BioMed Sample Sheets Documentation

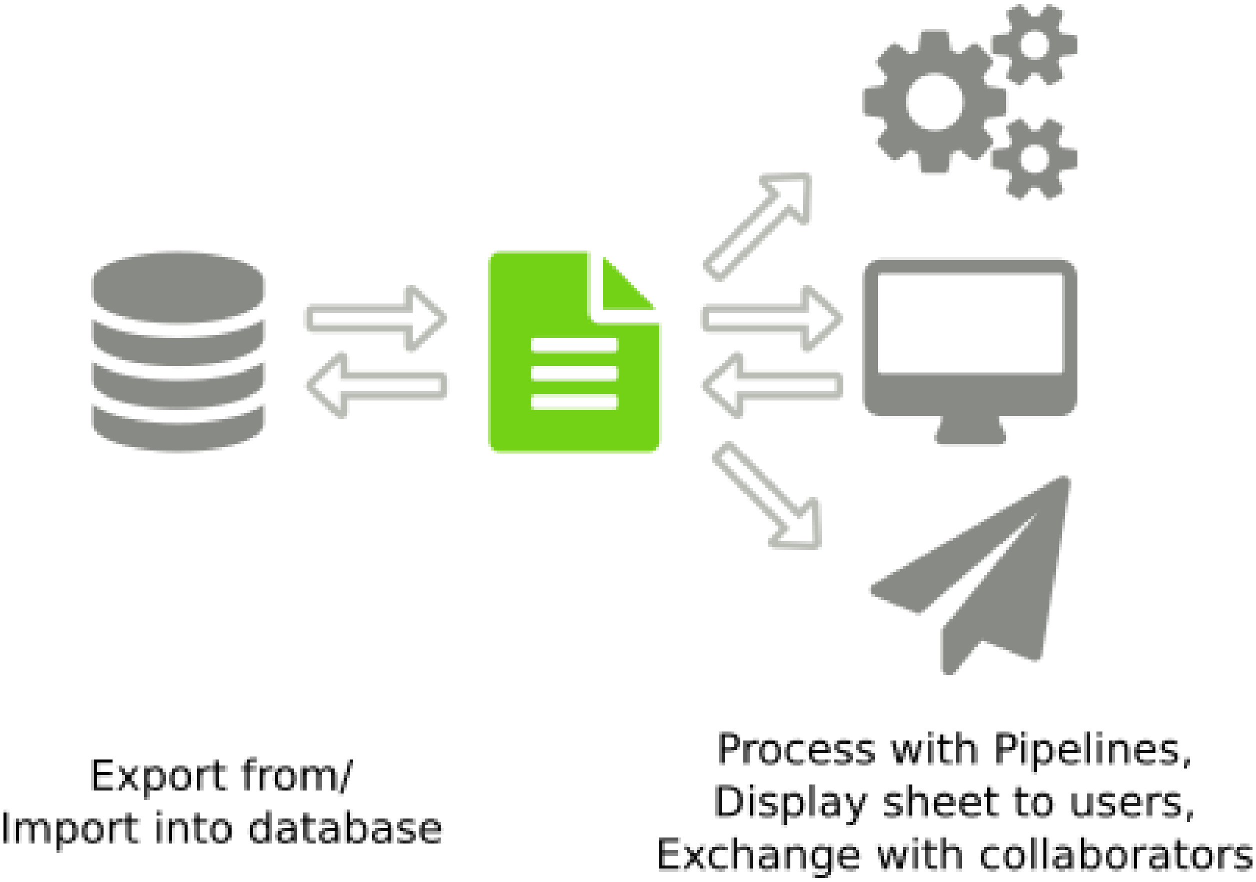
Release 0.1

Manuel Holtgrewe

Aug 16, 2018

Overview

This document describes the biomedical sample sheets file format and software.



Biomedical sample sheet files are files for saving the necessary information on biomedical experiments/studies that is required for the data analysis. For example, such a sheet might describe patients and their family relations and phenotypes for a raw disease NGS study. Another example is the description of a cohort of cancer patients, taken biopsies and subsequent sequencing.

Such files are useful for various tasks such as (1) information export from/import into databases, (2) required sample meta data for pipelines, (3) displaying information to users and allow them to enter data, (4) exchanging experiment meta data with collaborators.

The biomedical sample sheet file format (and the data schema used) was developed at the Core Unit Bioinformatics of the Berlin Institute of Health and is based on the work of Sven Nahnsen's group in Tubingen.

You are viewing the documentation of the `biomedsheets` Python package which contains the reference implementation for parsing/writing such files and the specification of the data schema and related file formats.

CHAPTER 1

Project Contents

The project consists of

- a JSON-based data format for the description of biomedical sample sheets as well as a TSV format that is easier to use for humans,
- pre-defined data types to be used in the sample sheets,
- a Python program for the validation thereof,
- a Python program for conversion between JSON and TSV-based sample sheets (for easier viewing and editing),
- a Python library for access of the JSON sheets, and
- a Python library for simplified access of the JSON sheets for the important cases of matched tumor/normal studies and rare disease studies with pedigree relationships.

CHAPTER 2

Project Status

We (CUBI) are actively using the file format and library but it is still under development.

CHAPTER 3

Open Questions

- Keep or drop globally unique PKs from the file names? Random pks make everything very hard to use as files, add complexity to shortcut schemas. Sequential pks at least keep a temporal order.

3.1 Introduction

A commonly recurring theme in the analysis of biomedical data is requiring the description of this data in machine-readable form. Two important areas where such a requirement arises are:

1. storing meta data for the results of an experiment/assay and
2. processing biomedical data sets in an automated and reproducible ways using some workflow engine.

This document describes a proposal for structuring the data, i.e., a data schema that allows for representing most important use cases that occurred for the Core Unit Bioinformatics (CUBI) at the Berlin Institute of Health (BIH). Further, this document proposes certain shortcuts/simplifications that make this data schema more easy to navigate and use for a handful of important use cases.

Originally, this is an adaption of the data schema used by Sven Nahnsen's group in Tübingen.

3.2 High-Level Overview

The figure below gives an overview of the overall data schema. Each box represents a table while the connections and labels specify the relation counts between them. The "core" schema is shown in yellow while the NGS-specific part is shown in blue.

