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Support Information

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4 An effective hybrid strategy for converting rice straw to furoic acid

5 by tandem catalysis via Sn-sepiolite combined with recombinant *E.*

coli whole cells harboring horse liver alcohol dehydrogenase

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1 Experimental section

2 Analytical methods

3 In-gel reduction, alkylation and digestion of HLADH proteins, extraction and
4 desalting of peptides, Mass Spectrum (MS) detection, and database searching were
5 carried out as below procedures:

6 (1) In-gel reduction, alkylation and digestion of proteins

7 (a) Cut excised bands (spots) from SDS-PAGE into cubes (ca. 1.0×1.0 mm), and
8 transfer them into a microcentrifuge tube;
9 (b) Rinse the gel cubes with water for 15 min, then remove water;
10 (c) Add 500 mL acetonitrile and incubate tubes for 15 min until gel pieces shrink,
11 then remove acetonitrile and speed-vacuum for total dryness;
12 (d) Add 100 μ L of the dithiothreitol (DTT) (10 mM DTT/50 mM NH₄HCO₃) solution
13 to completely cover gel pieces. Incubate for 30 min at 56 °C, then remove the
14 solution;
15 (e) Add 100 μ L of the iodoacetamide solution. Incubate for 30 min at room
16 temperature in the dark, then remove the solution;
17 (f) Shrink gel pieces with acetonitrile and remove all liquid, and speed-vacuum for
18 total dryness;
19 (g) Add 50 μ L trypsin buffer (0.025 μ g/ μ L trypsin/10 mM NH₄HCO₃) to cover the
20 dry gel pieces and leave it in an ice bucket for 1 h;
21 (h) Add 100 μ L of 10 mM ammonium bicarbonate buffer to cover the gel pieces and
22 keep them wet during enzymatic cleavage;
23 (i) Place tubes with gel pieces into an air circulation thermostat and incubate samples
24 overnight at 37 °C.

25 (2) Extraction and desalting of peptides

26 (a) Add 150 μ L extraction solution (60% acetonitrile/5% formic acid) to the tube and
27 incubate for 15 min at 30 °C in a shaker;

1 (b) Withdraw the supernatant into a new tube, and add additional 100 µL extraction
2 solution (60% acetonitrile/5% formic acid) to the gel pieces for further extraction,
3 then combine the extraction solution, and dry down in a vacuum centrifuge.

4 (c) Use StageTip C18 to desalt the extracted peptides.

5 **(3) Mass spectrum (MS) detection**

6 RPLC-ESI-MS/MS was used to determine the sample. LC-MS/MS data acquisition
7 was conducted on a Q Exactive HF-X mass spectrometer coupled with an Easy-nLC
8 1000 system (both Thermo Scientific). Peptides were first loaded onto a C18 trap
9 column and then eluted into a Acclaim PepMap C18 column (75 µm × 250 mm, 2 µm
10 particle size, 100 Å pore size, Thermo). Mobile phase A (0.1% formic acid) and
11 mobile phase B (80% acetonitrile, 0.1% formic acid) were used to establish a 30 min
12 gradient, which comprised of: 0 min in 8% mobile phase B, 20 min of 8-25% mobile
13 phase B, 4.5 min of 25-45% mobile phase B, 0.5 min of 45-90% mobile phase B, 90%
14 mobile phase B for 5 min. A constant flow rate was set at 300 nL/min. For DDA
15 mode analysis, each scan cycle consisted of one full-scan mass spectrum ($R = 120\text{ K}$,
16 AGC = 3e6, max IT = 50 ms, scan range = 350-1800 m/z) followed by 20 MS/MS
17 events ($R = 60\text{ K}$, AGC = 2e5, max IT = 110 ms). HCD collision energy was set to
18 32. Isolation window for precursor selection was set to 1.2 Da. Former target ion
19 exclusion was set for 40 s.

20 **(4) Database searching**

21 MS Raw data were assayed with Proteome Discoverer software. Data were searched
22 against the UniProtEcoli and horseprotein database using the following parameters:
23 Variable modifications, Oxidation (M) & Acetyl (Protein N-term) & Deamidated (N,
24 Q); Fixed modifications, Carbamidomethyl (C); Digestion, Trypsin/P.

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2 **Figure Captions**

3

4 **Fig. S1.** Time courses for the biological conversion of commercial FAL (25 mM)
5 with *E. coli* HL, IS, KG, and TS.

6

7 **Fig. S2.** Time courses for the biological conversion of commercial FAL (10-150 mM)
8 with *E. coli* TS cells.

9

10 **Fig. S3.** Scheme for the biotransformation of FAL into FOL and FA.

11

12 **Fig. S4.** Time courses for the biological conversion of FOL (10-150 mM) with *E. coli*
13 TS cells.

14

15 **Fig. S5.** HPLC image of FA liquor (a); HPLC image of FOL and FA (b); ¹H NMR of
16 isolated the product FA (c).

17

18 **Fig. S6.** Results of HPLC/MS for assaying the target proteins.

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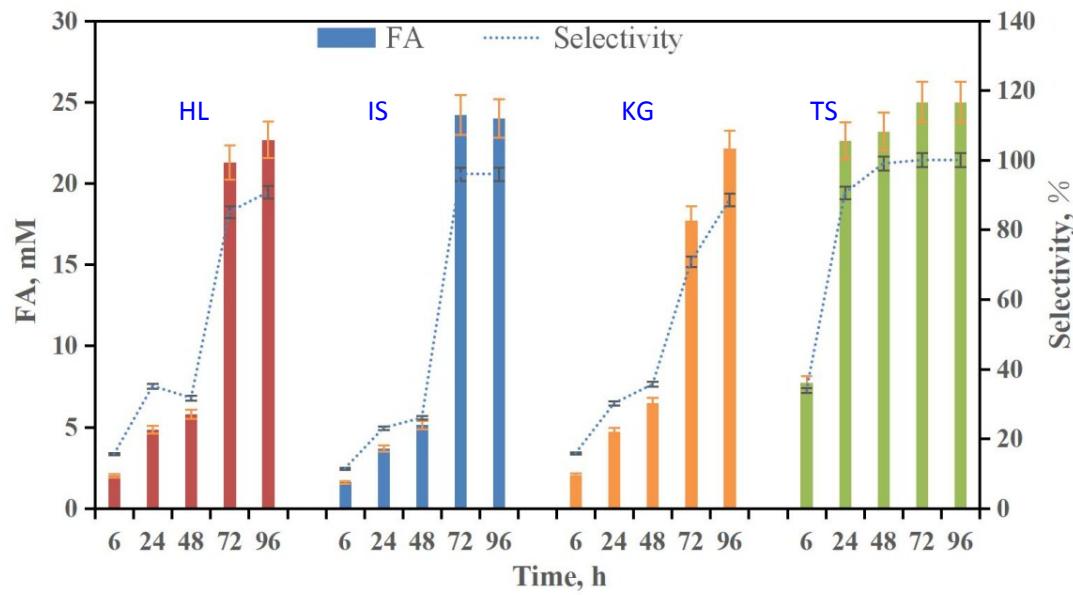
20 **Table S1.** The nucleotide sequences of optimized encoding genes.

21

22 **Table S2.** Strain, plasmids, and primer used in this study.

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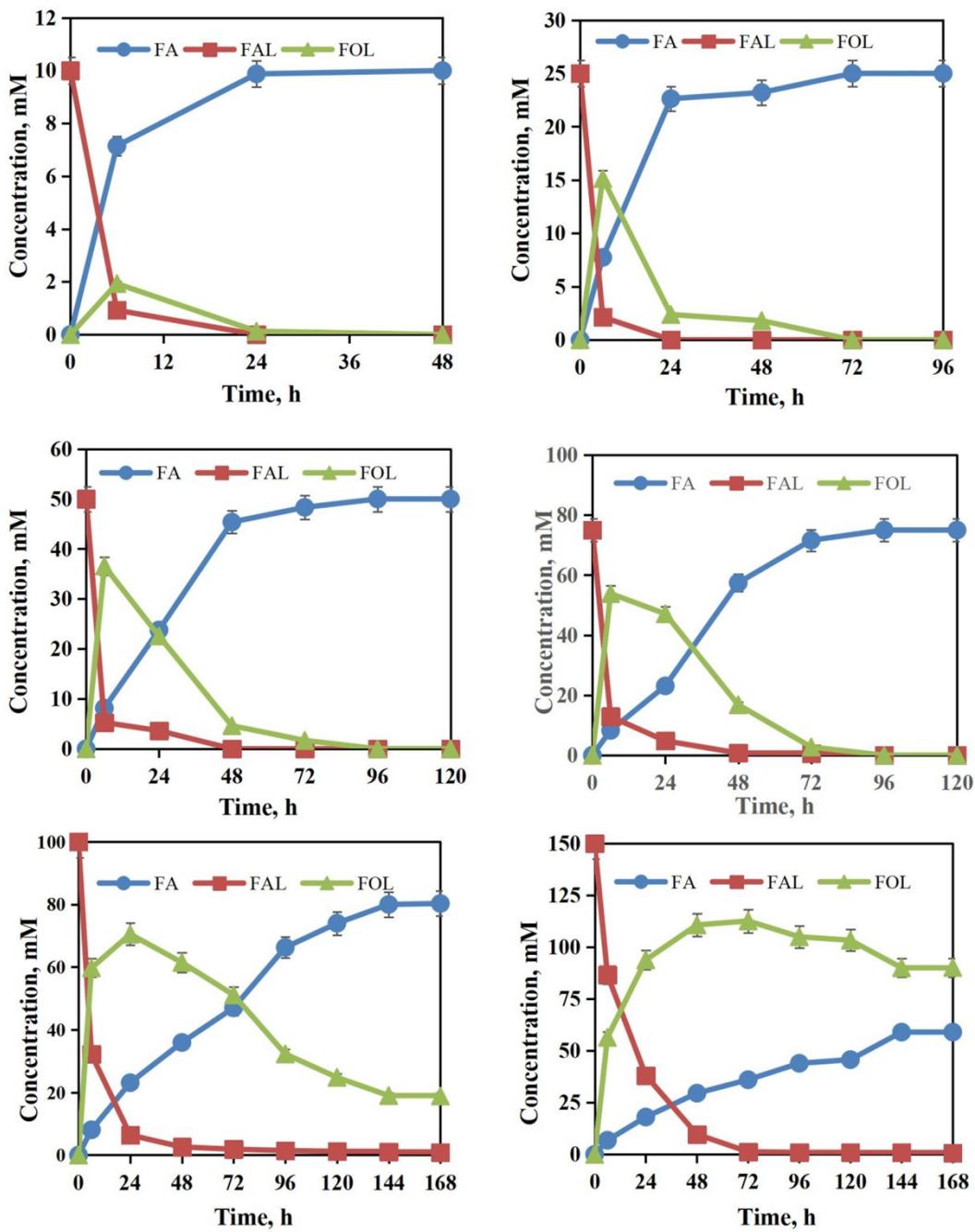
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Fig. S1

