
Segway Documentation

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For a conceptual overview see the paper:

Michael M. Hoffman, Orion J. Buske, Zhiping Weng, Jeff A. Bilmes, William Stafford Noble. 2012. [Un-supervised pattern discovery in human chromatin structure through genomic segmentation](#). *Nat Methods* 9:473-476. doi:10.1038/nmeth.1937.

[Manuscript version](#) available at PubMed Central requires no subscription.

For a preprint of a protocol on using Segway see:

Roberts EG, Mendez M, Viner C, Karimzadeh M, Chan RCW, Ancar R, Chicco D, Hesselberth JR, Kundaje A, Hoffman MM. 2016. [Semi-automated genome annotation using epigenomic data and Segway](#). Preprint: <http://doi.org/10.1101/080382>

QUICKSTART GUIDE

1.1 Installation and configuration

1.1.1 With Bioconda

```
conda install segway
```

1.1.2 Generic installation

1. To install Segway first install [GMTK](#), and install [HDF5](#), then run this command from **bash**:

```
pip install segway
```

2. If you are using SGE, your system administrator must set up a `mem_requested` resource for Segway to work. This can be done by installing Segway and then running `python -m segway.cluster.sge_setup`.

1.2 Acquiring data

3. Observation data is stored with the `genomedata` system. <http://pmgenomics.ca/hoffmanlab/proj/genomedata/>. There is a small Genomedata archive for testing that comes with Segway, that is used in the below steps. You can get it using:

```
wget http://pmgenomics.ca/hoffmanlab/proj/segway/2011/test.genomedata
```

1.3 Running Segway

4. Use the `segway train` command to discover patterns in the test data. Here, we specify that we want Segway to discover four unique patterns:

```
segway --num-labels=4 train test.genomedata traindir
```

5. Use the `segway identify` command to create the segmentation, which partitions the genome into regions labeled with one of the four discovered patterns:

```
segway identify test.genomedata traindir identifydir
```

Note: This example spawns jobs that will run sequentially due to small segment size. See the `--split-sequences` option for dividing segments into smaller pieces.

1.4 Results

6. The `identifydir/segway.bed.gz` file has each segment as a separate line in the BED file, and can be used for further processing.
7. The `identifydir/segway.layered.bed.gz` file is designed for easier visualization on a genome browser. It has thick lines where a segment is present and thin lines where it is not. This is not as easy for a computer to parse, but it is more useful visually.
8. You can also perform further analysis of the segmentation and trained parameters using Segtools <<http://pmgenomics.ca/hoffmanlab/proj/segtools/>>.

SEGWAY 3.0.3 OVERVIEW

2.1 Installation

With the [Conda](#) environment manager and the additional [Bioconda](#) channel, Segway can be installed with the command:

```
conda install segway
```

Alternatively without Bioconda, the following prerequisites must be installed for Segway:

You need Python 2.7, or Python 3.6 or later versions.

You need Graphical Models Toolkit (GMTK), which you can get at <https://github.com/melodi-lab/gmtk/releases>.

You need the HDF5 serial library and tools. The following packages are necessary for the OS you are running:

Ubuntu/Debian:

```
sudo apt-get install libhdf5-serial-dev hdf5-tools
```

CentOS/RHEL/Fedora:

```
sudo yum -y install hdf5 hdf5-devel
```

OpenSUSE:

```
sudo zypper in hdf5 hdf5-devel libhdf5
```

Afterwards Segway can be installed automatically with the command `pip install segway`.

Note: Segway may not install with older versions of pip (< 6.0) due to some of its dependencies requiring the newer version. To upgrade your pip version run `pip install pip --upgrade`.

2.1.1 Standalone configuration

Segway can be run without any cluster system. This will automatically be used when Segway fails to access any cluster system. You can force it by setting the `SEGWAY_CLUSTER` environment variable to `local`. For example, if you are using bash as your shell, you can run:

```
SEGWAY_CLUSTER=local segway
```

By default, Segway will use up to 32 concurrent processes when running in standalone mode. To change this, set the `SEGWAY_NUM_LOCAL_JOBS` environment variable to the appropriate number.

2.1.2 Cluster configuration

If you want to use Segway with your cluster, you will need the `drmaa>=0.4a3` Python package.

You need either Sun Grid Engine (SGE; now called Oracle Grid Engine), Platform Load Sharing Facility (LSF) and FedStage DRMAA for LSF, Slurm workload manager, or Torque/PBS/PBS Pro (experimental).

If FedStage DRMAA for LSF is installed, Segway should be ready to go on LSF out of the box.

If you are using the Slurm workload manager with versions past 18, it is recommended you install a DRMAA driver based on an updated fork since the original implementation is no longer updated or maintained. We currently test our Slurm support using <https://github.com/natefoo/slurm-drmaa>.

If you are using SGE, someone with cluster manager privileges on your cluster must have Segway installed within their `PYTHONPATH` or systemwide and then run `python -m segway.cluster.sge_setup`. This sets up a consumable `mem_requested` attribute for every host on your cluster for more efficient memory use.

2.2 The workflow

Segway accomplishes four major tasks from a single command-line. It–

1. **generates** an unsupervised segmentation model and initial parameters appropriate for this data;
2. **trains** parameters of the model starting with the initial parameters; and
3. **identifies or annotates** segments in this data with the model.
4. calculates **posterior** probability for each possible segment label at each position.

Note: The verbs “identify” and “annotate” are synonyms when using Segway. They both describe the same task and may be used interchangeably.

