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PSI Mass Spectrometry Standards Working Group

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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 1.0 FINAL

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 (spectral 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. Further detailed information, including any updates to this document, examples, and validators, is available at http://www.psidev.info/peff.

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1. Introduction

1.1 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 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. 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 including an identifier, taxonomy, description and sometimes other information such as alternative splicing variants, sequence processing leading to active forms and post-translational modifications (PTMs) in addition to the sequence itself. Most of the software convert the original format into a vendor-specific format to process the data. Currently available sequence databases are made available in FASTA format [PEARSON1] (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 information such as splicing forms, mutations or PTMs. To access information about these, 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). Yet, these files tend to be huge with vast amounts of information that is not needed by search engines.

MS-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.

There is also a need to be able to encode specific proteoforms for top-down proteomics platforms. Proteoforms represent protein sequences with a specific set of mass modifications at specific residues. The need cannot be fulfilled with FASTA alone since there is no capacity for encoding mass modifications on each sequence.

1.2 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.
- The format should be able to support encoding proteoforms.

1.3 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 to support all of these variations while consistently extracting the same information.
- The same database file is variably processed in different search engines. A given database entry can contain multiple identifiers, which can lead to variably interpreted identifiers, which renders difficult the mapping of identical entries in different tools (for instance the UniProtKB/Swiss-Prot AC: P02768 vs. UniProtKB/Swiss-Prot ID: ALBU_HUMAN).
- The same protein (and therefore also primary sequence) in different databases can have very different identifiers (for example, P02768 in UniProtKB/Swiss-Prot, NX_P02768 in neXtProt, gi|113576|sp|P02768.2|ALBU_HUMAN in NCBI, and ENSP00000295897 in Ensembl).
- 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.).
- Version name or date of a specific database is often requested for traceability purposes and to allow reproducibility of results obtained from the use of a given database (number of entries, protein or gene names, descriptions, sequences, PTMs, etc. vary from one version to another).
- 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 (using unique database prefixes) should be defined to avoid this.

2. 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].

3. The Format Implementation

3.1 The documentation

The documentation of the format is divided into 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). The CV keywords applicable for PEFF are in a branch of the PSI-MS CV (https://github.com/HUPO-PSI/psi-ms-CV/blob/master/psi-ms.obo), organized broadly as header keywords and individual entry keywords.
- Example files
- Reference to example implementations

3.2 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 MS-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. The only relevant MIAPE-MSI requirements regarding the database (aside from the fact that the database itself must be provided/identified) are the specification of a description, the version of the database, and the number of entries. All these concepts are supported in PEFF via the CV terms DbName, DbDescription, DbVersion, and NumberOfEntries.
2. *mzIdentML* (http://www.psidev.info/mzidentml). The mzIdentML specification is developed by PSI as a standard to capture the output of search engines that assign mass spectra to protein or peptide sequences. For searches performed using a PEFF file, the downstream result in mzIdentML MUST encode a reference to the PEFF file used. This is already supported in mzIdentML.
3. *mzTab* (http://www.psidev.info/mztab). The mzTab specification is developed by PSI as a standard to report proteomics and metabolomics results in a tab-delimited text file format. For searches performed using a PEFF file, the downstream result in mzTab will need to encode a reference to the PEFF file used. This is already supported in mzTab.

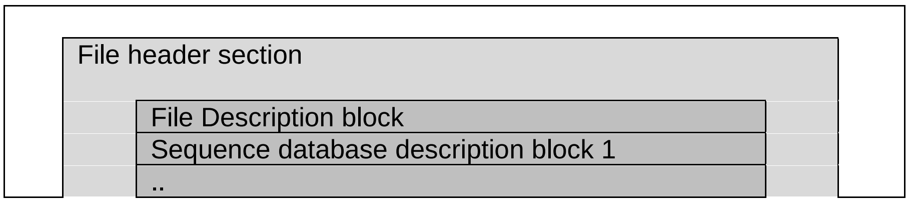
3.3 The common sequence database format description

The format has the form of a text file with two sections, a file header section and a section containing 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 MUST be ignored by parsers.

Descriptors of the information are defined as keywords in a special branch of the PSI-MS CV. The CV is available in OBO format at https://github.com/HUPO-PSI/psi-ms-CV/blob/master/psi-ms.obo.



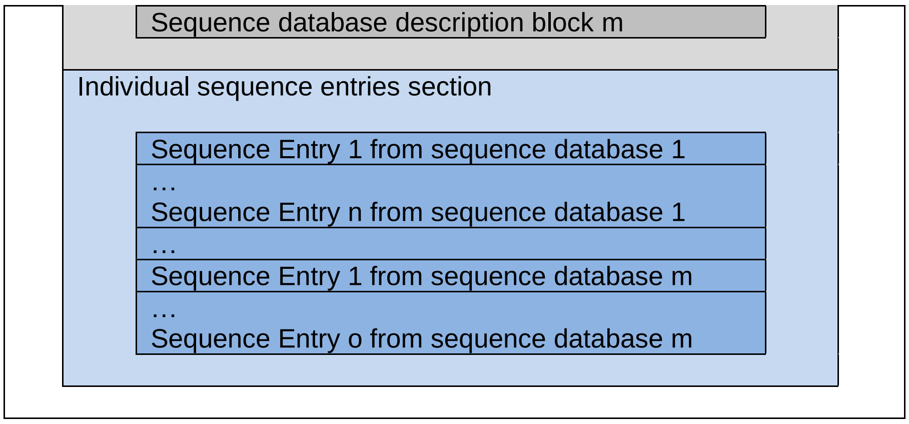

Sequence database description block m
Individual sequence entries section
Sequence Entry 1 from sequence database 1
…
Sequence Entry n from sequence database 1
…
Sequence Entry 1 from sequence database m
…
Sequence Entry o from sequence database m

