

Supplemental information

Top-down Mass Spectrometry Analysis of Membrane-bound Light-Harvesting Complex 2 from *Rhodobacter sphaeroides*

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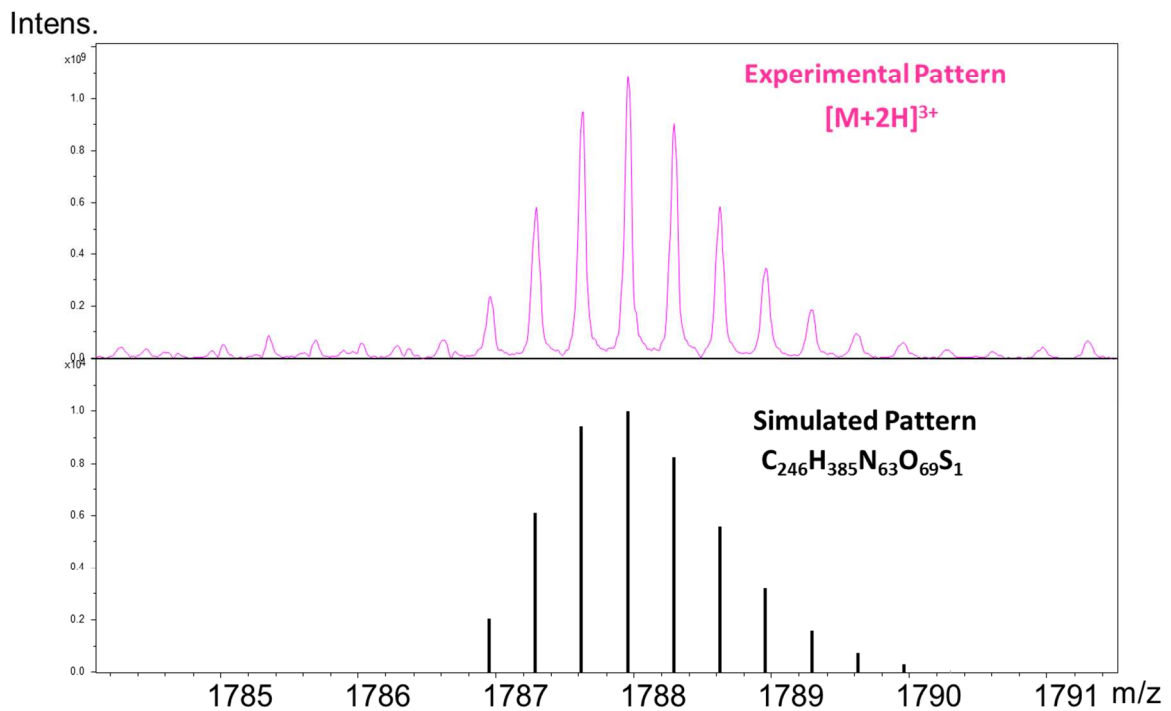
Material and methods

