attributed to copy number gains and losses in THCAS and CBDAS. Here we describe an alternative mechanism to ablate THCAS activity in 5 CBGA dominant cannabis varieties. Five cannabis cultivars from three unique cultivators surprisingly contain a THCAS gene but also share homozygous Pro333Arg and Ser355Asn mutation: in THCAS. Pro333Arg is commonly found in THCAS dominant varieties but is uniquely found in a homozygous state in CBGA varieties in conjunction with homozygous Ser355Asn missense mutations. These results hint at convergent evolution in cannabinoid synthesis where selection for and against THCA synthesis has been applied.

Figure 4. Allele frequency (Illumina read count ratio) in 103 samples with P333R mutations in THCAS. The samples with Allele ratio of 1 have their cultivar name added. Two samples are SRR#s NCBI.



THE EMERALD CONFERENCE

Produced by MJBizScience

Type IV (CBGA) that contain a THCA gene

Kannapedia
Library of Cannabis Genetics

Registered Strains Cannabis Phylotree Log in

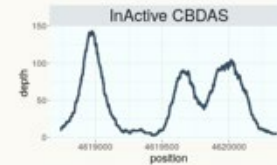
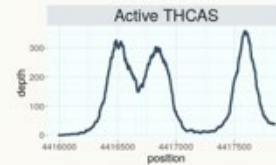
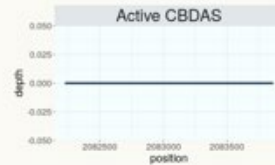
8 strains found

Sort by **Accession Date** ▾

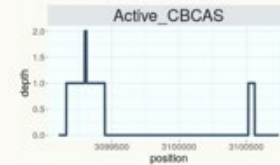
Search parameters
NGS Variant List contains
[+ Add parameter](#)

- CBG #30** Mar 31, 2020
Blue Forest Farms
- CBG Berry** Mar 31, 2020
Blue Forest Farms
- CBG Berry** Mar 31, 2020
Blue Forest Farms
- CBG-#40** Mar 31, 2020
Blue Forest Farms
- CBG Fiber** Mar 31, 2020
Blue Forest Farms
- Charlotte Dream** Jan 1, 2020
Altman Specialty Plants, LLC
- Medxotic** Jan 1, 2020
Altman Specialty Plants, LLC
- Doug's Varin** Aug 12, 2019
flower

Bt/Bd Allele Coverage



CBCAS Coverage



Variants (THCAS, CBDAS, and CBCAS)

search variants

GENE	HGVS.C	HGVS.P	ANNOTATION	ANNOTATION IMPACT	CONTIG	CONTIG POS	REF/ALT	VAR FREQ
THCAS	c.1064G>A	p.Ser355Asn	missense variant	moderate	contig741	4416764 IGV: Start_Jump	C/T	NGS: 0.018 C98: 0.000
THCAS	c.998C>G	p.Pro333Arg	missense variant	moderate	contig741	4416830 IGV: Start_Jump	G/C	NGS: 0.182 C98: 0.000

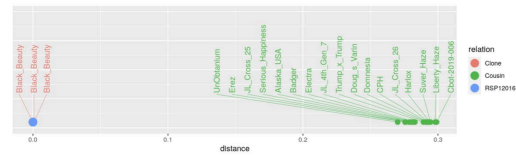
THE EMERALD CONFERENCE

Produced by MJBizScience

Many different tools

SS2 vs SS3, same samples: RSP12016

Nearest Genetic Relatives



	MGC_ID	Strain Name	Genetic Distance	Relationship
1	RSP11035	Black_Beauty	0.00%	Clone
2	RSP11175	Black_Beauty	0.08%	Clone
3	RSP11036	Black_Beauty	0.08%	Clone

SS1	Edit	RSP10697	10509 Medicinal Genomics Customer	Complete	3/1/2017 3:31 pm	Black Beauty
SS2	Edit	RSP11035	10509 Medicinal Genomics Customer	Complete	11/20/2017 4:55 pm	Black Beauty
SS2	Edit	RSP11036	10509 Medicinal Genomics Customer	Complete	11/20/2017 4:56 pm	Black Beauty
WGS	Edit	RSP11175	10509 Medicinal Genomics Customer	Complete	6/26/2019 3:51 pm	Black Beauty
SS3	Edit	RSP12016	9650 Medicinal Genomics	In Process	6/4/2021 10:24 am	Black Beauty

