

The Browser Extensible Data for modified RNA (bedRMod) format

1 Specification

bedRMod is tab-delimited file format, compatible with the standard BED (Browser Extensible Data) format. Data is stored in lines which describe the modification and the unmodified status of RNA sequences at certain positions. Metadata is stored in a header (comment lines starting with #) which appears in the beginning of the file. The file extension for the bedRMod format is `.euf.bed`.

1.1 Data Specification

| Col | BED Field | Type | Value | Brief description |
|-----|------------|-------------|---|---|
| 1 | chrom | String | <code>[[:alnum:]]_{1,255}</code> ¹ | Chromosome name |
| 2 | chromStart | Int | <code>[0, 2⁶⁴ - 1]</code> | Feature start position |
| 3 | chromEnd | Int | <code>[0, 2⁶⁴ - 1]</code> | Feature end position |
| 4 | name | String | <code>[[:alnum:]]_{1,255}</code> | Modification name (MODOMICS short name) |
| 5 | score | Int | <code>[0, 1000]</code> | Modification confidence scaled from 0 - 1000 |
| 6 | strand | String | <code>[-+.]</code> | Feature strand |
| 7 | thickStart | Int | <code>[0, 2⁶⁴ - 1]</code> | Thick start position |
| 8 | thickEnd | Int | <code>[0, 2⁶⁴ - 1]</code> | Thick end position |
| 9 | itemRgb | Int,Int,Int | <code>([0, 255], [0, 255], [0, 255]) 0</code> | Display color |
| 10 | coverage | Int | <code>[0, 2⁶⁴ - 1]</code> | Number of reads at this position |
| 11 | frequency | Int | <code>[0, 100]</code> | Percentage of modified reads at this position |
| 12 | refBase | Char | <code>[A, U, G, C, N]</code> | Reference base at this position |
| 13 | userData | String | <code>[key=value-pair(s);]</code> | Additional user provided data |

Table 1: **bedRMod Fields.**

In a bedRMod file, each data line must have 13 fields. The indices are 0-based. A comment line before the data lines denominates the column names (BED fields), specified in Tab. 1. It is possible to store different RNA modifications in one bedRMod file.

¹ `[[:alnum:]]_` is equivalent to the regular expression (regex) `[A-Za-z0-9_]`. It is also equivalent to the Perl extension `[[:word:]]`. `{1,255}` indicates the allowed length range between 1 and 255 characters.

1.1.1 Detailed Field Description

chrom Chromosome number or reference sequence name. This indicates on which reference sequence the (modified) position occurs. Should contain at least 1 and a maximum of 255 characters.

chromStart 0-based starting position of feature or unmodified base on the chromosome or reference sequence.

chromEnd The end position on the chromosome is excluded from the range of the feature. So, with features occurring at a single position on the chromosome this is **chromStart**+1.

name If a modified base it at the aforementioned position, this contains the short name as defined at Modomics². An unmodified base is denoted with "." (see 2 Example).

score The score denotes the modification confidence at this position with 1000 being the highest confidence and 0 being the lowest. Unmodified bases can have an arbitrary value.

strand Strandedness of the feature can be chosen from three values: + positive strand, - negative strand, or . unknown strand.

thickStart Usually the same as **chromStart** as this is used to display the sequence in a genome viewer.

thickEnd Usually the same as **chromend** as this is used to display the sequence in a genome viewer.

itemRgb An RGB value made up of three integers in range[0, 255]. For each modification a unique color can be saved while unmodified positions are usually left black (0, 0, 0).

coverage Number of reads at this position.

frequency Indicates the percentage of modified reads at the position.

refBase With a read mapped against a reference sequence, this contains the reference base at the feature position. Unmapped or unknown bases are indicated with "N".

userData Any further information that is relevant for this data. In predicting the modifications this could mean the p-value, or probability, or logpdf, etc. key-value pairs are passed as: key1=value1;key2=value2;...

² <https://iimcb.genesilico.pl/modomics/modifications>

1.1.2 Missing Data

Especially when converting into bedRMod from already existing data, some values to fill in an entry might not be available. Special values have been reserved to represent missing field in the bedRMod format:

| BED Field | Missing Value | Supplementary Info |
|-----------|-----------------|--|
| score | 0 | confidence in the occurrence of a modification at this position |
| coverage | 0 | number of reads at this position |
| refBase | N | usually obtained when RNA was aligned against reference sequence |
| userData | empty String "" | |

Table 2: **Missing Data Specification**

1.2 Header Specification

The header contains meta-information about the stored modification data. Each line starts with a "#" and contains the header field and the field description, separated by a "=", e.g. "#fileformat=bedRModv1.0". While some attributes are required (see Tab. 3), more can be added freely, such as the date, description which modifications are recorded, or further modification information. If the data is converted from already published data, add "#external_source=databank;ID of data" to the header.

| Header Field | Description |
|-------------------------|--|
| fileformat | fileformat and version e.g. bedRModv1.0 |
| organism | NCBI taxid |
| modification_type | RNA or DNA |
| sequencing_platform | sequencing platform, e.g. Illumina, ONT |
| basecalling | basecalling model, e.g. (*.cfg file) |
| assembly | genome/transcriptome assembly note |
| annotation_source | annotation source |
| annotation_version | annotation version |
| bioinformatics_workflow | reference to bioinformatics workflow (GitHub or GitLab) |
| experiment | information about experimental protocol, design, etc. ideally: openBIS instance |

Table 3: **Required Header Fields.**

2 Example

Example bedRMod file

```
#fileformat=bedRModv1.5
#organism=Escherichia coli str. K-12 substr. MG1655
#modification_type=RNA
...
#chrom chromStart chromEnd name score strand thickStart thickEnd itemRGB coverage frequency refBase userData
NC_000913.3 1089 1090 m5C 903 + 1089 1090 0,139,0 454 55 C p-value=0.836482645;
NC_000913.3 1090 1091 . 0 + 1090 1901 0,0,0 450 0 G
NC_000913.3 1091 1092 m5C 806 - 1091 1092 0,100,0 354 38 C p-value=0.6382873658;
...
NC_000913.3 1167 1168 m6A 839 + 1167 1168 0,0,155 468 61 A p-value=0.729376849;
...
```

3 Acronyms

| | |
|----------------|--|
| BED | Browser Extensible Data |
| bedRMod | Browser Extensible Data for modified RNA |
| ONT | Oxford Nanopore Technology |
| regex | regular expression |