PEFF: A Common Sequence Database Format for Proteomics

Status of this document

This document provides information to the proteomics community about a common sequence database format for proteomics. Distribution is unlimited.

Version Draft 15 - this is a draft of version 1.0

# Abstract

The Human Proteome Organisation (HUPO) Proteomics Standards Initiative (PSI) defines community standards for data representation in proteomics to facilitate data comparison, exchange and verification. This document presents a unified format for protein and nucleotide sequence databases to be used by sequence search engines and other associated tools (spectra library search tools, sequence alignment software, data repositories, etc). This format enables consistent extraction, display and processing of information such as protein/nucleotide sequence database entry identifier, description, taxonomy, etc. across software platforms. It also allows the representation of structural annotations such as post-translational modifications, mutations and other processing events. The proposed format has the form of a flat file that extends the formalism of the individual sequence entries as presented in a FASTA format and that includes a header of meta data to describe relevant information about the database(s) from which the sequence has been obtained (i.e., name, version, etc). The format is named PEFF (PSI Extended FASTA Format). Sequence database providers are encouraged to generate this format as part of their release policy or to provide appropriate converters that can be incorporated into processing tools.

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# Introduction

## Description of the need

One of the main goals of proteomics is to identify and quantify proteins in complex biological samples. This is achieved using mass spectrometry (MS) as a major analytical tool and so-called sequence search engines as the bioinformatics interpretation tool. Sequence search engines aim at matching experimental MS spectra with protein or peptide sequences from a protein or nucleotide sequence database. Typically, protein hits are reported with a database accession code, a description, a taxon, and some additional technical interpretation values such as matching score and sequence coverage reached by the procedure. Thousands of copies of sequence databases are searched by so called sequence search algorithms in proteomics labs all over the world. These algorithms regularly need to download the databases in the available formats; then they extract information like an identifier, taxonomy, description and sometimes other structural information such as alternative splicing variants, structural elements leading to active forms and post-translational modifications in addition to the sequence itself. Most of them need to convert the original format into a vendor-specific format to process the data. Currently available sequence databases are made available as FASTA format [Pearson88] (<http://en.wikipedia.org/wiki/FASTA_format>, <http://www.ncbi.nlm.nih.gov/BLAST/fasta.shtml>) or in other native formats (UniProtKB/Swiss-Prot and UniProtKB/TrEMBL in .dat or even XML for instance [THE\_UNIPROT\_CONSORTIUM1] [APWEILER1]). For the same database, the information might be richer or poorer according to the format. For instance, the current FASTA format does not generally store structural information such as splicing forms, mutations or post-translational modifications. To get access to this, one needs to choose another format, for instance a richer XML format or for UniProtKB the native .dat format (<http://www.expasy.org/sprot/userman.html>).

Mass spectrometry-based peptide identification software tools deliver, in their graphical interfaces or their export formats, protein and peptide hits with information such as a protein accession code, sequence coverage, matching score, taxonomy and description. The same entry identified by different tools is not necessarily displayed in a unique manner, which renders it difficult, if not impossible, to map results between the tools. One reason for this is that these tools do not “parse” and interpret the database content in a consistent manner. In order to create a standardized manner to represent a protein in a search engine result (entry identifier, description, taxonomy, etc), and to enable a consistent link to a protein from third party software, we are proposing a unified format for sequence databases that can be interpreted in a uniform manner by all sequence search software and other associated tools. Converters generated by the database providers or elsewhere have to be made available and maintained for the generation and parsing of these databases.

## Requirements

The main requirements to be fulfilled are:

* The format should allow more than one sequence database to be represented in one flat file.
* The format should require minimal changes to the existing parsers.
* The format should formalize the representation of all non-sequence associated information (identifiers, description, taxonomy, other structural or functional annotation data).
* The format should include meta-information about the database itself (name,version, type of content, etc).
* Controlled vocabularies (CVs) should be pragmatically used for keys and values (i.e. database names, prefixes, entry keys such as NcbiTaxId, Protein/Gene Name).
* The format should be compatible with MIAPE guidelines (<http://www.psidev.info/miape>), for instance MIAPE MSI.

