

Supplementary Materials

Table S1. Summary of the results from the cohort of 54 cannabis samples. Mean DNA yield is obtained from 54 samples with standard deviations. BLAST results from *THCAS* and *CBDAS* markers.

S/N	DNA yield (ng/mg)	Matched identity to known cannabis reference in GenBank (Blast results)	
		<i>THCAS</i>	<i>CBDAS</i>
1	Mean = 396 ± 266	<i>THCAS</i> (99.9%)	<i>CBDAS</i> -like (99.5%)
2		<i>THCAS</i> (99.9%)	<i>CBDAS</i> -like (98.8%)
3		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.9%)
4		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.3%)
5		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
6		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
7		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.3%)
8		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
9		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (100%)
10		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.1%)
11		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
12		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
13		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.4%)
14		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.2%)
15		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
16		<i>THCAS</i> (99.9%)	<i>CBDAS</i> -like gene (99.6%)
17		<i>THCAS</i> (99.9%)	<i>CBDAS</i> -like gene (98.4%)
18		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.2%)
19		<i>THCAS</i> (99.8%)	<i>CBDAS</i> pseudogene (99.4%)
20		<i>THCAS</i> (99.7%)	<i>CBDAS</i> pseudogene (99.2%)
21		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.9%)
22		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.9%)
23		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.8%)
24		<i>THCAS</i> (100%)	no data (PCR failure)
25		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.4%)
26		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (98.9%)
27		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.2%)
28		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (97.4%)
29		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (100%)
30		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (98.4%)
31		<i>THCAS</i> (99.9%)	<i>CBDAS</i> -like gene (98%)
32		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
33		<i>THCAS</i> (99.9%)	<i>CBDAS</i> -like gene (99.5%)
34		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
35		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
36		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.4%)
37		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
38		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
39		<i>THCAS</i> (99.8%)	<i>CBDAS</i> pseudogene (97.4%)
40		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.4%)
41		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.3%)
42		<i>THCAS</i> (99.9%)	<i>CBDAS</i> pseudogene (99.4%)
43		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.1%)
44		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (98.5%)
45		<i>THCAS</i> (100%)	<i>CBDAS</i> pseudogene (99.2%)

7:3	99.9% <i>Cannabis sativa</i>	99.1% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	99.0% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	99.3% <i>Cannabis sativa</i>
5:5	99.9% <i>Cannabis sativa</i>	98.9% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	98.7% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	99.1% <i>Cannabis sativa</i>
3:7	99.7% <i>Cannabis sativa</i>	99.1% <i>Cannabis sativa</i>	99.9% <i>Cannabis sativa</i>	99.0% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	99.1% <i>Cannabis sativa</i>
1:9	99.9% <i>Cannabis sativa</i>	99.1% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	99.3% <i>Cannabis sativa</i>	99.7% <i>Cannabis sativa</i>	99.2% <i>Cannabis sativa</i>

Table S4. Starting weight, DNA concentration and DNA yield of 27 blinded samples.

Sample	Starting material (mg)	DNA concentration (ng/mg)	DNA Yield (µg)
C1	27.8	56	2.8
C2	57	260	13
C3	52	68	3.4
C4	66	390	19.5
C5	52	310	15.5
C6	48	160	8
C7	58	200	10
C8	53	430	21.5
C9	54	130	6.5
C10	6	39	1.95
C11	30	160	8
C12	20	22.5	1.125
C13	5.2	28	1.4
C14	52	190	9.5
C15	50	403	20.15
C16	4.1	4.71	0.235

C17	1.2	23	1.15
C18	50	330	16.5
C19	50	360	18
C20	50	165	8.25
C21	50	189	9.45
C22	50	102	5.1
C23	5	0.747	0.03735
C24	50	312	15.6
C25	50	236	11.8
C26	50	158	7.9
C27	3.8	0.601	0.03

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Job Title

C26 matK

RID

DWB83H24114

Search expires on 10-03 15:44 pm

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Program

Blast 2 sequences

Citation

Query ID

lcl|Query_2423549 (dna)

Query Descr

C26 matK

Query Length

840

Subject ID

lcl|Query_2423551 (dna)