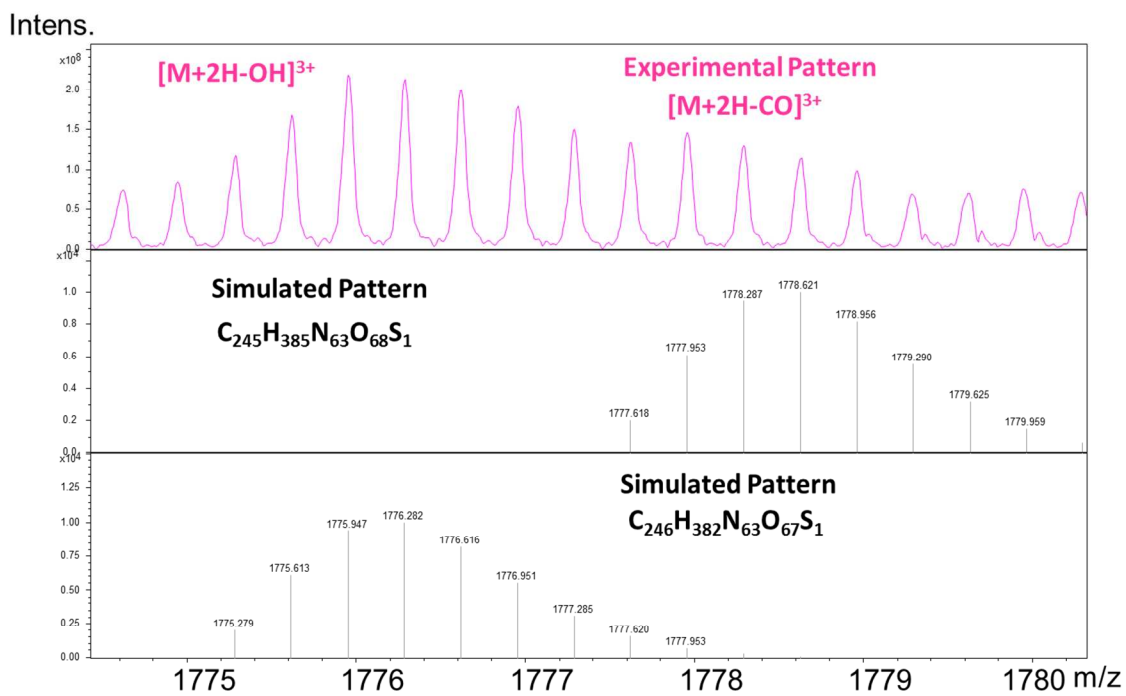
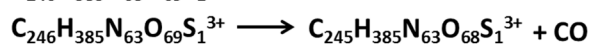
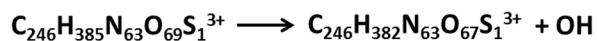
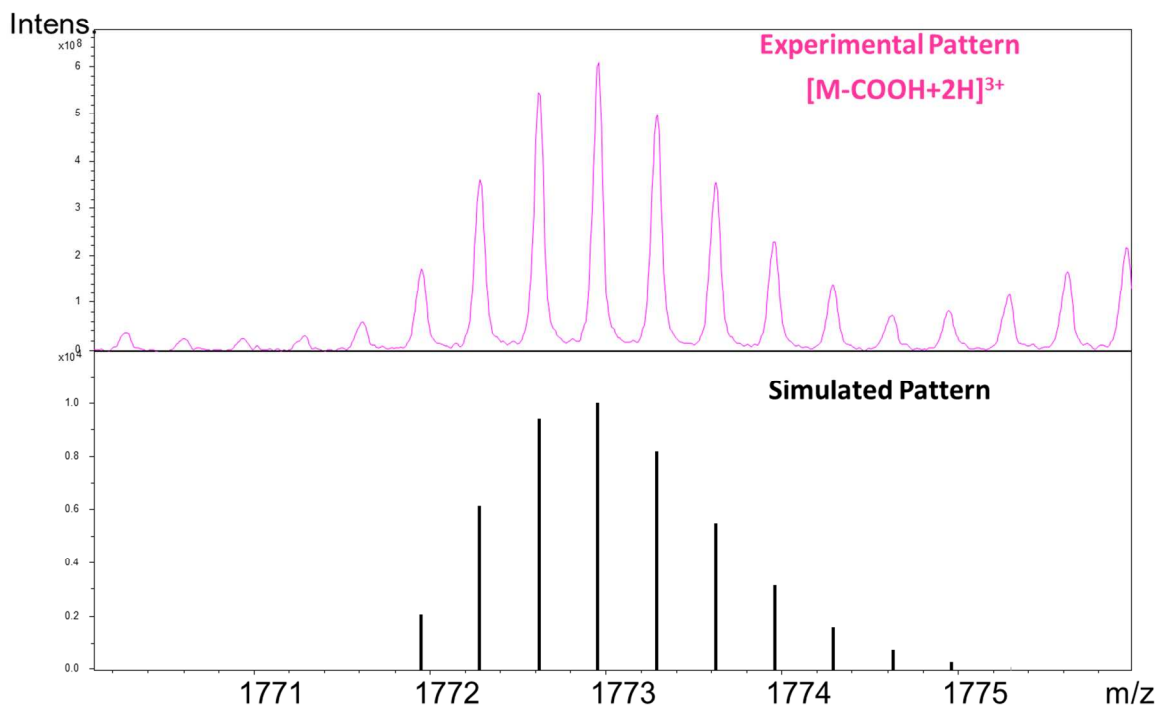
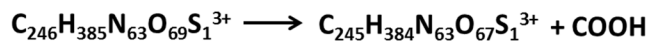
Bottom-up LC-MS analysis of LH2

