AFDAA, July 27, 2023

Development of a Next Generation Sequencing Panel Targeting Cannabinoid Synthase Genes to Distinguish Between Marijuana and Hemp

Ya-Chih (Jessica) Cheng, MS., Rachel Houston, PhD.

Department of Forensic Science
Sam Houston State University
Huntsville, TX 77340

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

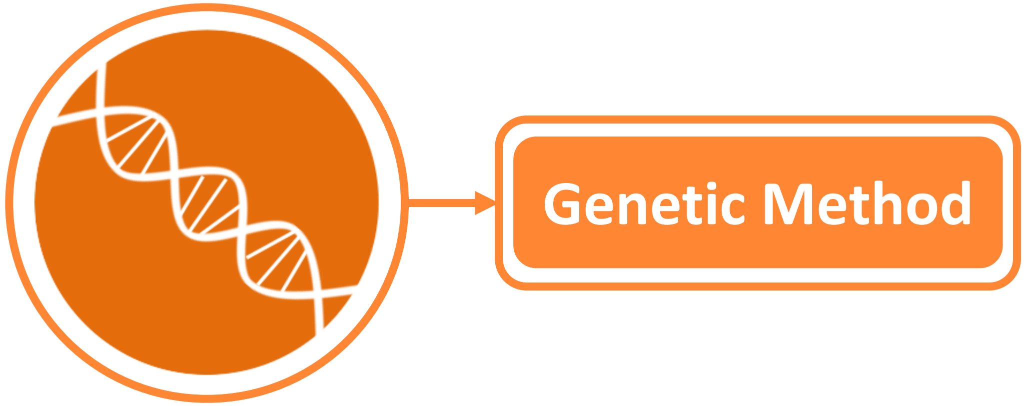
Role of Funding

- This project was funded by an award to Rachel Houston, PhD at Sam Houston State University through the Center for Advanced Research in Forensic Science (CARFS), an NSF-funded IUCRC at Florida International University (Award # 1739805).
- The opinions, finding, conclusions, or recommendations expressed in this presentation are those of the authors.

Differentiation of Marijuana and Hemp-Statement of Problems

➢ Traditional Method Limitations

- Microscopic, Color test→ cannot differentiate hemp and marijuana
- Chemistry instrumentation→ provide limited information, effected by age, storage, sample type and size



➢ Genetic Method

- Only need trace amount of sample available
- Analyzed non-traditional sample types
- Not effect by sample condition or storage
- Crop type differentiation
- Tracing geographic origin

Genetic Markers for Differentiating Hemp and Marijuana

Int J Legal Med (2016) 130:635–647

Legal Medicine 26 (2017) 33–40

Article

Developing and
(Hemp) for Thei

TECHNICAL NOTE

GENERAL

*Cristiane Barbosa D'Oliveira Mati
M.S.; Lilian de Oliveira Machado,
Dobbler,4 M.S.; Renata Machado
Roesch,4 Ph.D.; Flávio Anastácio*

Whole Plastome Seq
Drug-Type *Cannabis*: Insights into
Use of Plastid in Forensic Analyses

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Forensic Science International 159 (2006) 132–140
Forensic Science International
www.elsevier.com/locate/forsciint

DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug-type" and "fiber-type" *Cannabis sativa* L.

Mareshige Kojoma[a,b,*], Hikaru Seki[b], Shigeo Yoshida[b], Toshiya Muranaka[b]

[a] *JYUGEI Institute (The Arboricultural Research Institute), University Forests, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 457 Kano, Minami-izu, Shizuoka 451-0304, Japan*
[b] *Plant Science Center, RIKEN, 1-7-22 Suehirocho, Tsurumi-ku, Yokohama 230-0045, Japan*

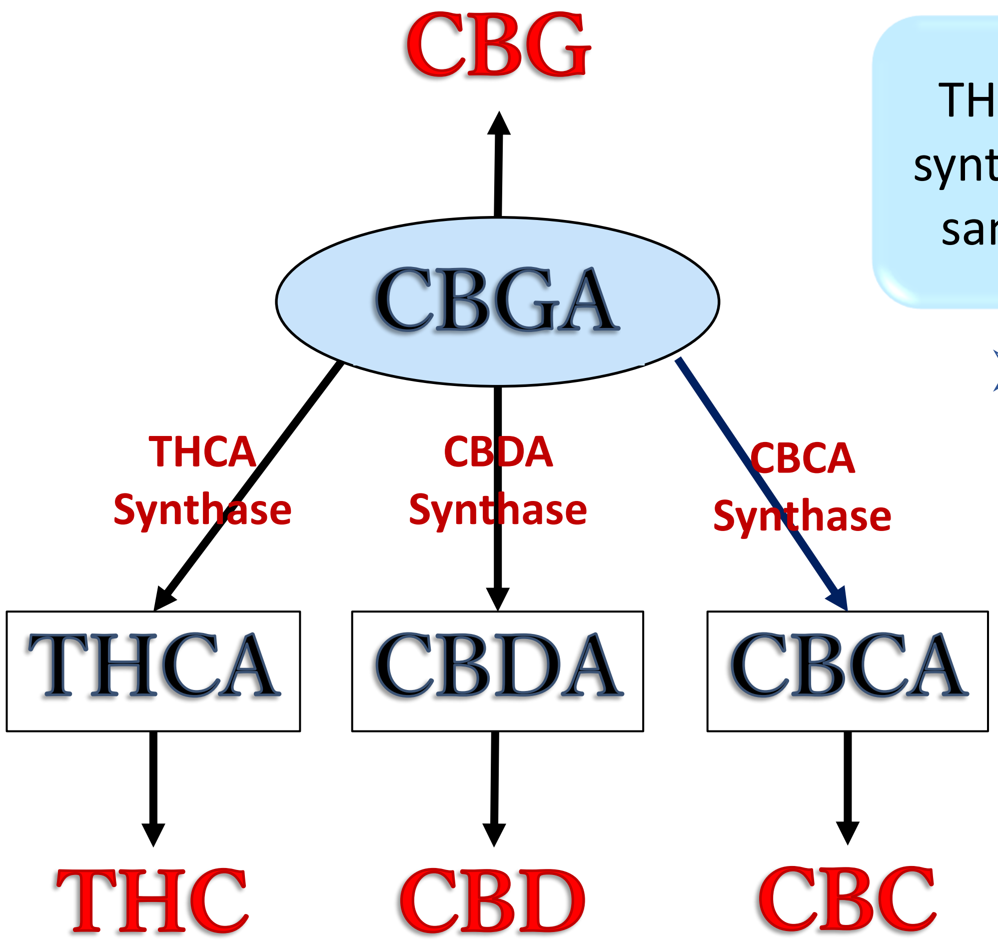
Received 20 May 2005; received in revised form 9 July 2005; accepted 18 July 2005
Available online 6 September 2005

Madeline G. Roman · David Gangitano · Rachel Houston

Received: 25 June 2019 / Accepted: 6 August 2019

Synthase Gene and Chemotype

Cannabis strains are usually differentiated by their chemical composition (chemotype)



THCA, CBDA, and CBCA synthase compete for the same substrate (CBGA) → The genotypes differences of the three synthase genes might influence cannabinoid content → Differentiation Markers

- **Synthase genes sequences**
  - CBCAS is reported to be 96% identical to THCAS sequence
  - Cannabinoid synthase genes copies may include functional genes as well as several pseudogenes
  - Pseudogene with 89%– 95% similar in identity to THCAS, CBDAS, and CBCAS

Study’s Goal and Approach

➢ Limitation of Current Genetic method

- **Need to incorporated CBCAS and pseudogene**
- **Limited sample varieties, small sample number (FP and FN still occurred)**

Limited sample varieties, small sample number (FP and FN still occurred)

- Lack of a comprehensive *C. sativa* database

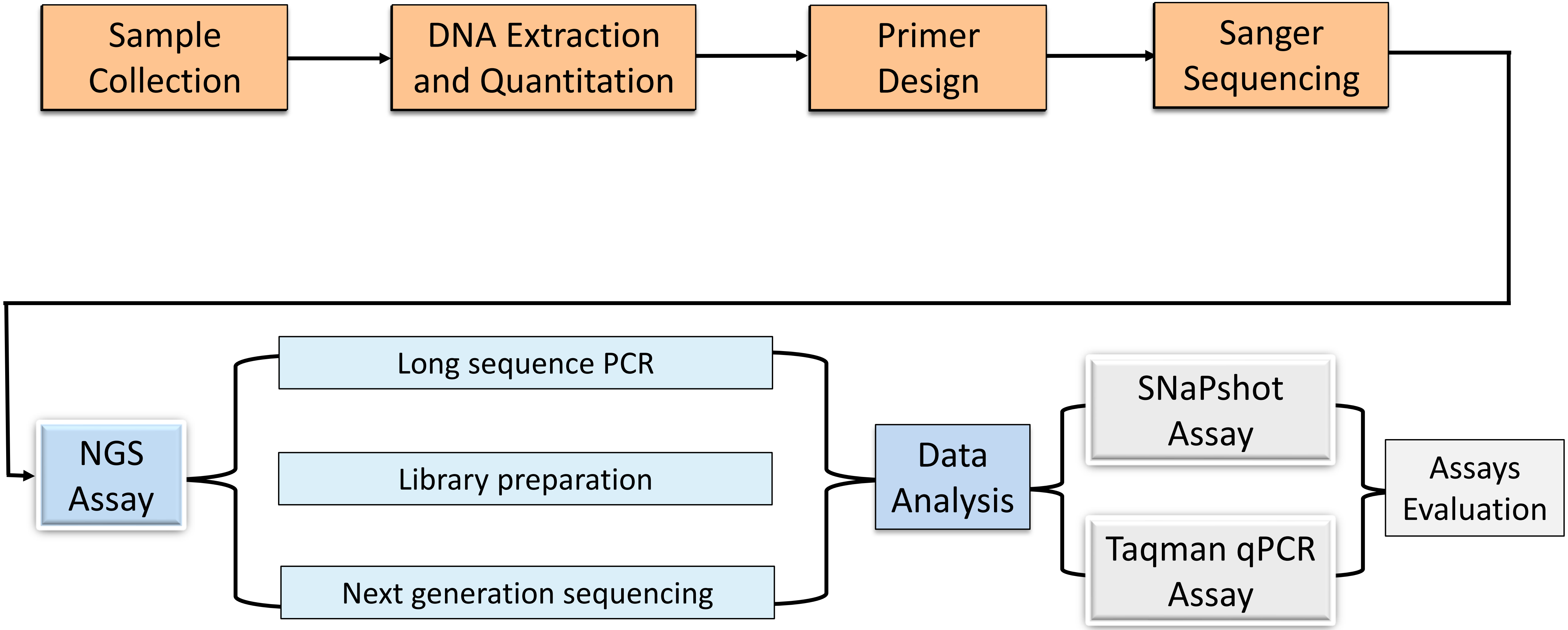
➢ Goal

Provide large scale evaluation of the synthase genes and pseudogenes on wide varieties of Cannabis to identify novel polymorphisms or pattern of genes’ present for crop type discrimination.

