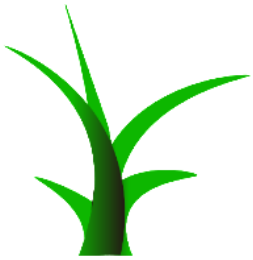
Jan 28, 2020

Proteomic protocol for Tibetan hulless barley under osmotic stress



DOI
dx.doi.org/10.17504/protocols.io.bbusinwe

Yulin Wang[1], Zha Sang[1], Shaohang Xu[2], Qijun Xu[1], Xingquan Zeng[1], Dunzhu Jabu[1], Hongjun Yuan[1]

[1]State Key Laboratory of Hulless Barley and Yak Germplasm Resources and Genetic Improvement, Lhasa 850002, China. Institute of Agricultural Research, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850002, China.;
[2]Deepxomics Co., Ltd., Shenzhen 518000, China.

Shaohang Xu

DOI: dx.doi.org/10.17504/protocols.io.bbusinwe

Protocol Citation: Yulin Wang, Zha Sang, Shaohang Xu, Qijun Xu, Xingquan Zeng, Dunzhu Jabu, Hongjun Yuan 2020. Proteomic protocol for Tibetan hulless barley under osmotic stress. **protocols.io https://dx.doi.org/10.17504/protocols.io.bbusinwe**

Protocol status: Working
We use this protocol and it's working

Created: January 28, 2020

Last Modified: January 28, 2020

Protocol Integer ID: 32370

Keywords: Tibetan hulless barley, osmotic stress, proteomics, DIA, quantification, abiotic stress

Abstract

This protocol describes the complete procedures for performing data-independent acquisition-based analysis from Tibetan hulless barley samples under osmotic stress.

Safety warnings

Wear labcoat and gloves at every step of the protocol. Formic acid and acetonitrile can be harmful, handle with care.

Before start

Prepare solutions as described in the steps.

Protein extraction

1 Weigh 1-g subsample and homogenized by grinding in liquid nitrogen, moved the powdered samples to 50 cm3 tubes;

2 Add 25 cm3 precooled acetone (-20 °C) containing 10% (v/v) trichloroacetic acid (TCA) and 10 mM dithiothreitol (DTT);

25 mL precooled acetone (-20 °C)

3 Mix for 2 min, the homogenate was precipitated overnight at -20 °C and then centrifuged (20,000×g, 4 °C) for 30 min;

Overnight homogenate at -20 °C

20000 x g, 4°C, 00:30:00

4 Wash the pellet with 20 cm3 chilled acetone (-20 °C) and left at -20 °C for 30 min followed by centrifugation (20,000×g, 4 °C) for 30 min, repeat this step for twice;

00:30:00

20000 x g, 4°C, 00:30:00

20000 x g, 4°C, 00:30:00

5 Remove the supernatant and add lysis buffer (4% SDS, 100 mM Tris-HCl, 10 mM DTT, pH 8.0) in the precipitate;

6 Sonicate for 5 min at 60 W (5 s sonication followed by 10 s break);

00:05:00 sonicate at 60 W

7 Centrifuge for 30 min (20,000×g, 20 °C) and collect the supernatant;

20000 x g, 20°C, 00:30:00

8 Estimate the protein concentration using a bicinchoninic acid (BCA) protein assay kit (Beyotime Institute of Biotechnology, China)

Protein digestion using the FASP (filter-aided sample preparation) procedure

9 Mix up to protein extract with 200 mm3 UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.0) and centrifuged at 14,000 g at 20°C for 40 min.

200 µL UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.0)

14000 x g, 20°C, 00:40:00

10 Add 200 mm3 of UA to the filter unit and centrifuge at 14,000 x g for 40 min. Discard the flow-through form the collection tube. Repeat this step twice.

200 µL UA

14000 x g, 00:40:00

11 Add 100 mm3 IAM solution (10 mM IAM in UA buffer) and incubate for 30 min.

100 µL IAM solution (10 mM IAM in UA buffer)

00:30:00 incubate

12 Centrifuge the filter units at 14,000 x g for 30 min.

14000 x g, 00:30:00

13 Add 100 mm3 of UA to the filter unit and centrifuge at 14,000 x g for 40 min. Repeat this step twice.

100 µL UA

14000 x g, 00:40:00

14000 x g, 00:40:00

14 Add 100 mm3 of ABC (0.05 M NH4HCO3 in water) and centrifuged at 14,000 g. Repeat this step twice.

100 µL ABC (0.05 M NH4HCO3 in water)

14000 x g, 00:40:00

14000 x g, 00:40:00

15 Add 40 mm3 ABC with trypsin (Promega, USA) and incubated for 18 h at 37°C;

40 µL ABC with trypsin (Promega, USA)

18:00:00 incubate at 37°C

16 Transfer the filter units to new collection tubes.

17 Centrifuge the filter units at 14,000 x g for 10 min and collect the filtration.
14000 x g, 00:10:00

Peptide fractionation by high-pH RP

18 Separate peptides using an LC-20AB HPLC system (Shimadzu, Kyoto, Japan) with a high-pH reversed-phase (high-pH RP) column (Phenomenon, Torrance, CA). Peptides were eluted at a flow rate of 0.8 cm3/min. Buffer A consisted of 10 mM ammonium acetate (pH 10.0), and buffer B consisted of 10 mM ammonium acetate and 90% v/v acetonitrile (pH 10.0). The following gradient was applied to perform separation: 100% buffer A for 40 min, 0-5% buffer B for 3 min, 5-35% buffer B for 30 min, 35-70% buffer B for 10 min, 70-75% buffer B for 10 min, 75-100% buffer B for 7 min, 100% buffer B for 15 min and, finally, 100% buffer A for 15 min. The elution process was monitored by measuring absorbance at 214 nm, and fractions were collected every 75 s. Finally, collected fractions (approximately 40) were combined into 12 pools. Each fraction was concentrated via vacuum centrifugation and reconstituted in 40 mm3 of 0.1% v/v formic acid.

19 Peptides were separated with a Dionex UltiMate 3000 RSLCnano system with an Acclaim PepMap C18 (3 µm, 100 Å, 75 µm x 50 cm) and emitted into a Thermo Q-Exactive HF tandem mass spectrometer. Solvent A was 0.1% formic acid in water, while solvent B was 0.1% formic acid in 98% acetonitrile. For each injection, 3 mm3 (approximately 3 µg) was loaded and eluted using a 90-minute gradient from 5 to 35% B followed by a 40 min washing gradient. Data were acquired using either data-dependent acquisition (DDA) or data-independent acquisition (DIA).