Subject Descr

JQ588584.1 Turnera diffusa voucher BioBot11480 maturase K (matK) gene, partial cds; chloroplast

Subject Length

772

Other reports

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to

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Sequences producing significant alignments

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
JQ588584.1 Turnera diffusa voucher BioBot11480 maturase K (matK) gene, partial cds; chloroplast		505	505	92%	9e-147	74.64%	772	Query_2423551

JQ588584.1 Turnera diffusa voucher BioBot11480 maturase K (matK) gene, partial cds; chloroplast

Sequence ID: Query_2423551 Length: 772 Number of Matches: 1

Range 1: 6 to 768

Graphics

Next Match

Previous Match

Score	Expect	Identities	Gaps	Strand
505 bits(559)	9e-147	574/769(75%)	6/769(0%)	Plus/Minus
Query 71	TCGATACAAAC	TCGATACCACTATAATGAGAAAGATTCTGTATAT		138
Sbjct 768	TCGATACAAAC	TCGATACCACTATAATGAGAAAGATTCTGTATAT		709
Query 131	ACGGCCAAAGCGCTCAATAATCAGAAATCGGCCAAATCGCCTTACCAAT			190
Sbjct 708	GTGGACAAATCGACGAATAATCAGAAATCGGCCAAATCGCCTTACCAAT			649
Query 191	AGGATGCCCAATGCGTTACAAATTCGATTTAGCCAATGATCCACAGAGGCATAAT			250
Sbjct 648	AGGATGCCCAATGCGTTACAAATTCGATTTAGCCAATGATCCACAGAGGCATAAT			589
Query 251	TGGAACAATACATCAAACTCTTAATAGCATTATCGATTAATAATGATTTCTAGCAT			318
Sbjct 588	TGGAATCCGTATCTAGATTAGCATCAATATATCTATTAACAGGAATTTCTAGCAT			529
Query 311	TTGACCGGTACCATTTGAGGCTTTAGCCGACACTGAACGATAACCCAGAAAGTCAAG			370
Sbjct 528	TTGAATCCGTACTACTGAAAGATTAGTCTAGACTGAAAGATAGCCAAAGATCAAG			469
Query 371	GGATGATTGGATAATTGGTTTATATAAATCTTCCGGTGAAGACACAGGTAAAAAGA			430
Sbjct 468	AGAAATGTTTGAAGAAAGTCCATATGGATCTTTCCGGTGAAGACACACATCAAAATG			489
Query 431	AGATTTCCAGAAATGACAAAGTAATTTTCCACTTATTCATCAAAAGAAACGTCCTTT			490
Sbjct 408	ACATTTGACATAAAATGACAAAGTAATTTTCCACTTATTCATCAAAAGAAACGTCCTTT			349
Query 491	TGAAGCAAGAATTGATTTTCTTGTACCTTAACATAATGATGAAGAAATCTTTGAATAA			550
Sbjct 348	TGAAGCCAGAATGGATTTTCTTGTATCGAATAATGATGTCAAAAATCTTTAAATAA			289
Query 551	CCAAAAATTCGCTTGAAGAGCCCTGGCAAGACTTTTGAAGATGCTCTATTTTCCATA			610
Sbjct 288	ACATAGGGTAGCCCCCAATCATATGACTATGCTGCAAAATGGTCTCTTTTCCATA			229
Query 611	GAATATATCGTTCAATAGGGCTCCAGAAATGTTGATTGTAAGTGAGAGATTGCTT			670
Sbjct 228	GAAGATATTTCCTCAAGAAAGACCCGATAATTAATTAATCGTAATGAGAGATTGCTT			169
Query 671	ACGGAGAAATAGGAAGCCAGATTCATATTCACATGCATAAGAAGTATATAGGAAGAAAA			730
Sbjct 168	ACGAATAAAACGAAGATGGATTCATATTCATATACATAAGAGTTATATAGGAATAGAA			109
Query 731	TAGTCTGTGATTGATTTTGAAGAAAGAAAGTGGCTTCTTTGAAATGAGATAAAG			790
Sbjct 108	TAATTTGGGTTCCCTTTTGAAGAAAGAAAGTGGCTTCTTTGAAATGAGATAAAG			55
Query 791	ACTATCCCAATTATGACATCATGGAGAAAGATCTTAATAATGCAAA	839		
Sbjct 54	CGGATTCGAATTCACACGCATGAAGAAAGATCGTAATAATGCAAA	6		

Figure S1. Sequence alignment of C26 *matK* sequence in comparison with *Turnera diffusa* *matK* sequence.