Figure 1: Graphical representation of the PEFF file structure. In this example, the file has *m* databases, database 1 has *n* entries, database *m* has *o* entries

3.3.1 PEFF file 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.

<u>Format of the file header section</u>

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 be:
  # PEFF N.N
  where N.N represents the version number of the PEFF format, currently 1.0. Parsers SHOULD check this value and compare it to what they are prepared to interpret;
- It MAY be followed by one or more general comment lines, which each have the following format:
  # GeneralComment=value (where *value* is a string of text)
- The description block MUST end with the following line:
  # //

If there is a GeneralComment, it MUST not be empty.

The format of the sequence database description blocks is as follows:

- All lines of a sequence database description block contain one piece of information.
- Each piece of information MUST have the following format:
  # key=value
  where the element *key* MUST be a keyword in the PSI-MS CV under the “PEFF File Header Section term” branch. The format of the *value* is defined for each key in the CV.
- The block MUST start with a sequence database line description and follow the following format:
  # DbName=value, (where *value* is the database name)
- The following five *key* elements MUST also be present:
  Prefix; DbVersion, DbSource, NumberOfEntries, SequenceType
  If there is no obvious independent DbSource and DbVersion (for example, if the PEFF file is the only persistence of the information), then the writing software or author may be named as the source, and sort form of version numbering is recommended.

- Additional key=values pairs that are used in the sequence description blocks later in the document MUST be defined here using the SpecificKey key or another key defined in the CV and dedicated to this section (such as IsProteoformDB and HasAnnotationIdentifier).
- A sequence database information block MUST begin and end with the following separation line:
  # //

One or more sequence description blocks MUST be present. This is an example of a PEFF header section:

```
# PEFF 1.0
# GeneralComment=This is a hand-crafted example comment
# //
# DbName=neXtProt-extract
# Prefix=nxp
# DbDescription=extract of neXtProt with manual modifications
# GeneralComment=A GeneralComment specific to one database is also legal here
# Decoy=false
# DbVersion=2018-01-11
# DbSource=http://www.nextprot.org
# NumberOfEntries=62
# SequenceType=AA
# //
# DbName=myDB
# Prefix=my
# DbDescription=FGF21 proteoforms from top-down experiment PXD123456
# DbVersion=1.1
# DbSource=PXD123456
# NumberOfEntries=2
# SequenceType=AA
# ProteoformDB=true
# HasAnnotationIdentifiers=true
# //
```

Non mandatory key-value pairs in the file header section MAY be used to add meta-data on the database description level (see section 3.3.2 and 3.3.3). They MUST be used to define keys that are not declared in the CV and used in the individual sequence database entries. This might include information such as protein function, ligands, links to experimental evidences, other custom-defined information. They also can imply an impact on the interpretation of the data provided in the individual sequence database section (sequence and annotation).

3.3.2 Defining custom keys in the sequence database description block for use in the sequence entries section

Most of the keys found in each of the individual sequence entries (described below in 3.3.3) are defined in the CV. However, it is possible to define custom keys that MAY be used within custom pipelines. The following rules SHOULD be applied:

- Do not create a custom key for a concept that is already in the CV. Check the CV carefully before creating a custom key
- For a PEFF file that will be exported publicly, concepts not already found in the PSI-MS CV should be proposed to psidev-ms-dev@lists.sourceforge.net for inclusion in the CV so that others who need to reference the same concepts may do so using the same term.

However, for internal use, a new key MAY be defined in the file header block:

# CustomKeyDef=(defKey=value|defKey=value|defKey=value…)

Where there defKeys may be any combination of:

• KeyName	Name of the custom key. CamelCase preferred
• Description	Free text description of the custom key. Enclose in ""
• ConceptCURIE	CURIE from an external CV defining the concept behind this custom key
• RegExp	Regular expression that can be used to parse this custom key. Enclose in ""
• FieldNames	Comma-separated list of names for the fields
• FieldTypes	Comma-separated list of data types of the fields

FieldTypes should use the xsd basic types without the xs: prefix. i.e.: string, decimal, integer, boolean, date, time. Additionally, type "enumeration(string1|string2|string3|etc.)" MAY also be used.

For example, a SecondaryStructure term MAY be defined in the header:

```
# CustomKeyDef=(KeyName=SecondaryStructure|Description="Secondary structure information"|ConceptCURIE=BAO:0000014|RegExp="([0-9]+)\|([0-9]+)\|([A-Za-z]+:[0-9]+)?\|(.*)\|?(.+)?"|FieldNames=StartPosition,EndPosition,CURIE,Description,OptionalTag|FieldTypes=integer,integer,string,string,string)
```

And then, if so defined, the term MAY be used in the sequence entry description lines like this:

```
\SecondaryStructure=(617|673|ncithesaurus:C47937|Helix)
```

3.3.3 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 MAY be empty lines between individual sequence entries.