Confidential

GOOD

CannSNP90 CHIP

FOR GENOTYPING PLANTS
[view the technical sheet](#)

SNP CHIP GENOTYPING
(PROBE SINGLE LOCATIONS)

✔ 89K+ SNPS

- ✔ GENDER DETERMINATION
- ✔ CHEMOTYPE DETERMINATION
- ✔ SNP LOCI FOR:

CANNABINOID SYNTHASE GENES
(THCA, CBDA, CBCA)

✔ DISEASE RESISTANCE

✔ TERPENES

✔ GENOME-WIDE TAG DISTRIBUTION

✔ StrainSEEK OVERLAP TAGS



DOWNLOADABLE VARIANT DATA

✔ KANNAPEDIA REPORT INCLUDED

BETTER

StrainSEEK®

FOR FINDING NOVEL VARIANTS IN KEY
GENOMIC AREAS

DNA SEQUENCING PANEL
(SEQUENCE AREAS OF INTEREST)

✔ 10M+ BASES SEQUENCED

✔ 300K-500K SNPS

✔ TAGS IN 7,500+ GENES

✔ GENDER DETERMINATION

✔ CHEMOTYPE DETERMINATION

✔ FULL/PARTIAL COVERAGE FOR:

CANNABINOID SYNTHASE GENES
(THCA, CBDA, CBCA)

✔ DISEASE RESISTANCE

✔ TERPENES

✔ FLAVINOIDS

✔ FLOWERING

✔ SEEDING

✔ OTHERS



DOWNLOADABLE VARIANT,
MAPPING, & SEQUENCE DATA

✔ KANNAPEDIA REPORT INCLUDED

BEST

WHOLE GENOME

FOR THE MOST COMPLETE, FUTURE-PROOF
GENOMIC PROFILE

SHOTGUN DNA SEQUENCING
(SEQUENCE ENTIRE GENOME)

✔ 876M BASES SEQUENCED

✔ 3M-15M SNPS

✔ 27K+ GENES

✔ GENDER DETERMINATION

✔ CHEMOTYPE DETERMINATION

✔ FULL COVERAGE FOR:

CANNABINOID SYNTHASE GENES
(THCA, CBDA, CBCA)

✔ DISEASE RESISTANCE

✔ TERPENES

✔ FLAVINOIDS

✔ FLOWERING

✔ SEEDING

✔ MANY, MANY OTHERS



DOWNLOADABLE VARIANT,
MAPPING, & SEQUENCE DATA

✔ KANNAPEDIA REPORT INCLUDED

Genes of Interest - compare coverage 10Mb vs 3.2Mb panel

- Genes of interest have been divided into 9 distinct categories (complete genes lists are [here](#))
1. [Flowering genes](#) - 23 genes that were pulled out of the annotation for Jamaican Lion Female matching the term “flower”
 2. [TLP1 genes](#) - 6 genes that were included in *Fig 6B* of MGC’s [40-genome pre-print](#) implicated in Powdery Mildew Resistance
 3. [TLP genes](#) - The 23 TLP genes included in [Fig6A](#)
 4. [Known Disease Associated](#) - 280 genes pulled out of JL annotation matching term “disease”
 5. [Kannapedia select genes of interest](#) - 27 genes manually curated by MGC scientists for which MGC report variants on in Kannapedia (there are also flowering genes on this list, but these have been pulled out into the *Flowering Genes* group for this analysis)
 6. [Chitinase](#) - 35 Chitinase genes included in Fig6A
 7. [MLO](#) - 23 genes included in Fig6A
 8. [CBCA “Pathogen Response” genes](#) - 35 genes on contig756 of the JL Female reference also included in Fig6A
 9. [Fig 6A](#) - all 90 genes included in Fig6A (includes TLP, Chitinase, MLO, and CBCA Pathogen response from above)