## Issues to be addressed

The main issues to be addressed by the format are:

* Definition lines in FASTA and other formats vary widely for no good reason. This causes problems for end users who want to use these files with protein identification tools. The creators of these tools are faced with a significant challenge of either supporting all of these variations or enabling a user to cope with them.
  + The same database file is variably processed in different search engines. A given database entry leads to variably interpreted identifiers, which renders difficult the mapping of identical entries in different tools (for instance the UniProtKB/Swiss-Prot AC: **P02768** vs. Swiss-Prot ID: ALBU\_HUMAN).
* The same protein (and therefore also primary sequence) in different databases can have very different identifiers (**P02768** in UniProtKB/Swiss-Prot vs gi|113576|sp|P02768.2|ALBU\_HUMAN in NCBI vs IPI:IPI00745872 in IPI).
* The identifier information extracted from the FASTA formats is heterogeneous (gi|113576 vs 113576 vs sp|P02768 vs gi|113576|sp|P02768.2|ALBU\_HUMAN etc). The definition and format description of the identifier should come from the DB provider (documentation).
* Description and availability of taxonomy are also heterogeneous and need to be properly interpreted (Latin names, common names, NCBI TaxID).
* Choice of the description string (variations include full or partial description, including or not taxonomy information, alternative names, truncation at a defined number of characters, etc).
* Because the content of an entry (protein or gene name, description, sequence, PTMs, etc.) might vary, support for versioning is required.
* It should be possible to store more than one sequence database in a single flat file. As identifiers might be identical in two or more “merged” databases, a mechanism should be defined to avoid this.

# Notational Conventions

The key words ‘MUST,” “MUST NOT,” “REQUIRED,” “SHALL,” “SHALL NOT,” “SHOULD,” “SHOULD NOT,” “RECOMMENDED,” “MAY,” and “OPTIONAL” are to be interpreted as described in RFC 2119 [BRADNER1].

# The Format Implementation

## The documentation

The documentation of the format is divided in several documents and files. These files are available from the main format description page on the HUPO-PSI website (<http://www.psidev.info/peff>).

* Main specification document (this document)
* Controlled Vocabulary (CV). It is currently an independent OBO file, but may be merged into the PSI-MS CV before release 1.0.
* Example files.

## Relationship to other specifications

The specification described in this document is not being developed in isolation; indeed, it is designed to be complementary to, and thus used in conjunction with, several existing and emerging models. Related specifications include the following:

1. *MIAPE MSI* (<http://www.psidev.info/miape>) The “Minimum Information About a Proteomics Experiment: Mass Spectrometry Informatics” document identifies the minimum information required to report the use of a mass spectrometry-based peptide and protein identification and characterization experiment. It is expected that the common sequence database format will be used to capture requirements specified in MIAPE MSI. However, the format does not enforce MIAPE compliance itself and may be valid and useful without being fully MIAPE compliant.
2. *mzIdentML* (<http://www.psidev.info/mzidentml>). The mzIdentML specification is being developed by PSI as a standard to capture the output of search engines that assign mass spectra to protein or peptide sequences.

## The common sequence database format description

The format has the form of a text file with two sections, a file header section and a section that contains the individual sequence entries. The two sections MUST be placed in the following order

* Section 1: The file header section.
* Section 2: The individual sequence entries section.

The characters allowed are the set of ASCII characters. A more constrained set of characters can be defined for specific sections of the file.