<u>Format of the individual sequence entries</u>.
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 sequence database description block, of the corresponding sequence database. This is the unique mandatory information of the description line.
  - Lines beginning with ";" (semicolon) are NOT permitted, although this was used in some early versions of FASTA (see https://en.wikipedia.org/wiki/FASTA_format)
  - The description line MAY include optional information, separated by at least one space character, each of them described as *\key=value* pairs.
    - The order of the *\key=value* pairs is not prescribed, but see section 3.3.5 for general recommendations.
    - The element *key* MUST be a CV term unless it is defined in the PEFF file itself. The format of the *value* is defined for each key in the CV repository.
    - The same key MUST NOT appear twice in a description line
    - The *value* for each key can contain one item or a list of items. In the latter case, items are placed in parentheses: *(item1)(item2)…* There MAY be spaces between items.
      Generic example: \key=(item1)(item2)
    - In case *item* contains multiple components, the "|" (pipe character) MUST be used as separator between components. The item therefore has the form
      (component1|component2)
    - If an item contains paired parentheses, brackets, or braces, they SHOULD be rendered without an escape character and parsers MUST properly handle the case of embedded parentheses, e.g. \ModRes=(380||N-linked (GlcNAc...)).
    - If one of the components contains the "|" (pipe character), a "\" (backslash character), or an unpaired parenthesis character (e.g., "(" without a corresponding ")" character) then it must be escaped by preceding it with a "\" (backslash character). For example, if an optionalTag component was "Abcg2|meta\x10", it should be written as "Abcg2\|meta\\x10" in PEFF.
    - Characters allowed for a key: Key: [A-Za-z0-9_]; use CamelCase.
    - Characters allowed for an item (if not complex) or a component of an item (unless the key is already controlled by a regular expression defined in the controlled vocabulary): [A-Za-z0-9_?]

  - The description line MUST contain only a single *>Prefix:DbUniqueId \key=value* block per sequence. 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. It is recommended either to split the entry and create one entry for each such differing header, or to make a selection of the most appropriate header for that PEFF file.
- The sequence block has the following structure:
  - The sequence block contains the actual sequence, coded as one-letter code for both protein and nucleotide sequences. Allowed characters are described in the table below [IUPAC1999; UniProtKB user manual]:
  - Amino acid table:

1 one-letter code	Amino acid name
A	Alanine
R	Arginine
N	Asparagine
D	Aspartic acid
C	Cysteine
Q	Glutamine
E	Glutamic acid
G	Glycine
H	Histidine
I	Isoleucine
L	Leucine
K	Lysine
M	Methionine
F	Phenylalanine
P	Proline
O	Pyrrolysine
S	Serine
U	Selenocysteine
T	Threonine
W	Tryptophan
Y	Tyrosine
V	Valine
B	Aspartic acid or Asparagine
Z	Glutamic acid or Glutamine
X	Any amino acid
J	Leucine or Isoleucine
*	Sequence interruption (stop codon, unknown linkage)

  - Nucleotide table

1 one-letter code	Nucleotide name
G	Guanine
A	Adenine
T	Thymine
C	Cytosine
U	Uracil
R	A or G
Y	C, T or U

K	G, T or U
M	A or C
S	C or G
W	A, T or U
B	not A
D	not C
H	not G
V	neither T nor U
N	Any nucleotide
-	Sequence interruption (i.e. gap)

- The sequence block MAY be a single long line with only a single line ending. We however suggest wrapping the sequences to 60-100 characters per line for better human readability.
- There MAY be blank lines in the individual sequence entries section, although this is discouraged.

Generic illustration:

>Prefix:DbUniqueID1 \key=value \key=value
SEQUENCESEQUENCE
>Prefix:DbUniqueID2 \key=value \key=value
SEQUENCESEQSEQUENCE

Real example:
```
>nxp: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 \Processed=(1|40|PEFF:0001021|signal
peptide)(41|890|PEFF:0001020|mature protein) \ModResPsi=(681|MOD:00048|O4'-
phospho-L-tyrosine)(685|MOD:00048|O4'-phospho-L-
tyrosine)(686|MOD:00048|O4'-phospho-L-tyrosine) (804|MOD:00048|O4'-phospho-
L-tyrosine)(64|MOD:00798|half cystine)(117|MOD:00798|half
cystine)(160|MOD:00798|half cystine)(203|MOD:00798|half cystine)
\ModRes=(63||N-linked (GlcNAc...)) (191||N-linked (GlcNAc...))(230||N-
linked (GlcNAc...))(240||N-linked (GlcNAc...))(293||N-linked
(GlcNAc...))(366||N-linked (GlcNAc...))(380||N-linked (GlcNAc...))
\VariantSimple=(21|L)(68|R)(74|M)(85|K)(90|H)(95|G)(114|G)(119|E)(119|L)(12
9|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)(4
55|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)(71
7|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)
MALRRSMGRPGLPPLPLPPPPRLGLLLAALASLLLPESAAAGLKLMGAPVKLTVSQGQPV
KLNCSVEGMEEPDIQWVKDGAVVQNLDQLYIPVSEQHWIGFLSLKSVERSDAGRYWCQVE
DGGETEISQPVWLTVEGVPFFTVEPKDLAVPPNAPFQLSCEAVGPPEPVTIVWWRGTTKI
GGPAPSPSVLNVTGVTQSTMFSCEAHNLKGLASSRTATVHLQALPAAPFNITVTKLSSSN
ASVAWMPGADGRALLQSCTVQVTQAPGGWEVLAVVVPVPPFTCLLRDLVPATNYSLRVRC
ANALGPSPYADWVPFQTKGLAPASAPQNLHAIRTDSGLILEWEEVIPEAPLEGPLGPYKL
SWVQDNGTQDELTVEGTRANLTGWDPQKDLIVRVCVSNAVGCGPWSQPLVVSSHDRAGQQ
GPPHSRTSWVPVVLGVLTALVTAAALALILLRKRRKETRFGQAFDSVMARGEPAVHFRAA
RSFNRERPERIEATLDSLGISDELKEKLEDVLIPEQQFTLGRMLGKGEFGSVREAQLKQE
DGSFVKVAVKMLKADIIASSDIEEFLREAACMKEFDHPHVAKLVGVSLRSRAKGRLPIPM
VILPFMKHGDLHAFLLASRIGENPFNLPLQTLIRFMVDIACGMEYLSSRNFIHRDLAARN
CMLAEDMTVCVADFGLSRKIYSGDYYRQGCASKLPVKWLALESLADNLYTVQSDVWAFGV
TMWEIMTRGQTPYAGIENAEIYNYLIGGNRLKQPPECMEDVYDLMYQCWSADPKQRPSFT
```

```
CLRMELENILGQLSVLSASQDPLYINIERAEEPTAGGSLELPGRDQPYSGAGDGSGMGAV
GGTPSDCRYILTPGGLAEQPGQAEHQPESPLNETQRLLLLQQGLLPHSSC
```