CannSNP90 bead chip

- Content
- Validation
- Applications

Molecular Breeding

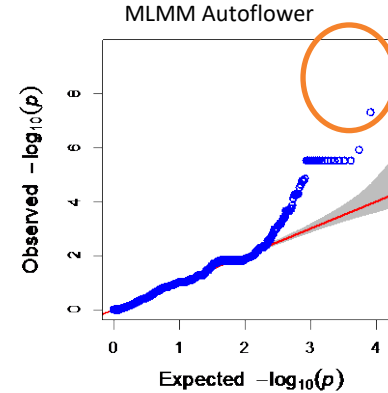
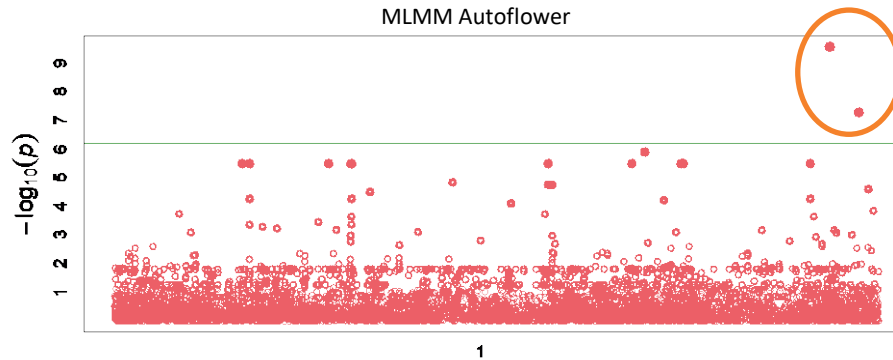
- Evolution of Molecular Markers
- Applications of Genotyping
- Construction of Hemp Genetic Map



Multiple Locus Mixed Linear Model (MLMM)



Dr. Hsuan Chen

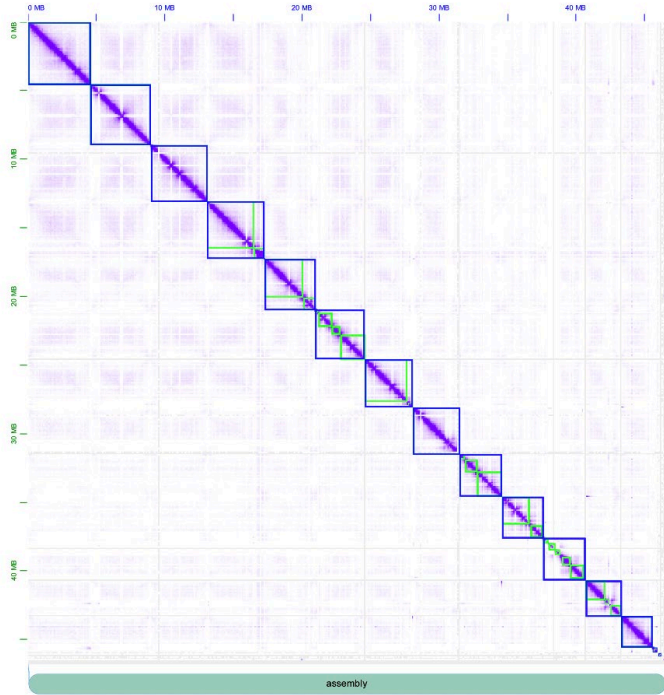




vs.



