AN INTEGRATED GALAXY-DRIVEN WORKFLOW FOR HOST-MICROBIOTA PROTEOMICS AND TARGETED ASSAY DEVELOPMENT TO STUDY HOST-MICROBE DYNAMICS IN CLINICAL CYSTIC FIBROSIS SAMPLES

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Poster #4

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Galaxy for proteomics (Galaxy-P) team
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Learn more at galaxyp.org
z.umn.edu/itcrgalaxyvideo
Background

- Airway microbiota composition correlates with cystic fibrosis (CF) progression, but microbial drivers of disease remain unclear.

- Mass spectrometry (MS)-based metaproteomics of bronchoalveolar lavage fluid (BALF) offers insights into host-microbe dynamics & potential interactions.

- We have developed a MS-based BALF analysis and Galaxy-driven bioinformatics processing of both host and microbial proteins, generating verified host and microbe peptide candidates suitable for targeted analysis within individual patient samples.

- We have utilized this workflow in our ongoing work to identify a promising host and microbe peptide panel for application to CF disease progression studies by comparison to disease control (DC).
Combined analytical and bioinformatic workflow

CF = cystic fibrosis
DC = disease control
CFL/H = cystic fibrosis with low/high microbial diversity
DCL/H = disease control with low/high microbial diversity
Results: 87 stringently filtered microbial peptides
Results: 87 stringently filtered microbial peptides

<table>
<thead>
<tr>
<th>Genus</th>
<th>Pseudomonas</th>
<th>Stenotrophomonas</th>
<th>Staphylococcus</th>
<th>Prevotella</th>
<th>Fusobacterium</th>
<th>Streptococcus</th>
<th>Sphingomonas</th>
<th>Moraxella</th>
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</thead>
<tbody>
<tr>
<td>CF/DC Ratio</td>
<td>109.7</td>
<td>49.0</td>
<td>21.1</td>
<td>16.0</td>
<td>8.8</td>
<td>5.8</td>
<td>4.5</td>
<td>0.001</td>
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<tr>
<td>Peptides Detected</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Function</td>
<td>Type II secretion system</td>
<td>SGL protein</td>
<td>Eap/MAP</td>
<td>ISS family transposase</td>
<td>Penicillin-binding prot.</td>
<td>LKG protein</td>
<td>Uncharacterized protein</td>
<td>Outer membrane prot.</td>
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Microbe MS Intensity in CF vs. DC

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What about the human proteins?

STRING-Db Pathway

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<tbody>
<tr>
<td>CF/DC Ratio (MS Intensity)</td>
<td>196.54</td>
<td>13.99</td>
<td>6.62</td>
<td>5.69</td>
<td>4.56</td>
<td>0.25</td>
<td>0.16</td>
<td>0.12</td>
<td>0.07</td>
<td>0.00</td>
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<tr>
<td>Protein Examples</td>
<td>Deoxyribonuclease-1</td>
<td>Dynein light chain Tctex-type 1</td>
<td>Protein S100-A8</td>
<td>Isoform 3 of Phosphorylase b kinase regulatory subunit beta</td>
<td>Neutrophil elastase</td>
<td>Guanylate-binding protein 2</td>
<td>Calcium-activated chloride channel regulator 1</td>
<td>Apolipoprotein E</td>
<td>Rootletin</td>
<td>Ankyrin-3</td>
</tr>
<tr>
<td>Pendrin</td>
<td>Intraflagellar transport protein 56</td>
<td>Neutrophil collagenase</td>
<td>Serine/threonine-protein phosphatase PP1-beta catalytic subunit</td>
<td>Azurocidin</td>
<td>Ubiquitin/ISG15-conjugating enzyme E2 LG</td>
<td>Chloride intracellular channel protein 3</td>
<td>Serum paraoxonase/arylesterase class 1</td>
<td>Ciliary rootlet coiled-coil protein 2</td>
<td>Cingulin</td>
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What’s next?

- Validate microbial and human peptides from proteins of interest via targeted mass spectrometry methods (Parallel reaction monitoring, PRM)
- Demonstrate the ability to detect and quantify microbial and host peptides in individual samples, as an assay for characterizing bacterial and human dynamics in CF

Interested in proteomics/metaproteomics in Galaxy?

“Introduction to Metaproteomics” training session,

1:20 pm today, Monday