3.3.4 Recommendations on and order of the keys in a description line

After the sequence identifier, which MUST start the description line, there is no mandatory order for placing the keys. However it is recommended to place the potentially longer keys at the end of the description lines. These are typically: *ModRes, ModResUnimod, ModResPsi, VariantSimple, VariantComplex*. The *Length* key SHOULD be provided.

In general, and by default, molecular features (such as *ModRes, ModResUnimod, ModResPsi, VariantSimple, VariantComplex, Processed*) encoded in keys SHOULD be considered as features that MAY be applied to the sequence. In the case where they MUST be present in the sequence, the sequence database section MUST contain a ProteoformDb=true *key-value* pair (see section 3.3.3).

3.3.5 Definition of OptionalTag elements

In all header keys that allow an optional tag component, this optional tag MAY be placed as the last component, example: (item|item|…|OptionalTag). The optional tag MAY be specified or not, as desired by the writer. If such a tag is not provided, the trailing pipe character ("|") MUST NOT be written. The tags are free text strings that are not constrained by a CV. The tags MAY be defined in the file header via the *OptionalTagDef=Tag:TagDescription* keyword (Tag is the text string of the tag and the TagDescription MAY be used to further describe the meaning of that tag). If several distinct tags are to be specified, each separate tag SHOULD be enclosed in square brackets ([]s). Examples of valid tags are "uncertain", "dbSNP", "in vitro", "[sample04][sample08]". In general, public PEFF providers are encouraged NOT to bloat PEFF files with copious optional tags filled with lots of metadata, but rather leave the optional tags for custom annotations initiated by the end user (i.e. either inserted directly by the user, or based on export options selected by the user at the provider web site). Nonetheless, this feature is somewhat experimental to see how the community wishes to use it.

3.3.6 Definition of complex header keys

Most keys in the CV are self-explanatory in the CV itself. However, some terms are sufficiently complex and central to the format that they are described in detail in this document in the following sections.

3.3.7 Variant header key

The header key "Variant" was deprecated in 2015 during the refinement of the format in favor of using "VariantSimple" and "VariantComplex". Some PEFF files, e.g. from neXtProt, were produced with the "Variant" header key before it was deprecated. This term MUST no longer be used.

3.3.8 VariantSimple header key

The header key "*VariantSimple*" is used to encode all single amino acid (or nucleotide) substitutions. The following description is heavily biased towards amino acid sequences, but single nucleotide substitutions are equally supported for nucleotide databases. The format of the value for this term is *(position|newAminoAcid|optionalTag),* e.g. "(223|A)" or "(225|C|dbSNP)". The first 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, and the second example shows that at position 225 the default amino acid in the sequence MAY be substituted with the amino acid C (and that change is tagged with the string "dbSNP"). The position MUST be greater than 0 and less than or equal to the length of the protein. This key MUST 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 MUST NOT be empty, or space, or any non-alphabetic character except asterisk. The asterisk is to be interpreted as a nonsense mutation (stop codon) over which a peptide sequence MUST NOT span. Regular expressions MUST 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.

3.3.9 VariantComplex header key

The header key *VariantComplex* is used to encode all sequence variations more complex than a single amino acid (or nucleotide) substitution. The format of the value for this key is (*startPosition*|*endPosition*|*newSequence*|*optionalTag*). Variations that can fit the description of a *VariantSimple* MUST NOT be encoded using this term. See the table below for a series of examples, both legal and illegal. Position counting begins with 1. For nucleotide databases, \VariantComplex should encode nucleotide-based variations.

Example Value	*Interpretation*
(100|100|)	Position 100 is nothing, signifying a single amino acid deletion. No character MUST be used to denote deletions, i.e. no dashes (-) or any other characters.
(100|100||10kexomes)	Same as above, but labeled with a tag "10kexomes".
(100|102|)	A 3-AA deletion starting at position 100.
(100|100|APT)	A replacement of the original residue at position 100 by APT. It represents X -> APT, where X can be any residue. Examples are: 1) A->APT where A is the residue at position 100 in the sequence line; this corresponds to an insertion of PT after A; 2) L->APT where L is the residue at position 100 in the sequence line; this corresponds to a replacement of L by APT (for instance in an alternative splicing between exons. For an insertion, the following convention SHOULD be used: inserted amino acids SHOULD come after the residue defined in the sequence line. In the example 1 above, a PT is inserted after A at position 100
(100|100|A) **ILLEGAL**	Not a legal VariantComplex. This MUST be encoded as a *VariantSimple*.
(100|102|KPA)	A 3-AA substitution as a cassette. If the AAs can be substituted individually, then they MUST be encoded as 3 separate *VariantSimple* entries.
(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) **ILLEGAL**	An insertion before the P originally at position 100 with any of A or E or Q. Not a legal *VariantComplex*. This MUST be encoded as three separate *VariantComplex*. No regular expressions are allowed in this item.