All lines in the file MUST end with LF (ASCII 10). A CR (ASCII 13) MAY precede the LF and should be ignored by parsers;

Descriptors of the information are defined as CV terms. The CV repository is available in obo format together with a file explicating the use of these terms at <http://psidev.cvs.sourceforge.net/viewvc/psidev/psi/psi-ms/mzML/controlledVocabulary/psi-ms.obo>.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
|  | File header section | | |  |
|  |  |  |  |  |
|  |  | File Description block |  |  |
|  |  | Database description block 1 |  |  |
|  |  | ..  Database description block n |  |  |
|  |  |  |  |  |
|  | Individual sequence entries section | | |  |
|  |  |  |  |  |
|  |  | Sequence Entry1 |  |  |
|  |  | …  Sequence Entry n |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

Figure 1: graphical representation of the PEFF file structure

### Section 1: The file header section

The file header section contains all necessary information to describe and reference the represented sequence database(s). This includes information such as the database(s) name, source, version, size, sequence type, etc. This meta-data section includes mandatory and optional elements.

General format of the file header section.

The file header section contains two types of information blocks: the file description block and the sequence database description block. The file header section MUST start with a file description block that MUST be followed by at least one sequence database description block. All lines in the file header section start with the character # followed by a space (ASCII 32) character.

The format of the file description block is the following:

* The first line of this section is also the first line of the file. It MUST start with “# PEFF”;
* It MAY be followed by one of more general comments lines, which have each the following format:

*# GeneralComment=value* (where the *value* is a string of text)

The format of the sequence database information blocks is the following:

* All lines of a sequence database description block contain one piece of information
* Each piece of information MUST have the following format:

*# key=value* (Note: Space character MUST be Space ASCII 32):

* The element *key* MUST be a CV term. The format of the *value* is defined for each key in the CV;
* The block MUST start with a database line description and follows the following format:
  + *# DbName=value,* (where *value* is the database name)
* The following five *key* elements MUST be present:

Prefix; DbVersion, DbSource, NumberOfEntries, SequenceType.

* Additional key=values pairs are allowed in a sequence description block
* A sequence database information block MUST end with the following separation line:
  + # // (Note: Space character MUST be Space ASCII 32).

One or more sequence description block MUST be present. Each sequence database description block has the following format:

Generic illustration:

# PEFF

# GeneralComment=This is an example comment

# DbName=*value*

# Prefix=*value*

# DbVersion=*value*

# DbSource=*value*

# NumberOfEntries=*value*

# SequenceType=*value*

# *key=value*

# …

# //

### Section 2: The individual sequence entries section

The individual sequence entries section contains the actual sequences, their associated identifiers and additional descriptors. The format is similar to a FASTA format. The informative elements appearing in the FASTA description lines are structured in the below described format. This section MUST immediately follow the file header section.

The format of each individual sequence entry is described below. The individual sequence entries are placed in one single block of individual sequence entries within a file. There MUST NOT be any empty lines between individual sequence entries.

General format of the individual sequence entries. For each sequence entry:

* A sequence entry is composed of a description line and a sequence block line.
* The description line has the following structure:

*>Prefix:DbUniqueId \key=value \key=value …*

* The header line MUST start with *>Prefix:DbUniqueId* where Prefix is the database Prefix, as defined in the database description block, of the corresponding sequence database. This is the unique mandatory information of the description line.
* The description line MAY include optional information, separated by at least one space character (Space ASCII 32), each of them described as *\key=value* pairs
  + The order of the *\key=value* pairs is not important.
  + The element *key* is a CV term. The format of the *value* is defined for each key in the CV repository.
  + The *value* can contain a list of items. In that case items are formatted under brackets: *(item1) (item2) …*
    - *Generic example: \key=(item1)(item2)*
  + In case *item* contains multiple components, “|” (pipe character) MUST be used as separator between components. In that case the item has the form *(component|component)*
  + Characters allowed for a key: Key: [A-Za-z0-9\_]+; Use CamelCase .
* The description line MUST contain only a single *>Prefix:DbUniqueId \key=value* block. Some FASTA files such as the NCBI non-redundant (nr) database have been seen to have multiple headers per sequence separated by delimiter ASCII 001 (CTRL+A). It has been decided that PEFF does not support this and readers therefore do not need to support this.
* The sequence block contains the actual sequence, coded as one-letter code for both protein and nucleotide sequences. Allowed characters follow the DDBJ/EMBL/GenBank Feature Table Definition, version 8 Oct2008 [DDBJ01, EMBL01].
* The sequence block MAY be a single long line with only a single line ending. When the database should be used with tools that do not handle long lines, we suggest to wrap the sequences.
* There MUST NOT be any blank lines in the individual sequence entries section.