➢ Designed NGS panel targeting three synthase genes and pseudogenes

- NGS can target specific genes of interest with high coverage and confidence
- Quickly screen and sequence several types of cannabis with its high-throughput capabilities

Flow Chart



Sample Collection

DNA Extraction and Quantitation

Primer Design

Sanger Sequencing

NGS Assay

Long sequence PCR

Library preparation

Next generation sequencing

Data Analysis

SNaPshot Assay

Taqman qPCR Assay

Assays Evaluation

Cannabis Sample Collection

Crop Types		Number	Source	Chemistry Data
1	**Reference Marijuana**	27	NIDA, NIST	Quant & TIC Data
2	**Reference Hemp**	3	NIDA, NIST, University of Kentucky	Quant & TIC Data
3	**Seized Marijuana**	61	DEA (University of Mississippi)	Quant Data
4	**CBD Hemp**	20	Online purchase	Quant & TIC Data
5	**CBG Hemp IH**	13	University of Mississippi	Not Available
	CBG Hemp	8	Online purchase	Quant & TIC Data
6	**Δ8-THC Hemp**	7	Online purchase	Quant & TIC Data
7	**THCO Hemp**	3	Online purchase	Quant & TIC Data
8	**Δ10-THC Hemp**	1	Online purchase	Quant & TIC Data
9	**CBDV Hemp**	1	Online purchase	Quant & TIC Data
10	**Hemp Seed**	4	Grocery Store purchase	Not Available
	Total	**148**	**Marijuana: 88**	**Hemp: 60**

Primer Design

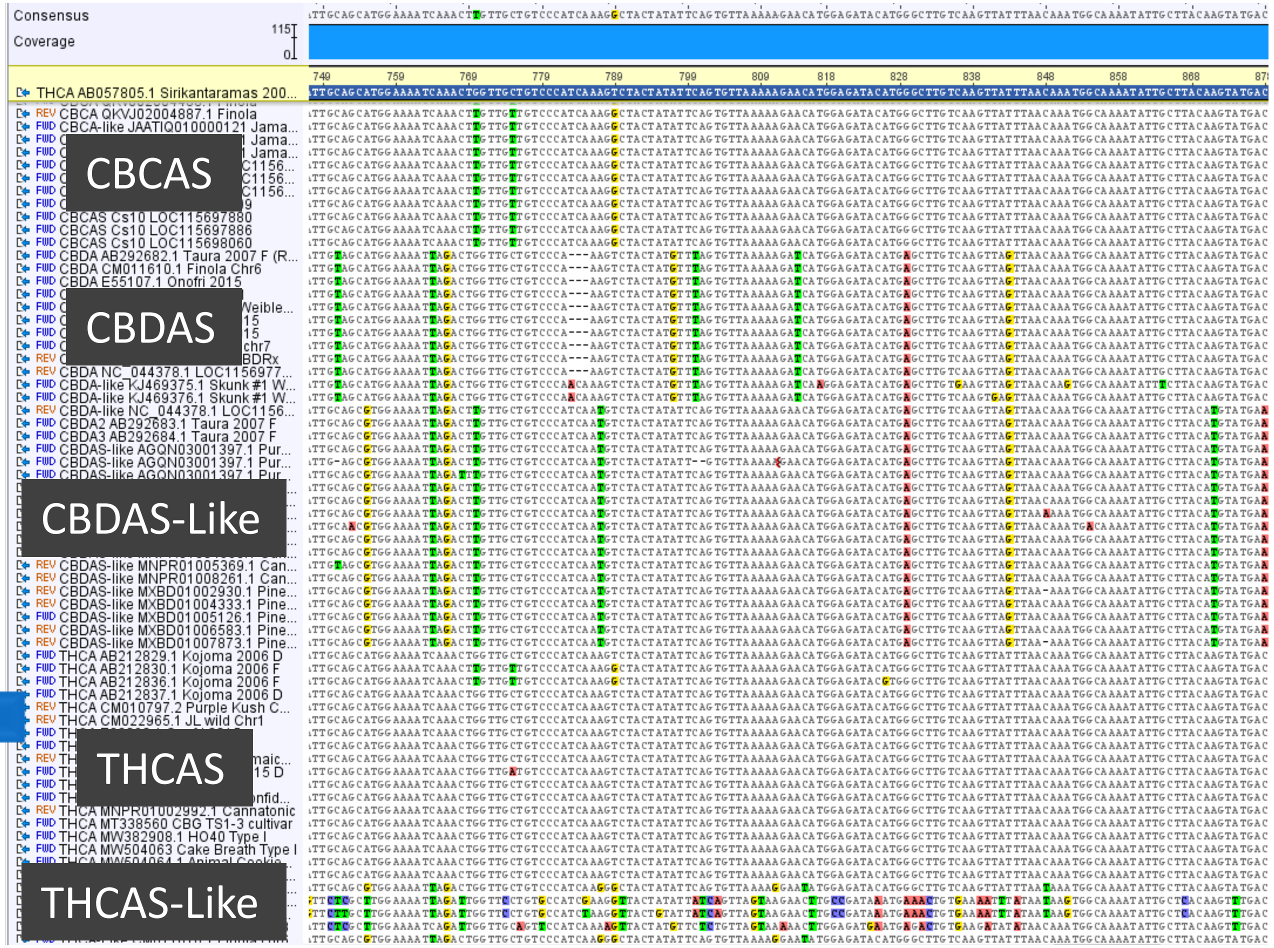
Approach 1: Using Published Primers

Name	Primer	Reference
THCAS F2	ACTGAAGAAAAATGAATTGCTCAG	**Cascini 2019**
THCAS R2	ATTTAAAGATAATTAATGATGATGCG	**Cascini 2019**
CBDAS F1	ATGAAGTGCTCAACATTCTC	**Cascini 2019**
CBDAS Lav F	CTGCAGGAATGAAGTACTCAACATTCTCCTTTTGG	**Laverty 2019**
CBDAS R1	TTAATGACGATGCCGTGG	**Cascini 2019**
CBDAS Lav R	AAGCTTTCATGGTACCCCATGATGATGCCGTGGAAGAG	**Laverty 2019**
CBCAS Lav F	CGGATGTACTGTTATGCTCCAA	**Laverty 2019**
CBCAS Lav R	CATTCTCCATTAAAATAAGAAAGACAA	**Laverty 2019**

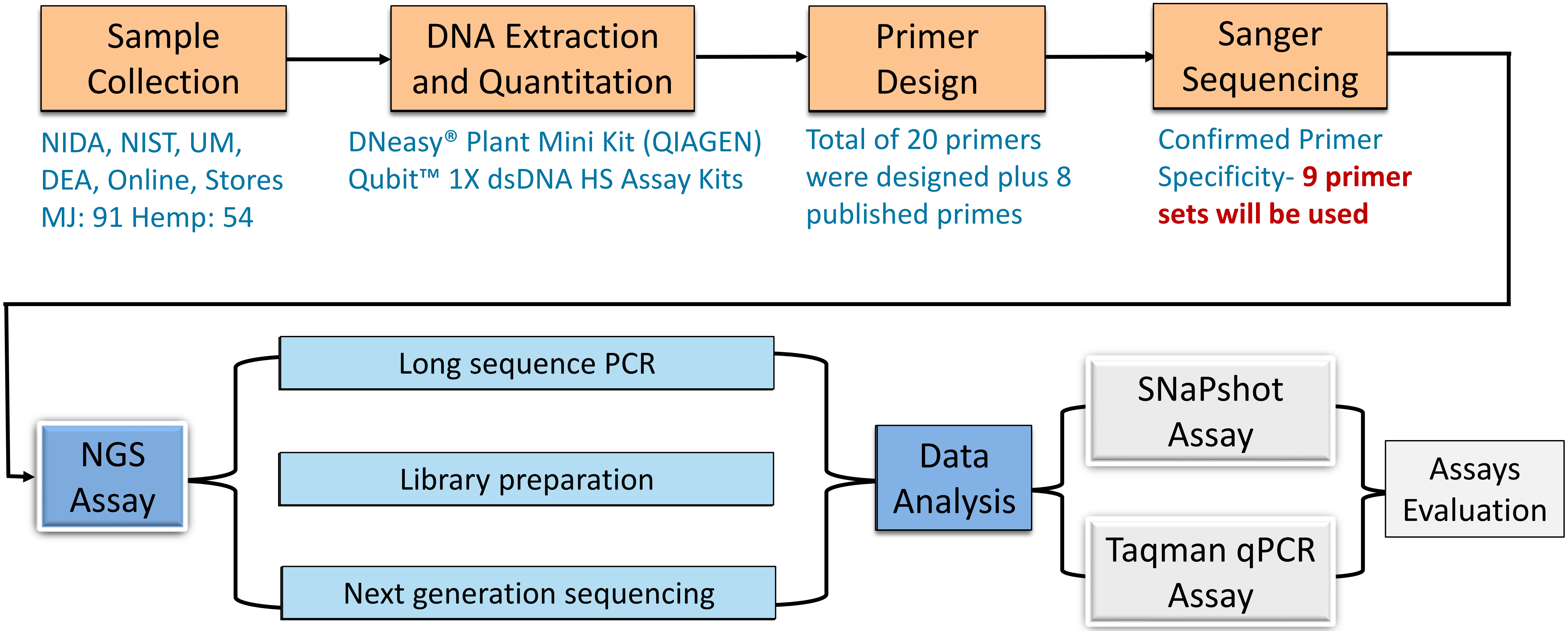
No.	Primer Name	No.	Primer Name	No.	Primer Name
1	THCAS F4	8	CBCAS R2	15	CBDA+Like F2
2	THCAS R4	9	THCA+Like R	16	CBDA+Like F3
3	CBDAS F3	10	Everyone R1	17	CBCA+Like F
4	CBDAS R3	11	CBDA+Like R1	18	THCA F5
5	CBCAS F1	12	THCA R5	19	Everyone R2
6	CBCAS F3	13	CBDA+Like R2	20	Everyone F1
7	CBCAS R1	14	CBDA+Like F1		

Approach 2: Aligned Published Synthases' Sequence

Align 121 Reference Sequences (including THCAS, THCAS-like, CBDAS, CBDAS-like, CBCAS)



Flow Chart



Sample Collection

NIDA, NIST, UM, DEA, Online, Stores
MJ: 91 Hemp: 54

DNA Extraction and Quantitation

DNeasy® Plant Mini Kit (QIAGEN)
Qubit™ 1X dsDNA HS Assay Kits

Primer Design

Total of 20 primers were designed plus 8 published primes

Sanger Sequencing

Confirmed Primer Specificity- **9 primer sets will be used**

NGS Assay

- Long sequence PCR
- Library preparation
- Next generation sequencing

Data Analysis

- SNaPshot Assay
- Taqman qPCR Assay

Assays Evaluation

General Workflow

Long-Range PCR

- LA PCR Kit Ver.2.1
- Single-plex PCR

PCR Product Pool

- Qubit quantitation
- PCR Products Pool (0.2 ng/µL)
- Pool all targets from the sample
- ExoI clean-up