Background in Open Source Fungal Genomics



Pacific Biosciences HiFi assembly
N50 3.3Mb, 97.6% BUSCO complete

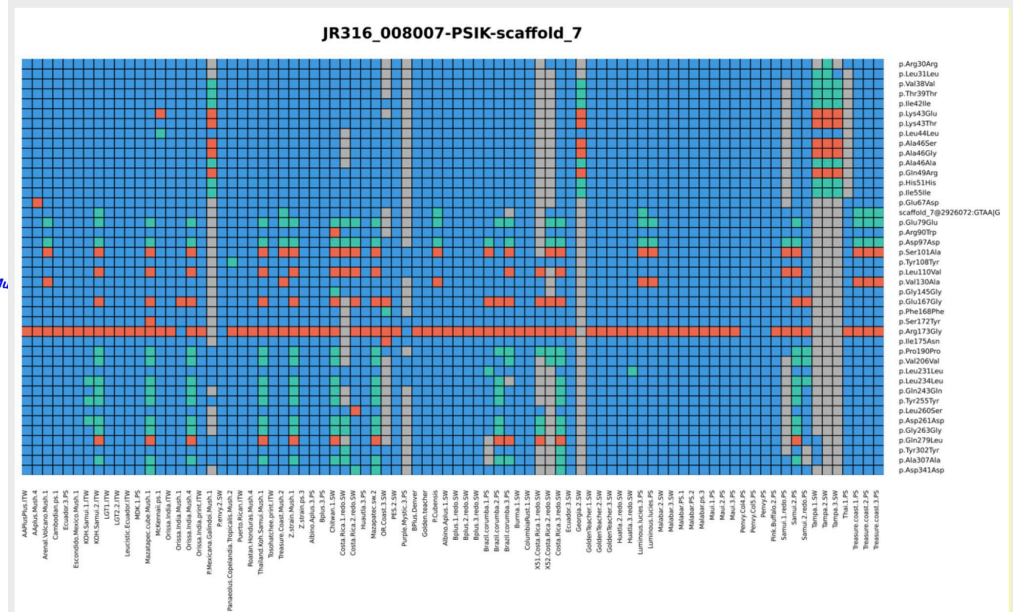
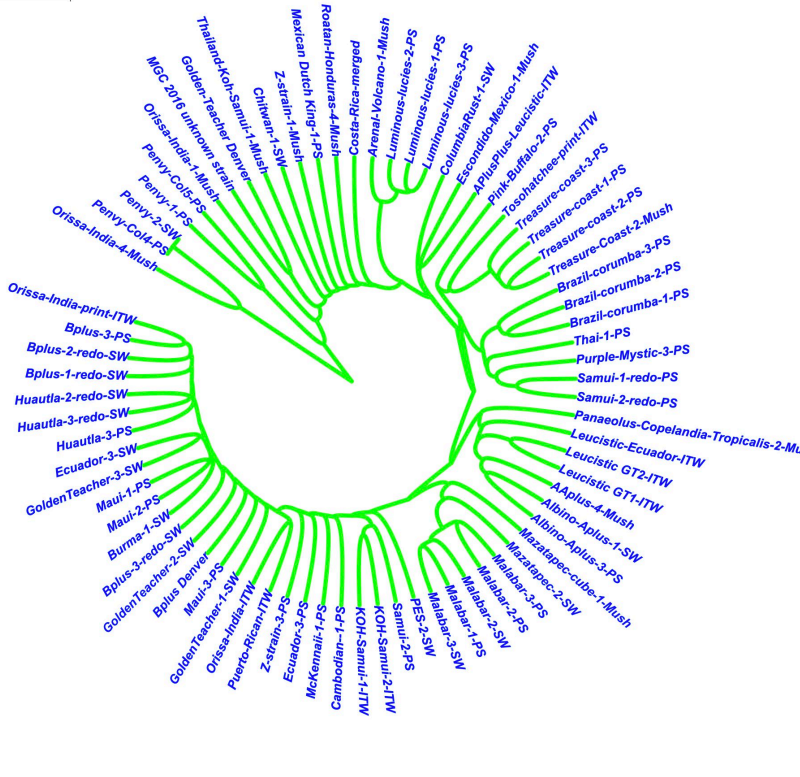
Phase Genomics Hi-C Chromatin Capture map of
Psilocybe cubensis

Psilocybe cubensis is now in
13 chromosomes

Figure 1. Hi-C contact map displayed in Juicebox. 13 large scaffolds emerge with total of 26 scaffolds. Mitochondria is on scaffold 20

Kannapedia.net: Open Source Fungal Genomics

81 Whole genome assemblies of *Psilocybe*
Damaging variants in the Pathway identified



Psilocybe Mating Type Loci identified on Chr. 1

Psilocybe has at least 4 genders

Mating Type Loci can guide which strains will make compatible crosses



Psilocydia- The Kannapedia fork for Psilocybe

Penvy-2-SW

PSP 10075 · Grower: [Medicinal Genomics](#)

General Information



Accession Date
June 13, 2021

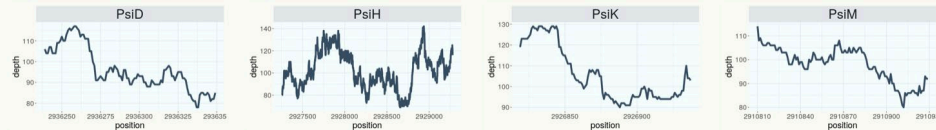
Report Type
Whole-Genome Sequencing

Genetic Information

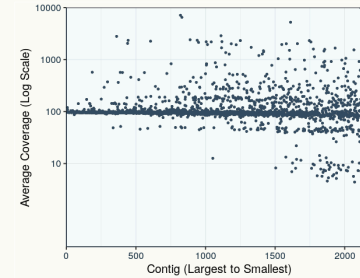
FILE DOWNLOADS

- [Annotated VCF](#)
- [Annotated VCF Index](#)
- [FASTQ Read 1](#)
- [FASTQ Read 2](#)
- [BAM](#)
- [BAM Index](#)
- [Assembled Genome, FASTA](#)
- [Assembled Genome, FASTA Index](#)
- [BAM \(Mapped to Assembly\)](#)
- [BAM \(Mapped to Assembly\) Index](#)