**Generic illustration:**

>*Prefix:DbUniqueID1 \key=value \key=value*

SEQUENCESEQUENCE

>*Prefix:DbUniqueID2 \key=value \key=value*

SEQUENCESEQSEQUENCE

**Real example:**

>nxp:NX\_Q06418-1 \DbUniqueId=NX\_Q06418-1 \Pname=Tyrosine-protein kinase receptor TYRO3 isoform Iso 1 \Gname=TYRO3 \NcbiTaxId=9606 \TaxName=Homo Sapiens \Length=890 \SV=135 \EV=357 \PE=1 \ModResPsi=(681|681|MOD:00048|O4'-phospho-L-tyrosine)(685|685|MOD:00048|O4'-phospho-L-tyrosine)(686|686|MOD:00048|O4'-phospho-L-tyrosine)(804|804|MOD:00048|O4'-phospho-L-tyrosine) \ModRes=(63|63||N-linked (GlcNAc...))(191|191||N-linked (GlcNAc...))(230|230||N-linked (GlcNAc...))(240|240||N-linked (GlcNAc...))(293|293||N-linked (GlcNAc...))(366|366||N-linked (GlcNAc...))(380|380||N-linked (GlcNAc...))(64|64||Disulfide)(117|117||Disulfide)(160|160||Disulfide)(203|203||Disulfide) \SimpleVariant=(21|L)(68|R)(74|M)(85|K)(90|H)(95|G)(114|G)(119|E)(119|L)(129|R)(144|K)(156|S)(178|M)(185|S)(187|L)(200|I)(208|P)(210|D)(215|H)(228|S)(235|R)(240|I)(251|S)(260|L)(265|D)(273|G)(277|L)(283|Y)(290|S)(299|H)(302|S)(302|K)(303|V)(306|S)(311|H)(314|L)(331|T)(333|C)(333|H)(346|N)(348|K)(351|S)(352|D)(353|S)(371|D)(392|I)(396|I)(399|T)(416|C)(433|F)(445|S)(452|Q)(455|Q)(455|W)(468|V)(470|Q)(487|K)(489|K)(511|M)(521|S)(522|Q)(523|L)(533|Q)(542|S)(545|G)(549|G)(566|F)(567|G)(580|L)(590|N)(596|R)(600|I)(605|L)(619|Q)(623|K)(635|L)(638|N)(647|R)(648|F)(659|W)(669|L)(675|R)(690|R)(705|V)(717|T)(719|R)(723|C)(723|L)(728|C)(734|S)(750|C)(756|Q)(759|D)(773|S)(776|L)(777|A)(785|K)(788|S)(797|F)(815|V)(817|D)(819|M)(824|G)(829|N)(831|T)(833|N)(842|D)(169|I)(343|K)(620|T)(819|Q)(848|W)(875|R) \Processed=(1|40|signal peptide)(41|890|mature protein)