Library Preparation

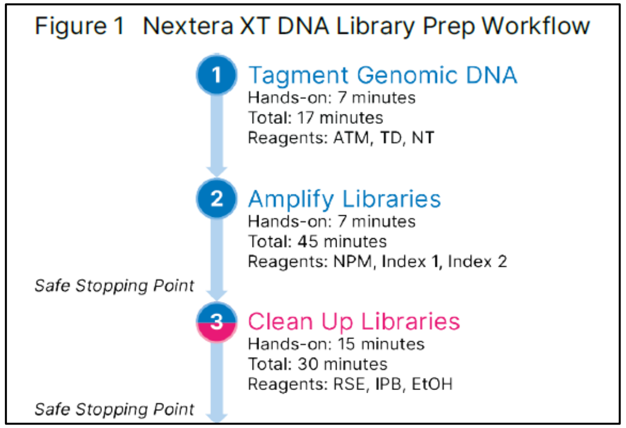


Figure 1 Nextera XT DNA Library Prep Workflow

1. Tagment Genomic DNA
   Hands-on: 7 minutes
   Total: 17 minutes
   Reagents: ATM, TD, NT
2. Amplify Libraries
   Hands-on: 7 minutes
   Total: 45 minutes
   Reagents: NPM, Index 1, Index 2

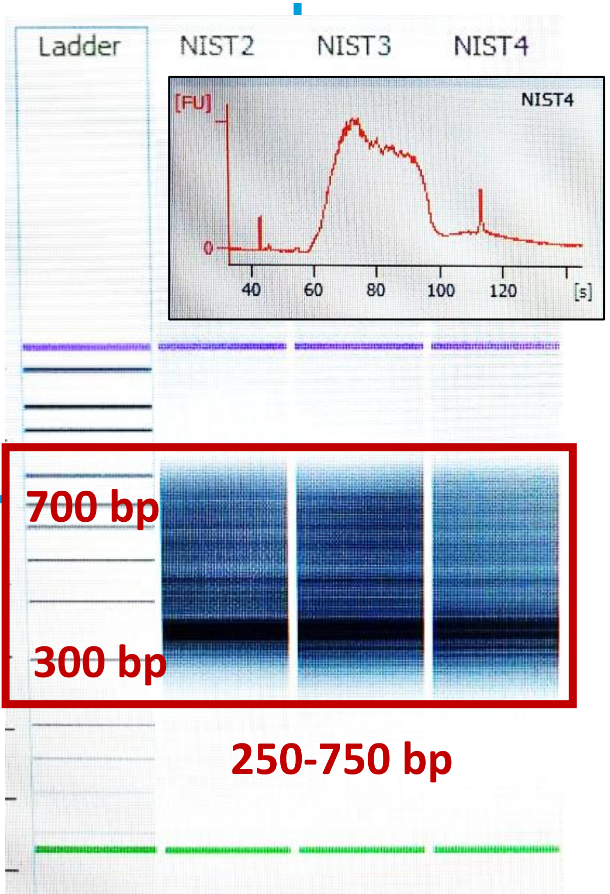
Safe Stopping Point

3. Clean Up Libraries
   Hands-on: 15 minutes
   Total: 30 minutes
   Reagents: RSE, IPB, EtOH

Safe Stopping Point

Libraries Quality Check

- Bioanalyzer



250-750 bp

Libraries Normalization

- Qubit quantitation
- Libraries Pool (1.32 ng/µL)

$$\text{Molarity} = \frac{\left(\frac{\text{ng}}{\mu\text{l}}\right)}{660\,\frac{\text{g}}{\text{mol}} \times\ 500\text{bp}}$$

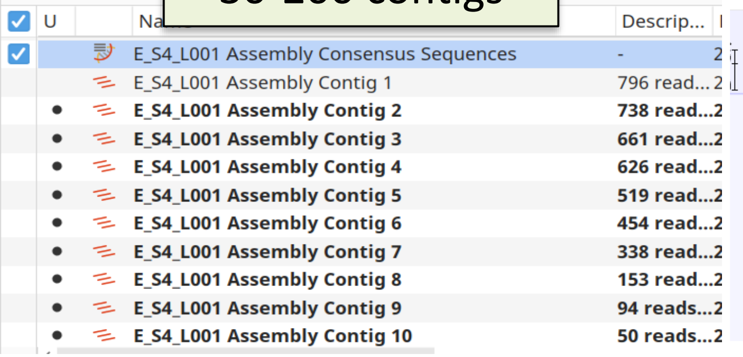
MiSeq FGx Run

- Denature libraries
- Dilute denature libraries
- Instrument loading

Data Analysis-Geneious Prime

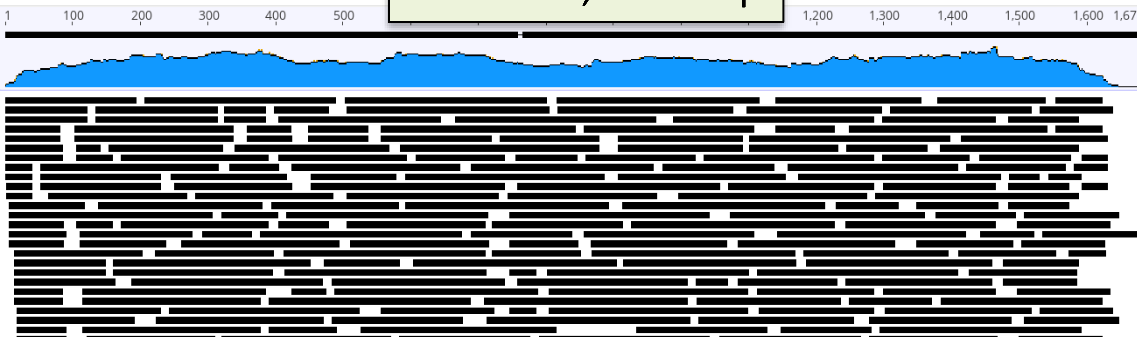
- Data Normalization
- De Novo Assembly
- Map to References
- Record Results

Assemble millions of reads generated by NGS platforms: align and merge overlapping fragments of a DNA sequence to reconstruct the original sequence.



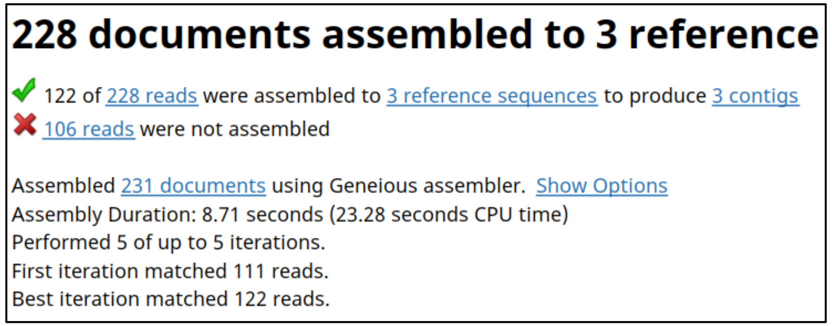
30-200 contigs

☑	U	Na...	Descrip...	I
☑		E_S4_L001 Assembly Consensus Sequences	-	2
		E_S4_L001 Assembly Contig 1	796 read...	2
	●	**E_S4_L001 Assembly Contig 2**	**738 read...**	**2**
	●	**E_S4_L001 Assembly Contig 3**	**661 read...**	**2**
	●	**E_S4_L001 Assembly Contig 4**	**626 read...**	**2**
	●	**E_S4_L001 Assembly Contig 5**	**519 read...**	**2**
	●	**E_S4_L001 Assembly Contig 6**	**454 read...**	**2**
	●	**E_S4_L001 Assembly Contig 7**	**338 read...**	**2**
	●	**E_S4_L001 Assembly Contig 8**	**153 read...**	**2**
	●	**E_S4_L001 Assembly Contig 9**	**94 reads...**	**2**
	●	**E_S4_L001 Assembly Contig 10**	**50 reads...**	**2**



796 read, 1677 bp

Map the contig to Reference Sequence: assemble contig sequences to THCAS, CBDAS, and CBCAS sequences to separate pseudo genes and locate differences



228 documents assembled to 3 reference

✓ 122 of 228 reads were assembled to 3 reference sequences to produce 3 contigs
✗ 106 reads were not assembled

Assembled 231 documents using Geneious assembler. Show Options
Assembly Duration: 8.71 seconds (23.28 seconds CPU time)
Performed 5 of up to 5 iterations.
First iteration matched 111 reads.
Best iteration matched 122 reads.

Excel Sheet: record present or absent of the synthase genes and pseudo synthase genes, as well as the polymorphisms on the synthase genes

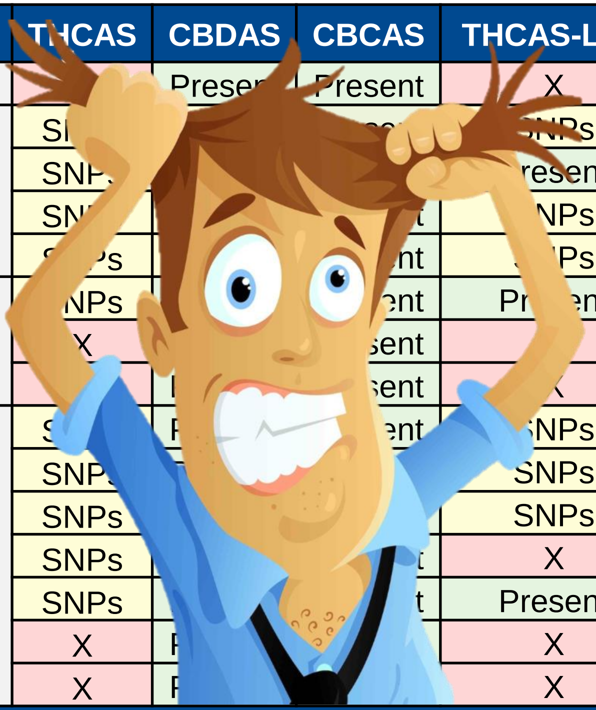
NGS Results Summary (Partial)

Sample	Strain	THCAS	CBDAS	CBCAS	THCAS-Like	CBDAS-Like
H18	**CBD Hemp**	X	Present	Present	X or SNPs	X
H20		X	SNPs	Present	X or SNPs	X
H24		X	Present	Present	X or SNPs	X
H14		X	Present	Present	X or SNPs	X
H19		X	Present	Present	X or SNPs	X
H11		X	Present	Present	X or SNPs	X
H10		X	Present	Present	X or SNPs	X
H9		X	Present	Present	X or SNPs	X
H12		X	Present	Present	X or SNPs	X
H47		X	Present	Present	X or SNPs	X
H45		X	Present	Present	X or SNPs	X
H44		X	Present	Present	X or SNPs	X
H46		X	Present	SNPs	X or SNPs	X