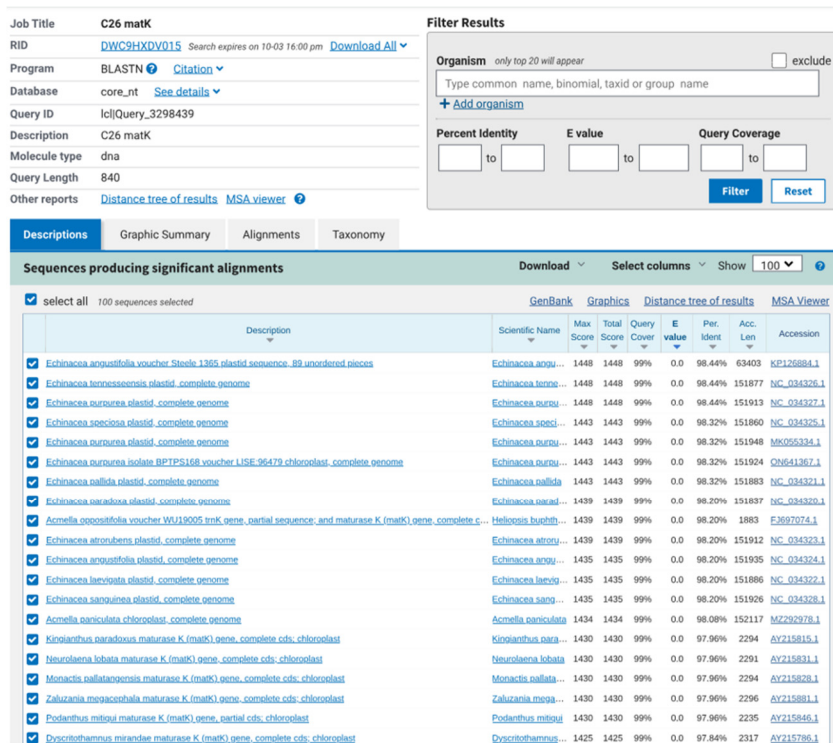


Figure S2. BLAST result and sequence alignment of C26 *matK* sequence in comparison with *Echinacea angustifolia* *matK* sequence.