BioEntity describes biological entities (or specimens or donors). This might be a human patient, a mouse, a cell culture, etc. **BioSample** describes one biological sample taken from the biological entity. This might be a blood sample, saliva swab, part of a primary tumor biopsy, part of a metathesis biopsy etc. **TestSample** describes a further processing of a BioSample, e.g., extracted DNA, RNA, etc. from a tumor biopsy. This data schema is taken from the Qbic tool by Sven Nahnsen's group.

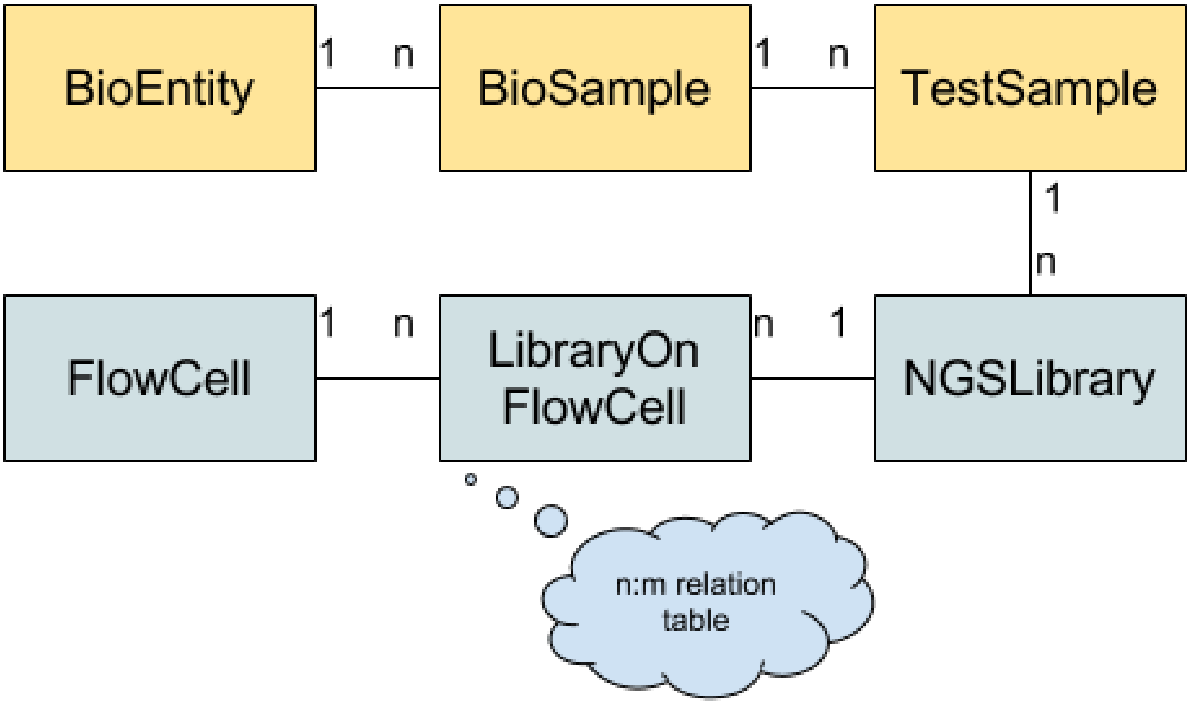


Fig. 1: Tables used in the BioMed Sheet data schema.

This data schema has the nice property that it models most of the relevant parts of many studies which are later analyzed by Bioinformaticians. For example, BioEntity records could describe cancer patients, BioSample describes the healthy samples and tumor biopsies taken from them (multiple samples can be modeled, e.g., for several saliva swabs at different time points, multiple biopsies, e.g., the primary tumor before regression, primary tumor after regression, and several metastases). TestSample then describes the result of the preparation of the sample, e.g., multiple DNA or RNA extractions can be modeled. Being able to describe multiple BioSamples and TestSamples has the advantage that certain batch effects as well as test- and sample-specific effects can be later taken into consideration based on the available meta data.

On the other hand, there are certain things that are not directly modeled, e.g., the link and order between tumors before and after regression is not modeled in the primary data schema. However, **arbitrary meta data** can be attached to each record and then be interpreted by a particular workflow.

Thus, the proposed data schema is useful enough such that generic software libraries can be written that can then be used for modeling many important specific cases and adjusted to the environment of its users.

For the NGS specialization, **NGSLibrary** represents on library prepared for sequencing (e.g. WES, WGS, RNA library). The various steps in the wet lab protocols are not modeled explicitely but each library record could be annotated by the user if he needs this information in a downstream analysis. FlowCell represents one flow cell run on a specific machine while **LibraryOnFlowCell** allows for assigning one library to multiple lanes on a given **FlowCell**.

Using this schema, possible flow cell–specific or lane-specific biases can later be incorporated into an analysis. In terms of mapping to files, each LibraryOnFlowCell then corresponds to one compressed FASTQ file in the case of single-end reads and two compressed FASTQ files in the case of paired reads (at least for bcl2fastq v2, it might correspond to a multiple ones when using v1).

3.2.1 Sample Sheets vs. Database Schemas

One important point here is the relation and separation between sample sheets and database schemas.

The aim of the sample sheets is to provide a useful computer-readable description of an experiment for processing the data with Bioinformatics workflows or further downstream analyses (e.g. in R or using Jupyter notebooks). This document describes a concept for such schemas and an implementation using JSON for the file format (plus a more human-focused TSV representation).

Of course, sample sheets could also be stored in a RDBMS (Relational database management system, e.g., Postgres), with a suitable database schema. Often such databases would be part of a LIMS system. A sample sheet could serve as a poor Bioinformatician's LIMS system, if only some meta information for samples and their relation is to be tracked. However, the more complex part of such a LIMS system or even a hospital information management system are mostly not of direct relevance to the Bioinformatics analysis.

Rather, the data from a laboratory or hospital IT system can be used to generate biomedical sample sheets. BioMed sample sheets further allow linking back from the bio entities, bio samples, test samples, ngs libraries to such systems by storing lists of URLs for each of the previous entities.