MALRRSMGRPGLPPLPLPPPPRLGLLLAALASLLLPESAAAGLKLMGAPVKLTVSQGQPV

KLNCSVEGMEEPDIQWVKDGAVVQNLDQLYIPVSEQHWIGFLSLKSVERSDAGRYWCQVE

DGGETEISQPVWLTVEGVPFFTVEPKDLAVPPNAPFQLSCEAVGPPEPVTIVWWRGTTKI

GGPAPSPSVLNVTGVTQSTMFSCEAHNLKGLASSRTATVHLQALPAAPFNITVTKLSSSN

ASVAWMPGADGRALLQSCTVQVTQAPGGWEVLAVVVPVPPFTCLLRDLVPATNYSLRVRC

ANALGPSPYADWVPFQTKGLAPASAPQNLHAIRTDSGLILEWEEVIPEAPLEGPLGPYKL

SWVQDNGTQDELTVEGTRANLTGWDPQKDLIVRVCVSNAVGCGPWSQPLVVSSHDRAGQQ

GPPHSRTSWVPVVLGVLTALVTAAALALILLRKRRKETRFGQAFDSVMARGEPAVHFRAA

RSFNRERPERIEATLDSLGISDELKEKLEDVLIPEQQFTLGRMLGKGEFGSVREAQLKQE

DGSFVKVAVKMLKADIIASSDIEEFLREAACMKEFDHPHVAKLVGVSLRSRAKGRLPIPM

VILPFMKHGDLHAFLLASRIGENPFNLPLQTLIRFMVDIACGMEYLSSRNFIHRDLAARN

CMLAEDMTVCVADFGLSRKIYSGDYYRQGCASKLPVKWLALESLADNLYTVQSDVWAFGV

TMWEIMTRGQTPYAGIENAEIYNYLIGGNRLKQPPECMEDVYDLMYQCWSADPKQRPSFT

CLRMELENILGQLSVLSASQDPLYINIERAEEPTAGGSLELPGRDQPYSGAGDGSGMGAV

GGTPSDCRYILTPGGLAEQPGQAEHQPESPLNETQRLLLLQQGLLPHSSC

### Most complex header keys

The header keys that may be used are dictated by the CV file. The addition of a new key term merely requires an addition to the CV file. If the purpose and format of the value for the term can be fully described in the CV, there is no need to further describe them in this section. However, some terms are sufficiently complex and central to the format that they are described in detail in this document.

### variant header key

The header key “variant” was deprecated in 2015 during final development of the format in favor of “simpleVariant” and “complexVariant”. Some PEFF files, e.g. from neXtProt, were produced with the “variant” header key before it was deprecated. This term should no longer be used.

### SimpleVariant header key

The header key “SimpleVariant” is used to encode all single-amino acid substitutions. The format of this term is (offset|newAminoAcid), e.g. “(223|A)”. This example indicates that at position 233 (count starting at 1) the default amino acid in the sequence may be substituted with the amino acid A. The offset MUST be equal to or greater than 1, and MUST be equal to or less than the length of the protein. This key may not be used to extend a protein. The “newAminoAcid” part of the value must be a valid amino acid code (ambiguity codes such as J or X are permitted) or an asterisk (\*). It may not be empty, or space, or any non-alphabetic character except asterisk. The asterisk is to be interpreted as a nonsense mutation over which a peptide sequence may not span. Regular expressions may not be used. Insertions or deletions (indels) MUST not be specified with this term. The rationale for separating these variants into a separate term from more complex variants is to more easily allow reader software and sequence search engines to support these simple variations in advance of more complex variations, which are considerably more difficult to implement.

### ComplexVariant header key

The header key “ComplexVariant” is used to encode all sequence variations more complex than a single-amino acid substitution. The format of this term is (startOffset|endOffset|newSequence). Variations that can fit the description of a SimpleVariant MUST NOT be encoded using this term. See the table below for a series of examples, both legal and illegal. Offset counting begins with 1.