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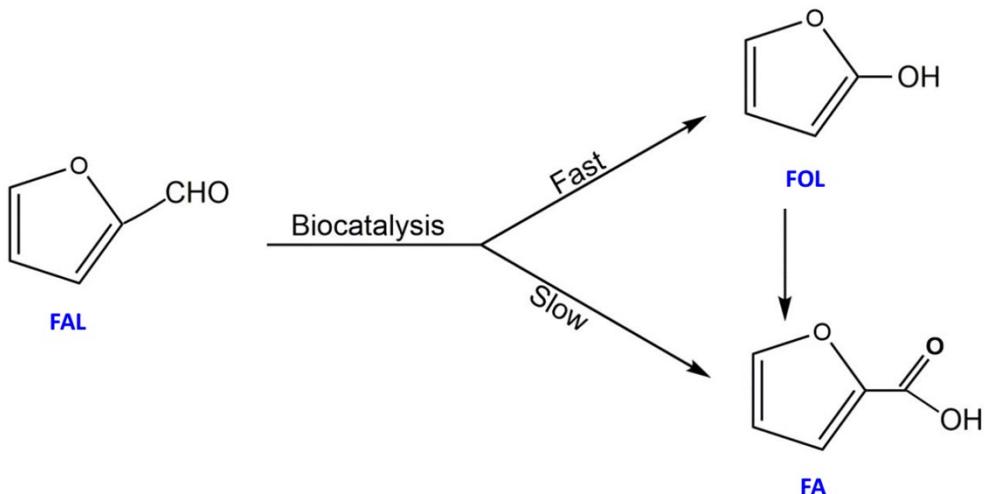


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Fig. S2

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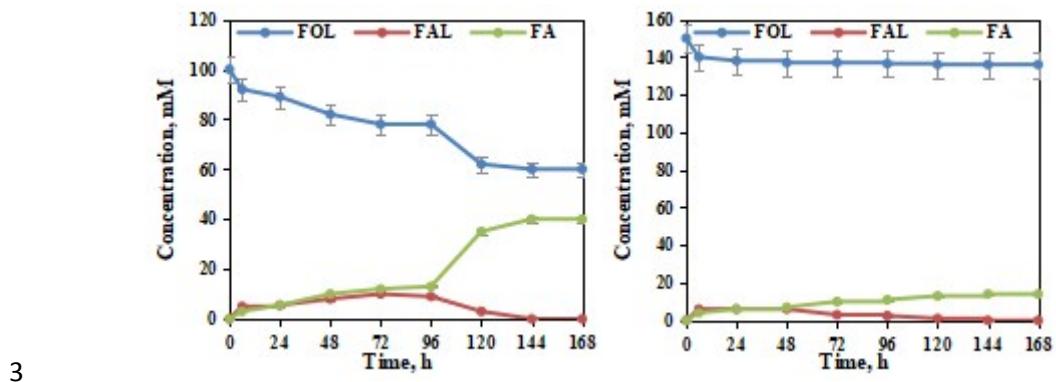
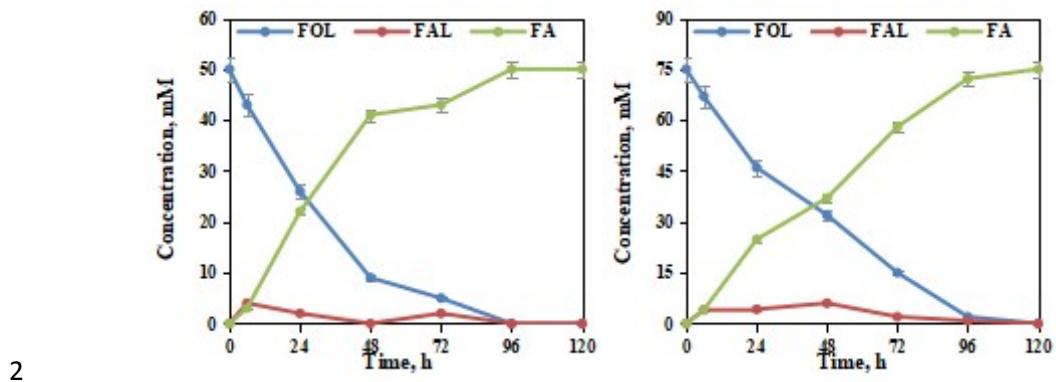
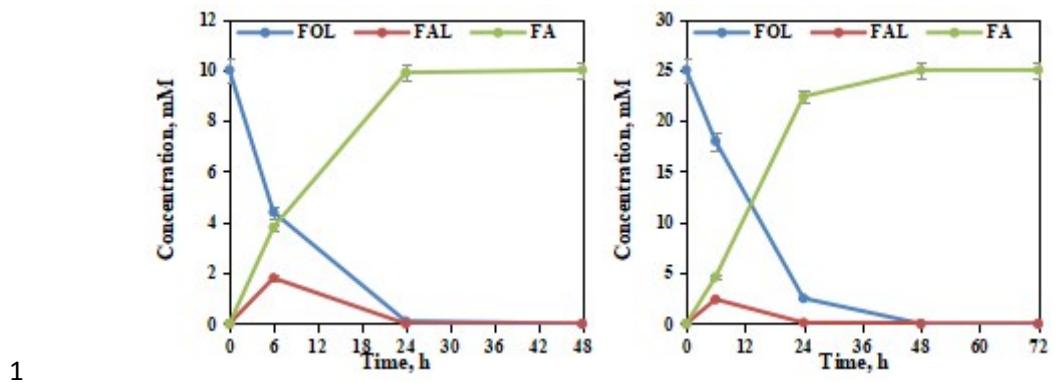


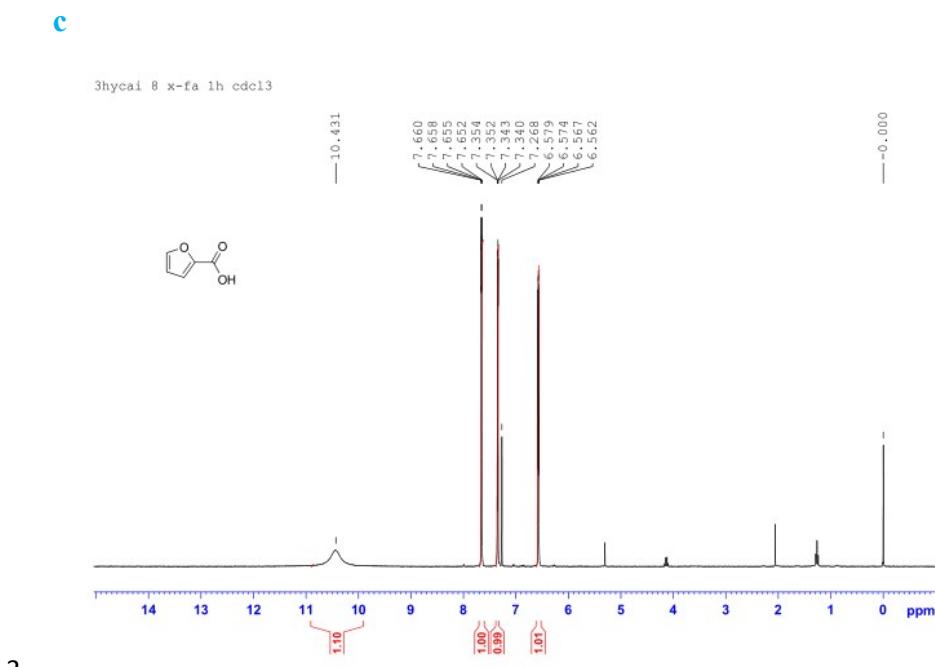
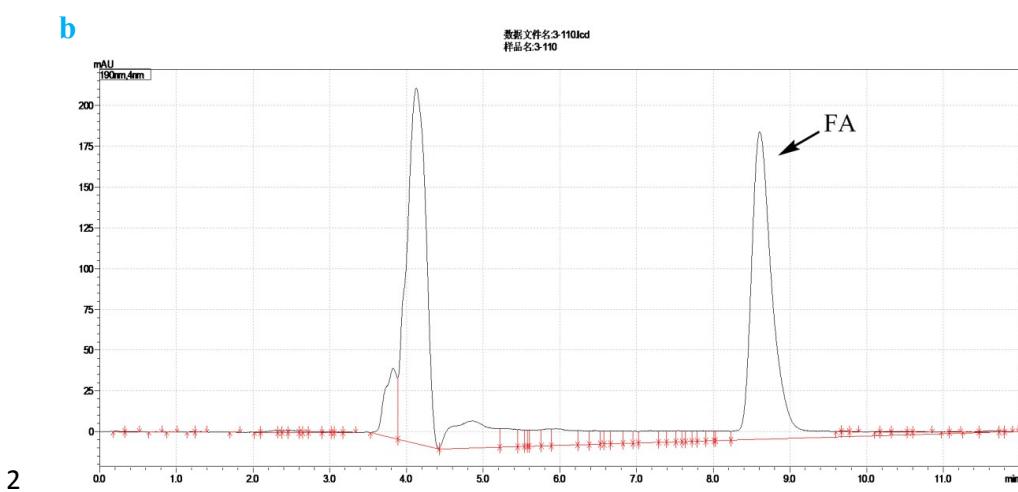
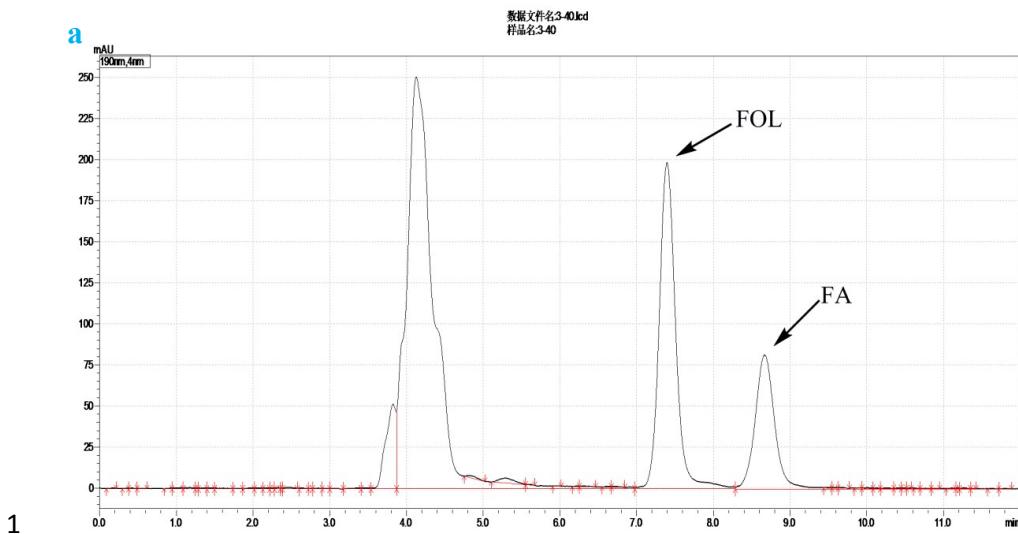
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Fig. S3