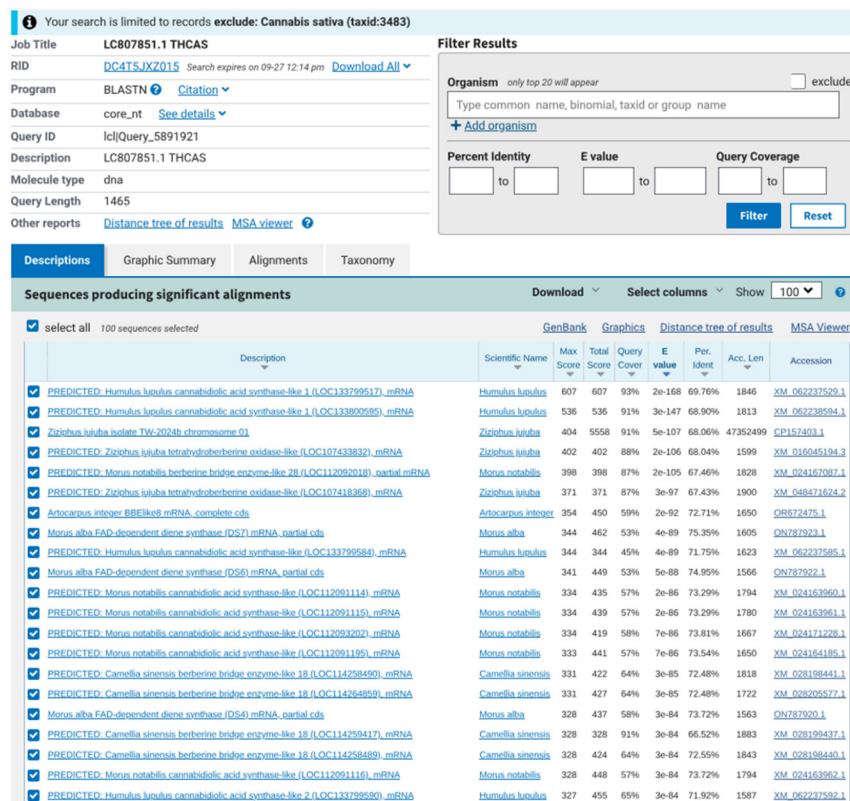
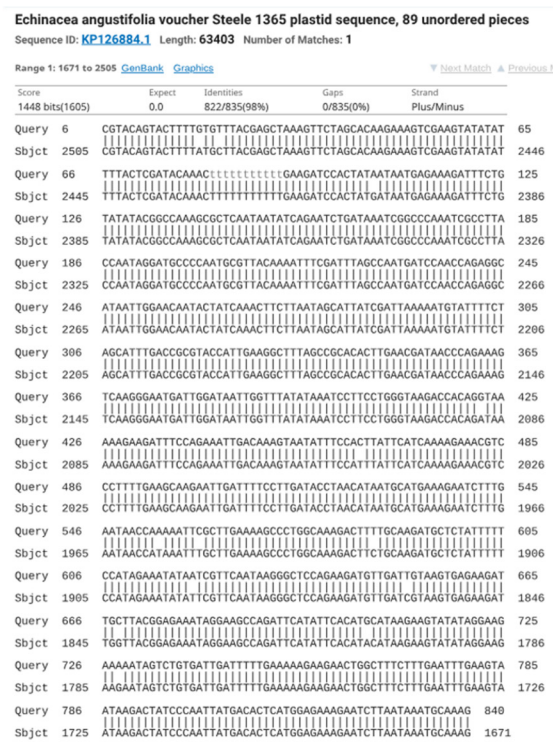


Figure S3. BLAST search of *Cannabis sativa* tetrahydrocannabinolic acid synthase (*THCAS*) sequence in Genbank excluding all DNA sequences relating to *Cannabis sativa*.



Your search is limited to records **exclude: Cannabis sativa (taxid:3483)**

Job Title **LC807867.1 CBDAS**
RID **DCSM7M88015** Search expires on 09-27 12:28 pm [Download All](#) ▼
Program **BLASTN** [Citation](#) ▼
Database **core_nt** [See details](#) ▼
Query ID **lcl|Query_6352057**
Description **LC807867.1 CBDAS**
Molecule type **dna**
Query Length **1485**
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Percent Identity to E value to Query Coverage to
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Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments

☒ select all 100 sequences selected

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	PREDICTED: Humulus lupulus cannabinolic acid synthase-like 1 (LOC133799517). mRNA	Humulus lupulus	603	603	97%	3e-167	69.36%	1846	XM_062237529.1
☒	PREDICTED: Humulus lupulus cannabinolic acid synthase-like 1 (LOC133800595). mRNA	Humulus lupulus	581	581	96%	3e-160	69.33%	1813	XM_062238594.1
☒	PREDICTED: Monus notabilis berberine bridge enzyme-like 28 (LOC112092018). partial mRNA	Monus notabilis	378	378	95%	2e-99	66.74%	1828	XM_024167087.1
☒	PREDICTED: Monus notabilis tetrahydrocannabinolic acid synthase-like (LOC112090781). partial mRNA	Monus notabilis	373	373	92%	8e-98	66.74%	1605	XM_024162862.1
☒	Artocarpus integer BBElike1 mRNA, complete cds	Artocarpus integer	370	512	56%	3e-97	73.67%	1650	OR672475.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like 1 (LOC112092227). mRNA	Monus notabilis	360	360	84%	5e-94	67.32%	1677	XM_061467777.1
☒	Zizophus juluba isolate TW-2024b chromosome 01	Zizophus juluba	359	5152	97%	2e-93	66.32%	47352499	CP157403.1
☒	PREDICTED: Zizophus juluba tetrahydroberberine oxidase-like (LOC107418368). mRNA	Zizophus juluba	359	359	97%	2e-93	66.32%	1900	XM_048471624.2
☒	Monus alba BBElike1 mRNA, complete cds	Monus alba	356	356	89%	6e-93	67.16%	1644	OR672471.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like (LOC112091114). mRNA	Monus notabilis	350	350	84%	9e-91	66.85%	1794	XM_024163960.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like (LOC112091115). mRNA	Monus notabilis	345	345	84%	1e-89	66.77%	1780	XM_024163961.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like 2 (LOC133799500). mRNA	Humulus lupulus	345	345	89%	1e-89	66.35%	1587	XM_062237592.1
☒	PREDICTED: Humulus lupulus tetrahydroberberine oxidase-like (LOC133801349). mRNA	Humulus lupulus	341	341	97%	5e-88	66.17%	2408	XM_062239532.1
☒	Monus alba FAD-dependent diene synthase (DS5). mRNA, partial cds	Monus alba	340	340	89%	5e-88	66.99%	1575	ON787921.1
☒	Monus alba FAD-dependent diene synthase (DS1). mRNA, partial cds	Monus alba	337	337	83%	6e-87	66.46%	1554	ON787917.1
☒	Monus alba FAD-dependent diene synthase (DS7). mRNA, partial cds	Monus alba	337	465	49%	6e-87	74.66%	1605	ON787923.1
☒	Monus alba FAD-dependent diene synthase (DS6). mRNA, partial cds	Monus alba	337	461	57%	6e-87	74.66%	1566	ON787922.1
☒	PREDICTED: Humulus lupulus cannabinolic acid synthase-like (LOC133799584). mRNA	Humulus lupulus	334	334	96%	7e-86	66.19%	1623	XM_062237585.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like (LOC112092387). mRNA	Monus notabilis	329	329	89%	9e-85	66.25%	1688	XM_024168490.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like (LOC112091207). mRNA	Monus notabilis	325	325	83%	1e-83	66.45%	1667	XM_024171228.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like (LOC112090895). mRNA	Monus notabilis	325	449	63%	1e-83	72.00%	1536	XM_024163213.1
☒	PREDICTED: Monus notabilis cannabinolic acid synthase-like (LOC112091116). mRNA	Monus notabilis	324	458	62%	4e-83	73.62%	1794	XM_024163962.1

Figure S4. BLAST search of *Cannabis sativa* cannabinolic acid synthase (CBDAS) sequence excluding all DNA sequences related to *Cannabis sativa*.

PREDICTED: Humulus lupulus cannabinolic acid synthase-like 1 (LOC133799517). mRNA