The LH2 precipitates were dissolved in 60% methanol in 100mM Tris 10mM CaCl₂ buffer (pH = 8.0). Chymotrypsin (Promega Corporation, Madison, WI) was added to the solution in a 1:100 enzyme to protein ratio. The digestion was carried out at 25°C for 3h. 1% TFA was added to quench the reaction and sep pak C18 (Waters Inco., Milford, MA) was used to desalt the sample before MS analysis. The peptides mixtures were trapped by a guard column (Acclaim PepMap100, 100 μm × 2 cm, C18, 5 μm, 100 Å; Thermo Fisher Scientific, Breda, Netherlands) and then fractionated on a home-packed Magic C 18 reverse phase column. The MS analysis was acquired on Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer. (Thermo Fisher Scientific, Breda, Netherlands)

Puc1a	MNQGKIWTVWDPVAVGIPLLLGSVAVTALLVHLAILQNTTWFPAFMQGG-----LKK
Puc2a	MNNSKMWLTVNPNLGVPLLLGSVAVASLVHGVAVLTTTPWIANYYQGSEWPVAAAPEE
Puc1a	AAAIVQVVG-----
Puc2a	AAAPVEAAAPADEAAAPVEEAAPVAEAAAPAEAAAPAAEAAVPAAEAAAPAAEAAAPAEAA
Puc1a	-----
Puc2a	APAAEAAAPAEAAAPAAEAAAPVEEAAPAAEAAAPAEAAAPVAEAAAPAEAAAPVAEPAA
Puc1a	-----
Puc2a	EPAPAAEAAAPVAEVSAPAAELAAPVAMSLVDIAAKLNGLGYSVQSVTKTEGGYVVMNTD
Puc1a	-----
Puc2a	ANGMPVAATLDPVTGLPFVPAAQ