Sample	Strain	THCAS	CBDAS	CBCAS	THCAS-Like	CBDAS-Like
DEA27A	**Seized MJ**	Present	X	Present	Present	Present
DEA5A		Present	X	Present	Present	Present
DEA36A		Present	X	Present	Present	Present
DEA31A		Present	X	Present	Present	Present
DEA44A		Present	X	Present	Present	Present
DEA42A		Present	X	Present	Present	Present
DEA32A		Present	X	Present	Present	Present
DEA25A		Present	X	Present	Present	Present
DEA60A		Present	X	Present	Present	Present
DEA58A		SNPs	X	Present	Present	Present

Sample	Strain	THCAS	CBDAS	CBCAS	THCAS-Like	CBDAS-Like
H23	**CBG Hemp**	SNP 1064G	X	X	Present	Present
CBG30A		SNP 1064G	Incomplete	Present	Present	Present
H22		SNP 1064G	X	X	Present	Present
H21		SNP 1064G	X	X	Present	Present
H41		SNP 1064G	X	X	Present	Present
H42		SNP 1064G	X	X	Present	Present
CBG59A		SNP 1064G	X	Incomplete	Present	Present
H26		SNP 1064G	X	X	Present	Present
CBG22A		SNP 1064G	X	X	Present	Present
CBG40A		SNP 1064G	Incomplete	X	Present	Present
CBG44A		SNP 1064G	Incomplete	X	Present	Present
CBG5A		SNP 1064G	X	X	Present	Present
CBG20B		SNP 1064G	Present	Present	Present	Present

Sample	Strain	THCAS	CBDAS	CBCAS	THCAS-Like	CBDAS-Like
H39	**CBDV Hemp**	[illegible]	Presen	Present	X	X
H1-J2	**Hemp Seed**	SI	[illegible]	[illegible]	NPs	Present
H2-5		SNP	[illegible]	[illegible]	resent	Present
H3-5		SN	[illegible]	t	NPs	Present
H4-4		Ps	[illegible]	nt	Ps	Present
H36	**THCO Hemp**	NPs	[illegible]	ent	Pr ent	Present
H37		X	[illegible]	sent	[illegible]	X
H35		[illegible]	[illegible]	sent	X	X
H28	**Δ8 Hemp**	S	[illegible]	ent	NPs	X
H29		SNP	[illegible]	[illegible]	SNPs	X
H31		SNPs	[illegible]	[illegible]	SNPs	X
H30		SNPs	[illegible]	t	X	Present
H34		SNPs	[illegible]	t	Present	Present
H34		X	[illegible]	[illegible]	X	Present
H32		X	[illegible]	[illegible]	X	Present



Differentiate Hemp and Marijuana

THCAS

Absent

- ~~Hemp~~

- CBD Hemp
- Other Hemp
- Low THC MJ

Present

- ~~Marijuana~~

- CBG Hemp
- Other Hemp
- Hemp Seed
- Marijuana

CBDAS

Absent

- ~~Marijuana~~

- CBG Hemp
- Marijuana

Present

- ~~Hemp~~

- CBD Hemp
- Other Hemp
- Hemp Seed
- High CBD MJ

CBCAS

Absent

- ~~N/A~~

- CBG Hemp
- Marijuana

Present

- ~~Hemp~~
- ~~Marijuana~~

- Everyone

Cannot differentiate hemp and marijuana by just synthase genes.

Cannot differentiate hemp and marijuana by looking at several genes “at the same time”.

Differentiation Flow Chart

THCAS 1064A SNP

- Present
  - Hemp
- Absent
  - **Marijuana**
  - Hemp
  - Hemp Seed

  THCAS-Like

  - Present
    - **Marijuana**
    - Hemp Seed

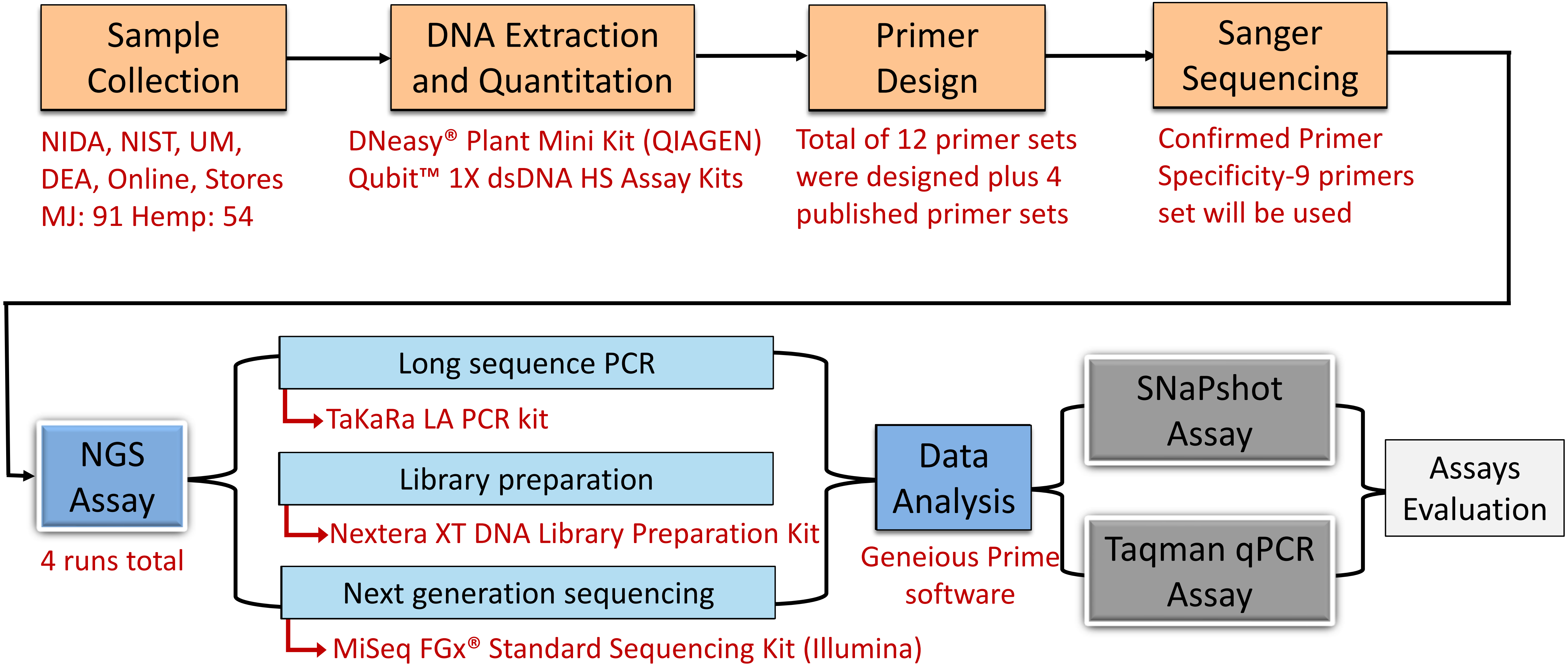
    CBDAS

    - Absent or Present
      - **Marijuana**
        - Present
          - High CBD **MJ**
        - Absent
          - Low CBD **MJ**
    - Present with 586G SNPs
      - **Marijuana**
      - Hemp Seed

      THCAS 366T or 1179T SNP

      - Present
        - Hemp Seed
      - Absent
        - **Marijuana**
  - Absent
    - Hemp

Flow Chart- Next Step



Sample Collection

NIDA, NIST, UM,
DEA, Online, Stores
MJ: 91 Hemp: 54

DNA Extraction and Quantitation

DNeasy® Plant Mini Kit (QIAGEN)
Qubit™ 1X dsDNA HS Assay Kits

Primer Design

Total of 12 primer sets were designed plus 4 published primer sets

Sanger Sequencing

Confirmed Primer Specificity-9 primers set will be used

NGS Assay

4 runs total

Long sequence PCR

TaKaRa LA PCR kit

Library preparation

Nextera XT DNA Library Preparation Kit

Next generation sequencing

MiSeq FGx® Standard Sequencing Kit (Illumina)