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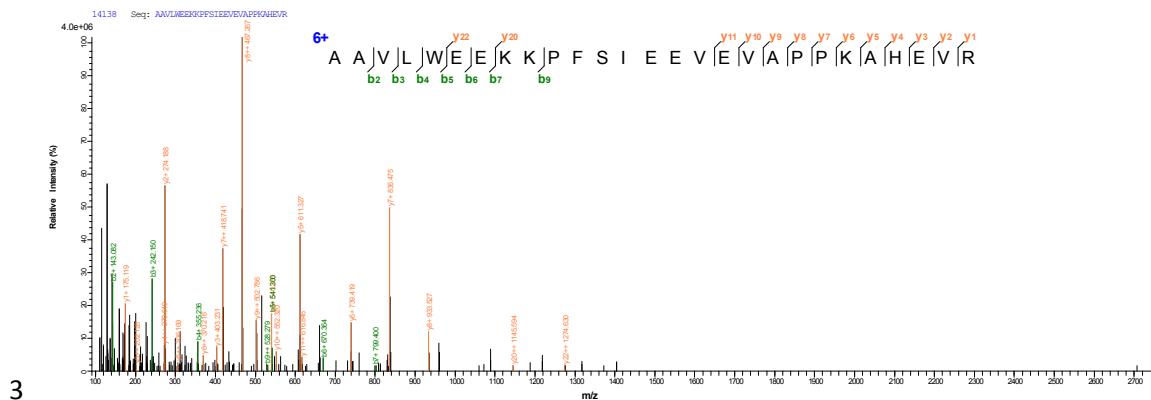


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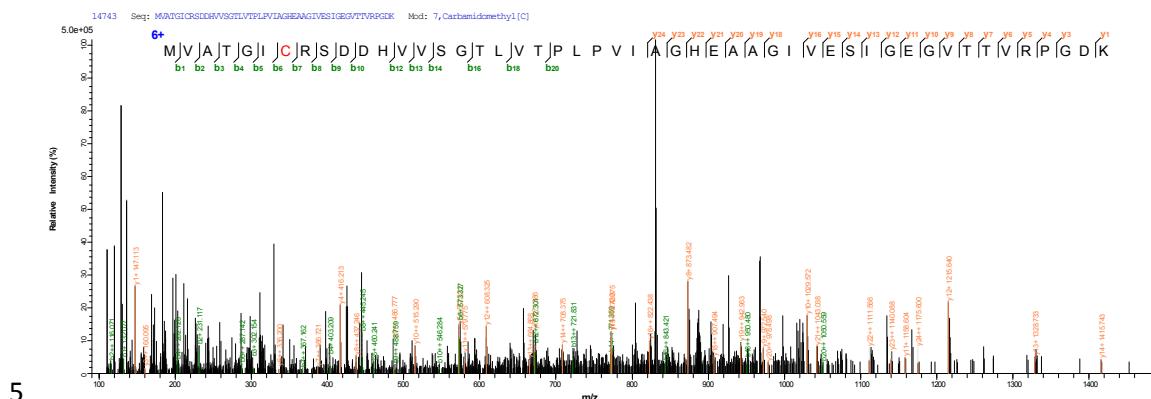
Fig. S5

1 HL

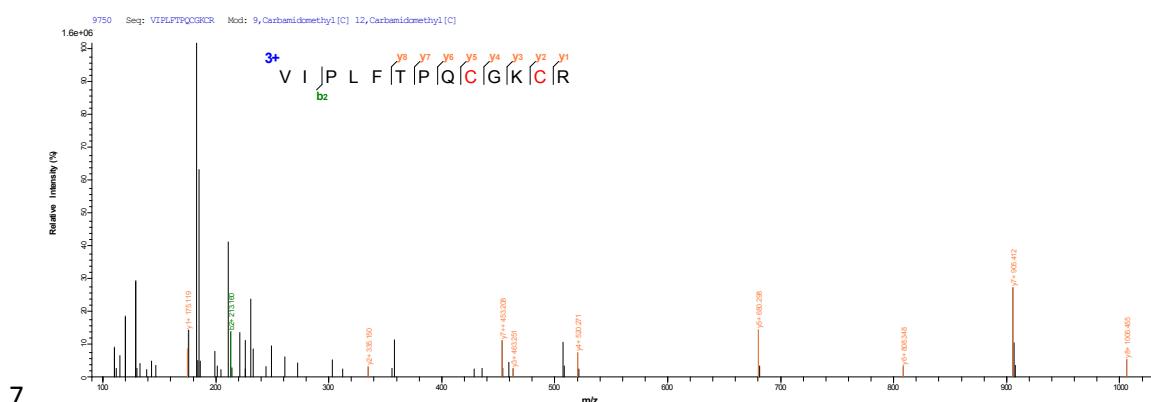
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4 HL₋ADH1E[41-89]



6 HL₋ADH1E[90-102]

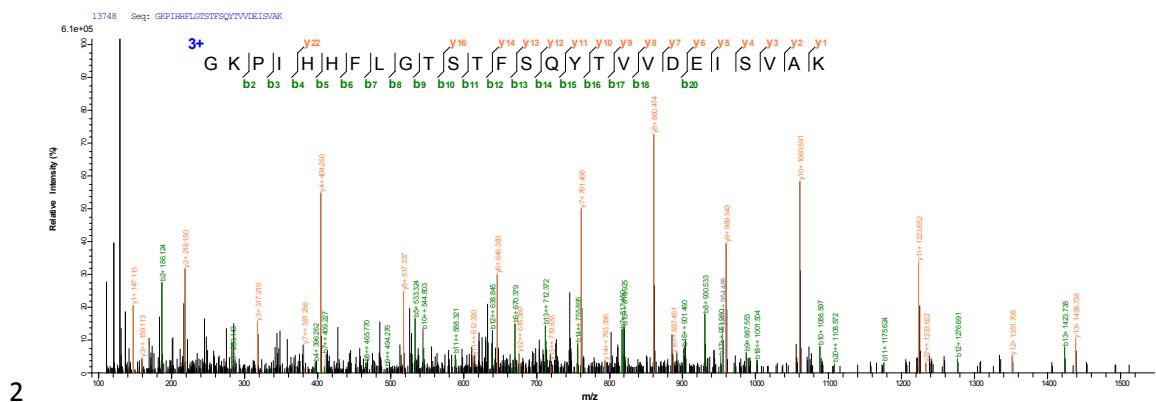


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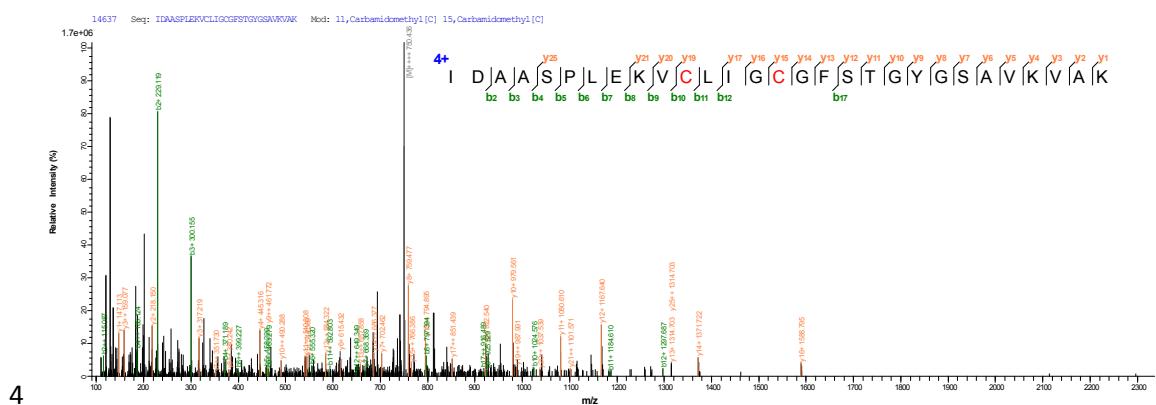
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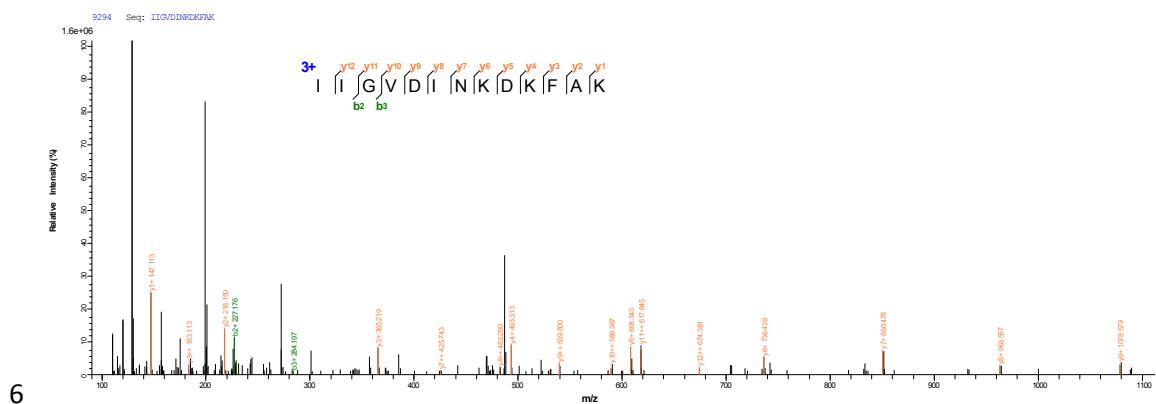
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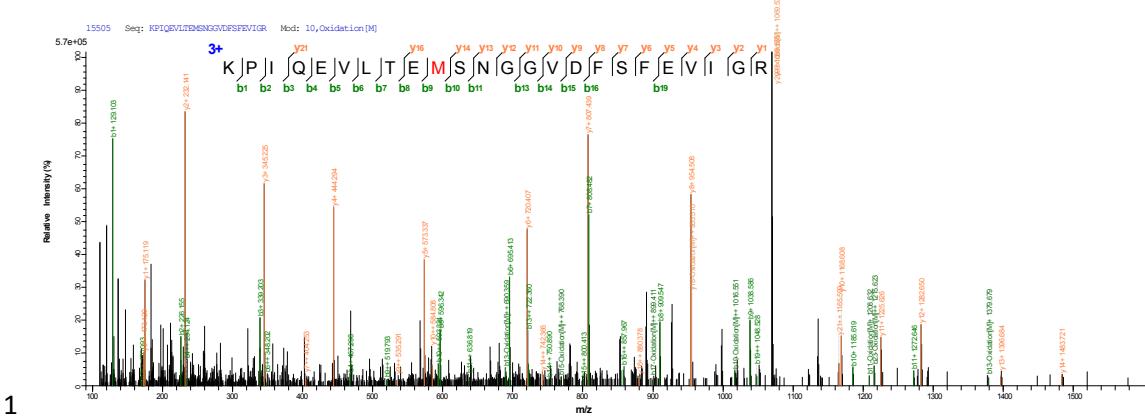
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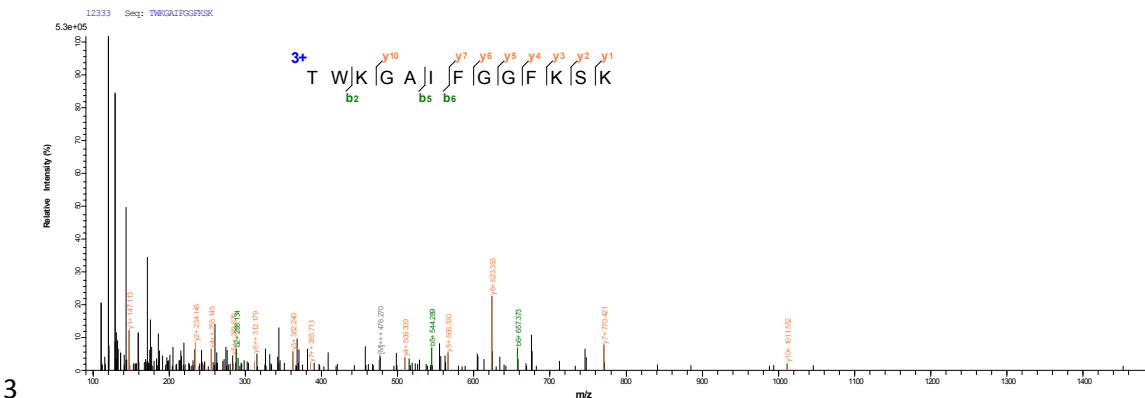
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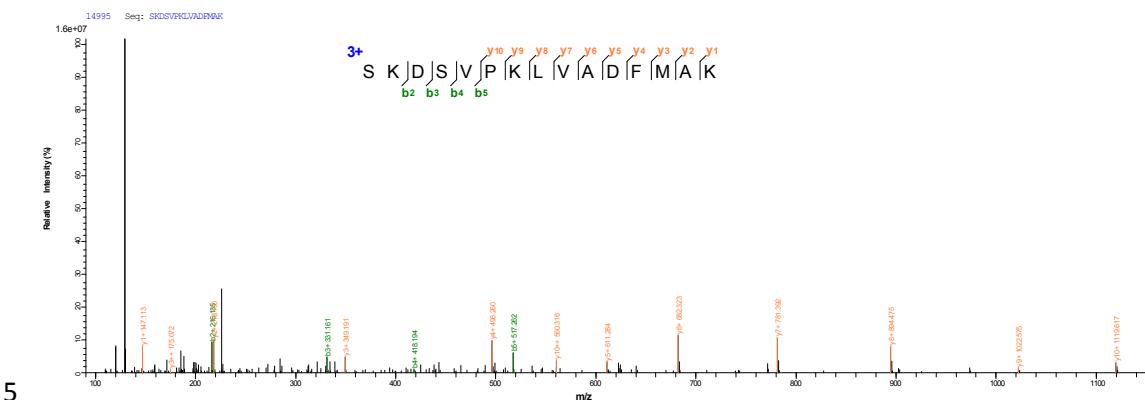
7 HL₋ADH1E[249-272]



