MIAPE: Gel Electrophoresis

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This module identifies guidelines for the minimum information to report about the use of *n*-dimensional gel electrophoresis in a proteomics experiment, in a manner compliant with the aims as laid out in the 'MIAPE Principles' document (latest version available from http://psidev.sf.net/miape/).

Introduction

Gel electrophoresis facilitates the separation of protein (or peptide) mixtures. These separations are effected in a gel matrix under the application of an electric field. Proteins with differing physical or chemical characteristics migrate at different speeds through the matrix and may become focused (*i.e.* cease to migrate) depending on the parameters of the gel matrix and applied electric field. Selecting particular physico-chemical properties for the matrix, chemically modifying the proteins themselves, or solubilising them with a detergent allows the separation to be further tuned. Electrophoresing a protein mixture along a single axis, on the basis of a single characteristic such as molecular weight, results in a one-dimensional separation. Higher-dimensional separations usually separate by different characteristics (for example, charge and mass) along orthogonal axes.

This document is a single module, as part of the MIAPE (Minimum Information about a Proteomics Experiment) family of reporting guidelines, produced by the Proteomics Standards Initiative. MIAPE is structured around a parent document that lays out the principles of a reporting guideline [1]. In brief, a MIAPE module represents the minimum information that should be reported about a dataset to allow a reader to interpret and critically evaluate the conclusions reached, and to support their experimental corroboration. In practice a MIAPE module comprises a checklist of information that should be provided (for example about the protocols employed) when a data set is submitted to a public repository. The MIAPE modules specify neither the format that data should be transferred in, nor the structure of the repository. However, PSI is not developing the modules in isolation; several compatible data exchange standards are now well established and supported by public databases. MIAPE Gel Electrophoresis (GE) will be implemented by public repositories such as PRIDE, Swiss2DPage and Gelbank and the PSI's GelML data format is designed to support MIAPE GE submission (for details see the PSI website www.psidev.info).

The guidelines for the gel electrophoresis family of techniques is prescriptive in some respects while maintaining flexibility, allowing the description of a wide range of protocols. For a full discussion of the

principles underlying this specification, please refer to the MIAPE 'Principles' document, which can be found on the MIAPE website (http://psidev.sf.net/miape/).

These reporting guidelines cover gel manufacture and preparation, running conditions, visualization techniques such as staining, the method of image capture and a technical description of the image obtained. They do not explicitly cover sample preparation, but do require the recording of which samples were loaded onto a gel. They do not include spot detection or other analyses of gel images, nor do they include protein identification procedures. Items falling outside the scope of this module may be captured in complementary modules, which can be obtained from the MIAPE website[†]. Note that subsequent versions of this document may evolve over time as will almost certainly be the case for all the MIAPE modules.

The following section, detailing the reporting requirements for the use of gel electrophoresis, is subdivided as follows:

1. General features
2. Sample
3. Gel matrix and electrophoresis
4. Inter-dimension process
5. Detection
6. Image acquisition
7. Image

The glossary provides a definition for each checklist item in the MIAPE: Gel Electrophoresis guidelines. Examples are given only to facilitate interpretation and are not intended to be a comprehensive list of the technologies that can or cannot be recorded under each section heading.

Reporting requirements for gel electrophoresis

1. General features
    1.1.1 Date stamp (as *yyyy-mm-dd*)
    1.1.2 Responsible person or institutional role
    1.1.3 Electrophoresis type

2. Sample
    2.1.1 Sample name(s)
    2.1.2 Loading buffer

3. Gel matrix and electrophoresis
There should be one description for each dimension. Each dimension must have a description for sections 3.1, 3.2 and 3.3. If a gel is composed from two or more matrices (e.g. stacking gel and resolving gel), each matrix must have a section 3.2 description.
3.1. Dimension details
    3.1.1 Ordinal number for this dimension
    3.1.2 Separation method employed
3.2 Gel matrix
    3.2.1 Description of gel matrix
    3.2.2 Gel manufacturer
    3.2.3 Physical dimensions
    3.2.4 The physicochemical property range and distribution (as appropriate)

[†] http://psidev.sf.net/gps/miape/

4. Inter-dimension process (not applicable for one-dimensional gel electrophoresis)
This section is used to record any process or processes applied to, or carried out between the dimensions described in section 3.
4.1 Inter-dimension process