Figure.S1 Sequence alignment of Puc1A- and Puc2B-encoded polypeptides





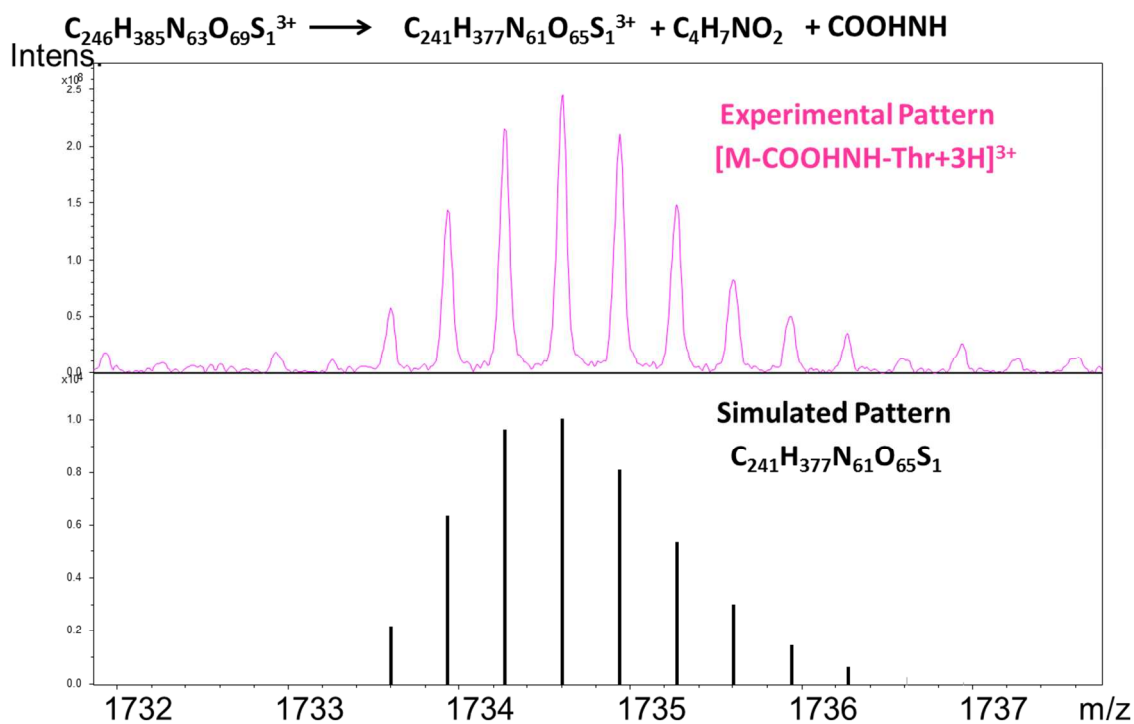
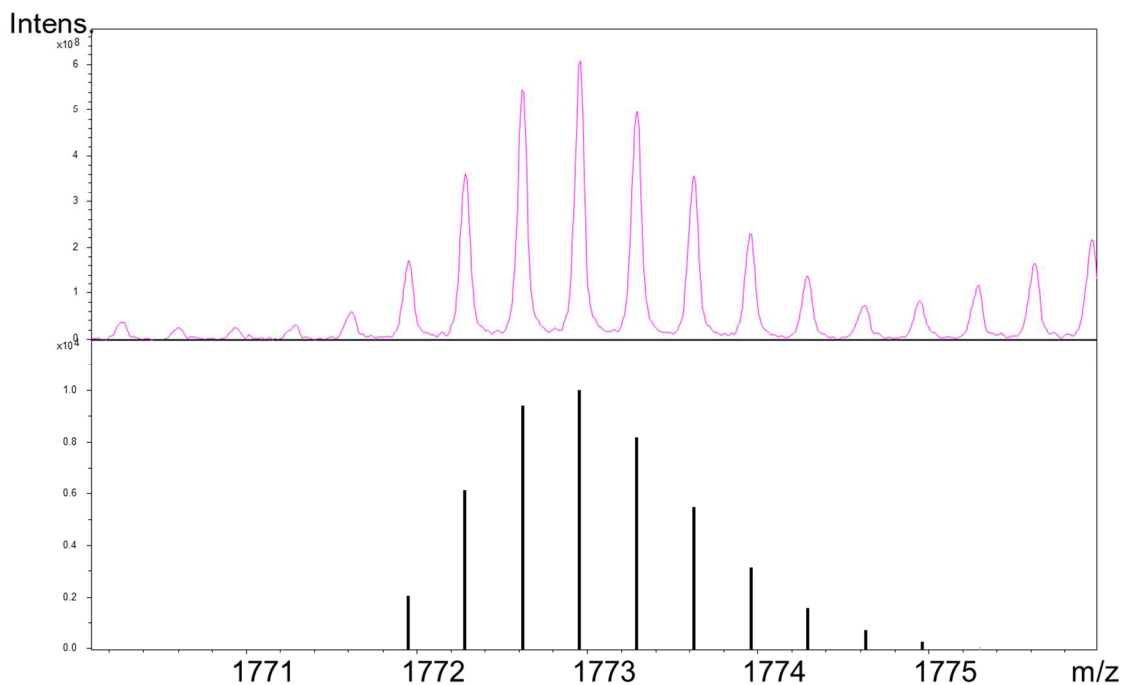
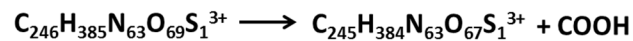