3.3 Table Fields

3.3.1 Core Table Fields

This section describes the common table fields. Generally, the `pk` field is an integer primary key that is to be automatically generated numbering each element uniquely despite its level in the hierarchy of all JSON elements (i.e. autoincrement in RDBMS). The field `secondary_id` is an identifier assigned by the "data owner" (e.g., the collaboration partner). This identifier has to be unique within a given project but can be ambiguous globally.

A possible best practice is to enforce the `secondary_id` to only consist of alphanumeric characters and underscores. Then, they should be constructed as (none of the `<Field>` values should contain a hyphen itself):

```
<BioEntity>-<BioSample>-<TestSample>-<NGSLibrary>
```

(of course only up to “BioSample” for BioSamples etc.).

Examples are:

- BioEntity secondary ids: 2355, BIH-234
- **BioSample secondary ids:**
  - 2355-B1 (first blood sample from patient 2355)
  - BIH_234-N1 (first normal sample from patient BIH-234)
  - BIH_234-T2 (second tumor sample from patient BIH-234)
- **TestSample secondary ids:**
  - 2355-B1-DNA1 (first DNA extraction from first blood sample)
  - BIH_234-T1-RNA1 (first RNA extraction from first tumor sample)
  - BIH_234-T2-DNA2 (second DNA extraction from second tumor sample)

Generally, the following are “core fields” (pk: primary key, fk: foreign key).

BioEntity

- pk: integer
- secondary_id: string

BioSample

- pk: integer
- bio_entity: fk to BioEntity.pk
- secondary_id: string

TestSample

- pk: integer
- bio_sample: fk to BioSample.pk
- secondary_id: string

NGSLibrary

- pk: integer
- test_sample: fk to TestSample.pk
- secondary_id: string

FlowCell

- pk: integer
- machine_name: string
- flowcell_name: string

NGSLibraryOnFlowCell

- pk: integer
- ngs_library: fk to NGSLibrary.pk
- flowcell: fk to FlowCell
- lane: int

3.3.2 Common Table Fields

For many major use cases, the following table fields are useful additions to get a list of "common fields".

For all tables, adding a list of strings with external IDs (e.g., called "external_ids") is recommendable. This way, external resources can be linked out to. A recommendation is to use URLs for giving reads an unambiguous prefix. These URLs can be pseudo URLs or real entry points in remote REST APIs. Further, each record has a meta_data field for structured data in JSON format.

BioEntity

- affected: boolean, optional field for specifying the "affected" state in rare disease studies
- sex: {'male', 'female', 'unknown'}, optional field for person's sex in germline studies
- father: fk to BioEntity.pk, optional fields for linking to father
- mother: fk to BioEntity.pk, optional fields for linking to mother

BioSample

- cell_type: string with controlled vocabulary, optional field for specifying cell type

TestSample

- extraction_type: controlled vocabulary with extraction type, e.g. {'DNA', 'RNA'} or a superset thereof; optional field for describing extracted data

NGSLibrary

- library_kind: controlled vocabulary with library preparation type, e.g., {'WES', 'WGS', 'RNA-seq', 'other'} or a superset thereof; required field for describing library type
- kit: controlled vocabulary describing kit and version used for targeted sequencing, or RNA amplifcation method

NGSLibraryOnFC

- adapter_name: string, optional field describing name of used adapter barcode(s)
- adapter_seq: string, optional field giving sequence of used adapter barcode(s)

3.4 JSON Sample Sheets

3.4.1 Rough Structure

Overall, a JSON sample sheet looks as follows.

The sheet is described as a JSON object and is given an ID, a title, and a description.

```
{
    "id": "https://omics.cubi.bihealth.org/experiments/33",
    "title": "Tumor/Normal Study Example",
    "description": "Example biomedical sheet for standard tumor/normal study",
```

This is followed by a section describing the optional additional fields for each of the objects.

```
    "extraInfoDefs": {
```

The extra fields can be described in each schema, e.g., as in the following example referring to the NCBI organism taxonomy.

```
            "ncbiTaxon": {
                "docs": "Reference to NCBI taxonomy",
                "key": "taxon",
                "type": "string",
                "pattern": "^NCBITaxon_[1-9][0-9]*$"
            }
        },
```

Or by refering to the built-in standard fields bundled with the `biomedsheets` module.

```
        "bioSample": {
            "uberonCellSource": { "$ref": "resource://biomedsheets/data/std_fields.
↪json#/extraInfoDefs/template/uberonCellSource" }
        },
```

There can be field definitions for each data type.

```
        "testSample": {},
        "ngsLibrary": {},
        "msProteinPool": {}
    },
```

Then, the bio entities are given. They are stored in a JSON object/map. The attribute name/key is the secondary ID that has to be unique within the project. Each BioEntity must have a primary key, can have some extra IDs and additional information (as described in `extraInfoDefs` above).

```
        "BIH_001": {
            "pk": "123001",
```

(continues on next page)

(continued from previous page)

```
            "extraIds": [
                "http://cancer-registry.hospital.de/PAT12345",
                "http://virtual-cuts.pathology.hospital.de/SMPL000021"
            ],
            "extraInfo": {
                "ncbiTaxon": "NCBITaxon_9606"
            },
            "bioSamples": {
```

Then, each BioEntity can have a number of BioSamples. Note that the secondary id is given without the prefix of the secondary ID of the containing BioSample. The BioSample must have a global ID `pk`, can have extra infos attached (and, of course extra IDs, omitted here).

```
                "N1": {
                    "pk": "234001",
                    "extraInfo": {
                        "uberonCellSource": "UBERON:0000178"
                    },
```

Recursively, each BioSample can have a number of TestSamples which can have a number of NGSLibrary's and MSProteinPool's.

```
                    "testSamples": {
                        "DNA1": {
                            "pk": "345001",
                            "extraInfo": { "extractionType": "DNA" },
                            "ngsLibraries": {
                                "WES1": {
                                    "pk": "567001",
                                    "extraInfo": { "libraryType": "WES" }
                                }
                            }
                        }
                    }
                }
            }
        }
    }
}
```

3.4.2 Sheet Validation

Validation of sample sheets has four steps:

1. the sheet must be valid JSON,
2. expansion of JSON pointers `{ "$ref": "<URL>" }` is performed,
3. the sheet must conform to the JSON schema bundled with `biomedsheets` (in the future it will be versionised at some URL),
4. additional validation based on `extraInfoDefs` is performed.