Sequence ID: [XM_062237529.1](#) Length: 1846 Number of Matches: 1

Range 1: 134 to 1576 [GenBank](#) [Graphics](#) [▼ Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand	
603 bits(668)	3e-167	1021/1472(69%)	64/1472(4%)	Plus/Plus	
Query 49	TCGTATACACTCAAAACACCCATGTATATGCTGCTCAATTCGACAAATACACAATC	188			
Sbjct 134	TCATATACACTCGAAAGCACTCGTGTTCCTGCTGTCGACGCTCCAACTACAAAC	193			
Query 109	TTAGATTCACCTTGACACACCCCAAAACCACTGTTATGCTACTCTCCACATGTCT	168			
Sbjct 194	CTCGATTCTCTTCCTCGCTGATTCAAAACCCCTGGTATCGTTACACTTCAAAGTCT	253			
Query 169	CTCATCCCAAGGCACTATTCTA-TGCTCCAAAGAGTTGGCTGCAGATTGCGAATCGA	227			
Sbjct 254	CCCATGTCGAAGCCTGCTG-CTACTGCTCGCAGAACATGGCTTGCAATTTCGACACGA	312			
Query 228	AGTGGTGCTGATGATTCGAGGCGATGCTCCACATATTCTCAAGTCCCATTTGTTAGTA	287			
Sbjct 313	ACGCGTGGCCATGATTTGAGGCTCTCTTACGCTGCCAAGTCCCATTTGCTCGTAATA	372			
Query 288	GACCTGAGAAACATCGGTTCAATCAAAATAGATGTTCTATAGCAAACTGCATGGGTTGAA	347			
Sbjct 373	CACTTGAGAAATCAATTCGATGATGTTGAGCTTGACGCGAAGAAACATGCTATGGGTTGA	432			
Query 348	GCCGAGCTACCTTGAGAAAGTTTATATTGGGTTATGAGAAAAATGAGATCTTAGT	497			
Sbjct 433	GCCGAGCTACCTTGAGAAAGTTTATATTGGGTTATGAGAAAAATGAGATCTTAGT	492			
Query 408	TTGCGCGTGGGATTTGCCCTACTGTTTGGCGAGGTGGACATTTGGTGGAGGAGCTAT	467			
Sbjct 493	TTTCTCTGGATTTTGGCCACGAGTGGGCTGGTGGCTGATTTAGTGGCGGTGGCTAT	552			
Query 468	GGACCATTTGAGAGAACTATGGCTCGGGCTGATATATCATTTGATGCACACTTGGCT	527			
Sbjct 553	GGACCTTTGGTGGAAATATGGCTTGCGACGTGATATCATTTGACGCTTATGATGCT	612			
Query 528	AACGTTTCATGAGAAAGTCTAGATCGAAATCATGGGGGAGATCTCTTTGGGCTTTA	587			
Sbjct 613	AATGTTGATGGGAAATTCCTTGACAGAGATCTATGGGGGAGATTTGTTCTGGGCCATA	672			
Query 588	CGTGGTGGGAGCAGAAAGTCTGGGAATCATTTAGCATGAGAAATAGACTGGTTGCT	647			
Sbjct 673	AGCTTTGGTGGAGCAAGTCTGCAATCATCTGTCAGACAGTTAGATTAAGGTTTCGCG	732			
Query 648	GTCC-CAAAGTCTATGTTAGTGTAAAGAGATCATGAGATACATGAGCTGTGCT	784			
Sbjct 733	GTCCTCAACGCTGACATCATCTGGTTGTTAATAGGACTTGACCAAGATGAGCAATG	792			
Query 705	AAGTAACTTAAACAAAGAAATATGCTTACAGATGACAAAGATTTATCTACTATG	824			
Sbjct 793	AGCTTTGGTGGAGCAAGTCTGCAATCATCTGTCAGACAGTTAGATTAAGGTTTCGCG	852			
Query 765	ACTCATTCTACATAGCAAGATACAGATATCAAGGAGAAATGAAGACAGCAATAC	893			
Sbjct 853	CTTAGTTCTACATGCTGATGATCTACAG-----AAG---ATAAAGATTAATATCCAA	924			
Query 825	ACTTACTCTCTTCAGTTTTCCTTGGTGGAGTGGATGCTAGTGCATGTTGATGAACAG	884			
Sbjct 904	GCTCAATTCATGCTCTGTTCTTCTTGGTGGAGTGAATGATCTCTCCATTTGATGGAAAG	963			
Query 885	AGTTTTCGATTTGGGATTTAAAAAACGAGATTCGACAGAAATGAGCTGGATTTGATAT	944			
Sbjct 964	AGTTTTCGATTTGGGATTTAAAAAACGAGATTCGATGATGATGATGATGATGATGAT	1003			
Query 945	ATCATCTCTATGAGTGGTGTGTAATATGACACCTGATGAATTTAAACAGGAAATTTTG	1024			
Sbjct 1024	GT-TCTTATTTGGCGGATTCCTCAAT---GGAGCTG-----AGATGGAAATTTTG	1071			
Query 1085	CTTATGATAGAT-----CGCTGGGCAAGAGCTGCT--TTCAAGATTAAATGATACGTT	1058			
Sbjct 1072	CTCAATGAACTCAACAATG-----CTTTCTTCAATTAAGGAAAGCTTGACTACTG	1125			

Figure S5. BLAST search of *Cannabis sativa* cannabidiolic acid synthase (CBDAS) pseudogene sequence excluding all DNA sequences relating to *Cannabis sativa*.

