MIRAGE Lectin Microarray Guidelines

Guidelines for reporting lectin microarray analysis

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	Description*
1. Sample: Glycan-containing sample (e.g. glycan, glycoprotein, cell lysate, cell, glycopeptide, etc.)	
Description of Sample	General description of samples (e.g. MIRAGE Sample Preparations Guidelines, DOI: 10.3762/mirage.1)
Sample preparation protocol	General methods for sample preparation. Describe the composition of solutions and the name(s) of any kits used for sample preparation.
Labeling protocol for sample detection	If the sample is directly labeled, describe the method and reagents (including sources) used for chemical labeling of the sample, e.g. fluorescein, biotin, etc.
Two-color reference (if used)	Define the nature of the reference used, if any (e.g. pooled reference of all samples labeled with AlexaFluor 647).
Assay protocol	The protocol used for microarray binding analysis of the sample. Describe the sample concentration used (and that of reference if added), the composition of solutions, time and temperature used for blocking (preventing nonspecific binding), binding, and washing, as well as the application of secondary reagent(s) required for the analyses. If the sample is pre-complexed with secondary detection reagents before adding to the array (e.g. anti-His antibody with his-tagged sample), give the ratio of reactants and pre-complexing time and temperature.
2. Lectin Library	
General description of the lectin library used in the array	Describe the array (custom or commercial). If commercial, provide supplier and model.

List of lectins and/or glycan-binding proteins, their source, concentration, and buffer	Could be provided as a separate table. Original source: Scientific name and abbreviation of organism the lectin was isolated from. Vendor: Name of commercial supplier and catalogue number (if applicable). Natural source (purified) or recombinant: If recombinant proteins are used, specify the source of proteins (e.g. references for protein), which carbohydrate-recognition domain (CRD) is used, and any mutations included in artificial lectins (if applicable). Lectin specificity: Provide a table giving the glycan-binding specificities of the lectins used in the array and relevant references used in designating the specificities of the lectins used (e.g. Bojar et al, ACS Chemical Biology, 2022, (DOI: 10.1021/acschembio.1c00689 or the Handbook of Plant Lectins or other sources).
Modification of lectins	Provide the modification of lectins (e.g. biotin).
3. Immobilization Surface; e.g., Microarray Slide	
Immobilization surface	Describe what type of surface is being used; i.e., microscope-glass slide, microtiter plate, or other formats.
Manufacturer	Provide the name of the manufacturer of coated microscope slides, microtiter plates, or other types of surfaces with a brief description of the product used for immobilization (e.g. catalog number).
Custom preparation of the surface	If the slides were custom prepared/coated, indicate the initial source and description of the starting surface; i.e., glass material, microtiter plate, etc., and the protocol used to prepare the surface before the sample deposition, i.e. streptadivin, etc.
4. Array Production	
Description of Arrayer	Describe the printing robot used to deliver the lectins onto the array surface - provide the name of the manufacturer and model of the instrument. If the instrument is not commercially available, provide sufficient information to indicate the instrument is comparable to a commercially available arrayer.

Lectin deposition	Indicate the approximate volume and number of replicates of each lectin that is deposited on the surface.
Printing conditions	Indicate the composition of the printing solution and the concentration of the lectin in the printing solution (single or more than one concentration). If applicable, the physical conditions reported for array production should include temperature, humidity, reaction time for covalent coupling or adsorption, and post-reaction treatment
Array layout	Specify the array geometry; e.g. single large array, subarrays, microtiter plate, etc. State the number of replicates of each lectin and the total number of printed spots in each array. Please note that the 'detailed lectin map' (physical layout of the lectins on the array) is not required at present. Such data should be retained by researchers for potential future use when new central storage facilities and analysis software are available.
Quality control	Describe methods to test the quality of the array.
5. Detector and Data Processing	
Instrument (scanner)	Describe the instrument for detecting the binding of lectins to the samples.
Instrument settings	The intensities of signals generated from sample binding to individual lectins are a reflection of affinities or avidities of the interactions. Indicate whether the scanner settings (scanning resolution, laser channel, PMT, and scan power) are such that signals are in a linear range of the scanner's detector (no 'saturation of signals') and whether the scanning resolution is adequate for the sizes of sample spots.
Image analysis software	Describe the software used to analyze (quantify) the output scanner image, indicating the name, version, and manufacturer used and any special features active in the software (i.e. data smoothing, normalization, etc.).
Data processing and statistical analysis	Provide details of how data in the table of microarray binding results are generated and calculated, i.e., specific software, normalization method, data selection procedures, and parameters, statistical analysis (including how the data from replicates on the array were handled in the statistical method), transformation algorithm and scaling parameters.

	Please note that the raw scan images and quantitative output files from the scanner software (e.g. proscan or gpr files) are not required at present. Such data should be retained by researchers for potential future use when new central storage facilities and analysis software are available.
6. Lectin Microarray Data Presentation	
Data presentation and interpretation	If software or algorithms are used to interpret processed data, e.g. for motif analysis, information or references to the software used, software version, name of the algorithm, and derived data (if available) should be included. If software or algorithms are not used, methods or criteria used for selecting binders/non-binders should be described.
7. Data Location	
Data Location	Give doi for data or indicate where data is hosted.

*Only items relevant to the experiment need to be completed. Descriptions in the checklist and references cited should complement rather than duplicate the content of the Materials and Methods.

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