Seed amino acid assay

Development of his method is described in the manuscript Gu, L., A.D. Jones and **R.L. Last**. 2007. Rapid LC-MS/MS assay for protein amino acids and metabolically related compounds. Anal. Chem. http://pubs.acs.org/cgi-bin/abstract.cgi/ancham/asap/abs/ac070938b.html. Please cite this paper if you publish data derived from all or part of this protocol.

Materials:
3 mm stainless steel balls (CCR Products, 302SS)
96-well 3mm bead dispenser (QIAGEN, 69973)
VWR deepwell plate and mat (40002-009, 40002-001)
Millipore filter plate (300 µl, Millipore, MSRL N0450)
Millipore collection plate (300 µl, Millipore, MSCP NPP00)
1.1-ml MicroTubes strips (Dot Scientific Inc, RN0946-08B)
MicroCaps strips (Dot Scientific Inc, RN0946-08C)
MicroRacks (Dot Scientific Inc, RN0946-08R)
96-well full-skirted plate (USA Scientific, 1402-9800)
0.2-ml PCR strip tubes (DENVILLE, C-18098-7)
Aluminum sealing film (Dot Scientific Inc, AF-100)
AXYMAT silicone sealing mat (VWR 10011-130, AXYGEN: AM-96-PCR-RD)
Memowell electronic pipetting aid (Matrix Technologies, 5000 and 5001)
Eppendorf tabletop centrifuge 5810R
Hero 2200 Dual Head paint shaker

Reagents:
1 mM Phe-d8
9 µM Val-d8 (Cambridge Isotope Laboratories, DLM-488-0.5 and DLM-372-1)
10 mM DTT

Procedures:

Create plate:
1. Create a Seed Amino Acid plate by loading one 3 mm stainless steel ball in each well of a 96-well deepwell plate and affixing the appropriate barcode sticker to the plate.
2. Using Seed Amino Acid database web form, aliquot 7-7.9 mg seed from each stock in a seed stock box into each well and seal with a sealing mat.

Extraction buffer:
45 ml ddI H_2O
45 µl of 1 mM Phe-d8
45 µl of 10 mM DTT
Keep chilled on ice.

Processing samples:

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1. Add 400 ul extraction buffer to each sample and completely seal the plate with a sealing mat. Keep on ice.
2. Shake the plate 5 min. using a paint shaker, flipping the plate after 2.5 min.
3. Centrifuge 10 seconds 4°C tabletop centrifuge.
4. Remove the sealing mat and incubate the plate 5 min, ~ 90°C. Reseal the plate.
5. Centrifuge 10 min. 4°C tabletop centrifuge. Keep on ice.
6. Transfer all the supernatant to 1.1 ml strip tubes in a 96-well rack and cap tubes.
7. Centrifuge the tubes in the racks 10 min. 4°C tabletop centrifuge. Keep on ice.

Filter, add internal standard, and transfer samples for storage:

1. Prewet filter plate by adding 100 ul dH_2O and centrifuging at 2000*xg*, Eppendorf 5810R tabletop centrifuge, 4°C, 5 min.
2. Place the prewetted filter plate on the collection plate, add 300 ul extraction supernatant. Centrifuge at 2000*xg* at 4ºC for 5 min.
3. 10 μl of 9 μM Val-d8 is added per 90 ul filtered extraction supernatant for mass spec analysis.
4. Store extraction aliquots in plates at – 80ºC, sealed with aluminum sealing film.

Set up LC-MS standards and samples

1. Carefully peel off the sealing film from sample plate and replace with a clean or new silicon mat.
2. Take one aliquot of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100 μM 21 amino acid standard stock from –80ºC (Sigma, LAA-21), thaw in room-temperature water bath, vortex, and keep on ice.
3. Take used LC vials (water, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100 μM, water) from -20ºC.
4. Add 1 ml of fresh ddH_2O to vial 1 and 11. Transfer amino acid stocks from 0.5 ml tubes to 100 ml LC vial inserts and place the inserts in the designated vials. Keep on ice.
5. Bring samples and 11 standard vials, on ice, to Mass Spec facility with protocols for LC-MS.

Reference:

Jander, G., Norris, S.R., Joshi, V., Fraga, M., Rugg, A., Yu, S.X., Li, L.L., and Last, R.L. 2004. Application of a high-throughput HPLC-MS/MS assay to Arabidopsis mutant screening; evidence that threonine aldolase plays a role in seed nutritional quality. Plant J. 39 (3): 465-475.

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