Steps 1 and 3 can be performed with standard tools or libraries. Step 2 is relatively easy and the `biomedsheets` module ships with code for performing this easily (the functionality is available as a Python program as well). Step 4 is not implemented yet.

On the one hand, custom fields allow for the definition of arbitrary "simple" values. Currently, it is possible to have boolean, numbers, strings, enums and lists of the atomic types. On the other hand, using JSON pointers, centrally defined field types can be used. This allows for easy sharing of data types and easier computation.

3.4.3 Predefined Fields

Note: Once stabilized, the common fields will be documented here.

3.5 TSV Sample Sheets

Writing sample sheets as TSV (tab-separated values) files is an alternative to writing JSON files. As they can be edited with text editors and spreadsheet programs such as Excel, viewing and modifying TSV files is more convenient than for JSON files.

However, TSV sample sheets do not have a full 1:1 relation to the JSON sample sheets. Rather, they serve as shortcuts for the most important practical cases (germline variants, matched cancer, and (later) relatively simple generic experiments).

3.5.1 Rough Structure

Overall, a TSV sample sheet looks as follows.

First, the sheet can start with a `[Metadata]` section. This can be used for defining meta data for the sheet. The following key/value pairs can be defined:

- `schema` – name of the schema (`germline_variants` or `cancer_matched`),
- `schema_version` – for versionizing the schema (currently only `v1`),
- `title` – title of the sample sheet,
- `description` – a description of the sample sheet.

```
[Metadata]
schema        germline_variants
schema_version        v1
title        Example for germline variants sheet file
description        This example shows how to add custom fields
```

Optionally, a `[Custom Fields]` section can follow. This can be used to define custom fields to place in addition to the core fields. The core fields will be described later when describing the germline variants and matched cancer sheet formats. Custom field definition is explained in *Custom Fields in TSV Schemas*.

```
[Custom Fields]
key        annotatedEntity        docs        type        minimum        maximum        unit
consentRetracted        bioEntity        boolean        Patient has retracted␣
↪consent        .        .        .        .        .
patientCenter        bioEntity        enum        Clinical center of patient␣
↪origin        .        .        .        .        New_York,Rio,Tokyo,Berlin
```

Finally, the data is placed in a `[Data]` section. If the Metadata and Custom Fields sections are missing, the file can also start with the column headers of the data section (omitting `[Data]`).

```
[Data]
patientName         fatherName        motherName        sex        affected        extractionType
12_345        12_346        12_347        1        2        DNA        WGS        12-
↪345        HP:0009946,HP:0009899        N        Berlin
12_348        12_346        12_347        1        1        DNA        WGS        12-
↪348        .        N        Berlin
12_346        0        0        1        1        .        .        .        .
↪        N        Berlin
```

3.5.2 TSV Sheet Processing

When reading TSV sample sheets, they will be converted to JSON sheets on the fly. This will be done with the algorithm described below.

For germline sample sheets, only the patient (`BioEntity`) is explicitely defined and named. It is assumed that only one bio sample it taken for each bio entity and is named `N1`. Further, it is assumed that only one test sample is extracted (implicitely using extraction type `DNA`) which is named `DNA1`. Multiple libraries can be specified by giving different lines with the same `patientName` and different `extractionType` and/or `folderName` is given. These will be named `WGS1`, `WGS2`, etc. (or `WES1`, `WES2`, etc.)

For matched cancer sample sheets, the patient (`BioEntity`) is explicitely defined and named as well as the `BioSample`. The name of the sample is given explicitely to allow for naming tumors differently, e.g., `T1` for the first sample from a primary tumor and `M1` for the first sample of a metastatic tumor. For each extraction type, (e.g., `DNA` or `RNA`), a new counter will be started (e.g., yielding `DNA1`, `DNA2`, `RNA1`, etc.) In addition, for each new `sampleType`, a new sample will be started and for each new `folderName`, a new `NGSLibrary` will be started.

3.6 Examples

3.6.1 Sheet Examples

Below is an example JSON file with a cancer sample sheet. Note that the sheet only contains one donor with two bio samples (normal sample `N1` and primary tumor `T1`).

```
{
    "identifier": "file://examples/example_cancer_matched.tsv",
    "title": "Cancer Sample Sheet",
    "description": "Sample Sheet constructed from cancer matched samples compact TSV␣
↪file",
    "extraInfoDefs": {
        "bioEntity": {
            "ncbiTaxon": {
                "docs": "Reference to NCBI taxonomy",
                "key": "taxon",
                "type": "string",
                "pattern": "^NCBITaxon_[1-9][0-9]*$"
            }
        },
        "bioSample": {
            "isTumor": {
                "docs": "Boolean flag for distinguishing tumor/normal samples",
                "key": "isTumor",
                "type": "boolean"
            }
```

(continues on next page)

(continued from previous page)

```
        },
        "testSample": {
            "extractionType": {
                "docs": "Describes extracted",
                "key": "extractionType",
                "type": "enum",
                "choices": [
                    "DNA",
                    "RNA",
                    "other"
                ]
            }
        },
        "ngsLibrary": {
            "seqPlatform": {
                "docs": "Sequencing platform used",
                "key": "kitName",
                "type": "enum",
                "choices": [
                    "Illumina",
                    "PacBio",
                    "other"
                ]
            },
            "libraryType": {
                "docs": "Rough classificiation of the library type",
                "key": "libraryType",
                "type": "enum",
                "choices": [
                    "Panel-seq",
                    "WES",
                    "WGS",
                    "mRNA-seq",
                    "tRNA-seq",
                    "other"
                ]
            },
            "folderName": {
                "docs": "Name of folder with FASTQ files",
                "key": "folderName",
                "type": "string"
            }
        }
    },
    "bioEntities": {
        "P001": {
            "pk": 1,
            "extraInfo": {
                "ncbiTaxon": "NCBITaxon_9606"
            },
            "bioSamples": {
                "N1": {
                    "pk": 2,
                    "extraInfo": {
                        "isTumor": false
                    },
                    "testSamples": {
```

(continues on next page)

