

IGVF CRISPR Jamboree 2024: MuData Proposal

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The goal of this document is to propose a **MuData** object structure for the IGVF CRISPR Jamboree 2024. It builds off of Lucas's [sample Gasperini MuData object](#) and the [per-guide metadata format](#). I will propose variants of the **MuData** structure that are appropriate as inputs and outputs for the gRNA assignment and inference modules. These structures will be exemplified by a subset of the Gasperini data, distinct from Lucas's. For each module, I will present minimal examples of **MuData** objects containing required fields, as well as more fleshed out examples containing optional fields. All of the **MuData** objects are available [on GitHub](#).

```
import mudata as md
import pandas as pd
pd.set_option('display.max_columns', None)
```

1 gRNA assignment

1.1 gRNA assignment (required fields)

1.1.1 Input

```
grna_assignment_input = md.read_h5mu("data/gasperini_grna_assignment_input_minimal.h5mu")
grna_assignment_input
```

```
## MuData object with n_obs × n_vars = 9704 × 167
##   uns:   'moi'
##   2 modalities
##   gene:   9704 x 112
##   guide:  9704 x 55
##   var:   'targeting', 'intended_target_name'
```

The minimal input **MuData** object for gRNA assignment contains two modalities: **gene** and **guide**. The **gene** modality just needs to have a **.X** matrix containing the gene RNA UMI counts. The **guide** modality needs to have a **.X** matrix containing the gRNA UMI counts, as well as a **.var** data frame containing at least the Boolean variable **targeting** and the string **intended_target_name**:

```
grna_assignment_input['guide'].var.iloc[[0, 1, 20, 21, 30, 31]]
```

```
##               targeting intended_target_name
## ATGTAGAAGGAGACACCGG      TRUE      ENSG00000012660
## GCGCAGAGGCGGATGTAGAG      TRUE      ENSG00000012660
## ACACCCTCATTAGAACCCAG      TRUE      candidate_enh_1
## TTAAGAGCCTCGGTTCCCT      TRUE      candidate_enh_1
## GACCTCCTGTGATCAGGTGG     FALSE      non-targeting
## ATTGGTATCCGTATAAGCAG     FALSE      non-targeting
```

Note that the **targeting** column is a string rather than a Boolean due to type compatibility issues involving

R, Python, and HDF5. It can be cast to a Boolean if desired. Finally, the gRNA assignment input MuData object must contain an `uns` field called `moi` (low or high).

1.1.2 Output

```
grna_assignment_output = md.read_h5mu("data/gasperini_grna_assignment_output_minimal.h5mu")
grna_assignment_output

## MuData object with n_obs × n_vars = 9704 × 167
##   uns:   'moi'
##   2 modalities
##     gene:   9704 x 112
##     guide:  9704 x 55
##       var:  'targeting', 'intended_target_name'
##     layers: 'grna_assignments'
```

The minimal gRNA assignment output MuData object is the same as the input object, except it now has a `.layer` called `grna_assignments`, which is a binary assignment matrix of guides to cells.

1.2 gRNA assignment (optional fields)

1.2.1 Input

```
grna_assignment_input = md.read_h5mu("data/gasperini_grna_assignment_input.h5mu")
grna_assignment_input

## MuData object with n_obs × n_vars = 9704 × 167
##   obs:   'prep_batch', 'within_batch_chip', 'within_chip_lane'
##   uns:   'moi'
##   2 modalities
##     gene:   9704 x 112
##     obs:    'num_expressed_genes', 'total_gene_umis'
##     var:    'symbol', 'gene_chr', 'gene_start', 'gene_end'
##     guide:  9704 x 55
##     obs:    'num_expressed_grnas', 'total_grna_umis'
##     var:    'targeting', 'intended_target_name', 'intended_target_chr', 'intended_target_start', 'i
```