      4.1.1 Step name

      4.1.2 Inter-Dimension buffer

      4.1.3 Additional reagents

      4.1.4 Equipment

      4.1.5 Protocol

5. Detection (if applicable)
If detection is carried out there should be a description for the appropriate direct (5.1) or indirect (5.2) detection method.
5.1 Direct detection

      5.1.1 Name of direct detection process

      5.1.2 Direct detection agents

      5.1.3 Additional reagents and buffers

      5.1.4 Equipment

      5.1.5 Direct detection protocol

5.2 Indirect detection

      5.2.1 Name of indirect detection process

      5.2.2 Transfer medium

      5.2.3 Detection medium

      5.2.4 Indirect detection agents

      5.2.5 Additional reagents and buffers

      5.2.6 Equipment

      5.2.7 Indirect detection protocol

6. Image acquisition (if applicable)
6.1 Acquisition equipment

      6.1.1 Type of equipment

      6.1.2 Name of equipment

      6.1.3 Software

      6.1.4 Calibration (if appropriate)

      6.1.5 Equipment specific parameters

6.2 Acquisition protocol

      6.2.1 Image acquisition process

      6.2.2 Reference to gel matrix

7. Image (as a result of section 6)

      7.1.1 Image name (or id)

      7.1.2 Dimensions

      7.1.3 Resolution

      7.1.4 Bit depth

      7.1.5 Image location

7.1.6 Standard image orientation

Summary

The MIAPE: Gel Electrophoresis minimum reporting guidelines for the use of n-dimensional gel electrophoresis specify that a significant degree of detail be captured about the gel, running conditions, visualization procedures and the acquisition of raw image data. However, it is clear that providing the information required by this document will enable the effective interpretation and assessment of gel electrophoresis data and metadata and potentially, support experimental corroboration. Much of the information required herein may already be stored in an electronic format, or exportable from instrumentation; we anticipate further automation of this process.

These guidelines will evolve. To contribute, or to track the process to remain 'MIAPE compliant', browse to the website at http://psidev.sf.net/miape

Appendix One. The MIAPE: Gel Electrophoresis glossary of required items.

Classification	Definition
1. General features	
1.1.1 Date stamp	The date on which the work described was initiated; given in the standard 'YYYY-MM-DD' format (with hyphens).
1.1.2 Responsible person or role	The (stable) primary contact person for this data set; this could be the experimenter, lab head, line manager *etc.*. Where responsibility rests with an institutional role (*e.g.* one of a number of duty officers) rather than a person, give the official name of the role rather than any one person. In all cases give affiliation and stable contact information, which consists of (*i*) Name, (*ii*) Postal address and (*iii*) Email address.
1.1.3 Electrophoresis type	The gel electrophoresis type; e.g. two-dimensional or one-dimensional.
2. Sample - The sample preparation should be documented in a MIAPE compliant manner and referenced from here.	
2.1.1 Sample name(s)	Name of sample(s) including any label, marker or tag applied that will be used for protein detection, such as radiolabels or fluorescent labels (by name only). From the sample described above identify control, standard and test samples.
2.1.2 Loading buffer	The components, with concentrations (excluding the sample) of the loading buffer that is to be loaded onto the gel matrix. In case of more than one loading buffer for the same dimension, describe one loading buffer for each sample.
3. Gel matrix and electrophoresis protocol — 3.1 Dimension details	
3.1.1 Ordinal number for this dimension	If this is a one-dimensional gel then the dimension is "First". For a two-dimensional gel there will be descriptions for the first dimension gel matrix "First" and the second dimension gel matrix "Second".
3.1.2 Separation method employed	Name of the separation technique employed for this particular dimension; *e.g.* isoelectric focusing (IEF), Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Non-equilibrium pH gradient electrophoresis (NEpHGE).
3. Gel matrix and electrophoresis — 3.2 Gel matrix	