2 HL₋ADH1E[314-326]



4 HL₋ADH1E[325-339]



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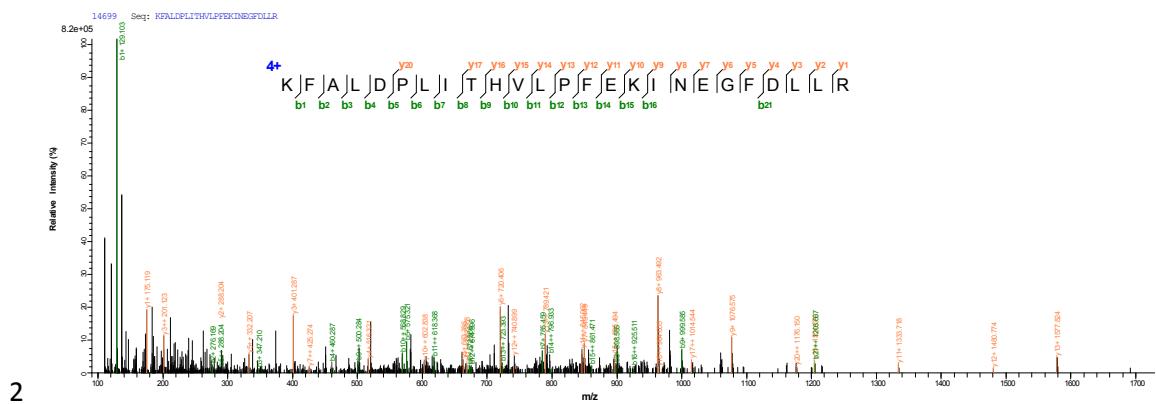
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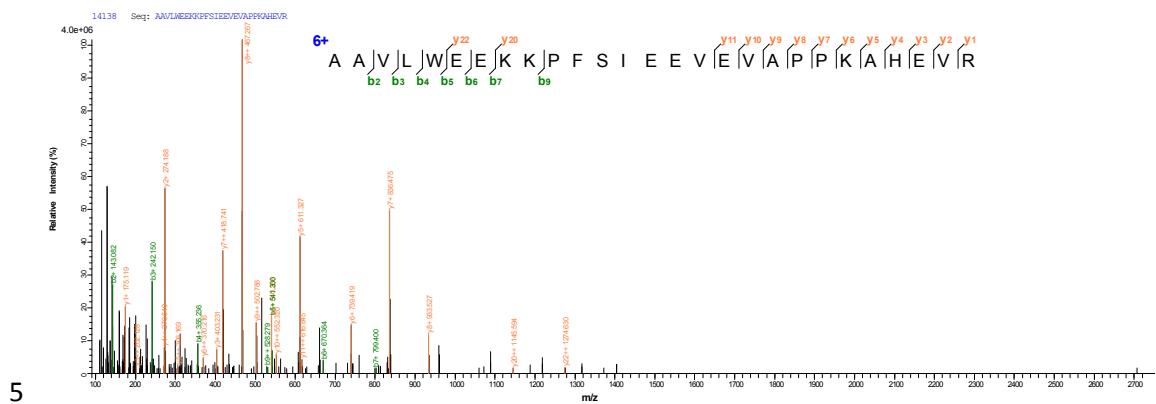
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1 HL₋ADH1E[340-364]