Psilocybin Synthase Gene Coverage



Coverage vs. Contig Size

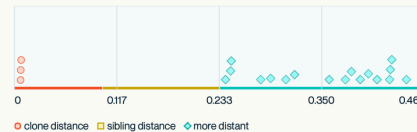


No. of Contigs (>= 25K bp) 414	Total Length (>= 50K bp) 21,463,896	Total Length 42,071,211
No. of Contigs (>= 50K bp) 198	Total Contigs 5,188	GC (%) 46.06
Total Length (>= 25K bp) 29,251,567	Largest Contig 432,312	N50 51,488

Variants

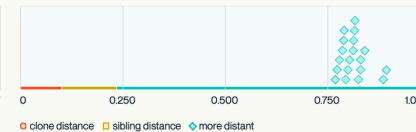
GENE	HGVS.C	ANNOTATION	ANNOTATION IMPACT	CONTIG	CONTIG POS	REF/ALT
PsiD	c.*1447A>G	downstream gene variant	modifier	scaffold_7	2934482 IGV: Start, Jump	T/C

NEAREST GENETIC RELATIVES (ALL SAMPLES)



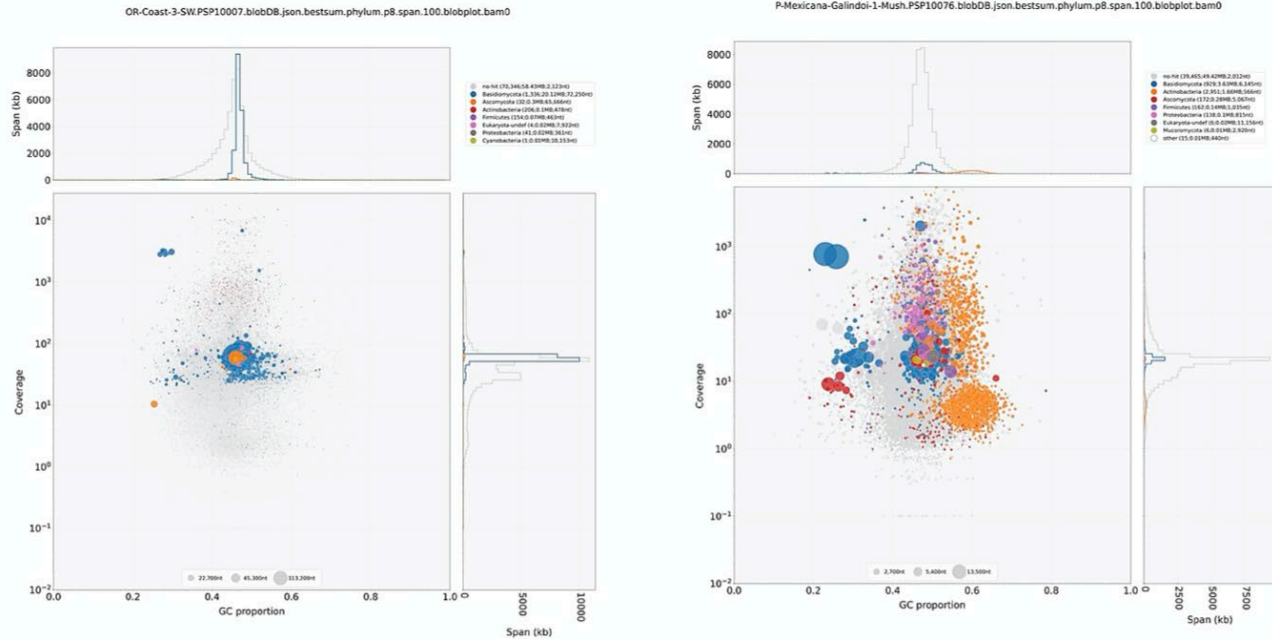
- 0.887 [Penvy-Col5-PS \(PSP10044\)](#)
- 0.887 [Penvy-I-PS \(PSP10045\)](#)
- 0.888 [Penvy-Col4-PS \(PSP10043\)](#)
- ◆ 0.240 [Tampa-I-SW \(PSP10050\)](#)
- ◆ 0.246 [Tampa-2-SW \(PSP10051\)](#)
- ◆ 0.247