|  |  |
| --- | --- |
| Example Value | Interpretation |
| (100|100|) | Position 100 may be nothing, signifying a single amino acid deletion |
| (100|102|) | A 3-AA deletion starting at position 100 |
| (100|100|AP) | A replacement of the original residue by AP. It represents X -> AP, where X can be any residue. |
| (100|100|A) | Not a legal ComplexVariant. This must be encoded as a simpleVariant. |
| (100|102|KPA) | A 3-AA substitution as a cassette. If the AAs can be substituted individually, then they should be encoded as 3 separate SimpleVariants. |
| (100|101|P) | A deletion and substitution. AAs at position 100 and 101 are both removed and replaced with a single P. Neither position was originally a P. If either position already had a P, then either (100|100|) or (101|101|) should be used. |
| (100|100|[AEQ]P) | An insertion before the P originally at position 100 with any of A or E or Q. |
| (100|100|[AEQ]) | Not a legal ComplexVariant. This must be encoded as three separate SimpleVariants. Any variants that can be encoded as a SimpleVariant must be encoded with SimpleVariant. |

### ModResPsi header key

The header key “ModResPsi” is used to encode mass modifications on amino acids (residues) using the PSI-MOD controlled vocabulary. Two other terms (ModResUnimod and ModRes) are used for other controlled vocabularies. The format of this term is (startOffset|endOffset|accession|name). See the table below for a series of examples, both legal and illegal. Offset counting begins with 1. Note that the ModResPsi CV entry encodes the amino acid that is modified, but it is possible that the amino acid referenced in the CV is not found at that position in the protein sequence. In cases where the position has a sequence variant, only the form which has the referenced residue should be potentially modified. Other forms are not modified. If the specified position cannot take on the specific amino acid in its default or variant form, this is an error in the file.

|  |  |
| --- | --- |
| Example Value | Interpretation |
| (100|100|MOD:00046|O-phospho-L-serine) | Potential phosphorylation of a serine at position 100 |
| (100|100||O-phospho-L-serine) | Not legal. The MOD:00046 accession must be provided |
| (100|100|MOD:00046|) | Not legal. The full name from the OBO file (or equivalent) must be provided |
| (100|102|MOD:00???|name) | Example of a multi-residue mass modification |

### ModResUnimod header key

The header key “ModResUnimod” is used to encode mass modifications on amino acids (residues) using the Unimod controlled vocabulary. Two other terms (ModResPsi and ModRes) are used for other controlled vocabularies. The format of this term is (startOffset|endOffset|accession|name). See the table below for a series of examples, both legal and illegal. Offset counting begins with 1. Note that a Unimod CV entry encodes a list of possible amino acids (or termini) that can be modified, but it is possible that the amino acid found at the specified position in the protein sequence (either by default or due to a variant) is not an allowed amino acid. In cases where the position has a sequence variant, only the form(s) which has an allowed residue ought to be potentially modified, although individual implementations may choose a different behavior. The specified modification name should be the one found in the “name:” field in the OBO file.

|  |  |
| --- | --- |
| Example Value | Interpretation |
| (100|100|UNIMOD:21|Phospho) | Potential phosphorylation on position 100 |
| (100|100||Phospho) | Not legal. The UNIMOD:nn accession must be provided |
| (100|100|UNIMOD:21|) | Not legal. The full name from the OBO file (or equivalent) must be provided |
| (100|102|MOD:00015|name) | Example of a multi-residue mass modification |

### ModRes header key

The header key “ModRes” is used to encode mass modifications on amino acids (residues) where a controlled vocabulary entry in neither PSI-MOD nor Unimod is available. Two other terms (ModResPsi and ModResUnimod) are preferred and should be used when possible. The format of this term is (startOffset|endOffset|accession|name). See the table below for a series of examples, both legal and illegal. Offset counting begins with 1. The accession field may be empty if no accession number is available. However, the name field must be provided. Since no amino acid may be specified, the modification is presumed to apply to all possible residues in that position.

|  |  |
| --- | --- |
| Example Value | Interpretation |
| (100|100||Floxilation) | The amino acid at position has floxilation. What could a search engine possibly do with this? |
| (100|100|CustomMod:2|Floxilation) | The amino acid at position has floxilation as described in a custom CV |
| (100|100||Phosphorylation) | The amino acid at position 100 has potential phosphorylation. Although this is permitted, use of either ModResPsi or ModResUnimod for well-known modifications is strongly encouraged. |