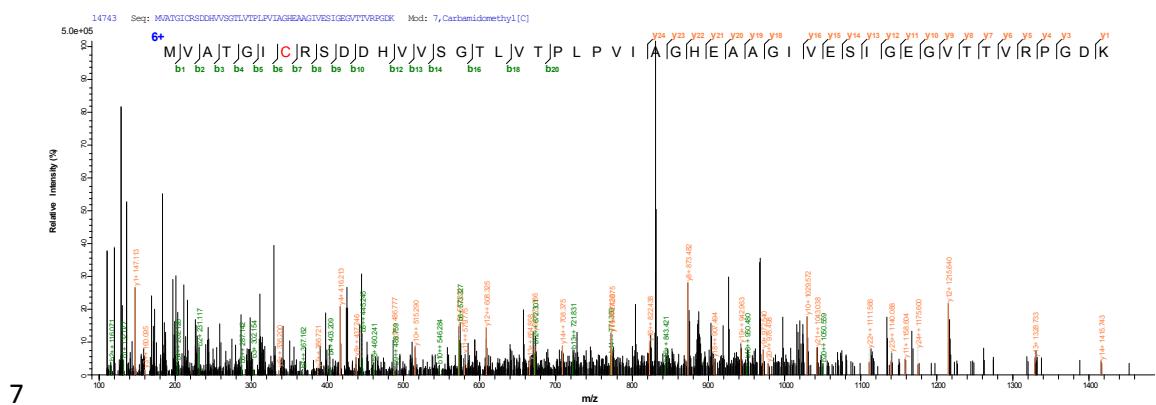


3 2. KG

4 KG_{_}ADH1E-mutation1[12-38]



6 KG_{_}ADH1E-mutation1[41-89]

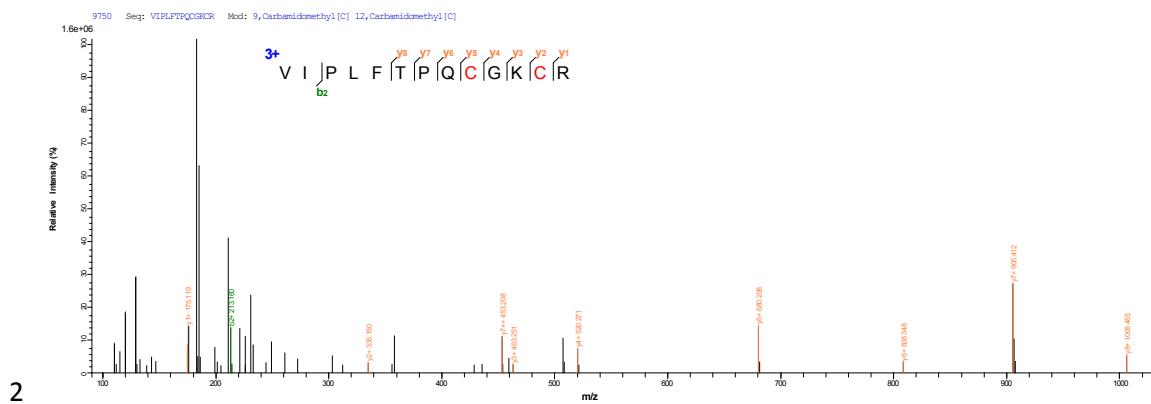


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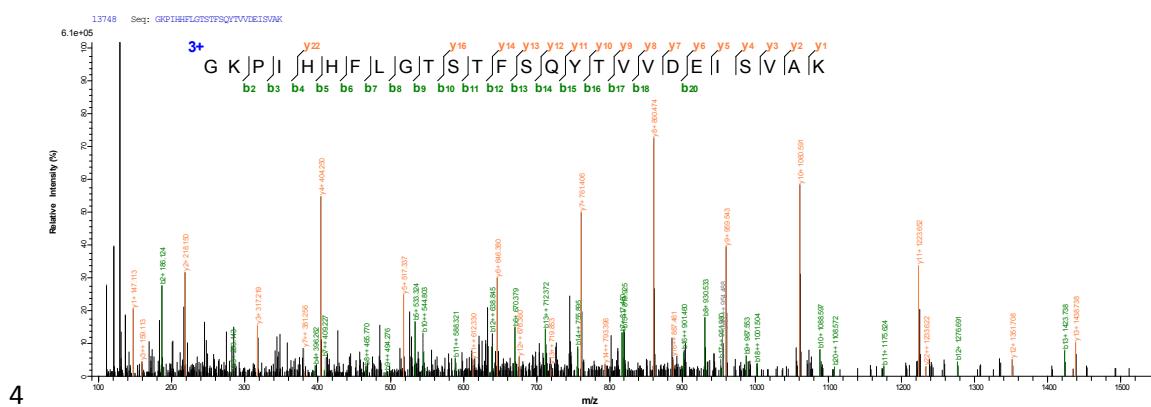
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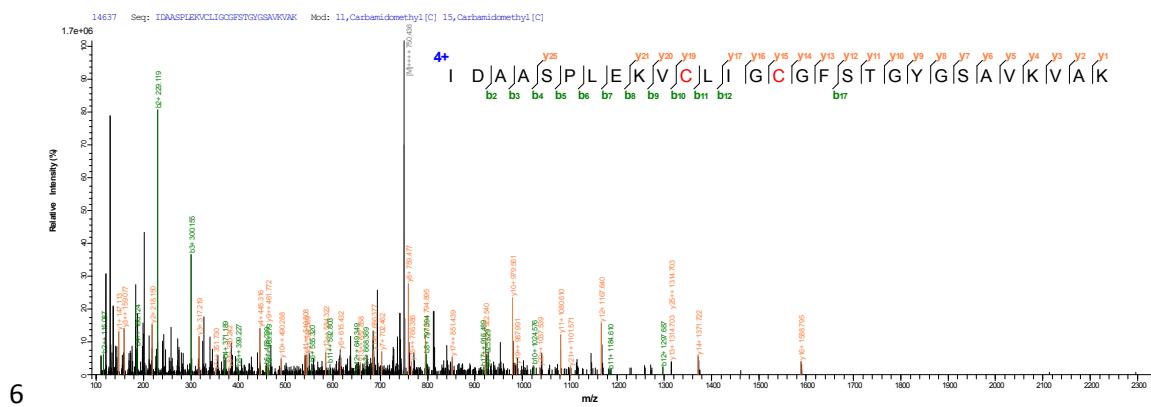
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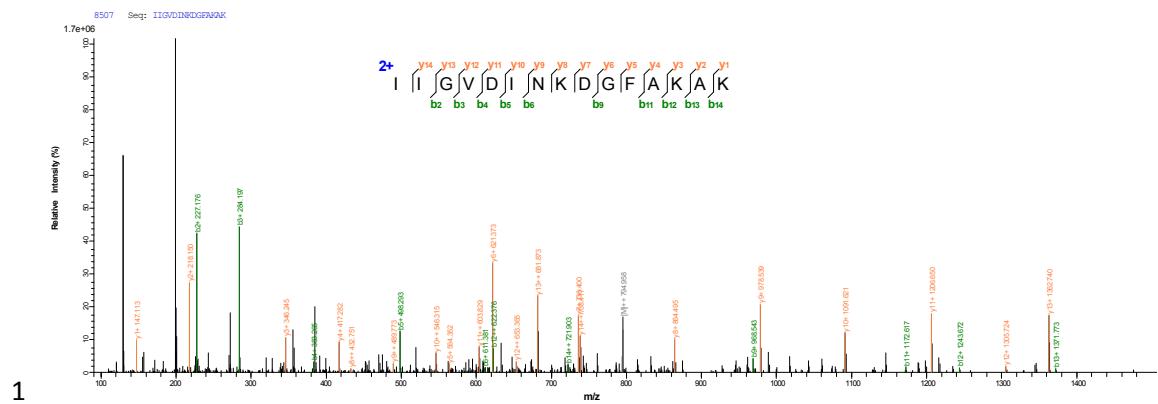
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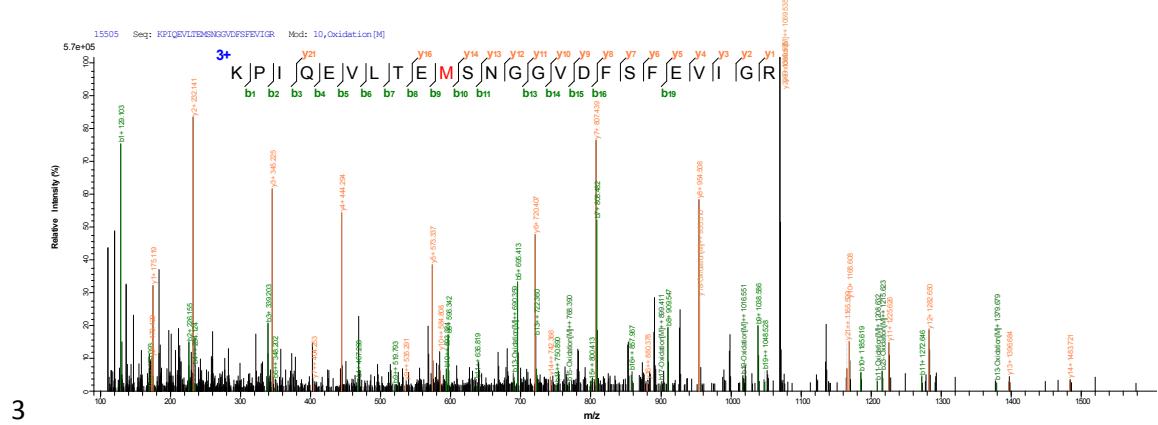
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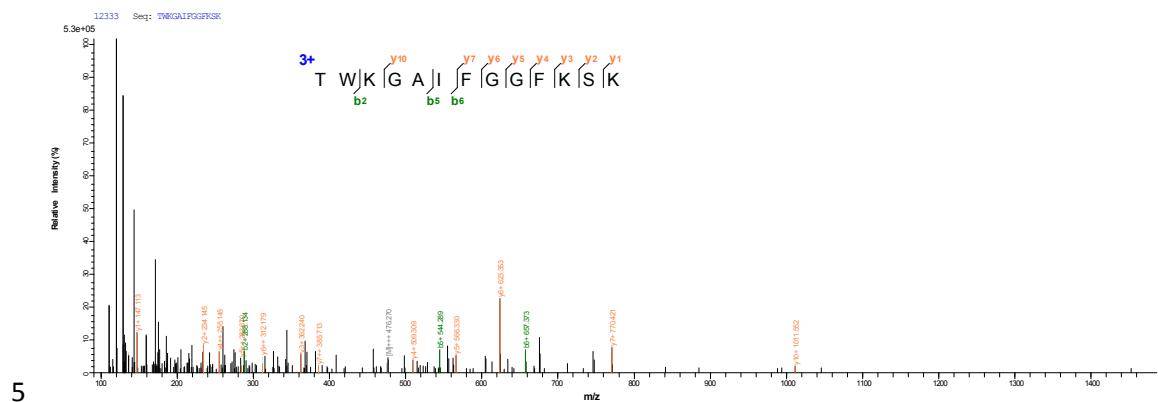
7 KG_ADH1E-mutation1[220-232]



2 KG_ADH1E-mutation1[249-272]



4 KG_ADH1E-mutation1[314-326]



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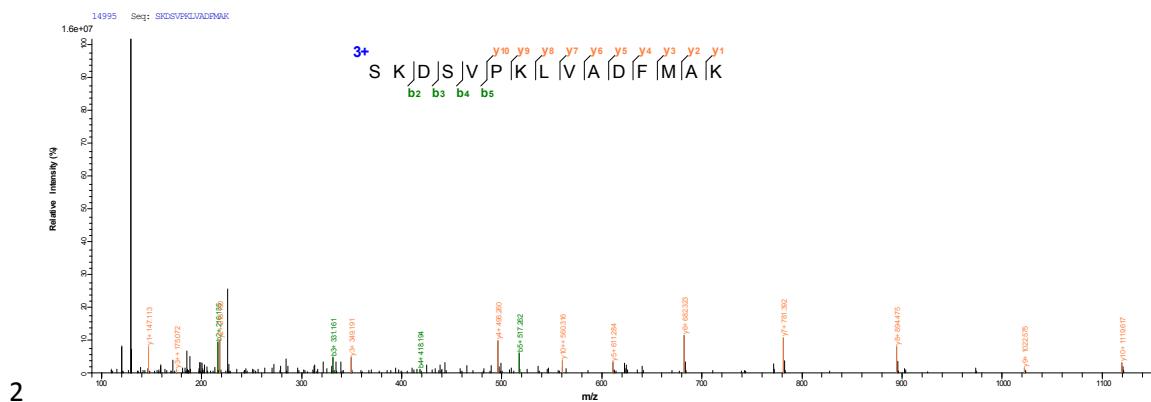
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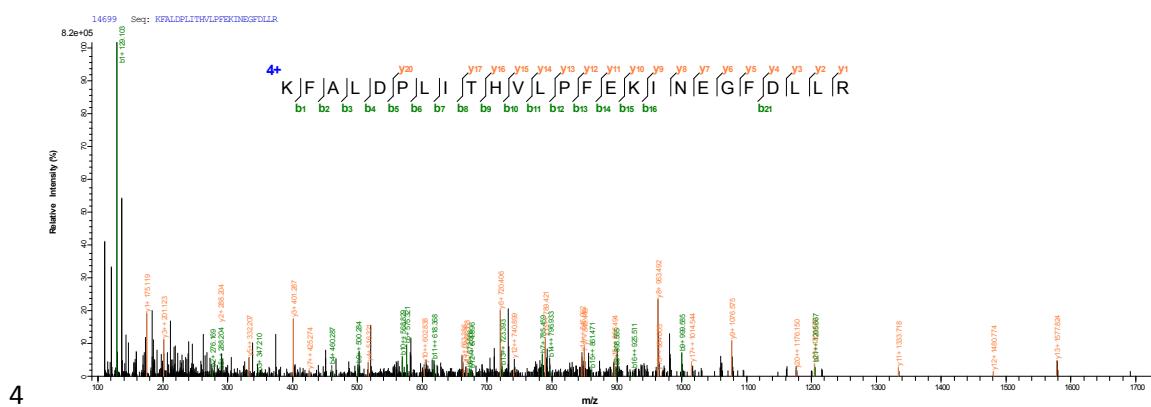
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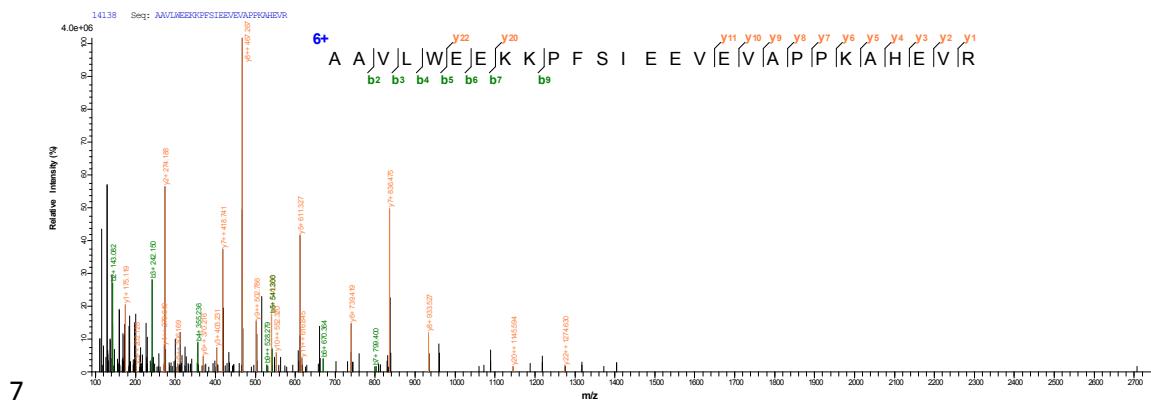