3.3.10 ModResUnimod header key

The header key *ModResUnimod* is used to encode mass modifications on amino acids (residues) using the Unimod CV [CREASY1]. Two other terms (ModResPsi and ModRes) are used for other CVs. The \ModRes* terms are not applicable to nucleotide databases. The format of this term is (*position*|*accession*|*name*|*OptionalTag*). An unknown position may be specified as a "?", and the "?" may be listed multiple times to denote multiple modifications of the same type at multiple unknown locations. If the specified position cannot take on the specific amino acid modification in its default or variant form, this is an error in the file. If the sequence entry has a variant that is modified (for instance an alanine -> phospho-Serine), a new protein entry MUST be created that contains this variant (i.e.

serine) in the main sequence. In this case the modified residue (O-phospho-L-serine) can be added in the new entry. The specified modification name MUST be the one found in the "name:" field in the OBO file, not a synonym. See the table below for a series of examples, both legal and illegal. The position counting begins with 1. The position element MAY be a comma-separated list of positions; for proteins with the same PTM on many residues, this can save substantial space. Unimod entries that specify an amino acid substitution MUST NOT be used. The \VariantSimple mechanism MUST be used instead. The tags MAY be defined in the file header via the *CustomTag* keyword as described in section 3.3.4.

Example Value	*Interpretation*
(100|UNIMOD:21|Phospho)	Potential phosphorylation on position 100 (required, not potential, if a proteoform database)
(100,157,214|UNIMOD:21|Phospho)	Potential phosphorylation on positions 100, 157, and/or 214
(100,157|UNIMOD:21|Phospho|invitro)	Potential phosphorylation on positions 100 and/or 157, with an optional tag (free text) of "invitro"
(100||Phospho) ILLEGAL	Not legal. The UNIMOD:nn accession MUST be provided
(100|UNIMOD:21|) ILLEGAL	Not legal. The full name from the OBO file (or equivalent) MUST be provided
(?|UNIMOD:21|Phospho)	A phosphorylation for which a position is unknown. If a position range is known, it MAY be encoded in the Optional tag component. However a reader is not supposed to be able to interpret this.

3.3.11 ModResPsi header key

The header key *ModResPsi* is used to encode mass modifications on amino acids (residues) using the PSI-MOD CV [MONTECCHI-PALAZZI1]. Two other terms (ModResUnimod and ModRes) are used for other CVs. The format of this term is (*position*|*accession*|*name*|*OptionalTag*). See the table below for a series of examples, both legal and illegal. Position counting begins with 1. The position element MAY be a comma-separated list of positions; for proteins with the same PTM on many residues, this can save substantial space. An unknown position may be specified as a "?", and the "?" may be listed multiple times to denote multiple modifications of the same type at multiple unknown locations. As explained in the previous section, note that the ModResPsi CV entry encodes the amino acid that is modified. If the specified position cannot take on the specific amino acid modification in its default or variant form, this is an error in the file. If the sequence entry has a variant that is modified (for instance an alanine -> O-phospho-L-serine), a new protein entry MUST be created that contains this variant (i.e. serine) in the main sequence. In this case the modified residue (O-phospho-L-serine) can be added in the new entry. The specified modification name MUST be the one found in the "name:" field in the OBO file, not a synonym. The tags MAY be defined in the file header via the *CustomTag* keyword as described in section 3.3.4.

Example Value	*Interpretation*
(100|MOD:00046|O-phospho-L-serine)	Potential phosphorylation of a serine at position 100 (required, not potential, if a proteoform database)
(12:100|MOD:00046|O-phospho-L-serine)	Potential phosphorylation of a serine at position 100, with an identifier of "12", that may be referenced in \Proteoform or in order contexts (see section 3.4.2 for details)
(100,157|MOD:00046|O-phospho-L-serine)	Potential phosphorylation of a serine at positions 100 and/or 157

(100,157,214|MOD:00046|O-phospho-L-serine|uncertain)	Potential phosphorylation of a serine at positions 100 157, and/or 214, with an optional tag of “uncertain”
(100||O-phospho-L-serine) ILLEGAL	Not legal. The MOD:00046 accession MUST be provided
(100|MOD:00046|) ILLEGAL	Not legal. The full name from the OBO file (or equivalent) MUST be provided.
(?|MOD:00046|O-phospho-L-serine)	A phosphoserine for which a position is unknown. If a position range is known, it MAY be encoded in the Optional tag component. However a reader is not required to interpret this.