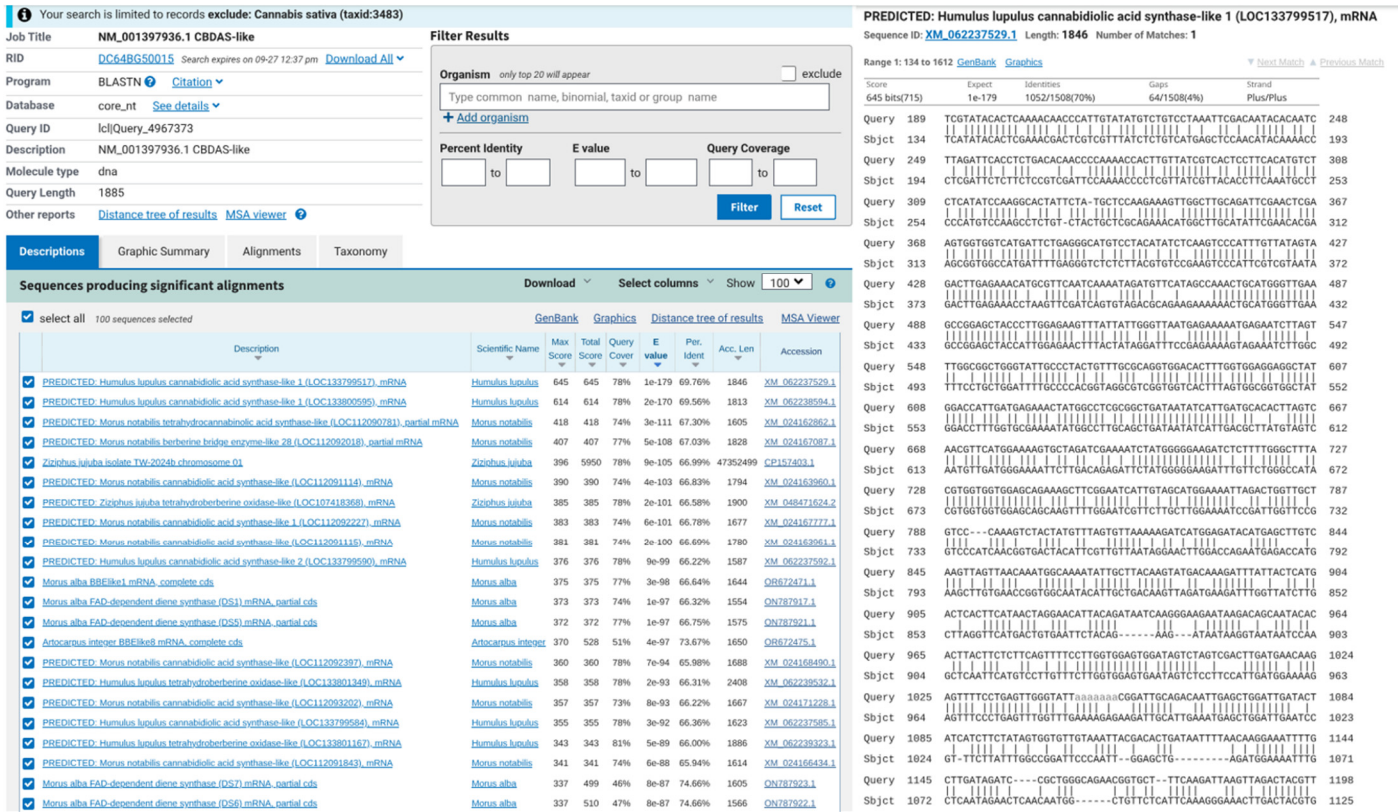


Figure S6. BLAST search of *Cannabis sativa* cannabidiolic acid synthase (CBDAS)-like gene sequence excluding all DNA sequences relating to *Cannabis sativa*.