3 KG_ADH1E-mutation1[340-364]



5 3. IS

6 IS_ADH1E-mutation2[12-38]

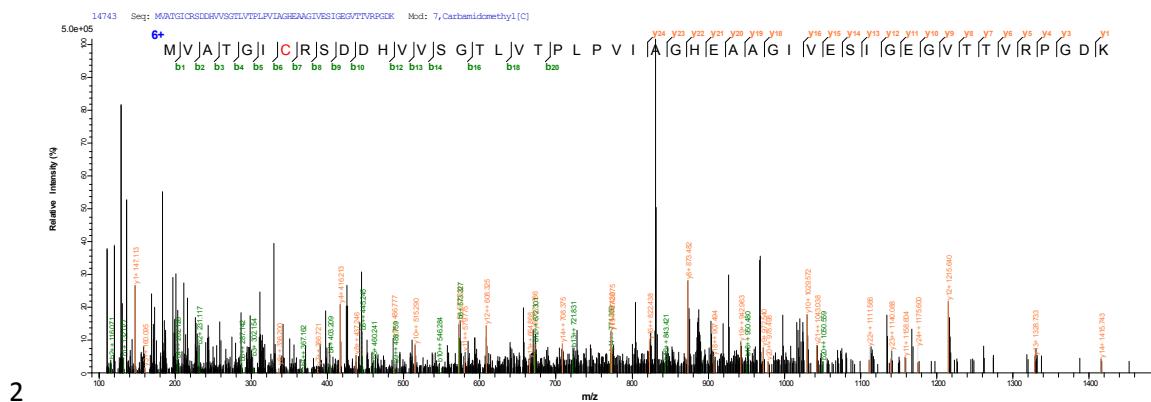


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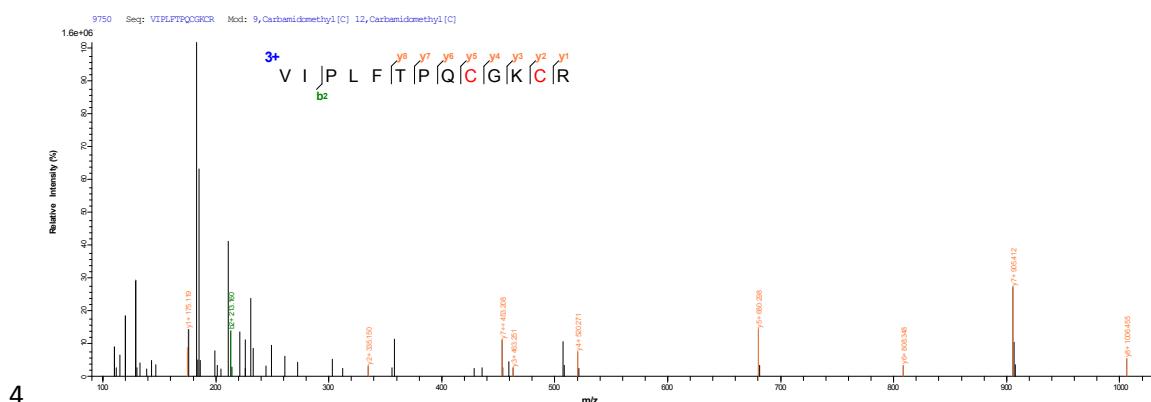
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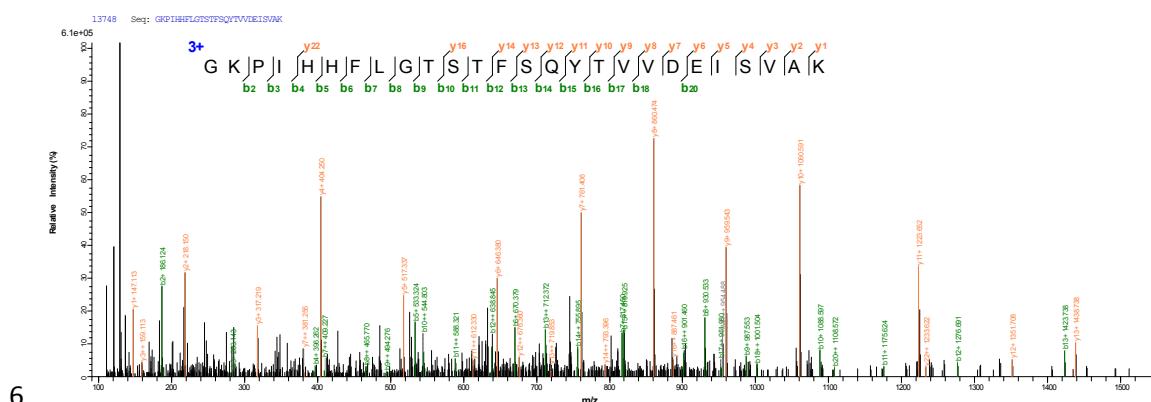
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3 IS_ADH1E-mutation2 [90-102]



5 IS_ADH1E-mutation2 [135-160]



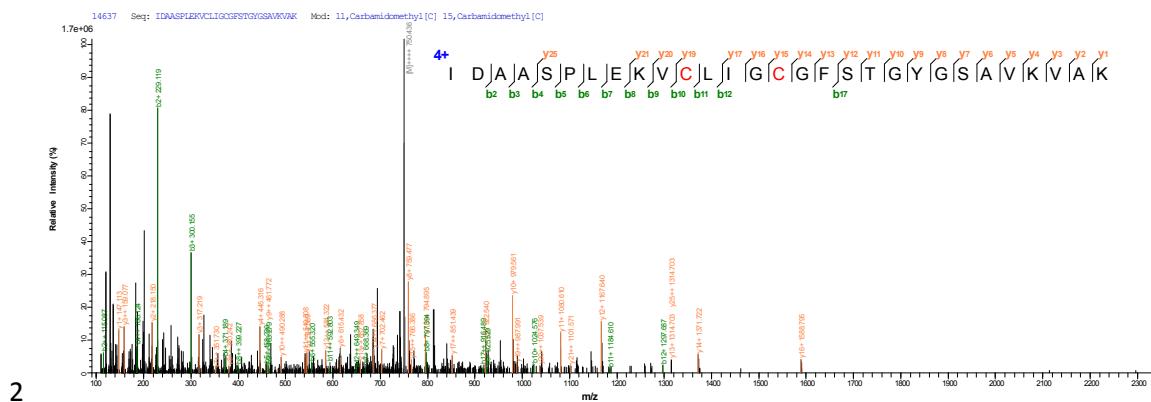
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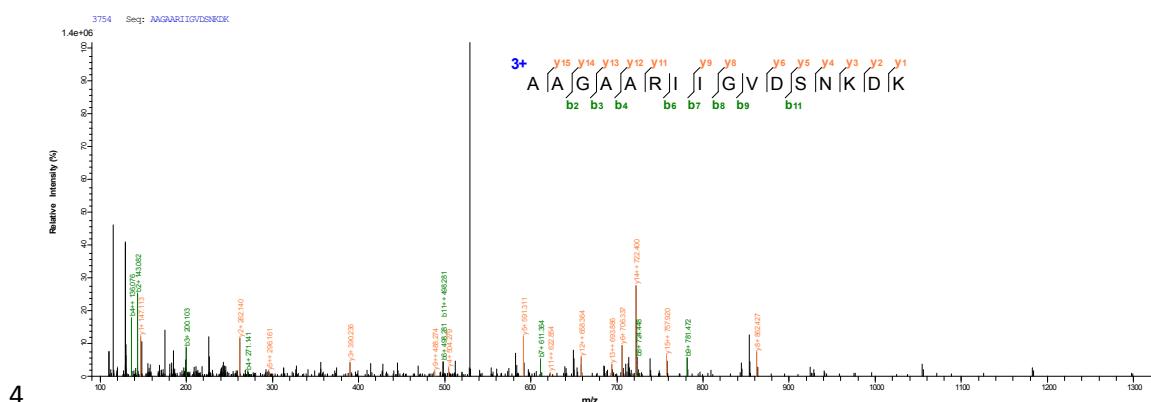
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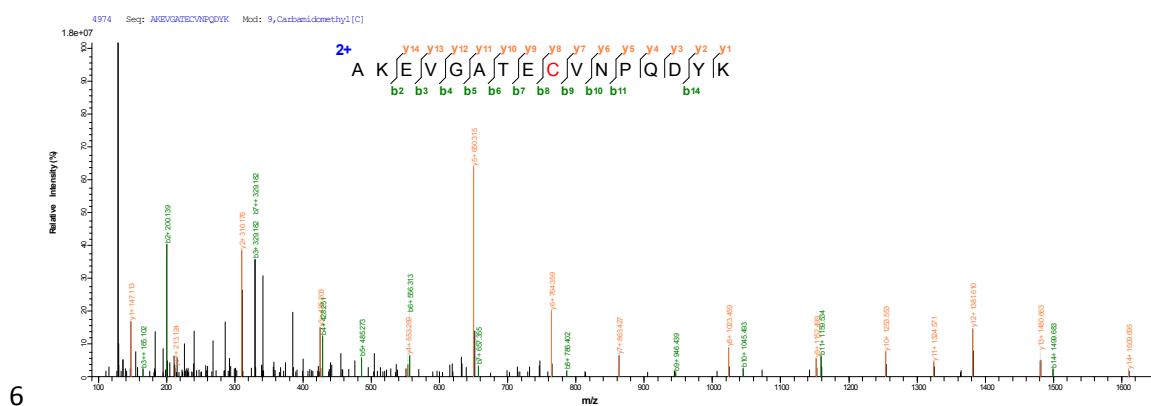
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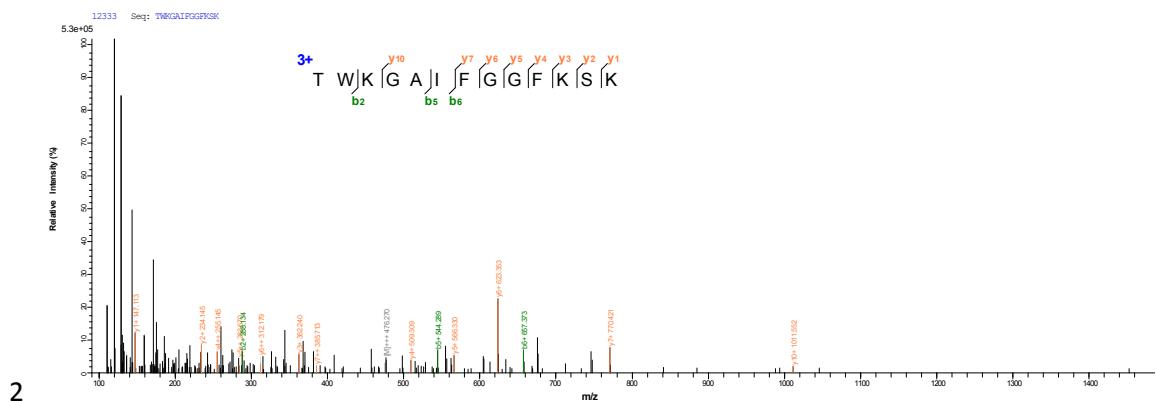
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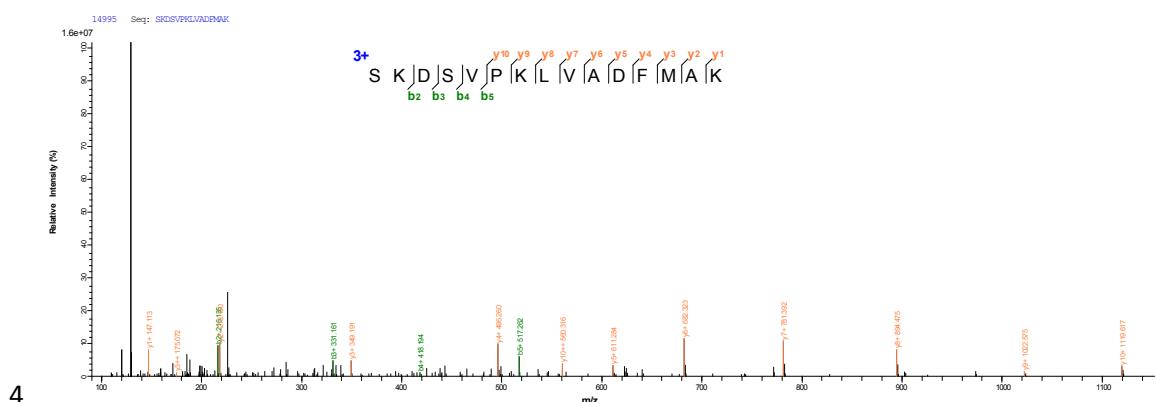
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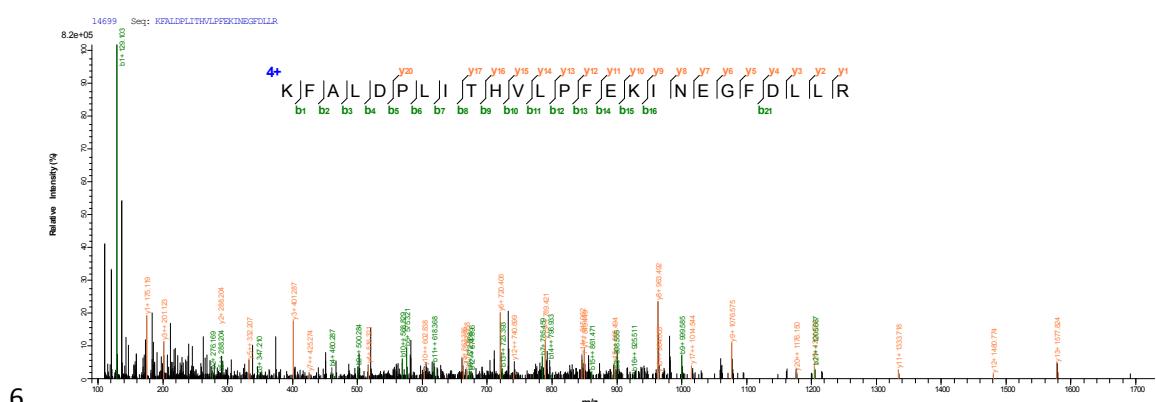
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3 IS_ADH1E-mutation2 [325-339]