Optionally, the gRNA assignment input object can contain a top-level `obs` field containing cell-level information that is not specific to modality, such as batch information. Here is what it looks like for the Gasperini data:

```
grna_assignment_input.obs[['prep_batch', 'within_batch_chip', 'within_chip_lane']]
```

```
##
##      prep_batch  within_batch_chip  \
## GCTTGAATCGAATGCT-1_1B_1_SI-GA-F2 prep_batch_1 within_batch_chip_B
## AGCTTGATCGAGAGCA-1_1A_2_SI-GA-E3 prep_batch_1 within_batch_chip_A
## CCCAATCTCCTCAATT-1_1B_1_SI-GA-F2 prep_batch_1 within_batch_chip_B
## CGCGGTACACTGTCGG-1_1A_2_SI-GA-E3 prep_batch_1 within_batch_chip_A
## GGACGTCTCATGTCTT-1_1B_8_SI-GA-F9 prep_batch_1 within_batch_chip_B
## ...
## CGCTATCTCTATCGCC-1_2A_4_SI-GA-G5 prep_batch_2 within_batch_chip_A
## TCACAAGCAGCCTTGG-1_2A_6_SI-GA-G7 prep_batch_2 within_batch_chip_A
## GCTGCAGGTGAAGGCT-1_2B_6_SI-GA-H7 prep_batch_2 within_batch_chip_B
## GGATTACCATGTTGAC-1_2A_4_SI-GA-G5 prep_batch_2 within_batch_chip_A
## GTGCTTCTCGGATGTT-1_2A_1_SI-GA-G2 prep_batch_2 within_batch_chip_A
##
##      within_chip_lane
```

```
## GCTTGAATCGAATGCT-1_1B_1_SI-GA-F2 within_chip_lane_1
## AGCTTGATCGAGAGCA-1_1A_2_SI-GA-E3 within_chip_lane_2
## CCCAATCTCCTCAATT-1_1B_1_SI-GA-F2 within_chip_lane_1
## CGCGGTACACTGTCGG-1_1A_2_SI-GA-E3 within_chip_lane_2
## GGACGTCTCATGTCTT-1_1B_8_SI-GA-F9 within_chip_lane_8
## ...
## CGCTATCTCTATCGCC-1_2A_4_SI-GA-G5 within_chip_lane_4
## TCACAAGCAGCCTTGG-1_2A_6_SI-GA-G7 within_chip_lane_6
## GCTGCAGGTGAAGGCT-1_2B_6_SI-GA-H7 within_chip_lane_6
## GGATTACCATGTTGAC-1_2A_4_SI-GA-G5 within_chip_lane_4
## GTGCTTCTCGGATGTT-1_2A_1_SI-GA-G2 within_chip_lane_1
##
## [9704 rows x 3 columns]
```

The gRNA assignment input object may also include cellwise covariates for the **gene** modality, such as number of expressed genes and total RNA UMIs:

```
grna_assignment_input['gene'].obs
```

```
##                                num_expressed_genes  total_gene_umis
## GCTTGAATCGAATGCT-1_1B_1_SI-GA-F2                41            280.0
## AGCTTGATCGAGAGCA-1_1A_2_SI-GA-E3                35            192.0
## CCCAATCTCCTCAATT-1_1B_1_SI-GA-F2                41            781.0
## CGCGGTACACTGTCGG-1_1A_2_SI-GA-E3                37            189.0
## GGACGTCTCATGTCTT-1_1B_8_SI-GA-F9                32            262.0
## ...
## CGCTATCTCTATCGCC-1_2A_4_SI-GA-G5                 23            203.0
## TCACAAGCAGCCTTGG-1_2A_6_SI-GA-G7                 30            173.0
## GCTGCAGGTGAAGGCT-1_2B_6_SI-GA-H7                 37            428.0
## GGATTACCATGTTGAC-1_2A_4_SI-GA-G5                 47            658.0
## GTGCTTCTCGGATGTT-1_2A_1_SI-GA-G2                 23            166.0
##
## [9704 rows x 2 columns]
```

Next, the gRNA assignment input object may contain additional information about the genes:

```
grna_assignment_input['gene'].var
```

```
##          symbol gene_chr  gene_start  gene_end
## ENSG00000008853  RHOTB2    chr8    22844930.0  22844931.0
## ENSG00000104679   R3HCC1    chr8    23145421.0  23145422.0
## ENSG00000104689  TNFRSF10A    chr8    23082573.0  23082574.0
## ENSG00000120889  TNFRSF10B    chr8    22926533.0  22926534.0
## ENSG00000120896   SORBS3    chr8    22409208.0  22409209.0
## ...
## ENSG00000114850   SSR3     chr3    156271913.0  156271914.0
## ENSG00000072274   TFRC     chr3    195808960.0  195808961.0
## ENSG00000134851  TMEM165    chr4     56262124.0  56262125.0
## ENSG00000198899                NaN            NaN
## ENSG00000228253                NaN            NaN
##
## [112 rows x 4 columns]
```