Data Analysis

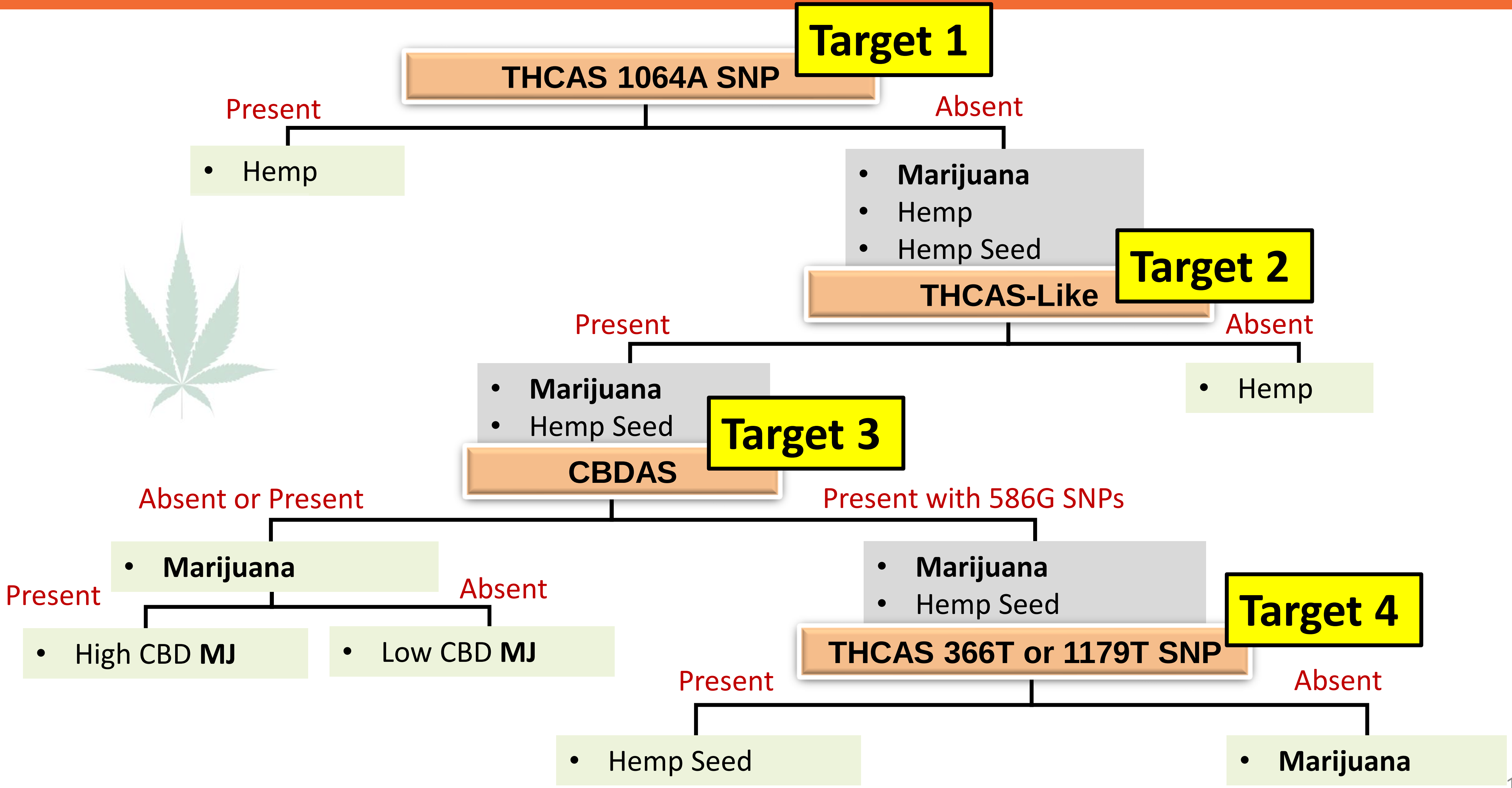
Geneious Prime software

SNaPshot Assay

Taqman qPCR Assay

Assays Evaluation

Differentiation Flow Chart-NGS



Target 1

THCAS 1064A SNP

Present

- Hemp

Absent

- **Marijuana**
- Hemp
- Hemp Seed

Target 2

THCAS-Like

Present

- **Marijuana**
- Hemp Seed

Absent

- Hemp

Target 3

CBDAS

Absent or Present

- **Marijuana**

Present

- High CBD **MJ**

Absent

- Low CBD **MJ**

Present with 586G SNPs

- **Marijuana**
- Hemp Seed

Target 4

THCAS 366T or 1179T SNP

Present

- Hemp Seed

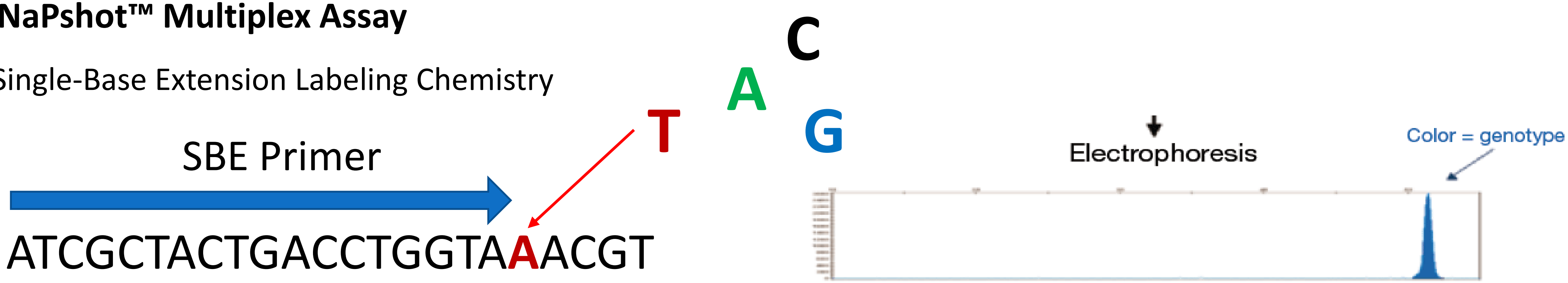
Absent

- **Marijuana**

Development of Fast Genotyping Assay

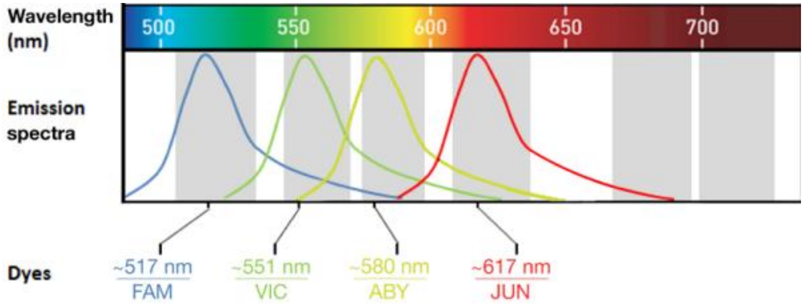
➢ SNaPshot™ Multiplex Assay

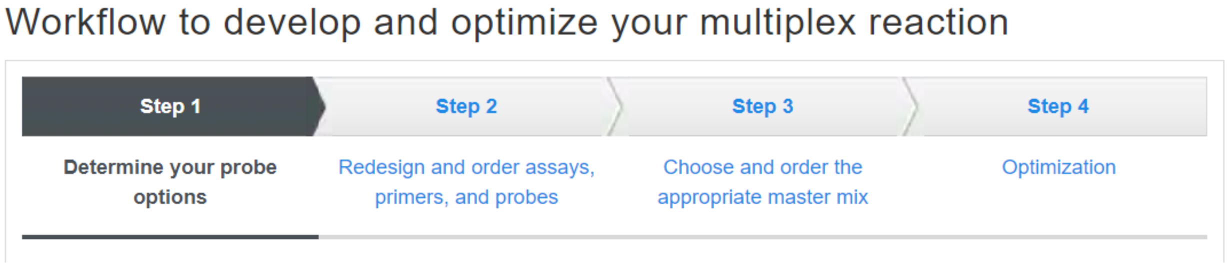
-Single-Base Extension Labeling Chemistry



➢ Custom TaqMan Probes qPCR Assay

Custom QSY probes—QSY probes are best-suited for multiplexed detection of more than two targets and pair with proprietary ABY and JUN dyes that are optimized for Applied Biosystems instruments.





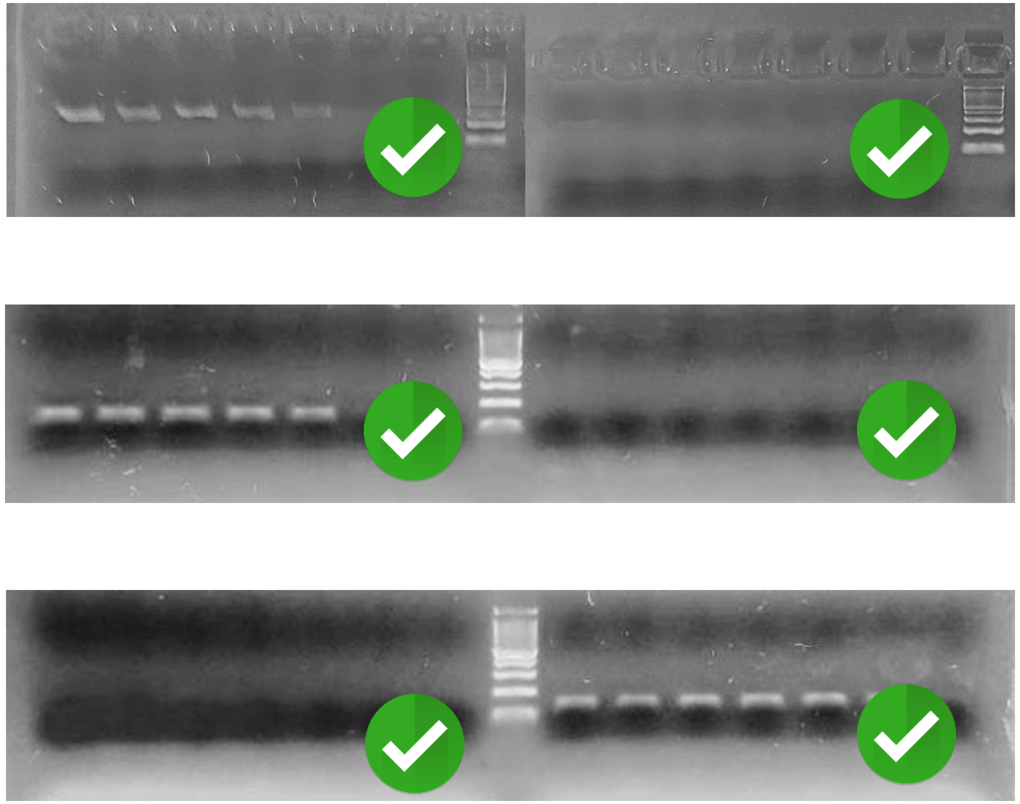
Workflow to develop and optimize your multiplex reaction

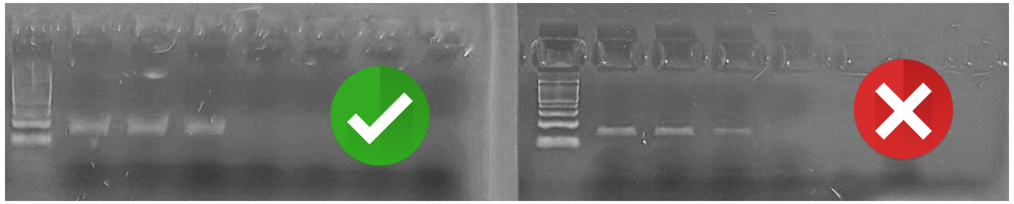
Step 1	Step 2	Step 3	Step 4
Determine your probe options	Redesign and order assays, primers, and probes	Choose and order the appropriate master mix	Optimization

- Gene Expression→4-plex
- SNP Genotyping→2-plex (1 SNP need 2 probes)