MOST GENETICALLY DISTANT STRAINS (ALL SAMPLES)



- ◆ 0.979 [Bplus-3-PS \(PSP10001\)](#)
- ◆ 0.893 [Huautla-3-PS \(PSP10005\)](#)
- ◆ 0.885 [Huautla-3-redo-SW \(PSP10031\)](#)
- ◆ 0.840 [Mauli-2-PS \(PSP10041\)](#)
- ◆ 0.838 [Bplus-2-redo-SW \(PSP10015\)](#)
- ◆ 0.838

Psilocybe microbomes



Supplementary Figure 2. Taxon annotated GC-Plot of *P.azurescens* (OR-Coast Left) and *P.galindoi* (Right) demonstrate some metagenomic bacterial contamination in the *P.galindoi* library. Contigs with Psilocybin producing genes in *P.galindoi* have been identified using tBLASTn.

14 Viruses/Viroid assays

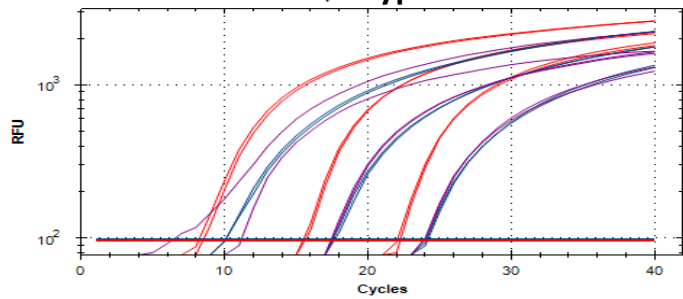
Released

1. Hop Latent Viroid
2. Lettuce Chlorosis Virus
3. Cannabis Cryptic Virus
4. Beat Curly Top Virus
5. Tobacco Mosaic Virus

Pipeline

1. Tobacco Streak Virus
2. Tobacco Ring Spot Virus
3. Arabis Mosaic Virus
4. Cucumber Mosaic Virus
5. Hop Mosaic Virus
6. Alfalfa Mosaic Virus
7. Tomato Ring Spot Virus
8. Raspberry Dwarf Virus
9. Hop Stunt Viroid

Triplex Virus/Viroid test
Hops Latent Viroid,
Lettuce Chlorosis Virus,
Cannabis Cryptic Virus 1