Finally, the gRNA assignment input object may contain cellwise covariates for the **guide** modality and additional information about the guides beyond the two required fields **targeting** and **intended_target_name**:

```
grna_assignment_input['guide'].obs
```

```
##                               num_expressed_grnas  total_grna_umis
## GCTTGAATCGAATGCT-1_1B_1_SI-GA-F2                1           9.0
## AGCTTGATCGAGAGCA-1_1A_2_SI-GA-E3                1          18.0
## CCCAATCTCCTCAATT-1_1B_1_SI-GA-F2                1          24.0
## CGCGGTACACTGTCGG-1_1A_2_SI-GA-E3                1          26.0
## GGACGTCTCATGTCTT-1_1B_8_SI-GA-F9                1          12.0
## ...                ...                ...
## CGCTATCTCTATCGCC-1_2A_4_SI-GA-G5                1           5.0
## TCACAAGCAGCCTTGG-1_2A_6_SI-GA-G7                1          39.0
## GCTGCAGGTGAAGGCT-1_2B_6_SI-GA-H7                1          21.0
## GGATTACCATGTTGAC-1_2A_4_SI-GA-G5                1          73.0
## GTGCTTCTCGGATGTT-1_2A_1_SI-GA-G2                1          12.0
##
## [9704 rows x 2 columns]
```

```
grna_assignment_input['guide'].var.iloc[[0, 1, 20, 21, 30, 31]]
```

```
##                               targeting intended_target_name intended_target_chr \
## ATGTAGAAGGAGACACCGGG          TRUE      ENSG00000012660             chr6
## GCGCAGAGGCGGATGTAGAG          TRUE      ENSG00000012660             chr6
## ACACCCTCATTAGAACCCAG          TRUE      candidate_enh_1             chr8
## TTAAGAGCCTCGGTTCCCCT          TRUE      candidate_enh_1             chr8
## GACCTCCTGTGATCAGGTGG          FALSE      non-targeting
## ATTGGTATCCGTATAAGCAG          FALSE      non-targeting
##
##                               intended_target_start  intended_target_end
## ATGTAGAAGGAGACACCGGG          53213723.0          53213738.0
## GCGCAGAGGCGGATGTAGAG          53213738.0          53213754.0
## ACACCCTCATTAGAACCCAG          23366136.0          23366564.0
## TTAAGAGCCTCGGTTCCCCT          23366564.0          23366992.0
## GACCTCCTGTGATCAGGTGG          -9.0                -9.0
## ATTGGTATCCGTATAAGCAG          -9.0                -9.0
```

2 Inference

2.1 Inference (required fields)

2.1.1 Input

Here is an example of a MuData object containing the minimal required fields for input to the inference module:

```
inference_input = md.read_h5mu("data/gasperini_inference_input_minimal.h5mu")
inference_input
```

```
## MuData object with n_obs × n_vars = 9704 × 167
##   uns:   'moi', 'pairs_to_test'
##   2 modalities
##     gene:   9704 × 112
##     guide:  9704 × 55
##     var:   'targeting', 'intended_target_name'
##     layers: 'grna_assignments'
```

This is the same as the minimal set of required fields for the output of the gRNA assignment module, except

there is an extra field in `uns` called `pairs_to_test`:

```
pd.DataFrame(inference_input.uns['pairs_to_test'])
```

```
##           gene_id intended_target_name
## 0  ENSG00000187109  ENSG00000187109
## 1  ENSG00000114850  ENSG00000114850
## 2  ENSG00000134851  ENSG00000134851
## 3  ENSG00000163866  ENSG00000163866
## 4  ENSG00000181610  ENSG00000181610
## ..           ...           ...
## 105 ENSG00000106789  candidate_enh_2
## 106 ENSG00000125482  candidate_enh_3
## 107 ENSG00000095380  candidate_enh_2
## 108 ENSG00000158941  candidate_enh_1
## 109 ENSG00000167123  candidate_enh_3
##
## [110 rows x 2 columns]
```

The minimal required fields in `pairs_to_test` are `gene_id` and `intended_target_name`. Each row specifies a test to be conducted between CRISPR perturbation of a given target and the the expression of a given gene.

2.1.2 Output

```
inference_output = md.read_h5mu("data/gasperini_inference_output_minimal.h5mu")
inference_output
```

```
## MuData object with n_obs × n_vars = 9704 × 167
##   uns:   'moi', 'pairs_to_test', 'test_results'
##   2 modalities
##     gene:   9704 x 112
##     guide:  9704 x 55
##     var:    'targeting', 'intended_target_name'
##     layers: 'grna_assignments'
```

The minimal required fields in the output of the inference module are the same as the minimal required fields for the input, except there is an extra field in `uns` called `test_results`:

```
pd.DataFrame(inference_output.uns['test_results'])
```

```
##           gene_id intended_target_name      p_value
## 0  ENSG00000187109  ENSG00000187109  3.217223e-85
## 1  ENSG00000114850  ENSG00000114850  2.414163e-79
## 2  ENSG00000134851  ENSG00000134851  4.309833e-50
## 3  ENSG00000163866  ENSG00000163866  4.704066e-49
## 4  ENSG00000181610  ENSG00000181610  3.766690e-42
## ..           ...           ...           ...
## 105 ENSG00000106789  candidate_enh_2  6.660000e-01
## 106 ENSG00000125482  candidate_enh_3  8.900000e-01
## 107 ENSG00000095380  candidate_enh_2  3.400000e-02
## 108 ENSG00000158941  candidate_enh_1  7.980000e-01
## 109 ENSG00000167123  candidate_enh_3  8.800000e-02
##
## [110 rows x 3 columns]
```

This is a data frame containing the same columns as the `pairs_to_test` data frame, plus at least one column containing a measure of the association for each pair. These columns can be `p_value`, `log2_FC`,

posterior_probability, or any other measure of association. We just have to standardize these column names.