5 IS_ADH1E-mutation2 [340-364]



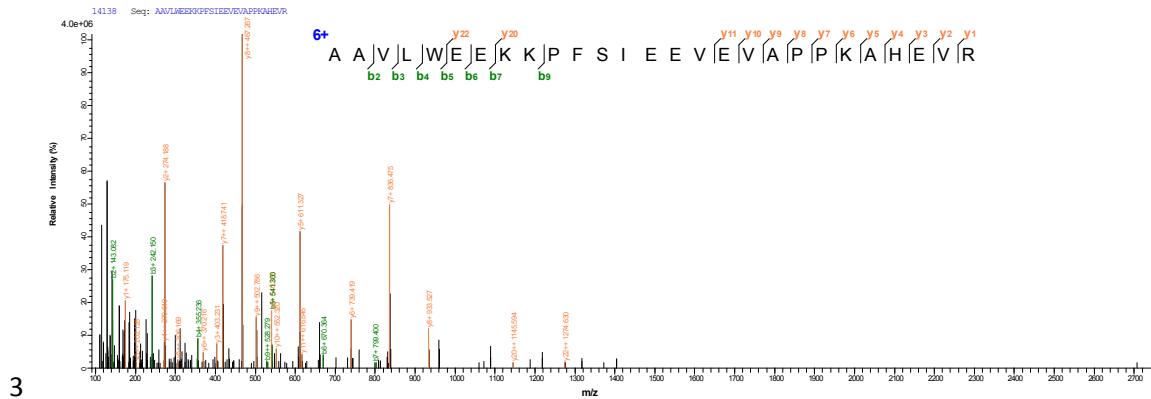
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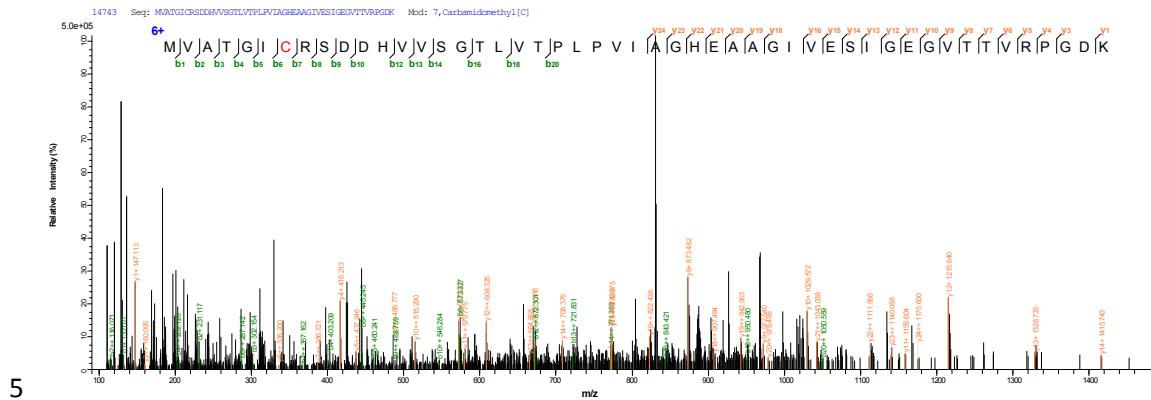
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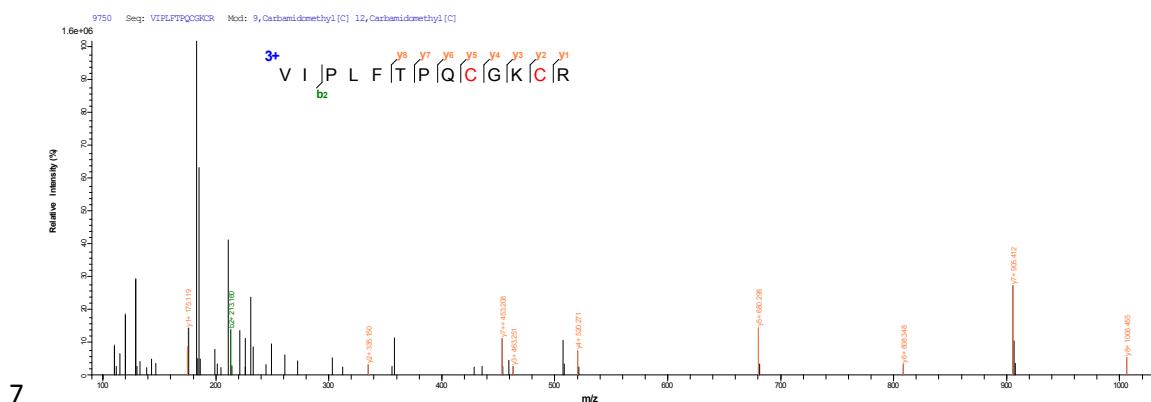
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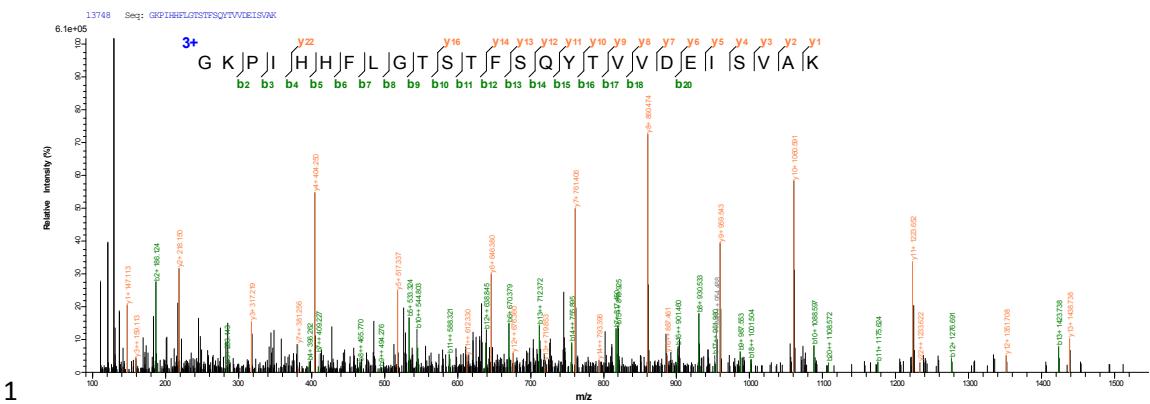
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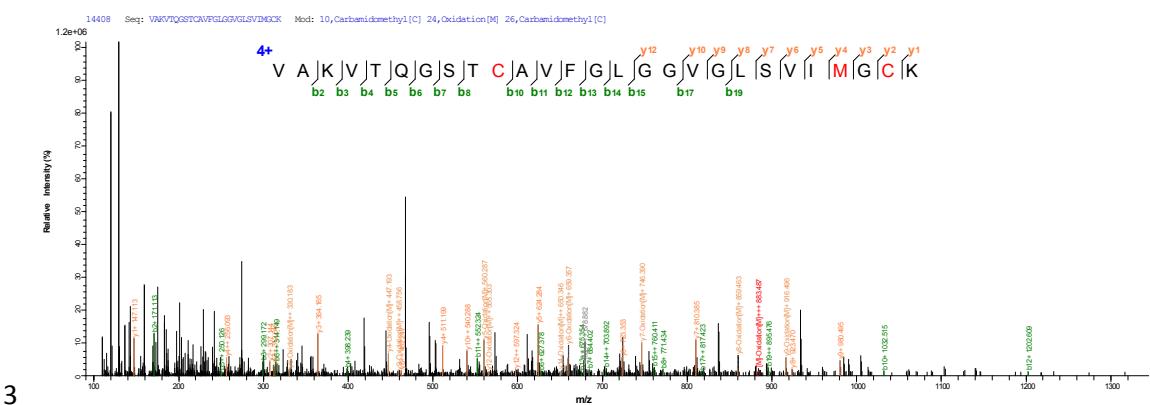