### Processed header key

The header key “Processed” is used to encode post-translational processing of the protein, such that the mature form of the protein is only a subset of the entire provided sequence. The format of this term is (startOffset|endOffset|accession|name). See the table below for a series of examples, both legal and illegal. Offset counting begins with 1. The coordinates are presumed to apply to the default sequence, not taking into account possible indels.

|  |  |
| --- | --- |
| Example Value | Interpretation |
| (1|40|PEFF:1027|signal sequence) | Residues 1-40 are a signal peptide sequence that is cleaved off after translation |
| (41|890|PEFF:1028|mature protein) | Residues 41-890 are the mature form of the protein after the signal sequence is removed |
| (1|40||signal sequence) | Not legal; an accession number from the PEFF CV must be provided. |
| (1|40|PEFF:1027|) | Not legal; the term name from the PEFF CV must be provided. |

## Additional considerations

### Representation of splicing variants

When splicing variants are to be represented for a given gene/protein in a sequence database, they SHOULD be represented in separate sequence entries; in this case, the DbUniqueId MUST be different for each of these sequence entries.

### Representation of processed sequences

### Processed sequences (removal of precursor peptide, active chain, …) SHOULD be represented with annotations in the sequence description line. In cases where reading software cannot interpret this annotation, or in case where the complexity of interpretation of additional annotation (such as active forms specific PTMs), processed sequences MAY be represented in separate sequence entries; in this case, the DbUniqueId MUST be different for each of these sequence entries.

### File extension

The suggested file extension is .peff (PSI Extended FASTA Format).

# Authors Information

Pierre-Alain Binz

CHUV Centre Universitaire Hospitalier Vaudois, CH-1011 Lausanne 14, Switzerland

pierre-alain.binz@chuv.ch

Sean L. Seymour

Applied Biosystems|MDS Analytical Technologies | 850 Lincoln Centre Drive | Foster City | CA 94404 | USA

sean.seymour@absciex.com

Eugene A. Kapp

Walter & Eliza Hall Institute of Medical Research and the University of Melbourne, Australia

kapp@wehi.edu.au

Jim Shofstahl

Thermo Fisher Scientific | 355 River Oaks Parkway | San Jose | CA 95134 | USA

jim.shofstahl@thermo.com

David Creasy

Matrix Science Ltd | 64 Baker Street | London W1U 7GB | UK

dcreasy@matrixscience.com

Lydie Lane

SIB Swiss Institute of Bioinformatics,, 1 Michel-Servet CH-1211 Genève 14 ,Switzerland

Lydie.Lane@isb-sib.ch

Harald Barnes

Proteomics Unit | Department of Biomedicine | University of Bergen | Norway

Harald.Barsnes@biomed.uib.no

Matt Chambers

Vanderbilt University, Nashville, Tennessee

matt.chambers@vanderbilt.edu

Robert Chalkley

University of California, San Francisco

chalkley@cgl.ucsf.edu

Eric W. Deutsch

Institute for Systems Biology, Seattle WA, USA

edeutsch@systemsbiology.org

# Contributors

In addition to the authors, a number of additional contributions have been made during the preparation process. The contributors who actively participated to the recommendation documentation are:

Members of the UniProt consortium that maped the proposal with UniProt :

Nicole Redaschi, Swiss Institute of Bioinformatics, Swiss-Prot group, Geneva, Switzerland

Maria Jesus Martin, European Bioinformatics Institute, Hinxton, UK

Claire O Donovan, European Bioinformatics Institute, Hinxton, UK

Peter McGarvey, Protein Information Resource, Washington, USA

Amos Bairoch, Swiss Institute of Bioinformatics, CALIPHO group, Geneva, Switzerland

Philip C Andrews, University of Michigan, Ann Arbor, MI, USA

Jason Falkner, University of Michigan, Ann Arbor, MI, USA

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# Glossary

Not used.

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