Figure.S2 Comparison of simulated and experimental isotopic pattern of parents and fragment ions on N-terminus of Puc2B-encoded polypeptide

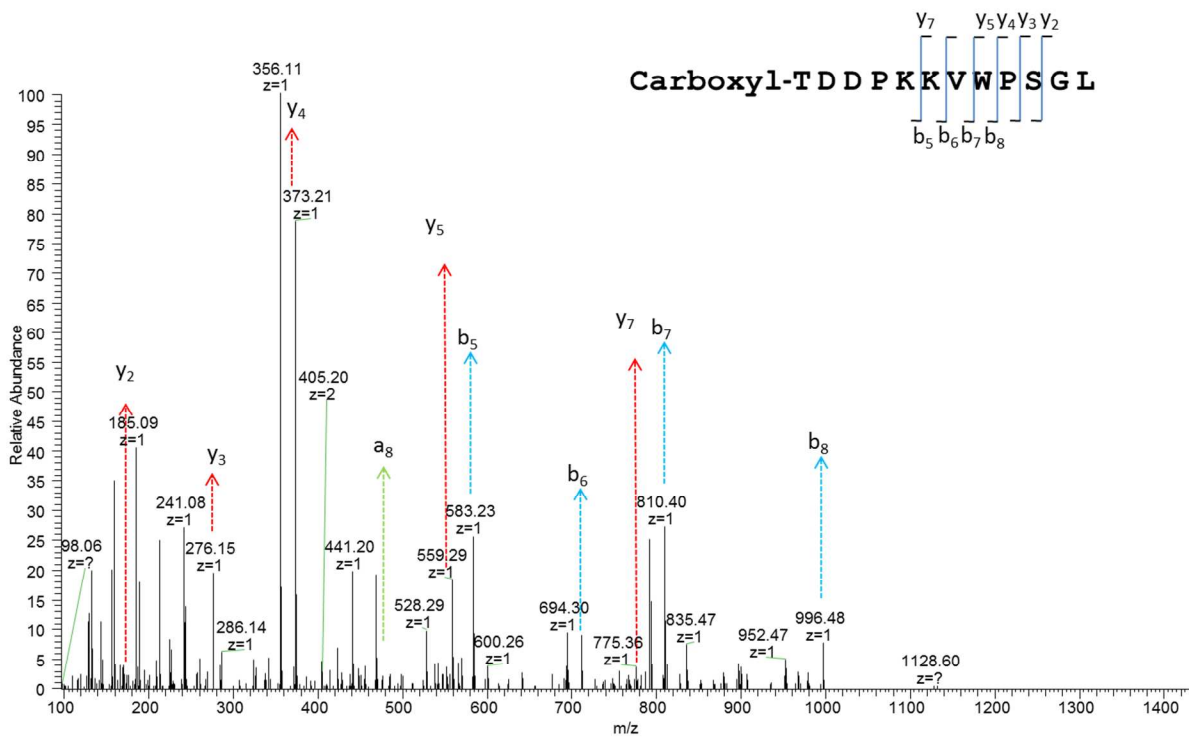
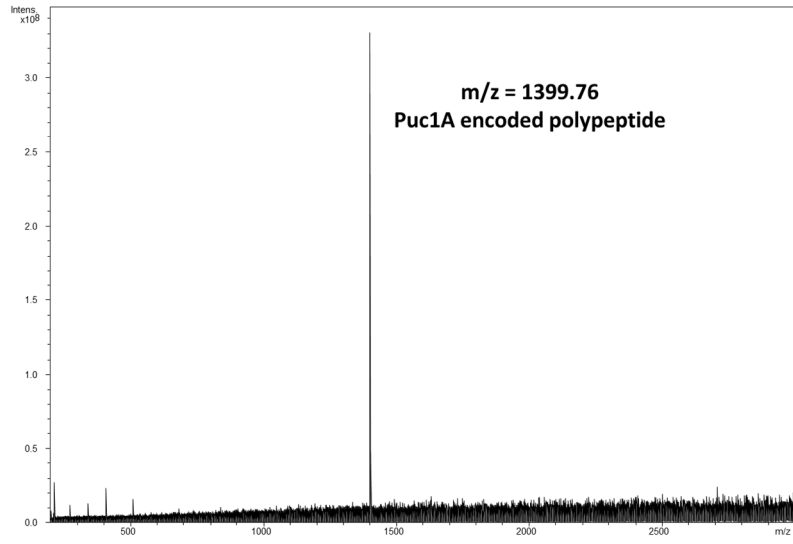
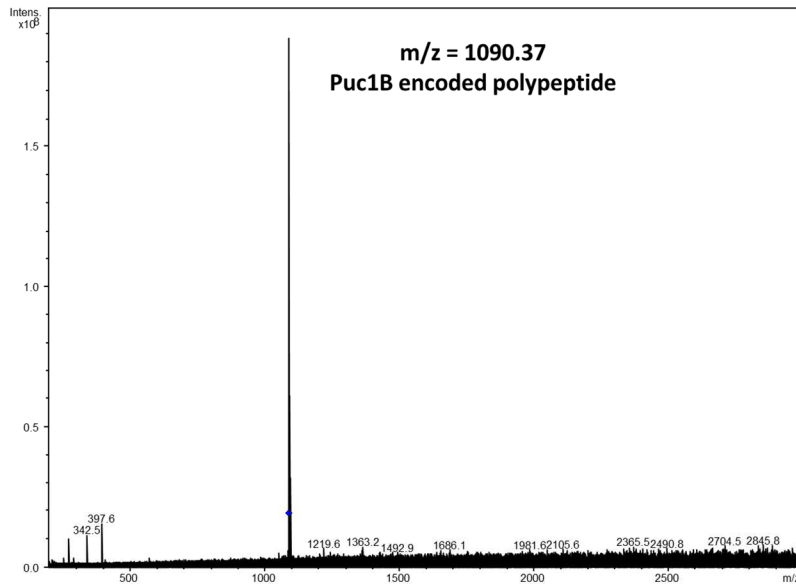


