FASP Kit Protocol-ORNL Developed for Bacteriophage

Nathan VerBerkmoes

Abstract

Collaborator, Nathan VerBerkmoes (Oak Ridge National Labs), worked with Tucson Marine Phage Lab to develop more sensitive proteomics assays for viral isolates which we are now also using for environmental viral concentrates. A new sample prep method (FASP) was optimized to maximize the signal from our commonly low-protein containing samples, and then run using 2d-LC-MS-MS to maximize detection across the large dynamic range in isolate and environmental samples.

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Guidelines

This method was optimized for bacteriophage by Kristen Corrier undergraduate research assistant at Oak Ridge National Laboratory (ORNL), Chemical Science Division using the commercial method from Protein Discovery (Knoxville, TN) as a starting point.

This method was written by Kristen Corrier using the commercial method from Protein Discovery (Knoxville, TN) as a starting point.

Dr. Nathan VerBerkmoes (ORNL, Chemical Science Division) oversaw the optimization process and creation of final method.

This method is designed to prepare small quantities (~1-10µg) of isolate bacteriophage or mixed bacterial/bacteriophage communities for shotgun proteomics via 2d-LC-MS/MS.

Please note this method is not effective for large quantities of material (>100µg), standard solution digest should be used for such samples. The minimum protein amount needed is estimated to be 500ng but this is highly sample dependent.

The main optimization of this method was to simplify it and greatly reduce the time required. This current method takes roughly half the amount of time (actually bench time) as the original commercial method, furthermore its now very straightforward to prepare 8, 16, or 24 samples at a time.

Protocol

Day 1

Step 1.
Prepare Urea/SDS Solution.

PROTOCOL

. Urea/SDS Solution

CONTACT: VERVE Team

Step 1.1.
In falcon tube, add 1 fleck DTT (10mM) to 5mL Tris HCl (provided with FASP kit) or Tris $CaCl_2$. Vortex briefly.

Step 1.2.
Add 1mL Tris buffer + DTT to 1 tube urea (75 µg, provided with FASP kit). Vortex until all powder dissolves.

Step 1.3.
Combine 300µL Urea solution and 150µL sample (sample can be in any form, mixture of viral proteins and bacteria, ionic or non-ionic detergents, buffers and/or CsCl).

Step 1.4.
Rock at room temperature for 30 min to 1 hr to lyse bacteria cells/phage particles.

DURATION
01:00:00

Day 1

Step 2.
Transfer Urea solution + sample to spin filter. Centrifuge at 14,000 x g for 15 min.

DURATION
00:15:00

Day 1

Step 3.
Add 200µL fresh Urea solution (**no DTT, no SDS**). Centrifuge at 14,000 x g for 15 min.

AMOUNT
200 µl Additional info:
DURATION
00:15:00

PROTOCOL

. Urea Solution

CONTACT: VERVE Team

Step 3.1.
1mL Tris $CaCl_2$ or Tris HCl added to one tube of urea. Vortex until all powder dissolves.

Day 1

Step 4.
Discard flow-through.

Day 1

Step 5.
Add 10µL iodoacetamide solution and 90µL Urea solution (no DTT, no SDS). Vortex for 1 min, then incubate without mixing for 20 min in the dark.

DURATION
00:20:00

PROTOCOL

. Iodoacetamide Solution

CONTACT: VERVE Team

Step 5.1.

100µL Urea solution (no DTT, no SDS) added to 1 tube iodoacetamide (provided with FASP kit). Pipette up and down 10-15 times to mix well and dissolve.

Day 1

Step 6.

Centrifuge at 14,000 x g for 10 min.

DURATION

00:10:00

Day 1

Step 7.

Add 100µL Urea solution (no DTT, no SDS). Centrifuge at 14,000 x g for 15 min. Repeat this step twice.

DURATION

00:15:00

Day 1

Step 8.

Discard flow-through.

Day 1

Step 9.

Add 100µL 50mM ammonium bicarbonate solution (provided with FASP kit). Centrifuge at 14,000 x g for 15 min.

DURATION

00:15:00

Day 1

Step 10.

Transfer filter to new collection tube.

Day 1

Step 11.

Add 75µL digestion solution. Vortex briefly. Incubate at 37°C for 4 – 18 hours (NO ROCKING)

AMOUNT

75 µl Additional info:

DURATION

18:00:00

PROTOCOL

. Digestion Solution

CONTACT: VERVE Team

Step 11.1.

75uL ammonium bicarbonate solution added to 20µg trypsin (1 tube).

Day 2

Step 12.

Add 40µL 50mM ammonium bicarbonate solution. Centrifuge at 14,000 x g for 10 min. Repeat this step once.

DURATION
00:10:00

Day 2

Step 13.

Add 50µL 0.5 M sodium chloride solution (provided with FASP kit). Centrifuge at 14,000 x g for 10 min.

DURATION
00:10:00

Day 2

Step 14.

Filtrate contains digested proteins. Add 170µL* H_2O + formic acid. Aliquot (150µL x 2)*, freeze

NOTES

VERVE Team 13 Aug 2015

*Volumes may be adjusted. Final filtrate volume = 130µL

*Generally two replicates can be obtained from one filter

*Sample is now ready for 2d-LC-MS/MS, remember a on-line desalting step is needed before SCX salt pulses start.