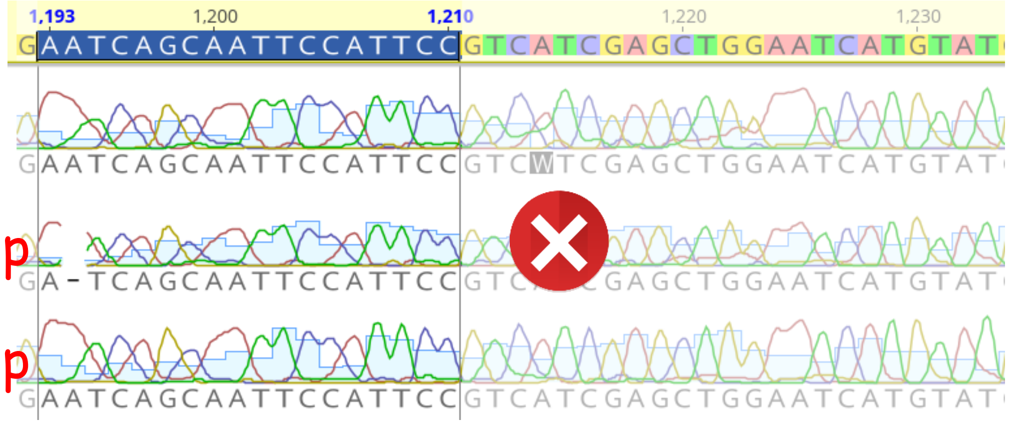
First Step-Primer Design

- Design 4 PCR primer sets for SNP





Try different primer sets, try sequencing



!!Hemp might actually all have THCAS-Like

NGS vs PCR

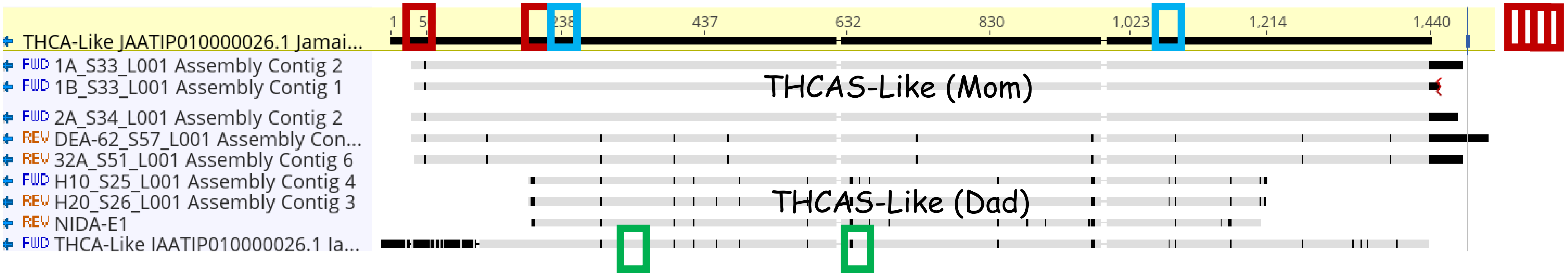
THCAS F4

CBDAS Like F3

Everyone F Primers

THCA-Like R Primers

Other R primers



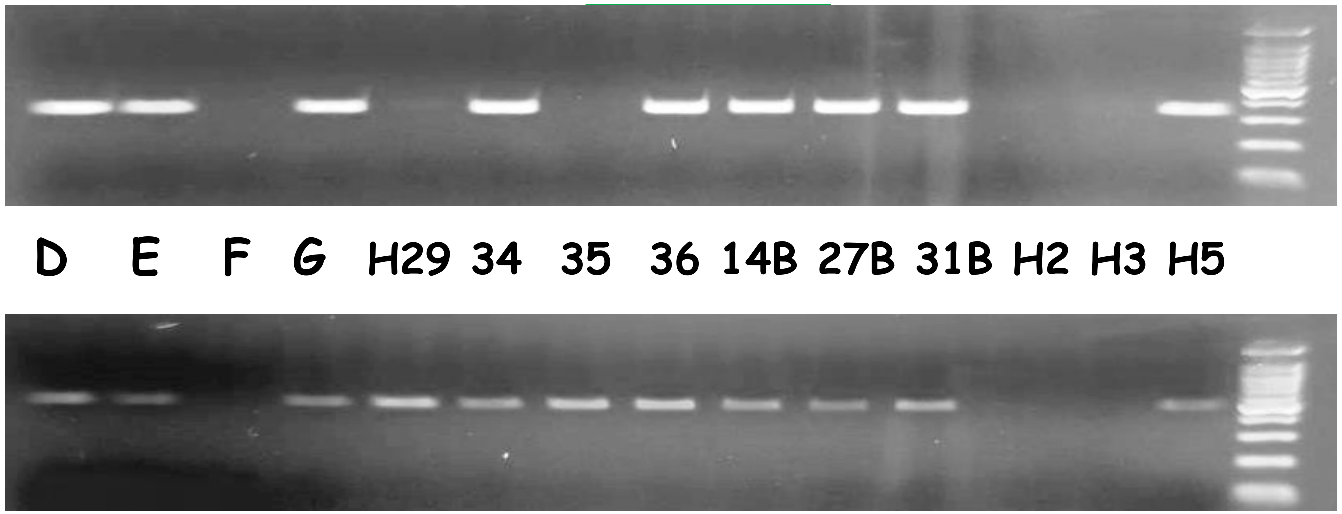
THCA-Like JAATIP010000026.1 Jamai...
FWD 1A_S33_L001 Assembly Contig 2
FWD 1B_S33_L001 Assembly Contig 1
FWD 2A_S34_L001 Assembly Contig 2
REV DEA-62_S57_L001 Assembly Con...
REV 32A_S51_L001 Assembly Contig 6
FWD H10_S25_L001 Assembly Contig 4
REV H20_S26_L001 Assembly Contig 3
REV NIDA-E1
FWD THCA-Like JAATIP010000026.1 Ja...

THCAS-Like (Mom)

THCAS-Like (Dad)

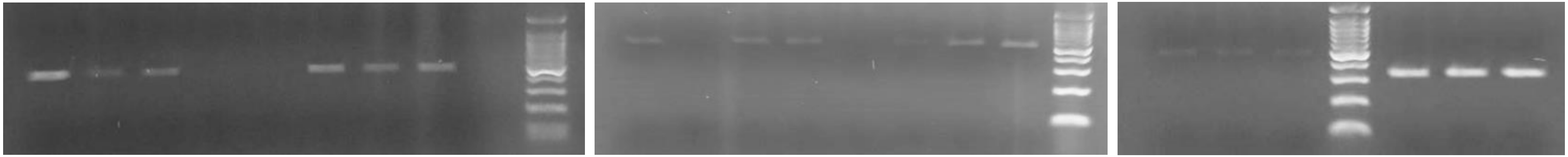
THCAS-Like (M)

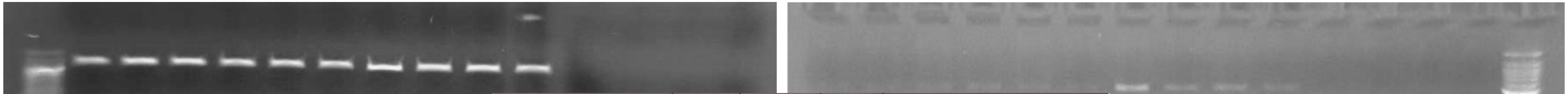
THCAS-Like (D)



THCAS?
THCAS-Like?
Mom or Dad?

Run Gel Run Gel Run Gel…Sequencing Confirmation



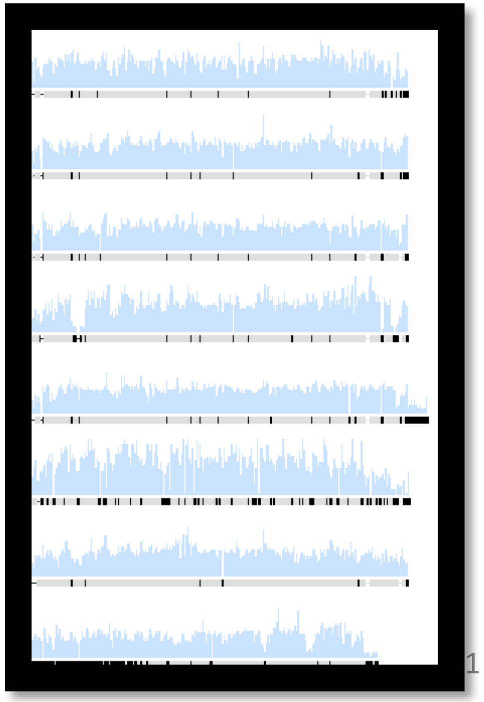


Testing, testing, testing…

Result	H31	H20	29	61	[illegible]	H2-5	H5-5
THCAS Like Mom	V	X	V	X	[illegible]	V	V
THCAS Like Dad	V	V	V	X	[illegible]	V	V

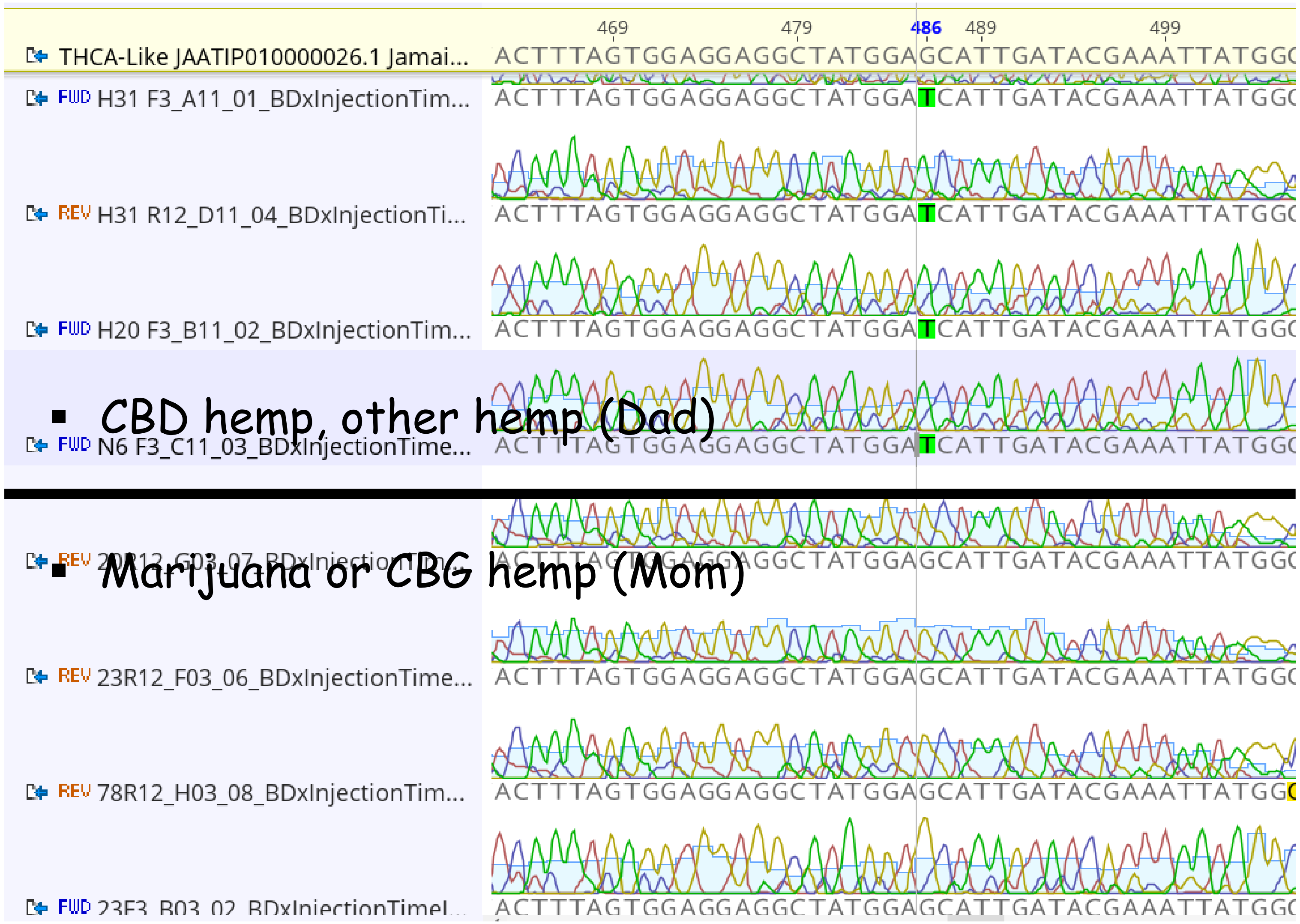
Results	IH1	IH2	IH3	IH4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	IH13	IH14
THCAS Like Mom	V	V	V	V	V	V	V	V	V	V	X	X	X	X
THCAS Like Dad	V	V	V	V	V	V	V	V	V	V	X	X	X	X

