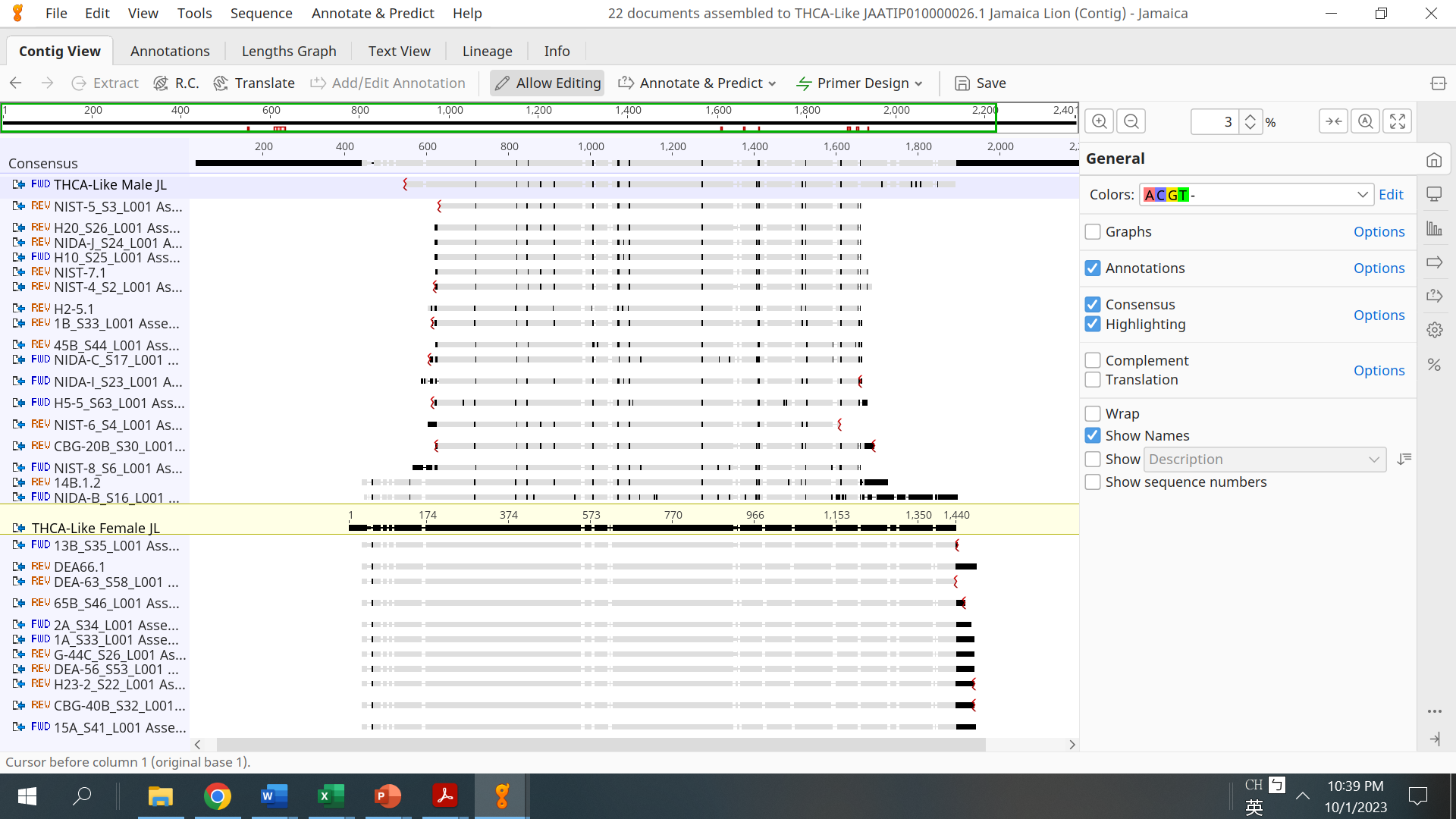
**Figure S1.** Alignment of NGS sequences and the Jamaican Lion THCAS-Like sequence from male parent (reference 1, GCA\_013030025.1) and female parent (reference 2, GCA\_012923435.1). Color boxes on the reference 2 sequence represent the primer position, purple and pink boxes were the primer designed by Cheng et al. (cite our paper), and blue boxes were the primer designed in this study



THCAS-Like sequences from NGS study   
(Cheng et al. 2024)

THCAS-Like sequences from NGS study   
(Cheng et al. 2024)