3.3.12 ModRes header key

The header key *ModRes* is used to encode mass modifications on amino acids (residues) where a CV entry is available in neither Unimod nor PSI-MOD, or for custom applications. Two other terms (ModResPsi and ModResUnimod) are preferred and SHOULD be used when possible. The format of this term is (*position*|*accession*|*name*|*OptionalTag*). See the table below for a series of examples, both legal and illegal. Position counting begins with 1. The position element MAY be a comma-separated list of positions; for proteins with the same PTM on many residues, this can save substantial space. An unknown position may be specified as a “?”, and the “?” may be listed multiple times to denote multiple modifications of the same type at multiple unknown locations. The accession field MAY be empty if no accession number is available. However, the name field MUST be provided. Since no amino acid can be specified, the modification is presumed to apply to all possible residues in that position, unless specified in the custom lookup file. If the reading software can understand the modification, it is up to the reading software to ensure that the modification is applicable to the target residue. The tags MAY be defined in the file header via the *CustomTag* keyword as described in section 3.3.4. If it is necessary to describe a modification of a specific mass but unknown identity, it is currently recommended that a suitable entry be placed in a custom Unimod-like controlled vocabulary and referenced that way. An alternative approach if this becomes a common necessity is to add a \ModMass key to the PEFF CV.

Example Value	*Interpretation*
(100||N-linked (GlcNAc...))	The amino acid at position 100 has N-linked glycosylation modification/s of unknown composition.
(100,178||N-linked (GlcNAc...)|invitro)	The amino acids at positions 100 and/or 178 have possible N-linked glycosylation modification/s of unknown composition, with an optional tag of “invitro”.
(100|CustomMod:22|Floxilation)	The amino acid at position 100 has a potential floxilation modification as described in a custom CV. This will not be usable by most reading software, but could potentially be used by custom workflows.
(100||Phosphorylation)	The amino acid at position 100 has a potential phosphorylation. Note that although this is permitted, the use of either ModResPsi or ModResUnimod CV when available is strongly encouraged.
(100|Phosphorylation) ILLEGAL	An empty region is permitted as the second element where the identifier should go, but skipping the second element is not permitted.

3.3.13 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 (*startPosition*|*endPosition*|*accession*|*name*|*OptionalTag*). See the table below for a series of examples, both legal and illegal. Position counting begins with 1. The coordinates are presumed to apply to the default sequence, not taking into account possible indels. Any term used in this context MUST be a child term of PEFF:0001032 "PEFF molecule processing keyword".

Example Value	*Interpretation*
(1|40|PEFF:0001021|signal peptide)	Residues 1-40 are a signal peptide sequence that is cleaved off after translation
(41|890|PEFF:0001020|mature protein)	Residues 41-890 are the mature form of the protein after the signal sequence is removed
(1|40||signal peptide) ILLEGAL	Not legal; an accession number from the PEFF CV MUST be provided.
(1|40|PEFF:0001021|) ILLEGAL	Not legal; the term name from the PEFF CV MUST be provided.

3.4 Advanced features for proteoforms and other combinations of annotations

A proteoform is defined as any one of the multitude of protein forms that can result from a single gene, including sequence variations, PTMs, and processing results [SMITH].

3.4.1 Long form recommendation for Proteoforms: The ProteoformDb=true key-value pair

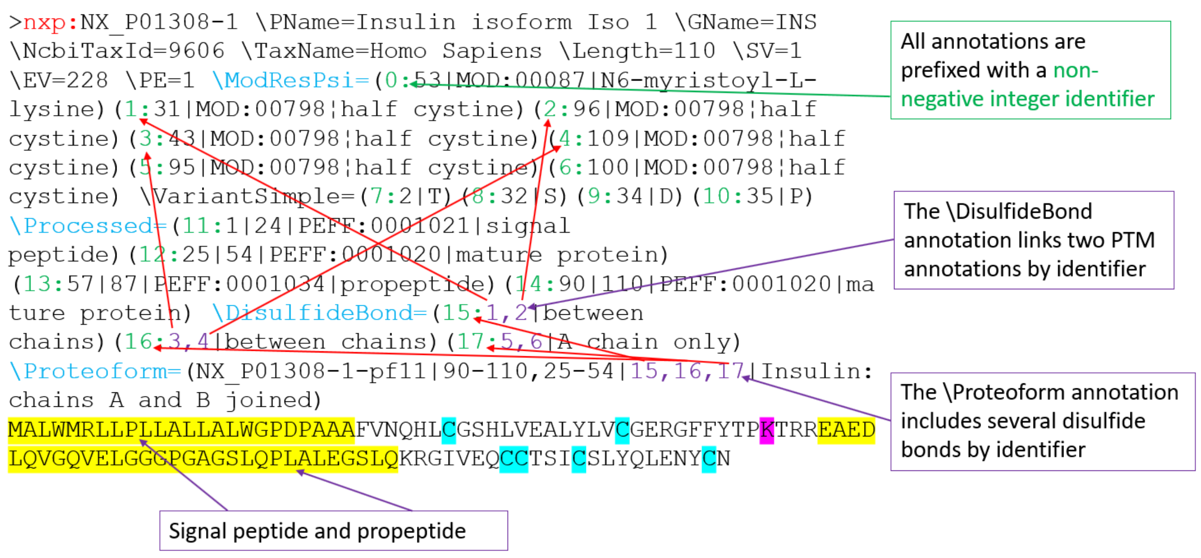
Specific proteoforms can be described in PEFF entries. When ProteoformDb=true is specified, structural annotations such as PTMs, sequence variations and maturation events are all to be considered as mandatory. The key-value pair ProteoformDb=true covers this use-case: If this key-value pair is provided in a sequence database description block, it indicates that the encoded proteins are to be considered as specific proteoforms. If structural annotations such as *ModRes*, *ModResUnimod*, *ModResPsi*, *VariantSimple*, *VariantComplex*, are provided in an entry, they all MUST be applied to the sequence, and not optionally applied. If a software package is not able to support this scenario, it SHOULD report to the user that the database has ProteoformDb=true, which is not supported.

