MIRAGE Sample Preparation Guidelines

Guidelines for reporting sample preparation descriptors for glycomics experiments

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Classification	Guidelines
1. Sample Origin	
Here "sample" is defined as any carbohydrate, polysaccharide, oligosaccharide or glycoconjugate that originates from any given starting material. The starting material may be a compound, mixture or cell product used to produce the oligosaccharide sample of interest. The source and/or methods used to produce the starting sample material can vary considerably but minimum information that describes its origin is outlined.	
General information	Describe how original starting sample material was generated or where it was obtained. Descriptions may include but are not limited to material produced in the laboratory (e.g. chemically synthesized), acquired from natural sources (e.g. human tissues) or purchased from a commercial manufacturer. In the case of commercial material, provide vendor and applicable item information. Starting material descriptions are further delineated by biologically or chemically derived material.
Biologically derived material	Biologically derived material includes recombinantly expressed proteins, cells, etc. as well as whole organisms or tissues. For recombinantly produced material, the cell type (e.g. CHO, HEK, NS0 etc.) and growth/harvest conditions should be specified. Any modifications to cells that influence the characteristics of the starting material (e.g. genetic manipulations) should also be stated. For material isolated from tissues, the tissue type, treatments and/or storage conditions should be reported. The species should also be provided.
Chemically derived material	Describe how material was generated. If samples were synthetically derived, provide information detailing synthesis steps or specify where the equivalent reaction protocol is available.
Description of starting material	Define the type of starting material used or produced that contains the oligosaccharide to be used/analysed in subsequent experiments. These may include glycoprotein(s), proteoglycan, glycolipid, GPI-anchored, free-oligosaccharides, sugar-nucleotides or synthetically derived material but are not limited to these definitions.

2. Sample Processing	
The sample processing is divided into 1) isolation, 2) modification and 3) purification subcategories. Required information for each is described below.	
2.1 Sample Processing - Isolation	
Processing may include methods to remove the oligosaccharide from the starting material prior to downstream experiments or conversely the starting material may also be altered so the oligosaccharide remains conjugated to non-carbohydrate material such as chemical (e.g. linker) or biological (e.g. peptides) components.	
Enzymatic treatments	Describe any enzymes used to for the purpose of oligosaccharide removal (e.g. PNGase F) or for modification of the starting material (e.g. trypsin protease). Specify where it was obtained (vendor) or for enzymes produced in-house, describe expression and purification procedure. Also state if sample material was treated in-solution or immobilized (SDS-PAGE, PVDF etc.) as well as temperature, duration, volume, enzyme concentration.
Chemical treatments	Define the technique for oligosaccharide release (hydrazinolysis, β-elimination etc.) or other chemical modifications. Provide details of reaction conditions (temperature, duration, volume and chemical concentrations).
2.2 Sample Processing - Modification	
Isolated material may be subject to a wide variety of chemical or enzymatic modifications. The type of modification and reaction protocols should be documented. If new protocols are used provide a thorough description.	
Chemical modifications	Describe any treatments made to the isolated material. Explain the type of modification employed (e.g. hydrolysis, sample tagging (including fluorescent labels), isotopic labelling, permethylation/peracetylation, etc.). Include source of materials, description of kits used, reaction conditions and detailed workflow.
Enzymatic modifications	Document enzyme concentration, supplier, biological source, incubation time and temperature. If novel glycosidase was used, provide information indicating the origin (i.e. species) of the enzyme.
2.3 Sample Processing - Purification	
The processing steps encompass any type of refining or clean-up strategies used to produce the purified final sample product.	
Purification step(s)	Specify all steps used to purify starting material after isolation/modification steps. Examples of procedures include solid phase extraction (SPE), liquid-liquid extraction or other chromatographic methods. For each method describe the all experimental materials (e.g. stationary phase) and methods (e.g. flow rates, fractionation etc.).

3. Defined Sample	
Provide a designation for the final sample in accordance with conventional terminology. Resources for glycoconjugate and oligosaccharide nomenclature are available in Essentials of Glycobiology (Varki, A., Cummins, R., et al. 2009) and Essentials of Carbohydrate Chemistry and Biochemistry (Lindhorst, T.K. 2003).	
Sample name	Name or specify the type of sample material to be analysed or used in other experiments. These may include but are not limited to glycoconjugates, glycosaminoglycans, N- or O-glycans, glycopeptides, glycolipids, monosaccharides, poly- and oligosaccharides