LCV Virus Phenotype More Pronounced in Flowering



Hop Latent Viroid is in the Roots, Stems and leaves

Sample Number	Sample Name	Target (FAM)	Ct (FAM)	Target (HEX)	Ct (HEX)
10.1	Chem 4 Mom	HLVd	ND	SCCG	29.79
10.1	Chem 4 Mom	HLVd	ND	SCCG	30.25
10.2	Chem 4 Clone 1	HLVd	ND	SCCG	30.60
10.2	Chem 4 Clone 1	HLVd	ND	SCCG	30.67
10.3	Chem 4 Clone 2	HLVd	ND	SCCG	31.83
10.3	Chem 4 Clone 2	HLVd	ND	SCCG	30.81
11.1	Motorbreath Clone 1	HLVd	ND	SCCG	31.10
11.1	Motorbreath Clone 1	HLVd	ND	SCCG	31.28
11.2	Motorbreath Clone 2	HLVd	ND	SCCG	30.22
11.2	Motorbreath Clone 2	HLVd	ND	SCCG	29.09
12.1	Old Family Purp Rooted, Leaf	HLVd	22.14	SCCG	31.07
12.1	Old Family Purp Rooted, Leaf	HLVd	20.78	SCCG	29.61
12.1	Old Family Purp Rooted, Root	HLVd	23.59	SCCG	29.59
12.1	Old Family Purp Rooted, Root	HLVd	22.59	SCCG	29.57
13.1	Sour Diesel Mom	HLVd	ND	SCCG	30.13
13.1	Sour Diesel Mom	HLVd	ND	SCCG	30.27
13.2	Sour Diesel Clone 1	HLVd	ND	SCCG	30.24
13.2	Sour Diesel Clone 1	HLVd	ND	SCCG	30.18
13.3	Sour Diesel Clone 2	HLVd	ND	SCCG	31.13
13.3	Sour Diesel Clone 2	HLVd	ND	SCCG	31.80
13.4	Sour Diesel Clone 3	HLVd	ND	SCCG	31.07
13.4	Sour Diesel Clone 3	HLVd	ND	SCCG	30.29
13.5	Sour Diesel Rooted, Leaf	HLVd	ND	SCCG	30.07
13.5	Sour Diesel Rooted, Leaf	HLVd	ND	SCCG	30.68
13.5	Sour Diesel Rooted, Root	HLVd	ND	SCCG	37.71
13.5	Sour Diesel Rooted, Root	HLVd	ND	SCCG	31.02
14.1	GMO Mom	HLVd	ND	SCCG	30.59
14.1	GMO Mom	HLVd	ND	SCCG	30.10
15.1	Strawberry Rooted, Leaf	HLVd	21.18	SCCG	28.04
15.1	Strawberry Rooted, Leaf	HLVd	21.31	SCCG	29.88
15.1	Strawberry Rooted, Root	HLVd	24.18	SCCG	30.05
15.1	Strawberry Rooted, Root	HLVd	25.44	SCCG	30.36
15.2	Strawberry Alive	HLVd	24.27	SCCG	31.74
15.2	Strawberry Alive	HLVd	25.56	SCCG	31.50
16.1	Strawana Rooted, Leaf	HLVd	20.52	SCCG	29.52
16.1	Strawana Rooted, Leaf	HLVd	21.21	SCCG	29.80
16.1	Strawana Rooted, Root	HLVd	21.12	SCCG	29.12
16.1	Strawana Rooted, Root	HLVd	22.16	SCCG	29.33
16.2	Strawana Alive	HLVd	22.81	SCCG	30.74
16.2	Strawana Alive	HLVd	22.02	SCCG	29.62
17.1	Triangle Wave Backup 2	HLVd	36.29	SCCG	33.04
17.1	Triangle Wave Backup 2	HLVd	37.78	SCCG	30.38
17.2	Triangle Wave Mom 2	HLVd	ND	SCCG	30.21
17.2	Triangle Wave Mom 2	HLVd	ND	SCCG	31.79
17.3	Triangle Wave Mom 3	HLVd	ND	SCCG	30.99
17.3	Triangle Wave Mom 3	HLVd	ND	SCCG	29.30
18.1	Trop Cookies Clone 1	HLVd	ND	SCCG	29.75
18.1	Trop Cookies Clone 1	HLVd	ND	SCCG	29.44
18.2	Trop Cookies Clone 2	HLVd	ND	SCCG	30.20
18.2	Trop Cookies Clone 2	HLVd	ND	SCCG	29.08
18.3	Trop Cookies Clone 3	HLVd	ND	SCCG	30.42
18.3	Trop Cookies Clone 3	HLVd	ND	SCCG	29.25
	Positive Control	HLVd	15.54	SCCG	39.26
	NTC	HLVd	ND	SCCG	37.64

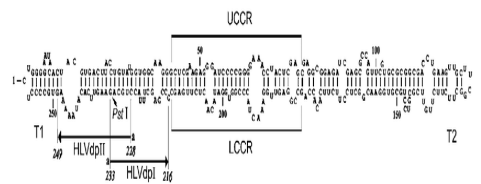
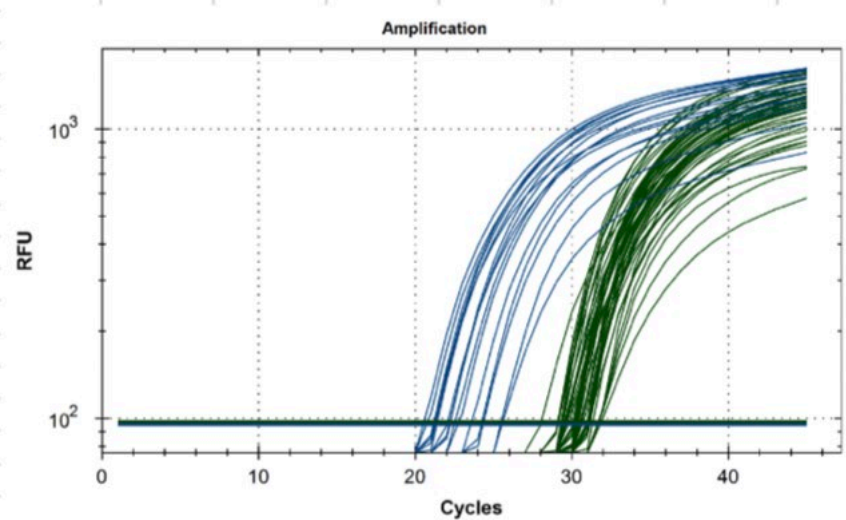
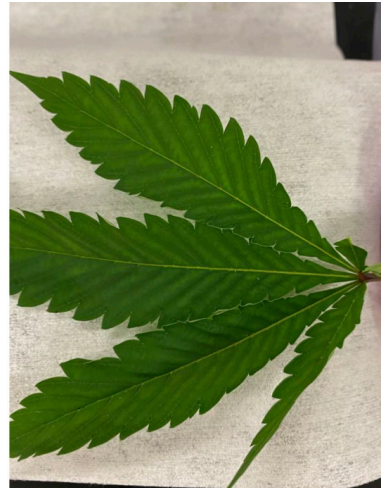


