Supplemental File 2

MIAPE: Gel Informatics

Version 1.0, July, 2009

Reporting requirements for gel informatics data

1. General features

1.1 Date stamp: 2013-03-26

1.2 Responsible person or institutional role: Todd M. Umstead, Senior Research Support Associate, Penn State Center for Host defense, Inflammation, and Lung Disease (CHILD) Research, Department of Pediatrics, P.O. Box 850, Hershey, PA 17033

1.3 Electrophoresis type: 2D-DIGE with PAGE electrophoresis

1.4 Electrophoresis context: Differences in the Mouse Alveolar Macrophage (mAM) proteome for vehicle treated SP-A (-/-) (knockout) male mice (KOM+VEH), vehicle treated SP-A (-/-) (knockout) female mice (KOF+VEH), KO male mice treated with 10 μg SP-A1 (KOM+SPA1), KO female mice treated with 10 μg SP-A1 (KOF+SPA1), KO male mice treated with 10 μg SP-A2 (KOM+SPA2), KO female mice treated with 10 μg SP-A2 (KOF+SPA2), KO male mice treated with 5 μg SP-A1 and 5 μg SP-A2 (KOM+5SPA1/2), KO female mice treated with 5 μg SP-A1 and 5 μg SP-A2 (KOF+5SPA1/2), KO male mice treated with 10 μg SP-A1 and 10 μg SP-A2 (KOM+10SPA1/2), KO female mice treated with 10 μg SP-A1 and 10 μg SP-A2 (KOF+10SPA1/2), vehicle treated hTG SP-A2 male mice (SPA2M+VEH), vehicle treated hTG SP-A2 female mice (SPA2F+VEH), hTG SP-A2 male mice treated with 10 μg SP-A1 (SPA2M+SPA1), hTG SP-A2 female mice treated with 10 μg SP-A1 (SPA2F+SPA1), vehicle treated wild-type male mice (WTM+VEH), and vehicle treated wild-type female mice (WTF+VEH)

1.5 Image(s): Available upon request

1.6 Image analysis software: Progenesis SameSpots v4.0 (Nonlinear Dynamics)

1.7 Statistical analysis software: Progenesis SameSpots v4.0 (Nonlinear Dynamics), Excel (Microsoft)

2. Gel analysis design

2.1 Type: Directed

2.2 Replicates: n=4 per group

2.3 Groups: 16 groups (vehicle treated SP-A (-/-) (knockout) male mice, vehicle treated SP-A (-/-) (knockout) female mice, KO male mice treated with 10 μg SP-A1, KO female mice treated with 10 μg SP-A1, KO male mice treated with 10 μg SP-A2, KO female mice treated with 10 μg SP-A2, KO male mice treated with 5 μg SP-A1 and 5 μg SP-A2, KO female mice treated with 5 μg SP-A1 and 5 μg SP-A2, KO male mice treated with 10 μg SP-A1 and 10 μg SP-A2, KO female mice treated with 10 μg SP-A1 and 10 μg SP-A2, vehicle treated hTG SP-A2 male mice, vehicle treated hTG SP-A2 female mice, hTG SP-A2 male mice treated with 10 μg SP-A1, hTG SP-A2 female mice treated with 10 μg SP-A1, vehicle treated wild-type male mice, and vehicle treated wild-type female mice)

2.4 Internal standard: Cy2 normalization pool of equal amount of protein from all study samples run on each analytical gel

2.5 External standard: Cy3/Cy5 counterbalancing to eliminate dye-based artefacts

3. Image preparation

3.1 Software: ImageQuant TL (GE)

3.2 Preparation steps: Images obtained using the Typhoon 9410 Variable Mode Imager (GE Healthcare) in the GEL file format (.gel)

3.2.1 Analytical (quantitative) gels: Laser voltages were optimized for each fluorophore prior to scanning to avoid signal saturation. Identical laser settings were then used to scan each gel

3.2.2 Preparative/picking gels: Fixed with ethanol/acetic acid and post-stained with Deep Purple Total Protein Stain (GE), scanned independently from analytical gels; or silver stained using SilverQuest Silver Stain Kit (Life Technologies) and photographed using a Nikon CoolPix 4500 digital camera

3.2.3 All gels were scanned at 100 μm resolution

3.2.4 See MIAPE Gel Electrophoresis supplement for more specific details of image collection

4. Image analysis pre-processing

4.1 Input image(s): Images obtained using the Typhoon 9410 Variable Mode Imager (GE) in the GEL file format (.gel)
4.2 Software*:* ImageQuant TL (GE)
4.3 Processing steps: See image preparation above

5. Data extraction process

5.1 Input image(s): Images obtained using the Typhoon 9410 Variable Mode Imager (GE) in the GEL file format (.gel) and are available upon request
5.2 Image quality control: Image QC done using Progenesis SameSpots v4.0 (Nonlinear Dynamics) to check images for bit depth, color, manipulation prior to analysis, proper file type, saturation, low dynamic range, and stretched contrast
5.3 Image alignment
  5.3.1 Automatic gel alignment using Progenesis SameSpots v4.0 (Nonlinear Dynamics) to allow for more accurate spot matching
  5.3.2 Gel alignment manually edited following automated alignment protocols
5.4 Feature detection
  5.4.1 Automatic spot detection using Progenesis SameSpots v4.0 (Nonlinear Dynamics)
  5.4.2 Features were manually edited following automated spot detection protocols
5.5 Matching
  5.5.1 Algorithm: Progenesis SameSpots v4.0 (Nonlinear Dynamics)
  5.5.2 Reference image(s) used: 13779 Standard Cy2 aligned
  5.5.3 Landmarks: Vectors were automatically and manually placed
  5.5.4 Match editing: Automatic spot matching using Progenesis SameSpots v4.0 (Nonlinear Dynamics) followed by manual edited to confirm matches
  5.5.5 One-hundred percent spot matching across all gels without missing values was set as a requirement for spot inclusion for data analysis
5.6 Feature quantitation
  5.6.1 Type: Normalized Volume
  5.6.2 Quantitation: Progenesis SameSpots v4.0 (Nonlinear Dynamics)
  5.6.3 Background subtraction: N/A
  5.6.4 Normalization: Progenesis SameSpots v4.0 (Nonlinear Dynamics)

6. Data analysis

6.1 Analysis intent: Features with ANOVA ($p<0.05$) and/or t-test ($p<0.05$) Software: Progenesis SameSpots v4.0 (Nonlinear Dynamics), Excel (Microsoft)
6.2 Type: ANOVA with false-discovery rates based on Progenesis assigned q-value, t-test, principal component analysis (PCA), power calculations, dendrogram
6.3 Parameters: Not blinded
6.4 Input data: Normalized volume (Cy3/Cy2 and Cy5/Cy2)

7. Data reporting

7.1 List of image features: Excel file available upon request
7.2 List of matches: Excel file available upon request
7.3 Description of analysis results: Excel file available upon request