CannMed2016
Molecular Phylotyping of *Cannabis*
Ryan Lynch PhD
Background

- Bacterial genomics since 2008
- *Cannabis* genomics since 2013, started in Nolan Kane’s Lab
- Medicinal Genomics scientist since late 2015
Cannabis diversity—phenotypic and genetic
What can we do with *Cannabis* DNA?

- Past, Present and Future
- Current efforts to unravel plant history
  - How many species?
  - ‘indica’ vs ‘sativa’? Hemp? *ruderalis*?
- Genetics of specific chemoprofiles of cannabinoids and terpenoids?
- Strain name issues
  - Is my OG Kush the real OG?
- Certifications set now
- Future prediction of traits from DNAseq
  - Custom strain development
DNA: genomes contain information about nearly all aspects of biology, even if we cannot yet interpret it.

Information is not knowledge: Al Einstein/ Frank Zappa
Step 1: Sample Collection
Restriction-site associated DNA sequencing (RAD seq)

http://www.floragenex.com/rad-seq/
Relatedness among strains--phylogenetics

Parts of a phylogenetic tree

New Results

Genomic and Chemical Diversity in Cannabis
Ryan C Lynch, Daniela Vergara, Silas Tittes, Kristin White, C.J. Schwartz, Matthew J Gibbs, Travis C Ruthenburg, Kymron deCesare, Donald P Land, Nolan C Kane

doi: http://dx.doi.org/10.1101/034314

Rooted v. Unrooted
The network of recent *Cannabis* breeding
Major groups of the Cannabis Family Tree (v3.1):

Lynch et al. Genomic and Chemical Diversity of Cannabis New Phytologist (2016) in review or pre-print is up on BioRxiv

= Jack Herer or Jack something

Lynched
Chemical Diversity of Genetic Groups

After removing the individuals with missing values, we had a total of 351 individuals: 94 BLDT, 229 NLDT, and 28 hemp.

Lynch et al. Genomic and Chemical Diversity of Cannabis New Phytologist (2016) in review or pre-print is up on BioRxiv
Scaling up with strainSEEK™

- CO, CA, MA, ME, WA, Spain samples... so far
- On average, our Rad seq protocol yields at least 10x coverage of 625,000 sites of the current reference genome. These sites contain 25,000 to 30,000 informative single nucleotide polymorphisms (SNPs)
- This April will witness over 100,000 genetically certified METRC tracked products hit the CO market (~60 strains)
Kannapedia – The Distributed Consensus on Cannabis Genetics

- Optional publication to Kannapedia
- Allows publishing of strain user comments/reviews
- Allows public distribution of Strain QR code
- Enables community verification of strain qualities
- Enables rating system for desired qualities
- Customer reviews
strainSEEK™ April update:

sisters from the same mister?

= CBD:THC of 2 < 31
Future Developments…
Summary

• DNA sequence analysis can tell you things about the plant (or plant microbial community) that are not otherwise apparent.

• DNA data is an additional layer of information, not a substitute for other types of data like chemical analytics or patient responses.

• We have just scratched the surface of Cannabis potential as it produces at least 113 phytocannabinoids and at least 120 terpenoids, yet what is available on the market or for research today is quite limited!
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- Orrin Devinsky
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- Russel Saneto
- University of Colorado:
  - Nolan Kane
Kannapedia – The Distributed Consensus on Cannabis Genetics

StrainSEEK™ Cannabis Certification Report

SAMPLE AND TEST INFORMATION
- Strain Name: Witches Weed
- Sex: Female
- RSP ID: 10165
- FFR ID: FR11059738
- CMR ID:
- Registrant: Doctor’s Orders
- Customer: 10795 The List Exchange
- Report Date: April 01, 2016
- Test Version: v1.4

STRAIN CHEMOPROFILE

<table>
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<tr>
<th>Compounds</th>
<th>THC+THCA</th>
<th>CBD+CBDA</th>
<th>CBG</th>
<th>CBN</th>
<th>Total Cannabinoids</th>
<th>Total Terpenoids</th>
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NEAREST GENETIC RELATIVE

- CERTIFIED STRAIN ID: Witches Weed
- NEAREST RELATIVE: Serious
- GENETIC DISTANCE: 6.01
- CLONE DISTANCE: N

If genetic distance from sample to its nearest relative is below the allowable threshold for clonally propagated plants, clone distance is listed as Y. Siblings from highly inbred lineages may be indistinguishable from clones.

HETEROZYGOITY:

- CERTIFIED STRAIN ID: Witches Weed
- PERCENT HETEROZYGOUS SNPs: 88.8379

When each of the two homologous SNPs from the sample diploid genome display different genetic variants, they are called heterozygous. If both SNPs are identical they are homozygous. Heterozygosity levels reflect aspects of hybridization and potential trait stability in offspring.
Male Female qPCR testing

- Works on a 2\textsuperscript{nd} Leaf
- Provides XY answer in hours
- $10$/Test
Whole genome shotgun method for 43 *Cannabis* samples
Nolan Kane at work on the farm...