(continued from previous page)

```
                        "DNA1": {
                            "pk": 3,
                            "extraInfo": {
                                "extractionType": "DNA"
                            },
                            "ngsLibraries": {
                                "WES1": {
                                    "pk": 4,
                                    "extraInfo": {
                                        "seqPlatform": "Illumina",
                                        "folderName": "P001-N1-DNA1-WES1",
                                        "libraryType": "WES"
                                    }
                                }
                            }
                        }
                    }
                },
                "T1": {
                    "pk": 5,
                    "extraInfo": {
                        "isTumor": true
                    },
                    "testSamples": {
                        "DNA1": {
                            "pk": 6,
                            "extraInfo": {
                                "extractionType": "DNA"
                            },
                            "ngsLibraries": {
                                "WES1": {
                                    "pk": 7,
                                    "extraInfo": {
                                        "seqPlatform": "Illumina",
                                        "folderName": "P001-T1-DNA1-WES1",
                                        "libraryType": "WES"
                                    }
                                }
                            }
                        },
                        "RNA1": {
                            "pk": 8,
                            "extraInfo": {
                                "extractionType": "RNA"
                            },
                            "ngsLibraries": {
                                "mRNA_seq1": {
                                    "pk": 9,
                                    "extraInfo": {
                                        "seqPlatform": "Illumina",
                                        "folderName": "P001-T1-RNA1-mRNAseq1",
                                        "libraryType": "mRNA_seq"
                                    }
                                }
                            }
                        }
                    }
```

(continues on next page)

(continued from previous page)

```
                    }
                }
            }
        }
}
```

3.6.2 Code Examples

The following Python program uses the `biomedsheets` module for loading the JSON sheet from above. It then prints the names of the donors and the names of the NGS libraries for the tumor/normal pairs.

```python
# -*- coding: utf-8 -*-
"""Demonstrate shortcuts for cancer sample sheet
"""

import collections
import os

from biomedsheets import io, ref_resolver, shortcuts


def load_sheet():
    """Return ``Sheet`` instance for the cancer example"""
    path = os.path.join(os.path.abspath(
        os.path.dirname(__file__)), 'example_cancer_matched.json')
    sheet_json = io.json_loads_ordered(open(path, 'rt').read())
    resolver = ref_resolver.RefResolver(dict_class=collections.OrderedDict)
    return io.SheetBuilder(
        resolver.resolve('file://' + path, sheet_json)).run()


def main():
    """Main program entry point"""
    cancer_cases = shortcuts.CancerCaseSheet(load_sheet())
    print('Donors\n')
    for donor in cancer_cases.donors:
        print('  {}'.format(donor.name))
    print('\nLibraries of all tumor/normal pairs\n')
    for pair in cancer_cases.all_sample_pairs:
        print('  {}'.format(pair.donor.name))
        print('    normal DNA: {}'.format(
            pair.normal_sample.dna_ngs_library.name))
        if pair.normal_sample.rna_ngs_library:
            print('    normal RNA: {}'.format(
                pair.normal_sample.rna_ngs_library.name))
        print('    tumor DNA:  {}'.format(
            pair.tumor_sample.dna_ngs_library.name))
        if pair.tumor_sample.rna_ngs_library:
            print('    tumor RNA:  {}'.format(
                pair.tumor_sample.rna_ngs_library.name))


if __name__ == '__main__':
    main()
```

3.6.3 Output

The output of the program is as follows:

```
Donors

P001-000001

Libraries of all tumor/normal pairs

  P001-000001
    normal DNA: P001-N1-DNA1-WES1-000004
    tumor DNA:  P001-T1-DNA1-WES1-000007
    tumor RNA:  P001-T1-RNA1-mRNA_seq1-000009
```

3.7 Frequently Asked Questions

3.7.1 Why JSON?

While YAML is easier to process by human beings, JSON has better tool support and is used more widely. In particular, JSON schema is widely accepted as are JSON pointers, and RDBMS such as Postgres have good support for JSON fields as well.

Further, JSON is valid YAML, so YAML parsers can be used for reading the JSON files and the resulting structures can then be validated by JSON validators.

3.7.2 Why only secondary ID parts in JSON?

Here, the idea is to increase readability a bit and remove redundancy. The prefix is implicitely defined by the secondary ids on the path to the root.

3.8 Workflow Assumptions

One aim for the biomedical sheets is to drive workflow engines. Currently, the main aim is supporting Snakemake but the described data structures and file formats are straightforward enough so they can be used by any engine. Further, the schemas and workflows described below focus on high-throughput sequencing data. Adaption to proteomics, metabolomics, etc. should be

One central assumption is that the overall workflow is modularized and each module deals with one “well-defined” processing step with “homogeneous data”. While this is not clearly defined, some examples are:

- A module that aligns HTS data from FASTQ files, supporting both RNA/DNA sequencing data and paired/single read data. Depending on the annotated test sample extraction type, read length, and paired/single mode, the aligner is chosen (BWA-MEM, BWA-ALN, STAR). The alignments are post-processed for masking duplicates, converted to BAM, sorted, indexed, and basic statistics are computed. The module is smart enough to create appropriate read groups in the BAM file for each pair of/single FASTQ files for correcting for lane bias.
- A module that takes HTS alignments in BAM data and performs “simple” variant calling, i.e., each sample is considered independently. The resulting VCF file is appropriately postprocessed (normalize indels, sort, bgzip, index). The module can also interpret donor-based pedigree information from the sample sheet and call variants for all samples from a family independently. If the pedigree mode is used then there is the restriction that there must be only one sample from each person in the family. Based on configuration, one or multiple of a set of

supported variant callers can be used. A "cancer" mode performs variant calling of all samples of one donor together.

- A module that takes HTS alignments in BAM data and performs "somatic" variant calling. Each donor must have exactly one sample flagged as "is not cancer" and one or more samples flagged as "is cancer". For each cancer sample, the module performs paired somatic variant calling with one or more tools from a supported list. Only one library for each the control and the cancer samples is supported. The result is a VCF file for each tool and each cancer sample with the somatic variants. The VCF is appropriately preformatted.

Such typical modules are much easier to write and use if certain assumptions about the sample sheets hold. Generally, the aim is to define a small core set of information that is required for the proper processing in workflows. Schema-specific settings are described in the following chapters. Core information is described here (somewhat redundant with the chapter *High-Level Overview*).