The Proforma notation [LEDUC] for annotating proteoforms has recently been proposed. Proforma uses a different style of notation that embeds the annotations into the sequence. For various reasons, the ProForma notation was not found ideal for a FASTA-replacement file format, but is still useful for other applications. ProForma is meant to describe actually measured proteoforms and does not relate to annotated databases such as UniProtKB. On the other hand, PEFF is useful to describe possible proteoforms and handle details about the proteins not captured so far. Conversion from the PEFF format to the ProForma notation and vice versa is possible for some annotations, although there may be loss of information in some cases. As of this writing, this has been tried carefully.

3.4.2 Annotation identifiers enabling compact form recommendation for Proteoforms: The HasAnnotationIdentifiers=true key-value pair

Specifying proteoforms with ProteoformDB=true as described in the previous section is precise but can be highly repetitive, potentially leading to enormous files. A far more compact form is supported via annotation identifiers and references. In this form, each annotation (PTM or sequence variant or other kind of annotation) MAY be prefixed with a non-negative consecutive integer identifier (0,1,2,3,…) unique within each protein entry. This enables additional keywords to encode references to previous or combinations of previous annotations. For example, the \DisulfideBond keyword MUST refer to two previous \ModResPsi entries describing the PTM, e.g. \DisulfideBond=(15:1,2|between chains). In this example, the bond is between \ModResPsi entries with identifiers 1 and 2. The disulfide bond itself receives an identifier of 15 in this example, and may be referred to later as part of a \Proteoform definition. See Figure 2 for a simplified depiction of how \ModResPsi, \DisulfideBond, and \Proteoform can inter-reference each other.

When a database in a PEFF file uses this advanced feature, the HasAnnotationIdentifiers=true flag must be set in the database header. If a software package is not able to support this scenario, it SHOULD report to the user that the database has HasAnnotationIdentifiers=true, which is not supported. Note that in one PEFF file, one database MAY have HasAnnotationIdentifiers=true enabled, while another database does not.



```
>nxp:NX_P01308-1 \PName=Insulin isoform Iso 1 \GName=INS
\NcbiTaxId=9606 \TaxName=Homo Sapiens \Length=110 \SV=1
\EV=228 \PE=1 \ModResPsi=(0:53|MOD:00087|N6-myristoyl-L-
lysine)(1:31|MOD:00798¦half cystine)(2:96|MOD:00798¦half
cystine)(3:43|MOD:00798¦half cystine)(4:109|MOD:00798¦half
cystine)(5:95|MOD:00798¦half cystine)(6:100|MOD:00798¦half
cystine) \VariantSimple=(7:2|T)(8:32|S)(9:34|D)(10:35|P)
\Processed=(11:1|24|PEFF:0001021|signal
peptide)(12:25|54|PEFF:0001020|mature protein)
(13:57|87|PEFF:0001034|propeptide)(14:90|110|PEFF:0001020|ma
ture protein) \DisulfideBond=(15:1,2|between
chains)(16:3,4|between chains)(17:5,6|A chain only)
\Proteoform=(NX_P01308-1-pf11|90-110,25-54|15,16,17|Insulin:
chains A and B joined)
MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED
LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN
```

All annotations are prefixed with a non-negative integer identifier
The \DisulfideBond annotation links two PTM annotations by identifier
The \Proteoform annotation includes several disulfide bonds by identifier
Signal peptide and propeptide

Figure 2. Simplified depiction of how annotation identifiers can be referenced by other annotations to link them, such as for disulfide bonds and for proteoform definitions.

The following more extensive example shows how annotation identifiers can encode 11 different proteoforms of insulin:

```
>nxp:NX_P01308-1 \PName=Insulin isoform Iso 1 \GName=INS \NcbiTaxId=9606 \TaxName=Homo Sapiens
\Length=110 \SV=1 \EV=228 \PE=1 \ModResPsi=(0:53|MOD:00087|N6-myristoyl-L-
lysine)(1:31|MOD:00798|half cystine)(2:96|MOD:00798|half cystine)(3:43|MOD:00798|half
cystine)(4:109|MOD:00798|half cystine)(5:95|MOD:00798|half cystine)(6:100|MOD:00798|half
cystine) \VariantSimple=(7:2|T)(8:6|C)(9:6|G)(10:6|H)(11:8|Q)(12:9|S)(13:12|V)(14:18|R)
(15:21|L)(16:22|V)(17:23|S)(18:23|T)(19:24|D)(20:24|V)(21:29|D)(22:29|P)(23:32|R)(24:32|S)(25:
34|D)(26:35|P)(27:38|V)(28:42|A)(29:43|G)(30:44|R)(31:45|K)(32:46|Q)(33:47|V)(34:48|C)(35:48|S
)(36:49|L)(37:51|I)(38:52|R)(39:53|E)(40:53|T)(41:55|C)(42:55|H)(43:56|W)(44:58|V)(45:63|A)(46
:63|L)(47:64|W)(48:65|L)(49:68|M)(50:70|R)(51:71|V)(52:73|C)(53:75|D)(54:76|N)(55:76|R)(56:79|
L)(57:81|V)(58:83|K)(59:84|R)(60:85|Y)(61:89|C)(62:89|H)(63:89|L)(64:89|P)(65:90|C)(66:90|D)(6
7:92|L)(68:93|K)(69:94|K)(70:96|S)(71:96|Y)(72:98|R)(73:101|C)(74:103|C)(75:106|D)(76:108|C)
\Processed=(77:1|24|PEFF:0001021|signal peptide)(78:25|54|PEFF:0001020|mature
protein)(79:57|87|PEFF:0001034|propeptide)(80:90|110|PEFF:0001020|mature protein)
\DisulfideBond=(81:1,2|between chains)(82:3,4|between chains)(83:5,6|A chain only)
\Proteoform=(NX_P01308-1-pf1|1-110||preproinsulin)(NX_P01308-1-pf2|25-
110||proinsulin)(NX_P01308-1-pf3|25-110|1,2,3,4,5,6|proinsulin with disulfide mods)(NX_P01308-
1-pf4|90-110||Insulin A chain cleaved)(NX_P01308-1-pf5|90-110|3,4,5,6|Insulin A chain
modified)(NX_P01308-1-pf6|25-54||Insulin B chain cleaved)(NX_P01308-1-pf7|25-54|5,6|Insulin B
chain cleaved)(NX_P01308-1-pf8|25-53|0,1,3|B chain in an extracellular region)(NX_P01308-1-
pf9|57-87||C peptide cleaved)(NX_P01308-1-pf10|57-87||C peptide cleaved)(NX_P01308-1-pf11|90-
110,25-54|81,82,83|Insulin: chains A and B joined)
MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED
LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN
```