Result	29B	61B	NIST4	NIST5	NIST11	NIST13	NIST17	NIST10	NIDA H	NIDA I
THCAS Like Mom	V	V	X	X	V	V	F	V	V	V
THCAS Like Dad	V	F	V	V	F	V	V	V	V	V



Need to Differentiate the Two Variants of THCAS-Like (Mom and Dad)

- The new primer set will catch Mom in MJ and CBG hemp; catch Dad in CBD hemp



THCA-Like JAATIP010000026.1 Jamai... ACTTTAGTGGAGGAGGCTATGGAGCATTGATACGAAATTATGGC

FWD H31 F3_A11_01_BDxInjectionTim... ACTTTAGTGGAGGAGGCTATGGATCATTGATACGAAATTATGGC

REV H31 R12_D11_04_BDxInjectionTi... ACTTTAGTGGAGGAGGCTATGGATCATTGATACGAAATTATGGC

FWD H20 F3_B11_02_BDxInjectionTim... ACTTTAGTGGAGGAGGCTATGGATCATTGATACGAAATTATGGC

FWD N6 F3_C11_03_BDxInjectionTime... ACTTTAGTGGAGGAGGCTATGGATCATTGATACGAAATTATGGC

REV 20R12_G03_07_BDxInjectionTim... ACTTTAGTGGAGGAGGCTATGGAGCATTGATACGAAATTATGGC

REV 23R12_F03_06_BDxInjectionTime... ACTTTAGTGGAGGAGGCTATGGAGCATTGATACGAAATTATGGC

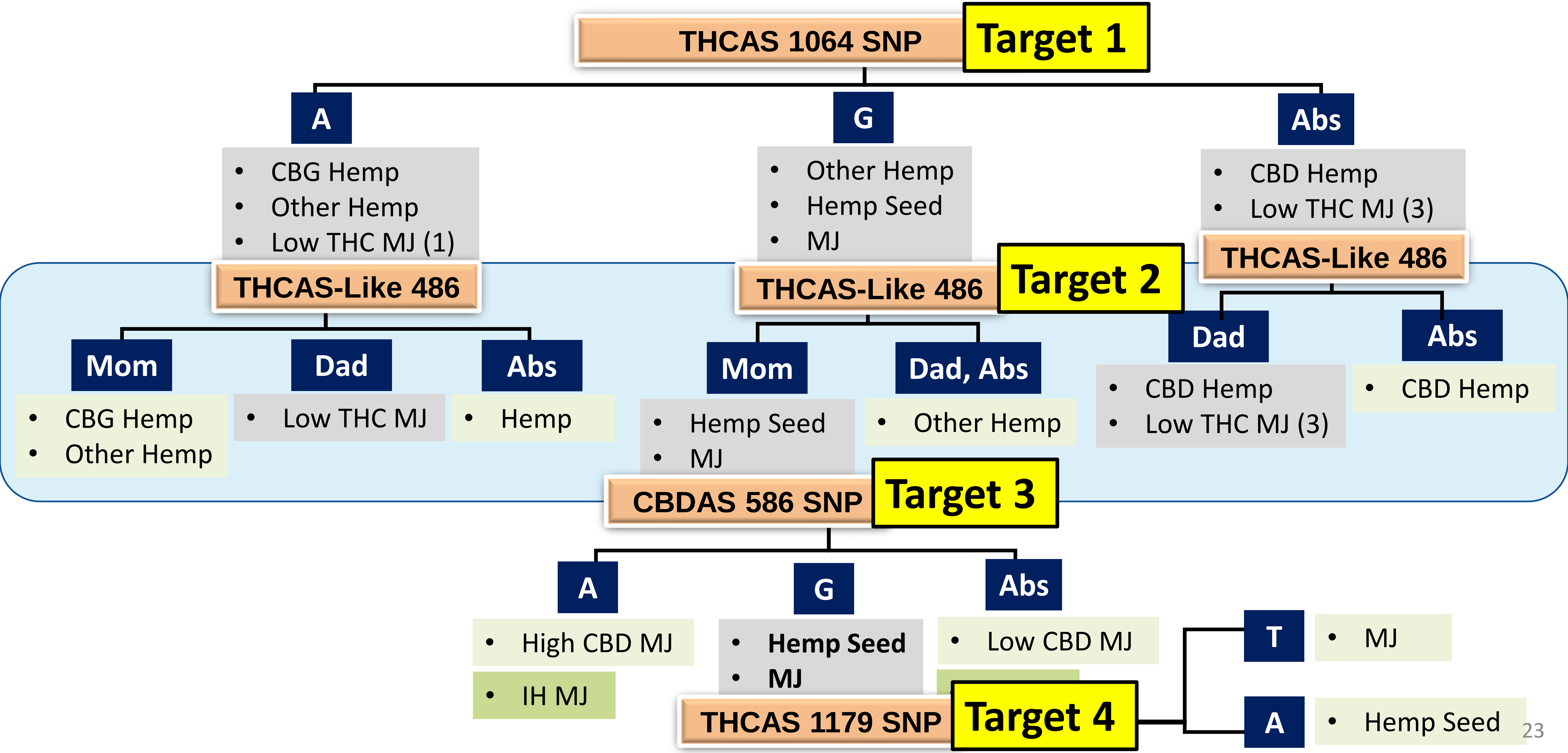
REV 78R12_H03_08_BDxInjectionTim... ACTTTAGTGGAGGAGGCTATGGAGCATTGATACGAAATTATGGC

FWD 23F3_B03_02_BDxInjectionTimel... ACTTTAGTGGAGGAGGCTATGGAGCATTGATACGAAATTATGGC

- CBD hemp, other hemp (Dad)
- Marijuana or CBG hemp (Mom)

Using THCAS-Like 486 SNP to differentiate Mom and Dad Variants

Differentiation Flow Chart-PCR



THCAS 1064 SNP **Target 1**

A
- CBG Hemp
- Other Hemp
- Low THC MJ (1)

THCAS-Like 486

Mom
- CBG Hemp
- Other Hemp

Dad
- Low THC MJ

Abs
- Hemp

G
- Other Hemp
- Hemp Seed
- MJ

THCAS-Like 486 **Target 2**

Mom
- Hemp Seed
- MJ

CBDAS 586 SNP **Target 3**

A
- High CBD MJ
- IH MJ

G
- **Hemp Seed**
- **MJ**

THCAS 1179 SNP **Target 4**

T
- MJ

A
- Hemp Seed

Abs
- Low CBD MJ

Dad, Abs
- Other Hemp

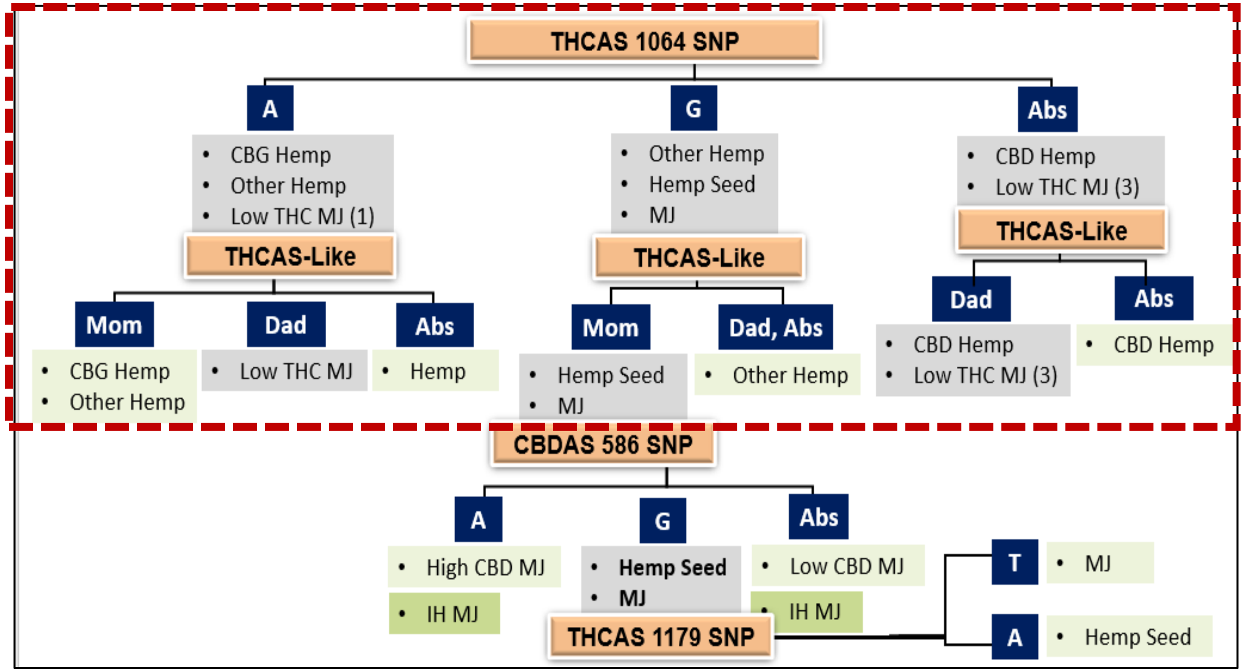
Abs
- CBD Hemp
- Low THC MJ (3)

THCAS-Like 486

Dad
- CBD Hemp
- Low THC MJ (3)

Abs
- CBD Hemp

Fast Genotyping Assays



THCAS 1064 SNP

A
- CBG Hemp
- Other Hemp
- Low THC MJ (1)

THCAS-Like

Mom
- CBG Hemp
- Other Hemp

Dad
- Low THC MJ

Abs
- Hemp

G
- Other Hemp
- Hemp Seed
- MJ

THCAS-Like

Mom
- Hemp Seed
- MJ

Dad, Abs
- Other Hemp

Abs
- CBD Hemp
- Low THC MJ (3)

THCAS-Like

Dad
- CBD Hemp
- Low THC MJ (3)

Abs
- CBD Hemp

CBDAS 586 SNP

A
- High CBD MJ
- IH MJ

G
- **Hemp Seed**
- **MJ**

THCAS 1179 SNP

T
- MJ

A
- Hemp Seed

Abs
- Low CBD MJ
- IH MJ

➢ **SNaPshot Assay (Can have all targets)**

Expectation:
- Separate most Marijuana form all strains of Hemp
- Separate marijuana from hemp seed
- Giving CBD information
- Might be able to estimate THC conc