3.8.1 Core Fields

- **BioEntity**
  - pk
  - secondaryId
- **BioSample**
  - pk
  - secondaryId
- **TestSample**
  - pk
  - secondaryId
  - extraction_type
- **AssaySample**
  - pk
  - secondaryId

3.8.2 HTS Core Fields

The following fields are required by all workflow steps that process HTS data.

- **TestSample**
  - extractionType – extracted sample, currently one of {`DNA`, `RNA`, `other`}
- **AssaySample**
  - libraryType – library type, currently one of {`WES`, `WGS`, `Panel_seq`, `mRNA_seq`, `total_RNA_seq`, `other`}

3.9 Entity Names

The term **name** is used for strings/tokens that represent a bio entity, bio sample, test sample, NGS library, etc. uniquely as part of file names. Further, they are used where sample names or similar are required in file contents, e.g., in VCF or BAM files. There are many possible strategies for this.

One important point is that the name must be unique, ideally globally. For this reason, it is recommended to include the primary key in the name, such that read alignment files can be named `{sample_name}.bam`, for example, and the file name is then unique throughout all systems.

3.9.1 Primary Key as Name Parts

The recommendation is to construct sample names etc. as `{secondary_id}-{pk}` where `{secondary_id}` is the **full** secondary id (e.g., `PATIENT-T1-DNA1-WES1`) and `pk` is the integer primary key from the database.

Example file names:

- `bwa.PATIENT-T1-DNA1-WES-0000001.bam`
- `mutect.PATIENT-T1-DNA1-WES-0000001.vcf.gz`

3.9.2 Secondary IDs as Names

If no primary key has been assigned yet, a possible alternative strategy is to only use the secondary id. This allows stable file names even if no stable primary key can be assigned.

Note: This is the recommendation for cubi_pipeline until we have a good id assignment system available.

Example file names:

- `bwa.PATIENT-T1-DNA1-WES.bam`
- `mutect.PATIENT-T1-DNA1-WES.vcf.gz`

3.10 Matched Tumor Samples

A relatively simple schema for the analysis of matched tumor/normal samples from cancer studies. The assumed setting is as follows.

- Each bio entity is a patient/donor.
- Each donor gives one normal (bio) sample (e.g., blood or saliva) and at least one (bio) sample from the cancer (e.g., primary tumor or metastesis).
- For each tumor and non-tumor sample, there is at least one DNA HTS library sequenced.
- For each tumor sample, there can be RNA HTS libraries.
- Only the first seen DNA/RNA library is considered for each sample (the "primary one")

Note: The requirement of one DNA HTS library for each sample and RNA only for tumor can be dropped in the future.

3.10.1 Matched Tumor Fields

The following fields must be present for matched tumor sample sheets.

- **BioSample**

– isTumor – a boolean defining whether the sample was taken from tumor cells

3.10.2 Matched Tumor TSV Schema

Additionally, there is an alternative to defining schemas in JSON format for matched tumor sample sheets. Instead, a TSV-based schema can be used.

Optionally, the schema can contain meta data, starting with `[Metadata]` INI-style section header (the data section has to start with `[Data]`).

```
[Metadata]
schema          cancer_matched
schema_version          v1
title           Example matched cancer tumor/normal study
description             The study has two patients, P001 has one tumor sample, P002 has two

[Data]
```

The `schema` and `schema_version` lines are optional.

If the file does not start with an INI-style section header, it starts with tab-separated column names. An example is shown below:

```
patientName         sampleName          isTumor         extractionType          libraryType             folderNa
P001         N1         N         DNA         WES         P001-N1-DNA1-WES1
P001         T1         Y         DNA         WES         P001-T1-DNA1-WES1
P001         T1         Y         RNA         mRNA-seq         P001-T1-RNA1-mRNAseq1
P002         N1         N         DNA         WES         P001-N1-DNA1-WES1
P002         T1         Y         DNA         WES         P001-T1-DNA1-WES1
P002         T1         Y         DNA         WES         P001-T1-RNA1-RNAseq1
P002         T2         Y         DNA         WES         P001-T2-DNA1-WES1
P002         T2         Y         RNA         mRNA-seq         P001-T2-RNA1-mRNAseq1
```

They are as follows:

1. `patientName` – name of the patient, used to identify the patient in the sample sheet. This value will be used as the secondary id of the `BioEntity` of the patient.
2. `sampleName` – name of the sample, used to identify the sample for the patient in the sample sheet. The secondary ID of the `BioSample` will be generated from the `patientName` and `sampleName`.
3. `isTumor` – a flag identifying a sample as being from tumor, one of {`Y`, `N`, `1`, `0`}
4. `extractionType` – a valid extraction type as in the JSON schema, which is one of `DNA`, `RNA` or `other`. Based on the `extractionType` (and the secondary ID of the `BioSample`), the secondary ID of the `TestSample` will be generated.
5. `libraryType` – a valid libraryType, as in the JSON schema, e.g., `WES` or `mRNA-seq`. Based on the `libraryType` (and the secondary ID of the `TestSample`), the secondary ID of the `NGSLibrary` will be generated.
6. `folderName` – a folder name to search the library's FASTQ files for. The list of base folders to search for is given in the configuration and this folder is searched for a folder with the name given here. Thus, no absolute path is given here, only the folder name.

Optionally, the following fields can be added:

- `seqPlatform` can be one of `Illumina` and `PacBio`, default is `Illumina`

3.11 Germline Variants Samples

A relatively simple schema with the essential information for genetic germline variant samples with support for pedigree information. The assumed setting is as follows.

- Each bio entity is a patient/person/donor.
- Each donor gives one normal (bio) sample (e.g., blood or saliva) and each sample has at one DNA library.
- The pedigree has to be described in a way such that all members who donated a sample are connected.
- Within each pedigree, the flag for "affected" specifies the same genetic disease (i.e., in a polydactily family, only members affected with this phenotype are flagged as affected).

Note: The requirement of one DNA HTS library for each donor can be dropped in the future.

3.11.1 Germline Fields

The following fields must be present for germline variants sample sheets.

- **`BioSample`**
  - `fatherPk` – pk value of the father
  - `motherPk` – pk value of the mother
  - `sex` – sex of the patient
  - `affected` – "is affected" flag

3.11.2 Germline TSV Schema

Additionally, there is an alternative to defining schemas in JSON format for germline variants sample sheets. Instead, a TSV-based schema can be used.