Figure.S3 MS/MS fragmentation of N terminus peptide from Puc2B-encoded subunit

(A)



(B)



(C)

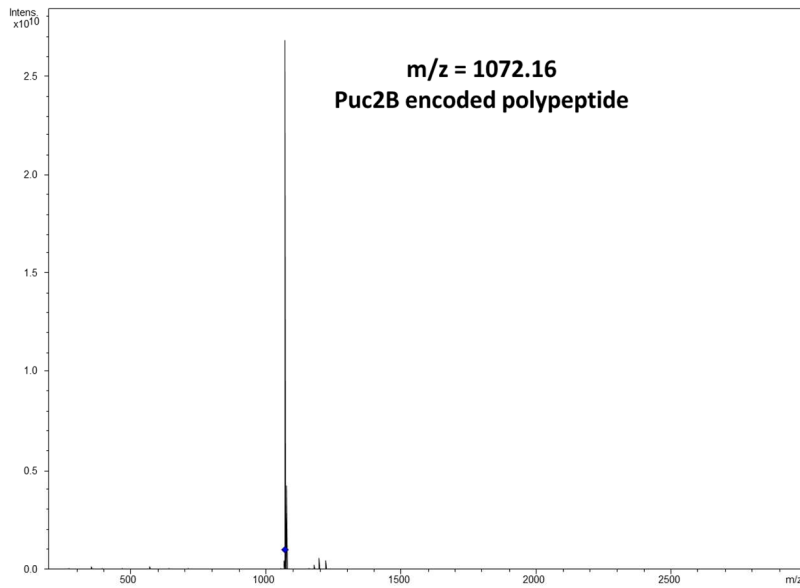
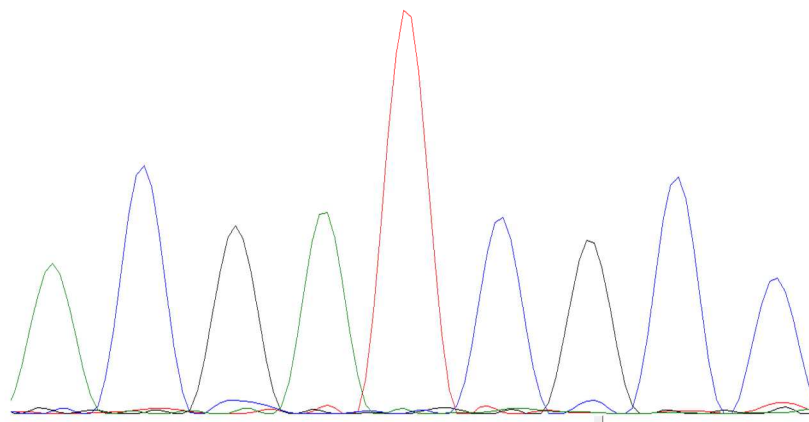