Figure 4 Secondary structure (Puchta et al., 1988) of HLVd and localization of primers for RT-PCR analysis of viroid infection in pollen. UCCR, upper part of the central conserved region; LCCR, lower part of the central conserved region; T1 and T2, left and right terminal domains. Primer binding sites are localized in the lower part of the viroid structure and cover a unique PstI restriction site. Primers are represented by arrows and positions are designated by numbers; a, position of a non-specific nucleotide.



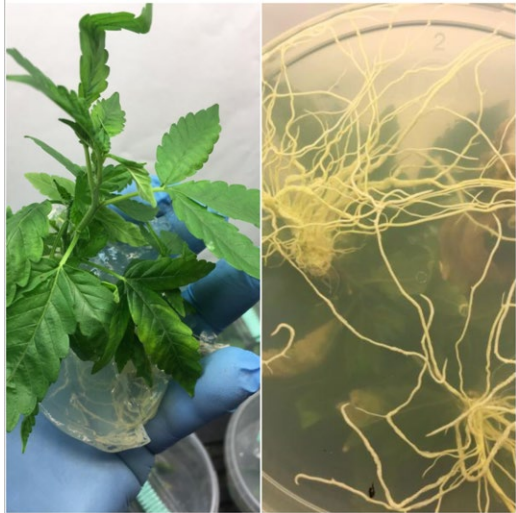
Vendors are consolidating on the Jamaican Lion references

Tissue Culture has been optimized for JL.

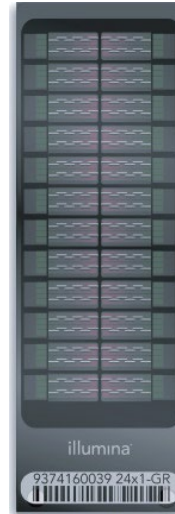
SNP Chips are Validated on JL. August 2020 Launch.

Mini-Exomes are available targeting Cannabinoid, Terpene, Chitinase, Y-chromosome, TLP, Flowering, CannFlavin, Edestin related genes.

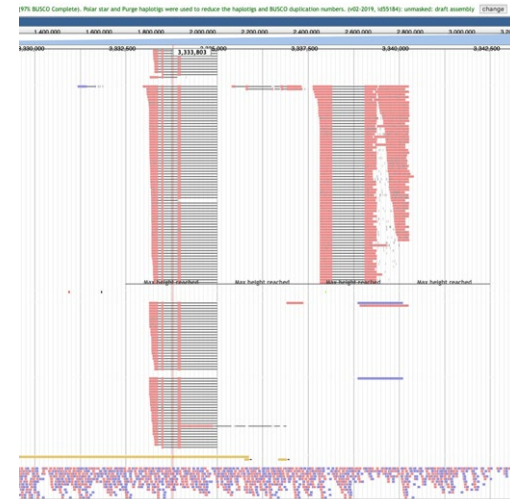
Doug Klier (IGBR)- Tissue
Cultured Jamaican Lion



85K SNP array



Mini-Exomes (5-10Mb Seq panels)





- Blockchain experts and cryptocurrency



- Single Molecule Sequencing Pioneers



- Methylation, Enzymes and Sequencing Pioneers. Founded by Nobel Laureate Sir Richard Roberts



- Experts in qPCR and Hyb Capture

- Experts in GPU acceleration/AI



- Experts in Field Portable LAMP assays



- Cannabis Genomic Pioneers. Sequencing veterans. Leaders from the Human Genome Project

Yvonne Helbert

Lei Zhang

Heather Ebling

Anne Bennett

Juliana Silva

Liam Kane

Nathan Houde

Steve McLaughlin

Nick Lawson

Matthew Brown

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Scan the QR code below to provide your feedback on the presentation.