Optionally, the schema can contain meta data, starting with `[Metadata]` INI-style section header (the data section has to start with `[Data]`).

```
[Metadata]
schema        germline_variants
schema_version        v1
title        Example germline study
description        The [Data] section is similar to a PEDigree file

[Data]
```

The `schema` and `schema_version` lines are optional.

If the file does not start with an INI-style section header, it starts with tab-separated column names. An example is shown below:

```
patientName        fatherName        motherName        sex        affected        libraryType
12_345        12_346        12_347        1        2        WGS        12-
↪345        HP:0009946,HP:0009899
12_348        12_346        12_347        1        1        WGS        12-348        .
12_346        0        0        1        1        .        .        .
12_347        0        0        2        1        WGS        12-347        .
```

They are as follows:

1. patientName – name of the patient, used for identifying the patient in the sample sheet. This value will be used as the secondary id of the BioEntity of the patient.
2. fatherName – name of the patient's father, use 0 or . for founder. This refers to the patientName of the patient's father.
3. mother – name of the patient's mother, use 0 or . for founder. This refers to the patientName of the patient's mother.
4. sex – flag for sex, one of M: male, F: female, .: unknown/missing, or 0, 1, 2, as in PED.
5. affected – flag for being affected, one of Y: yes, N: no, .: unknown/missing, or 0, 1, 2, as in PED.
6. folderName – a folder name to search the library's FASTQ files for, . if not sequenced. The list of base folders to search for is given in the configuration and this folder is searched for a folder with the name given here. Thus, no absolute path is given here, only the folder name.
7. hpoTerms – a comma-separated list of HPO terms to label the patient with, . if empty.
8. libraryType – a rough classification of the library type {WGS, WES, Panel-seq}, . if not sequenced or unknown
9. kitName/kitType – type/name of the kit used (free text/controlled vocabulary), . if not sequenced or unknown; optional. This column can be left out.
10. kitVersion – version of the kit used, . if not sequenced or unknown; optional. This column can be left out.

Optionally, the following fields can be added:

- seqPlatform can be one of Illumina and PacBio, default is Illumina

3.12 Custom Fields in TSV Schemas

When using TSV cancer_matched or germline_variants schemas, you can use custom fields by filling the [Custom Fields] header in the TSV file.

3.12.1 Example

In the following example, we add the fields shimada4Class (an enum with two possible values), patientStatus (an enum with three different value), to the bio entity/patient, the field mycnCn (a floating point number greater than or equal to 0) to the test sample, and one boolean flag for each the test sample and NGS library (testSampleFlag and ngsLibraryFlag).

```
[Metadata]
schema        cancer_matched
schema_version        v1
title        Example matched cancer tumor/normal study
description        This example shows how to add custom fields

[Custom Fields]
key        annotatedEntity        docs        type        minimum        maximum        unit
shimada4Class        bioEntity        Shimada 4 classification        enum        .
↪        .        .        favorable,unfavorable        .
patientStatus        bioSample        Status of patient        enum        .        .
↪        .        Deceased_from_disease,No_disease_event,Relapse_progression        .
mycnCn        bioSample        MYCN copy number        number        0.0        .
↪        .        .        .
```

(continues on next page)

(continued from previous page)

```
testSampleNice        testSample        Is test sample nice?        boolean        .
↪        .        .        .        .
ngsLibraryNice        ngsLibrary        Is NGS library nice?        boolean        .
↪        .        .        .        .

[Data]
patientName        sampleName        isTumor        libraryType        folderName        shimada4Clas
P001        N1        N        WES        P001-N1-DNA1-
↪WES1        favorable        Deceased_from_disease        2.0        Y        N
P001        T1        Y        WES        P001-T1-DNA1-
↪WES1        favorable        Deceased_from_disease        2.0        N        Y
P001        T1        Y        mRNA_seq        P001-T1-RNA1-
↪mRNAseq1        favorable        Deceased_from_disease        2.0        Y        Y
```

3.12.2 Field Types

The valid field types are given together with whether additional restrictions/information is allowed (Y) or even required (R).

Type	Summary	minimum	maximum	unit	choices	pattern
string	a string value					
integer	an integer	Y	Y	Y		
number	a floating point value	Y	Y	Y		
boolean	boolean flag					
enum	one of several strings				R	
regex	regex-checked string					R

3.13 Generic Sample Sheets

We describe call using a sample sheet not following the matched cancer or germline variants schema **generic sample sheets**. These can be used to describe arbitrary biomedical experiments. There is no restriction on the structure of such a sample sheet.

However, there also is a shortcut TSV format to describe such sample sheets that has some restrictions.

3.13.1 Generic TSV Schema

The following assumptions must hold when using the generic TSV schema:

- Each bio entity must have at least one bio sample, each bio sample at least one test sample, and each test sample at least one NGS library.
- You have to explicitely assign a secondary ID to each object in this tree.
- The files of each NGS library are below the same folder.
- You have to define the extraction type (e.g., DNA or RNA) and the librar type (e.g., mRNA_seq or WES).

A minimal generic TSV file (with header) looks as follows:

```
[Metadata]
schema        generic_experiment
schema_version        v1
title        Example generic experiment
description        This is an example for a generic experiment.

[Data]
bioEntity        bioSample        testSample        ngsLibrary        extractionType        libraryTy
E001        BS1        TS1        LIB1        RNA        total_RNA_seq        E001-
↪BS1-TS1-LIB1
E001        BS2        TS1        LIB1        RNA        total_RNA_seq        E001-
↪BS2-TS1-LIB1
E002        BS1        TS1        LIB1        RNA        total_RNA_seq        E002-
↪BS1-TS1-LIB1
E002        BS1        TS1        LIB2        RNA        total_RNA_seq        E001-
↪BS1-TS1-LIB2
```