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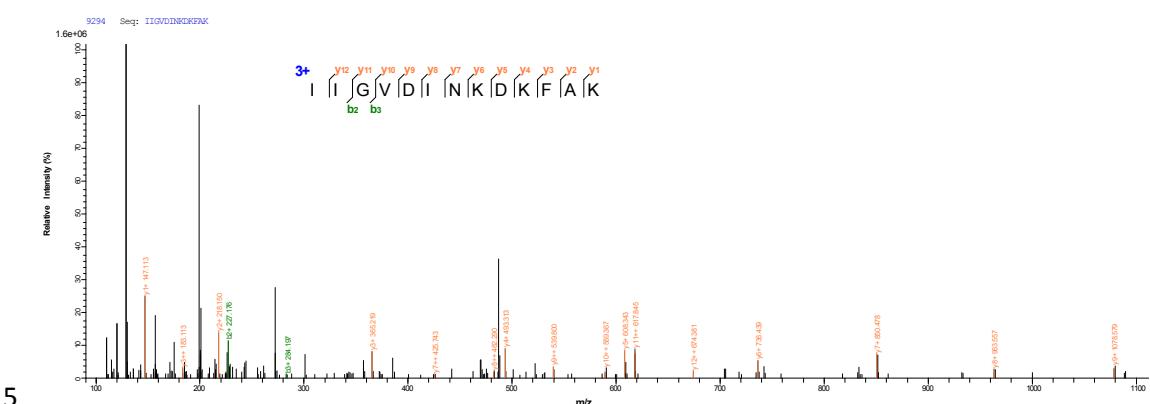
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2 TS_ADH1E-mutation3[187-213]



4 TS ADH1E-mutation3[220-232]



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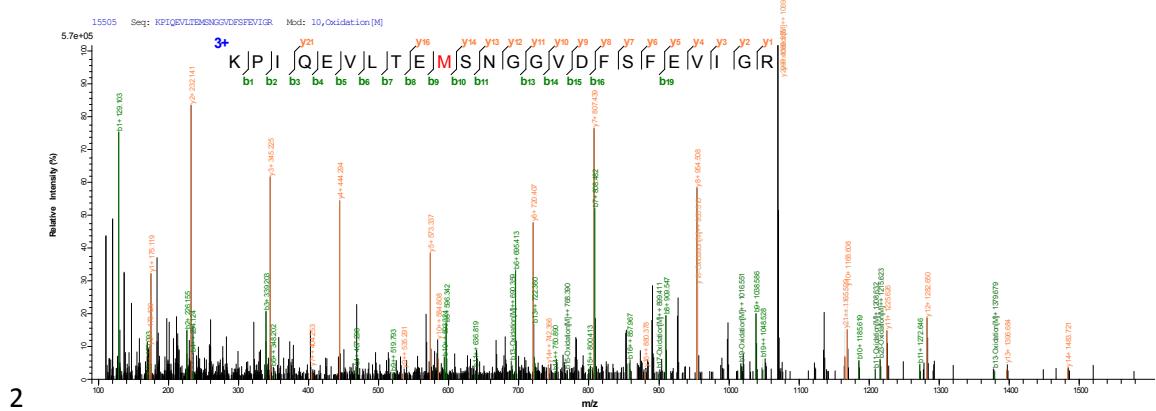
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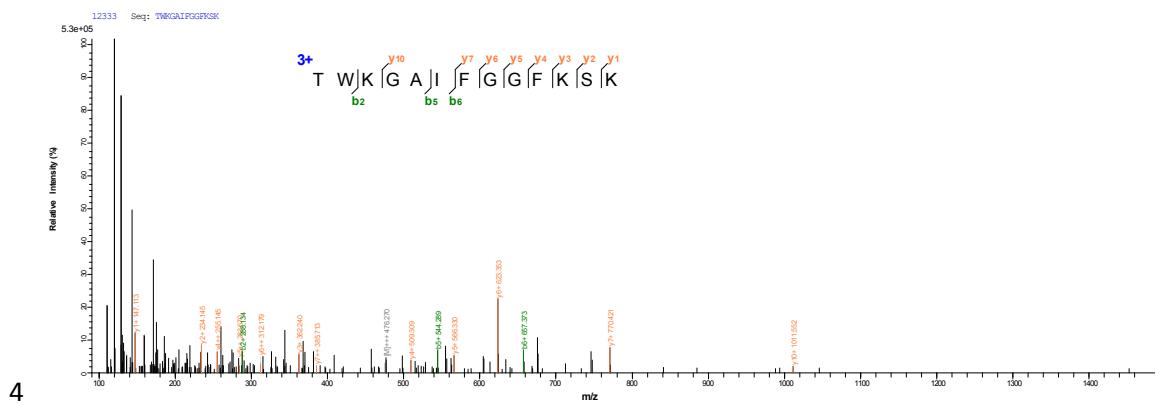
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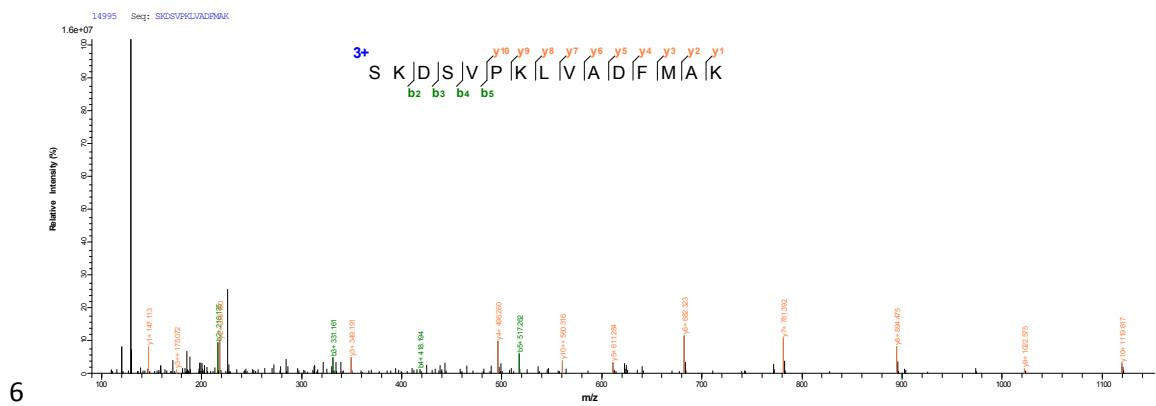
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3 TS_—ADH1E-mutation3[314-326]



5 TS_—ADH1E-mutation3[325-339]



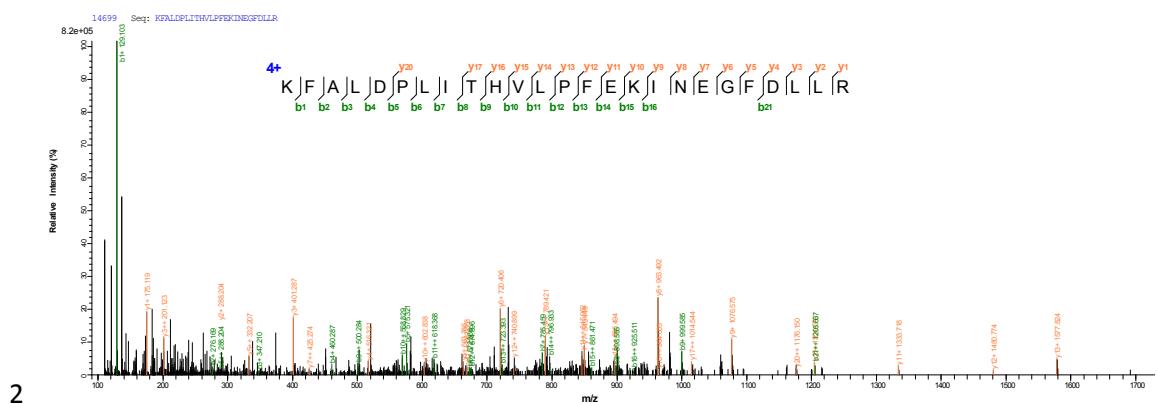
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1 TS_ADH1E-mutation3[340-364]



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Table S1. The nucleotide sequences of optimized encoding genes.

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Table S2. Strain, plasmids, and primer used in this study.

Symbol	Primer sequence (5' →3')	Template
HLADH + homologous sequence	Forward: <u>tcatcaccacagccaggatcgatgagcacgcagg</u> Reverse: <u>gcattatcgccgcgaagctttaaaaggtcagaatggta</u> <u>cacatcttac</u>	DNA (HLADH)
pRSFDuet-1+homologous sequence	Forward: <u>gcggccgcataatgctaagtgcac</u> Reverse: <u>Tggctgtggatgatggatggc</u>	Plasmid pRSFDuet-1
T178S	Forward: <u>ggtgtgttttagcagcggttatggtagc</u> Reverse: <u>Acacattcggtgcgcacac</u>	Plasmid pRSFDuet-1- HLADH
K228G	Forward: <u>gttgtatattaacaaagatggcttgcaagccaaaggagg</u> Reverse: <u>cggaacacccaacaataacgctaaccatatg</u>	Plasmid pRSFDuet-1-HLADH
I269S or I224S	Forward: <u>gcagcacgtattattgggtgttagcaacaaagataattgc</u> <u>g</u> Reverse: <u>ggtaaccatggtatccagacgaccgctaactcaaag</u>	Plasmid pRSFDuet-1-HLADH

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