3.2.1 Description of gel matrix	Gel matrix being used for this dimension. Include the descriptive name of the matrix (*e.g.* IPG strip, slab gel) and the type of the matrix used for this dimension (*e.g.* a native gel, denaturing gel, gradient gel, *etc.*). State whether the matrix is composed of more than one kind of gel and name the parts (*e.g.* stacking gel). Give the dimensions of the matrix and associated parts, under the physical dimension section, below.
3.2.2. Gel manufacture	If the gel was purchased pre-cast, then include the model name, model number, batch number and manufacturer. If the gel has been manufactured 'in house' then a reference to published protocol should be given. If no published protocol is available a recipe should be given.
3.2.3 Physical dimensions	The physical dimensions of the gel matrix and of any sub-matrices described in section 3.2.1. The measurements must be in the form of the Cartesian Coordinate system (x,y,z). According to the standard image orientation described in section 7.1.6, x represents the distance from the anode (+) to the cathode (−). For example in an IPG strip x = the strip length, for a standard slab gel, x = the width. z = the matrix depth.
3.2.4 Physicochemical property range and distribution	As applicable, the details of the pH distribution of the matrix, including the overall pH range of the gel, if known. Details of the molecular weight distribution of matrix with appropriate measurement unit. Examples include linear pH 4-7, logarithmic apparent molecular mass 200-10 kDa.
3.2.5 Acrylamide concentration	The acrylamide concentration of the gel, or each matrix as described in section 3.2.1. In the form of a single percentage (1%) or gradient (1-2%). For gradients include the gradient distribution if appropriate, (e.g. fixed, stepped or linear).
3.2.6 Acrylamide : Crosslinker ratio	The acrylamide to crosslinker ratio of the gel, or parts described in 3.2.1 (Name of crosslinker and ratio described as "acrylamide : crosslinker").
3.2.7 Additional substances in gel	The details of any additional substances or reagents contained within the gel (not given in section 3.2.2), recording the concentration or molarities for each (and pH if appropriate).
3.2.8 Gel lane	The number of lanes on the gel matrix.
3.2.9 Sample application	Description of the sample (referenced from section 2.1.1) as applied to the matrix, giving: 1. Name of sample (reference 2.1.1) 2. Volume of loading buffer (reference 2.1.2) 3. Sample loaded per lane (if applicable for quantification, in SI measurement) 4. Lane designation (as defined in section 3.2.8) Include a description of how the sample was applied to the matrix; *e.g.* well loading, rehydration loading, cup loading *etc.* If the sample is applied using the cup-loading method also state the application point as either anode or cathode.

3. Gel matrix and electrophoresis — 3.3 Protocol. If the protocol is a MIAPE compliant published protocol then provide a reference to the appropriate protocol(s). If no published protocol is available then record the running conditions as outlined	
3.3.1 Buffer	Description of the running buffers used in terms of name of buffer, components with concentrations. State if this is the anode or cathode buffer (if appropriate).
3.3.2 Electrophoresis conditions	The running conditions applied to the gel described in 3.2. To be given in terms of voltages versus time/kilovolt hours, (or appropriate measurements) and temperature. Include voltage mode profile (step and hold, or gradient).
4. Inter-dimension Process — 4.1 Protocol (not applicable for one-dimensional gel electrophoresis). This section is used to record any process or processes applied to, or carried out between the dimensions described in section 3. This includes processes such as equilibration, reduction and alkylation. If the protocol is MIAPE compliant and published then provide a reference to the appropriate protocol(s) in the standard manner. If no published protocol is available then record the running conditions as outlined	
4.1.1 Step name	A descriptive name of the steps involved in the inter-dimension process. For example, equilibration, or reduction and alkylation.
4.1.2 Inter-dimension buffer	The details of the buffer should be recorded with name, components and concentrations.
4.1.3 Additional reagents	Any additional reagents used should be recorded with name, components, and concentrations. For example, reduction and alkylation agents.
4.1.4 Equipment	Record the Model Name, Model Number and Manufacturer for specialised equipment (note that equipment such as glassware and shakers should not be included unless deemed integral to the result).
4.1.5 Protocol	For the steps named above including, duration and temperature, if appropriate.
5. Detection (if applicable) — this section documents the process and the methods employed to allow the proteins which have been separated along the matrix, or chemical groups on such proteins, to be detected. .This can involve such process as; staining the proteins on the gel (direct detection), exposing the gel which contains radiolabel sample to photographic film, or transfer of proteins to a matrix such as in immunoblotting (indirect detection). If detection is carried out there should be a description for the appropriate direct 5.1 or indirect detection method 5.2.	
5. Detection — 5.1 Direct detection	
5.1.1 Name of direct detection process	The detection process applied to the gel; for example, visible staining or fluorescent staining.