2.2 Inference (optional fields)

2.2.1 Input

Here is an example of a MuData object containing some optional fields for input to the inference module:

```
inference_input = md.read_h5mu("data/gasperini_inference_input.h5mu")
inference_input

## MuData object with n_obs × n_vars = 9704 × 167
##   obs:   'prep_batch', 'within_batch_chip', 'within_chip_lane'
##   uns:   'moi', 'pairs_to_test'
##   2 modalities
##     gene:   9704 x 112
##       obs:   'num_expressed_genes', 'total_gene_umis'
##       var:   'symbol', 'gene_chr', 'gene_start', 'gene_end'
##     guide:  9704 x 55
##       obs:   'num_expressed_grnas', 'total_grna_umis'
##       var:   'targeting', 'intended_target_name', 'intended_target_chr', 'intended_target_start', 'intended_target_end'
##       layers: 'grna_assignments'
```

The additional fields are the same as those described for the gRNA assignment module. The only additional optional field is in the `pairs_to_test` data frame:

```
pd.DataFrame(inference_input.uns['pairs_to_test'])

##           gene_id intended_target_name      pair_type
## 0      ENSG00000187109      ENSG00000187109  positive_control
## 1      ENSG00000114850      ENSG00000114850  positive_control
## 2      ENSG00000134851      ENSG00000134851  positive_control
## 3      ENSG00000163866      ENSG00000163866  positive_control
## 4      ENSG00000181610      ENSG00000181610  positive_control
## ..           ...
## 105  ENSG00000106789      candidate_enh_2      discovery
## 106  ENSG00000125482      candidate_enh_3      discovery
## 107  ENSG00000095380      candidate_enh_2      discovery
## 108  ENSG00000158941      candidate_enh_1      discovery
## 109  ENSG00000167123      candidate_enh_3      discovery
##
## [110 rows x 3 columns]
```

Note the third column: `pair_type`. This optional column classifies pairs based on whether they are intended to be positive controls (an association is known to exist), negative controls (an association is known not to exist), or discovery pairs (pairs where it is unknown whether an association exists). This information need not be used by the inference module, but it is useful for downstream analysis.

2.2.2 Output

Here is an example of a MuData object containing some optional fields for output from the inference module:

```
inference_output = md.read_h5mu("data/gasperini_inference_output.h5mu")
inference_output

## MuData object with n_obs × n_vars = 9704 × 167
##   obs:   'prep_batch', 'within_batch_chip', 'within_chip_lane'
```

```
## uns: 'moi', 'pairs_to_test', 'test_results'
## 2 modalities
## gene: 9704 x 112
## obs: 'num_expressed_genes', 'total_gene_umis'
## var: 'symbol', 'gene_chr', 'gene_start', 'gene_end'
## guide: 9704 x 55
## obs: 'num_expressed_grnas', 'total_grna_umis'
## var: 'targeting', 'intended_target_name', 'intended_target_chr', 'intended_target_start', 'intended_target_end'
## layers: 'grna_assignments'
```

The only difference from before is in `test_results`:

```
pd.DataFrame(inference_output.uns['test_results'])
```

```
##          gene_id intended_target_name  log2_fc  p_value \
## 0  ENSG00000187109  ENSG00000187109 -0.774367  3.217223e-85
## 1  ENSG00000114850  ENSG00000114850 -1.849572  2.414163e-79
## 2  ENSG00000134851  ENSG00000134851 -0.893860  4.309833e-50
## 3  ENSG00000163866  ENSG00000163866 -1.223700  4.704066e-49
## 4  ENSG00000181610  ENSG00000181610 -1.314285  3.766690e-42
## ..          ...          ...          ...          ...
## 105 ENSG00000106789  candidate_enh_2  0.079632  6.660000e-01
## 106 ENSG00000125482  candidate_enh_3  0.144014  8.900000e-01
## 107 ENSG00000095380  candidate_enh_2 -0.165492  3.400000e-02
## 108 ENSG00000158941  candidate_enh_1  0.117617  7.980000e-01
## 109 ENSG00000167123  candidate_enh_3 -0.482057  8.800000e-02
##
##          pair_type
## 0  positive_control
## 1  positive_control
## 2  positive_control
## 3  positive_control
## 4  positive_control
## ..          ...
## 105  discovery
## 106  discovery
## 107  discovery
## 108  discovery
## 109  discovery
##
## [110 rows x 5 columns]
```

Now, the output includes the optional `pair_type` as well as a `log2_fc` in addition to the `p_value` column. This illustrates how an inference method may output multiple measures of association for each pair.