In this example, the annotation identifiers are colored green. Each potential annotation (PTM, variant, processing, disulfide bond) has a non-negative integer identifier unique to this protein entry. Optional keywords such as \DisulfideBond and \Proteoform make use of the identifiers to describe entities that combine multiple annotations. In this example, 11 different proteoforms of insulin are described as combinations of potential annotations, applied to a single base sequence. The variations encoded in \VariantSimple are not included in the proteoform descriptions, but could be. This format could easily be translated (expanded) to the ProteoformDb=true format. The reverse is also possible, although much more difficult. One advantage of this format is that although complex proteoform information is encoded and available, search engines can easily treat the database as an ordinary search database with some minimal code modifications that ignore the unsupported optional constructs.

Note that ProteoformDb=true and HasAnnotationIdentifiers=true are not compatible with each other and MUST NOT both be set for a database, since ProteoformDb=true indicates that all specified annotations are required. When these flags are not true, they may either have the value of false or be omitted entirely.

3.5 Additional considerations

3.5.1 Representation of splicing variants

When splicing variants (alternative exon splicing products) 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. Such corresponding sequences MAY be discriminated by a different suffix (>sp:P01234-1 and >sp:P01234-2).

3.5.2 Representation of processed sequences

Processed sequences (removal of precursor peptide, active chain, etc.) SHOULD be represented with annotations in the sequence description line using the \Processed keyword. In cases where reading software cannot interpret this annotation, or in cases where the complexity of interpretation of additional annotations (such as active forms of 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.

3.5.3 File extension

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

3.5.4 PEFF File Validation

As new PEFF writers are developed, it is important to have a consistent validator to check the results. At the time of this writing there is one online reference PEFF validator available. The hyperlink to the validator is available from the main PSI PEFF page. Candidate PEFF files may be uploaded and validated online. If additional validators become available, they will be linked from the PSI PEFF page:

http://www.psidev.info/peff

3.5.5 PEFF Reference Implementation

One of the benefits of PEFF is that it is quite similar to the FASTA format. Since most relevant tools already have a FASTA parser, it seems likely that developers of many software packages will simply update their FASTA parser to extract whatever additional information they wish from a PEFF file into existing data structures, rather than using an external package. Nonetheless, we provide the Perl package Proteomics::PEFF as the reference implementation. It provides a set of classes that correspond to the major components of PEFF, supports all the features of PEFF, and most importantly provides substantial validation to detect problems with existing files. In addition to the validator, the `alterPEFF` tool in Proteomics::PEFF provides a relatively simple mechanism to convert FASTA files to PEFF and alter the annotations in a PEFF file based on a easily managed columnar file of annotations to change. Access to this reference implementation is described at the PEFF web site:

http://www.psidev.info/peff

Links to additional implementations in Python, PHP, Java, etc. are provides as links on this same web page.

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7. Copyright Notice

8. Glossary

In this specification, the following special terms are used throughout:

8.1 Key

The term “Key” corresponds to a annotation concept, and is used to denote that the value following the “Key” provides one or more instances of that concept. In the file header, “Keys” are preceded by “# “. In the entry description lines, “Keys” are preceded by a “\” character.

8.2 Item

The term “Item” corresponds to an instance of an annotation. If a single “Item” is present, then it is not enclosed in parentheses. If there are multiple items, they are enclosed in parentheses. For example:

\Key=Item
\Key=(Item1)(Item2)

8.3 Component

The term “Component” corresponds to an individual element of an Item. If there is more than one component in an Item, they are separated by “|” characters. For example:

\Key=Component1|Component2
\Key=(Component1|Component2)(Component1|Component2|Component3)

8.4 Tag / OptionalTag

The term “Tag” or “OptionalTag” corresponds to the last component is some items that provide a user-attached description to an item. For example:

\Key=(Component1|Component2|OptionalTag)(Component1|Component2|OptionalTag)

See section 3.3.5 for additional discussion about the OptionalTag

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