5.1.2 Direct detection agents	The detection agents employed for the described process above. Such as the type of stain employed or the type of radiolabel. Give model and manufacturer, if appropriate, else describe origin. If any antibodies are used also describe them in terms of polyclonal, monoclonal, species, target and specificity. Identify antibodies as primary/secondary *etc.*. The amount of detection agent employed during the detection protocol. In terms of volume and concentrations (if appropriate).
5.1.3 Additional reagents and buffers	Additional reagents used other than the detection agent described above; components with concentrations.
5.1.4 Equipment	Record the Model Name, Model Number and Manufacturer for specialised equipment (note that equipment such as glassware and shakers should not be included unless deemed integral to the result).
5.1.5 Direct detection protocol	Outline of the direct detection protocol, including, duration and temperature, if appropriate. If the protocol is a MIAPE compliant and published protocol then provide a reference the appropriate protocol(s).
5. Detection — 5.2 Indirect detection	
5.2.1 Name of indirect detection process	The indirect detection process applied to the gel. Such as immunoblot, exposure to photographic film.
5.2.2 Transfer medium	If the proteins separated along the gel are transferred to another medium to aid detection (such as a nitrocellulose or PVDF membrane, as used in immunoblots) then give details of the name and type of medium, models and manufacturers, where appropriate.
5.2.3 Detection medium	If the proteins on the gel matrix must be exposed to another medium to permit detection (*e.g.* photographic film for radiolabel samples) then give details of name, model, manufacture, of detection medium.
5.2.4 Indirect detection agents	The indirection detection agents employed for the described process above. Such as the type of stain employed or the type of radiolabel. Give model and manufacturer, if appropriate, else describe origin. If any antibodies are used also describe them in terms of polyclonal, monoclonal, species, target and specificity. Identify antibodies as primary/secondary *etc.*. The amount of detection agent employed during the detection protocol. In terms of volume and concentrations (if appropriate).
5.2.5 Additional reagents and buffers	Additional reagents used other than the detection agent described above; components with concentrations.
5.2.6 Equipment	Record the Model Name, Model Number and Manufacturer for specialised equipment (note that equipment such as glassware and shakers should not be included unless deemed integral to the result).
5.2.7 Indirect detection protocol	Outline of the indirect detection protocol, including, duration and temperature, if appropriate. If the protocol is a MIAPE compliant and published protocol then provide a reference the appropriate protocol(s).

6. Image Acquisition — 6.1 Acquisition Equipment	
6.1.1 Type of equipment	The type of equipment used in image acquisition, (e.g. laser scanner, camera, fluorescent imager)
6.1.2 Name of equipment	Image acquisition equipment. Give model name and manufacturer.
6.1.3 Software	The software used in the acquisition process such as scanning software. Give manufacturer, name and version.
6.1.4 Calibration	Is the equipment calibration Automatic? Value of "Yes" or "No". If "No" describe the calibration processes applied to the image acquisition equipment in section 6.1.5
6.1.5 Equipment specific parameters	Any equipment settings that are necessary to reproduce measurements, such as manual calibration or photomultiplier voltages on imagers, filters used. Include files if appropriate.
6. Image Acquisition — 6.2 Acquisition Protocol	
6.2.1 Image acquisition process	The protocol for image acquisition; *e.g.* exposure times, laser types, wavelengths. If any pre-processing of scanner equipment other than calibration is performed such as noise filters, give details.
6.2.2 Reference to gel matrix	A reference to section 3 to indicate which gel matrix is being used for the image acquisition process
7. Image (The raw image file resulting from section 6, no post-cropped images should be included)	
7.1.1 Image name (or id)	The name or identifier for the image file; include image format (*e.g.* tiff).
7.1.2 Dimensions	The *x*, *y* dimensions of the image (in pixels for a digitized image).
7.1.3 Resolution	The resolution of the digitized image (*e.g.* micrometers per pixel [μm/pixel])
7.1.4 Bit-depth	The bit-depth of the digitized image (*e.g.* 8bit, 16bit).
7.1.5 Image Location	The image file location should be made available when the experiment is published, for example, using a Uniform Resource Identifier (URI) or a Digital Object Identifier (DOI).
7.1.6 Standard image orientation	The orientation of all gels must be high molecular weight at the top, low at the bottom. For gels with lanes, the lane numbers must increase from left to right. For other gels the lowest value of the physicochemical distribution must be on the left with the highest value to the right.

Acknowledgements

References

[1] Taylor CF, et al,. The minimum information about a proteomics experiment (MIAPE). Nat Biotechnol. 2007 Aug;25(8):887-93.