Main advantage:
- Fast, easier interpretation, provide more info

➢ **Tagman qPCR Assay (only allowed two SNPs)**

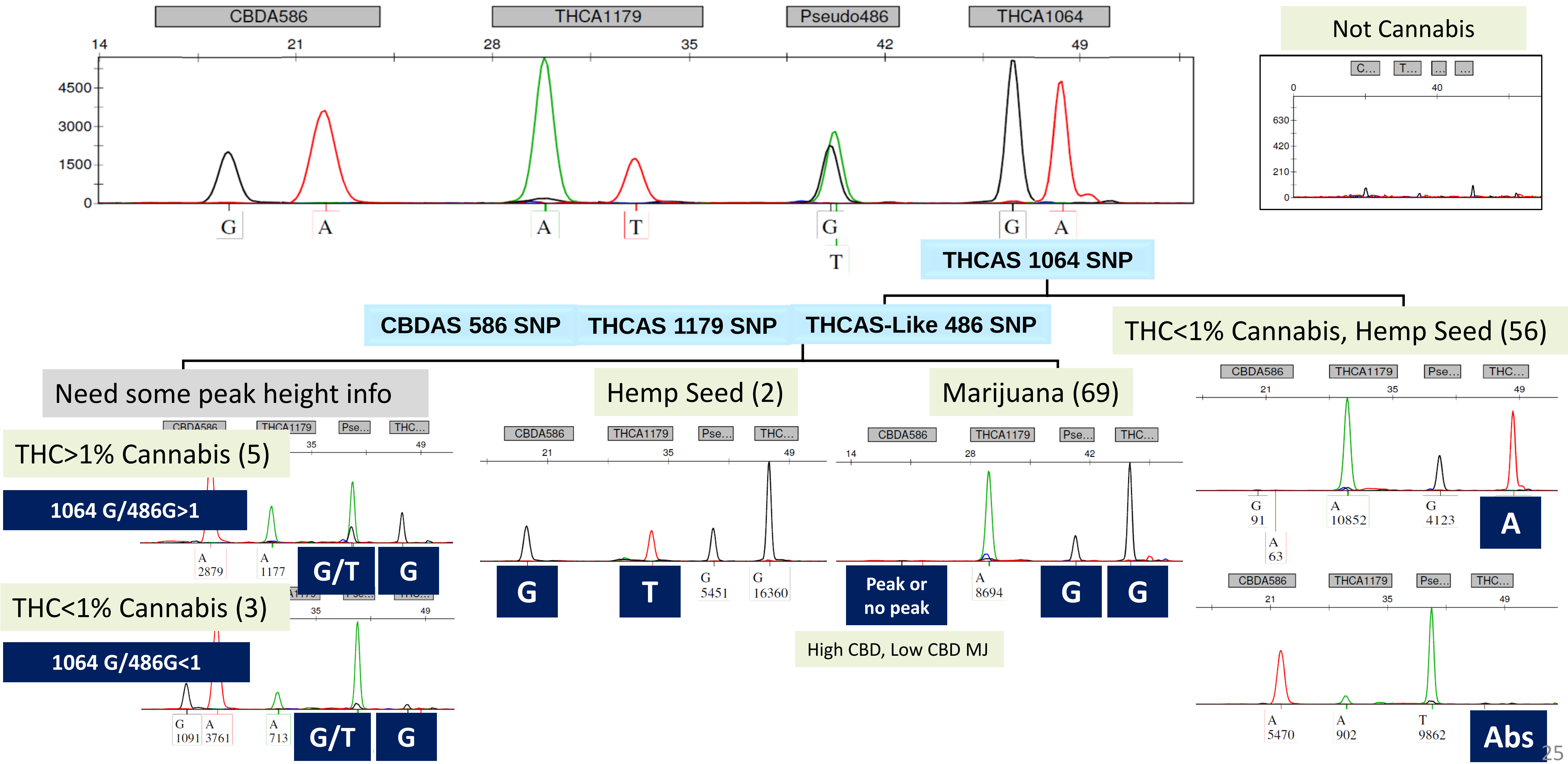
Expectation:
- Separate most MJ form all strains of Hemp
- Might be able to estimate THC conc

Main advantage:
- Faster than SNaPshot, low cost

Limitation:
- Unable to separate MJ from hemp seed
- No CBD information

Limitation of both:
- Unable to separate some low THC (0.3%-1%) marijuana form hemp

SNaPshot Assay (n=138)



Not Cannabis

THCAS 1064 SNP

CBDAS 586 SNP **THCAS 1179 SNP** **THCAS-Like 486 SNP**

THC<1% Cannabis, Hemp Seed (56)

Need some peak height info

THC>1% Cannabis (5)

1064 G/486G>1

G/T **G**

THC<1% Cannabis (3)

1064 G/486G<1

G/T **G**

Hemp Seed (2)

G **T**

Marijuana (69)

Peak or no peak

G **G**

High CBD, Low CBD MJ

A

Abs

Next Step

Tagman qPCR Assay

Next Step-Assay Validation

Blind testing Cannabis sample to validate the application of new genotyping assay

Strain	Number	Source	Chemistry Data
US-Mexico Marijuana	60	CBP LSSD	Not Available
Chile Marijuana	20	PDI	Not Available
Chile Medical Marijuana	4	PDI	Not Available
Commercial Hemp	Need More!	Shops	Hopefully Available
Seized Marijuana	Need More!		Hopefully Avaliable
Reference Sample	Need More!		Hopefully Avaliable
Hemp Seed	Need More!	Shops	Hopefully Avaliable
Other Type of Sample	Needed		Hopefully Available
Total	150-200		

Summary

- Demonstrated the usage of the custom targeted NGS panel that serves as a comprehensive analysis of the three synthase genes and their pseudogenes to discover the relationship between chemotype and gene copies.
- With wider variety of sample types, hemp and marijuana cannot be differentiated look at only THCAS and CBDAS gene.
- Potential comprehensive workflow to differentiate cannabis crop types with not only synthase genes but the pseudogene.
- Develop a rapid SNaPshot assay for the differentiation of hemp and marijuana (validation need in future study).

Acknowledgements

- Center for Advance Research in Forensic Science (CARFS)
- National Institute on Drug Abuse (NIDA) – Drug Supply Program
- National Institute of Standards and Technology (NIST)
- University of Mississippi

CENTER FOR ADVANCED RESEARCH
IN FORENSIC SCIENCE

THE UNIVERSITY of
MISSISSIPPI

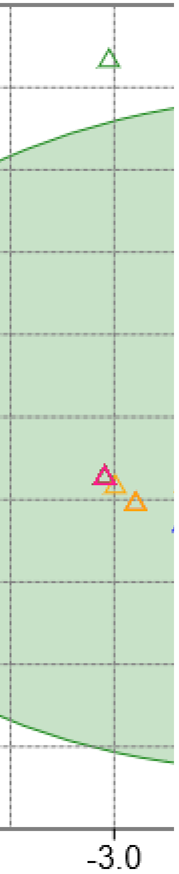
NIST
National Institute of
Standards and Technology

NIDA
NATIONAL INSTITUTE
ON DRUG ABUSE

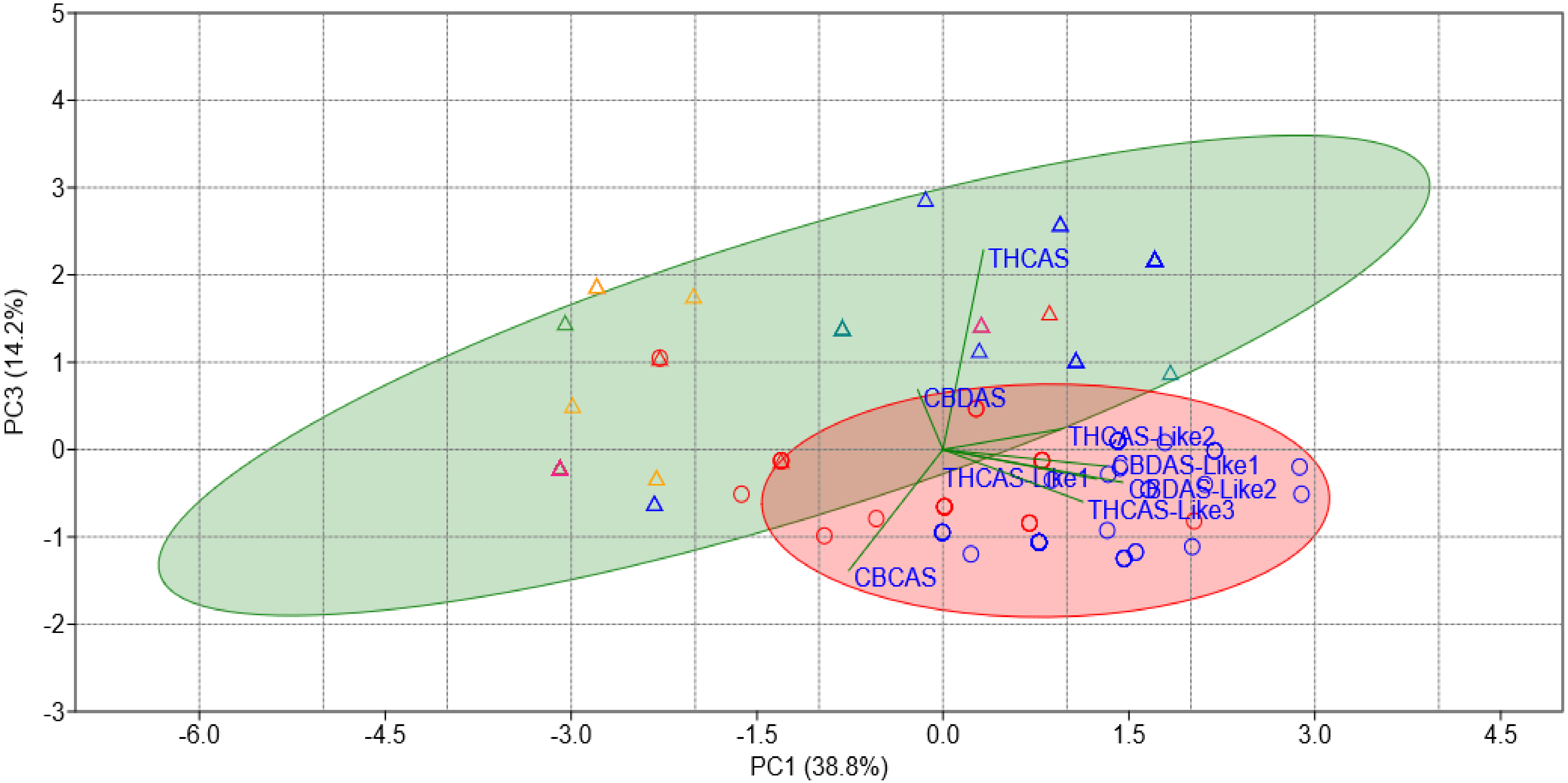
THANK YOU FOR YOUR ATTENTION

Jessica (Ya-Chih) Cheng

yxc059@shsu.edu


-3.0

PC1 (38.8%)


5
4
3
2
1
0
-1
-2
-3
PC3 (14.2%)
-6.0
-4.5
-3.0
-1.5
0.0
1.5
3.0
4.5
PC1 (38.8%)
THCAS
CBDAS
THCAS-Like2
CBDAS-Like1
THCAS-Like1
CBDAS-Like2
THCAS-Like3
CBCAS