Figure.S4 Isolation spectrums of (a) Puc1A- (b) Puc1B- (c)Puc2B- encoded polypeptides

(A)

```
gtgaccgatgatccgaaaaaggctctggccgagcggcctgacgatcgccgaagcggaagag
V T D D P K K V W P S G L T I A E A E E
gtgcataagcagctcatcctgggcacgcgcgtgttcggcggcatggccctgatcgcgat
V H K Q L I L G T R V F G G M A L I A H
ttcctggcggcggcggcgcacgccgtggctcggctga
F L A A A A T P W L G -
```

Below the sequence, a horizontal line indicates the positions of the amino acids corresponding to the highlighted sequence: A, C, G, A, T, C, G, C, 300, C.



(B)

```
gtgaccgatgatccgaaaaaggtctggccgagcggcctgacgatcgccgaagcggaagag
V T D D P K K V W P S G L T I A E A E E
gtgcataagcagctcatcctgggcacgcgcgtgttcggcggcatggccctgatcgcgcat
V H K Q L I L G T R V F G G M A L I A H
ttcctggcggcggcggcgacgcctggctcggctga
F L A A A A T P W L G -
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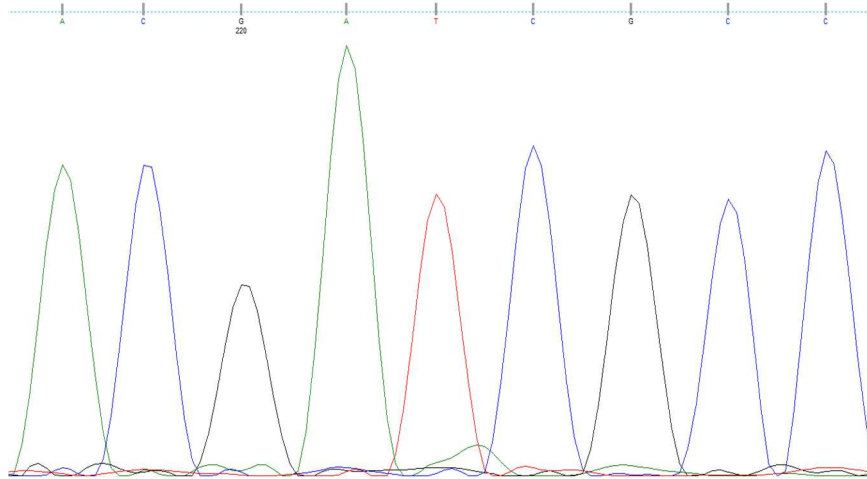


Figure. S5 DNA (a) and RNA (b) *puc2B* operon sequencing result and trace files around 14th valine region.