THCA+Like R

Everyone F

Reference 2

Other R primers

THCAS-Like R3

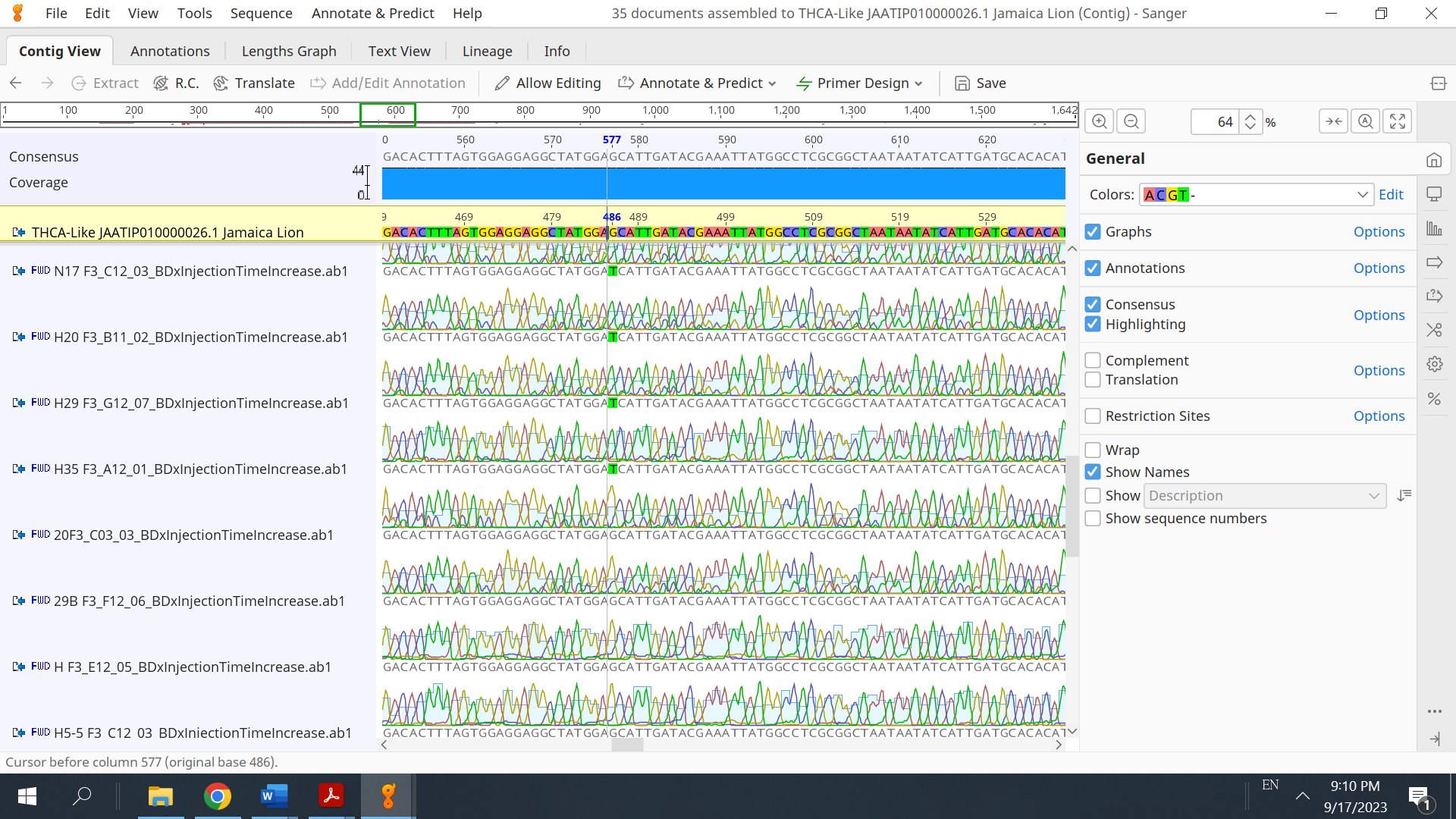
THCAS-Like R2

THCAS F4

CBDAS Like F3

Reference 1

**Figure S2.** Example of the SNP at position 486 on the THCAS-Like sequence separated sample with two variants (GCA\_012923435.1 and GCA\_013030025.1) of the THCAS-Like gene from cultivar JAATIP010000026.1



Hemp Seed

MJ

MJ

MJ

MJ THC<1%

Hemp

Hemp

Hemp

Sample with male variant (GCA\_013030025.1)

Sample with female variant (GCA\_012923435.1)

Reference Sequence (GCA\_012923435.1)

**Figure S3.** Examples of each genotype and their corresponding profile and interpretations

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Profile Example** | **Interpretation** |
| 1 |  | THCA 1064→A  **Hemp, Hemp Seed, or Cannabis with THC<1%** |
| 2 |  | THCA 1064→Absent  **Hemp, Hemp Seed, or Cannabis with THC<1%** |
| 3 |  | 1. THCA 1064→G 2. Pseudo486→G  3. CBDA586/THCA1179 →not G/T  4. CBDA586→Absent  **Low CBD Marijuana** |
| 3 |  | 1. THCA 1064→G 2. Pseudo486→G  3. CBDA586/THCA1179 →not G/T  4. CBDA586→Present  **High CBD Marijuana** |
| 4 |  | 1. THCA 1064→G 2. Pseudo486→G  3. CBDA586/THCA1179 →G/T  **Hemp Seed** |
| 5 |  | 1. THCA 1064→G 2. Pseudo486→G  3. 1064 G/486G>1  **Marijuana** |
| 5 |  | 1. THCA 1064→G 2. Pseudo486→G  3. 1064 G/486G<1  **Cannabis with THC<1%** |
| N/A |  | No peak  **Not Cannabis** |