Again, the header can be omitted, then the file starts after the `[Data]` line. All further information has to be described using custom fields as described in the section *Custom Fields in TSV Schemas*. For example with additional information:

```
[Metadata]
schema        generic_experiment
schema_version        v1
title        Example generic experiment
description        This is an example for a generic experiment.

[Custom Fields]
key        annotatedEntity        docs        type        minimum        maximum        unit        c
cellLine        bioEntity        Cell line name        enum        .        .        .
↪         HELA,HEK        .
treatment        bioEntity        Cell line treatment        enum        .        .
↪        .        Ibrutinib,Nivolumap,control        .
replicate        bioEntity        Replicate identifier        string        .        .
↪        .        .        .

[Data]
bioEntity        bioSample        testSample        ngsLibrary        extractionType        libraryTy
HELA_control_A        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_control_A-BS1-TS1-LIB1        HELA        control        A
HELA_control_C        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_control_C-BS1-TS1-LIB1        HELA        control        C
HELA_Ibrutinib_A        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_Ibrutinib_A-BS1-TS1-LIB1        HELA        Ibrutinib        A
HELA_Ibrutinib_B        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_Ibrutinib_B-BS1-TS1-LIB1        HELA        Ibrutinib        B
HELA_Nivolumap_A        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_Nivolumap_A-BS1-TS1-LIB1        HELA        Nivolumap        A
HELA_Nivolumap_B        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_Nivolumap_B-BS1-TS1-LIB1        HELA        Nivolumap        B
HELA_Nivolumap_C        BS1        TS1        LIB1        RNA        mRNA_
↪seq        HELA_Nivolumap_C-BS1-TS1-LIB1        HELA        Nivolumap        C
HEK_control_A        BS1        TS1        LIB1        RNA        mRNA_seq        HEK_
↪control_A-BS1-TS1-LIB1        HEK        control        A
HEK_control_B        BS1        TS1        LIB1        RNA        mRNA_seq        HEK_
↪control_B-BS1-TS1-LIB1        HEK        control        B
HEK_control_C        BS1        TS1        LIB1        RNA        mRNA_seq        HEK_
↪control_C-BS1-TS1-LIB1        HEK        control        C
```

(continues on next page)

(continued from previous page)

```
HEK_Ibrutinib_A        BS1        TS1        LIB1        RNA        mRNA_
↪seq         HEK_Ibrutinib_A-BS1-TS1-LIB1        HEK        Ibrutinib        A
HEK_Ibrutinib_B        BS1        TS1        LIB1        RNA        mRNA_
↪seq         HEK_Ibrutinib_B-BS1-TS1-LIB1        HEK        Ibrutinib        B
HEK_Nivolumap_B        BS1        TS1        LIB1        RNA        mRNA_
↪seq         HEK_Nivolumap_B-BS1-TS1-LIB1        HEK        Nivolumap        B
HEK_Nivolumap_C        BS1        TS1        LIB1        RNA        mRNA_
↪seq         HEK_Nivolumap_C-BS1-TS1-LIB1        HEK        Nivolumap        C
```

3.14 Models

3.14.1 biomedsheets.models.Sheet

3.14.2 biomedsheets.models.BioEntity

3.14.3 biomedsheets.models.BioSample

3.14.4 biomedsheets.models.TestSample

3.14.5 biomedsheets.models.NGSLibrary

3.14.6 biomedsheets.models.CrawlMixin

3.15 Shortcuts

Contents

3.15.1 biomedsheets.shortcuts.TestSampleChildShortcut

3.15.2 biomedsheets.shortcuts.NGSLibraryShortcut

3.15.3 biomedsheets.shortcuts.TestSampleShortcut

3.15.4 biomedsheets.shortcuts.GermlineCaseSheet

3.15.5 biomedsheets.shortcuts.CancerCaseSheet

3.15.6 biomedsheets.shortcuts.CancerDonor

3.15.7 biomedsheets.shortcuts.CancerMatchedSamplePair

3.15.8 biomedsheets.shortcuts.CancerBioSample

3.15.9 biomedsheets.shortcuts.SampleSheet

3.16 Input/Output

Contents

3.16.1 biomedsheets.io.SheetSchema

3.16.2 biomedsheets.io.SheetBuilder

3.17 TSV Input/Output

Contents

3.17.1 biomedsheets.io_tsv.DELIM

3.17.2 biomedsheets.io_tsv.LIBRARY_TYPES

3.17.3 biomedsheets.io_tsv.LIBRARY_TO_EXTRACTION

3.17.4 biomedsheets.io_tsv.EXTRACTION_TYPE_DNA

3.17.5 biomedsheets.io_tsv.EXTRACTION_TYPE_RNA

3.17.6 biomedsheets.io_tsv.EXTRACTION_TYPES

3.17.7 biomedsheets.io_tsv.KEY_TITLE

3.17.8 biomedsheets.io_tsv.KEY_DESCRIPTION

3.17.9 biomedsheets.io_tsv.PLATFORM_ILLUMINA

3.17.10 biomedsheets.io_tsv.PLATFORM_PACBIO

3.17.11 biomedsheets.io_tsv.PLATFORM_OTHER

3.17.12 biomedsheets.io_tsv.PLATFORM_DEFAULT

3.17.13 biomedsheets.io_tsv.PLATFORM_NAMES

3.17.14 biomedsheets.io_tsv.BOOL_VALUES

3.17.15 biomedsheets.io_tsv.NCBI_TAXON_HUMAN

3.17.16 biomedsheets.io_tsv.CANCER_DEFAULT_TITLE

3.17.17 biomedsheets.io_tsv.CANCER_DEFAULT_DESCRIPTION

3.17.18 biomedsheets.io_tsv.CANCER_BODY_HEADER

3.17.19 biomedsheets.io_tsv.CANCER_EXTRA_INFO_DEFS

3.17.20 biomedsheets.io_tsv.GERMLINE_DEFAULT_TITLE

3.17.21 biomedsheets.io_tsv.GERMLINE_DEFAULT_DESCRIPTION

3.17.22 biomedsheets.io_tsv.CANCER_BODY_HEADER

3.17.23 biomedsheets.io_tsv.GERMLINE_EXTRA_INFO_DEFS

3.17.24 biomedsheets.io_tsv.SEX_VALUES

3.17.25 biomedsheets.io_tsv.AFFECTED_VALUES

3.17.26 biomedsheets.io_tsv.SheetIOException

3.17.27 biomedsheets.io_tsv.TSVSheetException

3.17.28 biomedsheets.io_tsv.std_field