2.2.1 Technical description

More specifically, Segway performs the following steps:

1. Acquires data in `genomedata` format
2. Generates an appropriate model for unsupervised segmentation (`segway.str`, `segway.inc`) for use by GMTK
3. Generates appropriate initial parameters (`input.master` or `input.*.master`) for use by GMTK
4. Writes the data in a format usable by GMTK
5. Call GMTK to perform expectation maximization (EM) training, resulting in a parameter file (`params.params`)
6. Call GMTK to perform Viterbi decoding of the observations using the generated model and discovered parameters
7. Convert the GMTK Viterbi results into BED format (`segway.bed.gz`) for use in a genome browser, or by Segtools <<http://pmgenomics.ca/hoffmanlab/proj/segtools/>>, or other tools
8. Call GMTK to perform posterior decoding of the observations using the generated model and discovered parameters
9. Convert the GMTK posterior results into bedGraph format (`posterior.seg*.bedGraph.gz`) for use in a genome browser or other tools
10. Use a distributed computing system to parallelize all of the GMTK tasks listed above, and track and predict their resource consumption to maximize efficiency

11. Generate reports on the established likelihood at each round of training (`likelihood.*.tab`)

The **identify** and **posterior** tasks can run simultaneously, as they depend only on the results of **train**, and not each other.

2.3 Data selection

Segway accepts data only in the Genomedata format. The Genomedata package includes utilities to convert from BED, wiggle, and bedGraph formats. By default, Segway uses all the continuous data tracks in a Genomedata archive. Multiple Genomedata archives can be specified to be used in data selection as long as each archive refers to the same sequence and do not have overlapping track names.

Note: Segway does not allow multiple genomedata archives to contain equivalent tracks names. However if your archives have tracks with matching track names, you may explicitly specify to Segway the track names that do not overlap in other genomedata archives and Segway will run as normal.

2.3.1 Tracks

You may specify a subset of tracks using the `--track` option which may be repeated. For example:

```
segway --track dnasei --track h3k36me3
```

will include the two tracks `dnasei` and `h3k36me3` and no others.

You can run a concatenated segmentation by separating tracks with a comma. For example:

```
segway --track dnasei.liver,dnasei.blood --track h3k36me3.liver,h3k36me3.blood
```

2.3.2 Positions

By default, Segway runs analyses on the whole genome. This can be incredibly time-consuming, especially for training. In reality, training (and even identification) on a smaller proportion of the genome is often sufficient. There are also regions as the genome such as those containing many repetitive sequences, which can cause artifacts in the training process. The `--exclude-coords=file` and `--include-coords=file` options specify BED files with regions that should be excluded or included respectively. If both are specified, then inclusions are processed first and the exclusions are then taken out of the included regions.

Note: BED format uses zero-based half-open coordinates, just like Python. This means that the first nucleotide on chromosome 1 is specified as:

```
chr1    0    1
```

The UCSC Genome Browser and Ensembl web interfaces, as well as the wiggle formats use the one-based fully-closed convention, where it is called `chr1:1-1`.

For example, the ENCODE Data Coordination Center at University of California Santa Cruz keeps the coordinates of the ENCODE pilot regions in this format for `(GRCh37/hg19) http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/referenceSequences/encodePilotRegions.hg19.bed` _

and

``(NCBI36/hg18) http://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/encodeRegions.txt.gz`_`. For human whole-genome studies, these regions have nice properties since they mark 1 percent of the genome, and were carefully picked to include a variety of different gene densities, and a number of more limited studies provide data just for these regions. All coordinates are in terms of the GRCh37 assembly of the human reference genome (also called hg19 by UCSC).

After reading in data from a Genomedata archive, and selecting a smaller subset with `--exclude-coords` and `--include-coords`, the final included regions are referred to as *windows*, and are supplied to GMTK for inference. There is no direction connection between the data in different windows during any inference process—the windows are treated independently.

An alternative way to speed up training is to use the `--minibatch-fraction=frac` option, which will cause Segway to use a fraction *frac* or more of genomic positions, chosen randomly at each training iteration. The `--exclude-coords=file` and `--include-coords=file` options still apply when using minibatch. The fraction will only apply to the resulting chosen coordinates. For example, using `--minibatch-fraction=0.01` will use a different random one percent of the genome for each training round. This will allow training to have access to the whole genome for training while maintaining fast iterations. Using this option will select on the basis of windows, so the fraction of the genome chosen will be closer to the specified fraction if the windows are small (but the chosen fraction will always be at least as large as specified). Therefore, it is best to combine `--minibatch-fraction` with `--split-sequences`. The likelihood-based training stopping criterion is no longer valid with minibatch training, so training will always run to `--max-train-rounds` (100, by default) if `--minibatch-fraction` is set.

An alternative way to choose the winning set of parameters is available through the `--validation-fraction=frac` or `--validation-coords` options. Specifying a fraction *frac* to `--validation-fraction` will cause Segway to choose a fraction *frac* or more of genomic positions as a held-out validation set. `--validation-coords=file` allows one to explicitly specify genomic coordinates in a BED-format file, to be used as a validation set. When using either of these options, Segway will evaluate the model after each training iteration on the validation set and will choose the winning set of parameters based on whichever set gives the best validation set likelihood across all instances.

Note: `--exclude-coords` is applied to `--validation-coords` but `--include-coords` is not. This allows the user to easily specify regions of the genome that should not be considered by Segway overall, while also allowing them to specify a set of validation coordinates in a straightforward manner.

2.3.3 Resolution

In order to speed up the inference process, you may downsample the data to a different resolution using the `--resolution=res` option. This means that Segway will partition the input observations into fixed windows of size *res* and perform inference on the mean of the observation averaged along the fixed window. This can result in a large speedup at the cost of losing the highest possible precision. However, if you are considering data that is only generated at a low resolution to start with, this can be an appealing option.

Warning: You must use the same resolution for *both* training and identification.

In semi-supervised mode, with the resolution option enabled, the supervision labels are also downsampled to a lower resolution, but by a different method. In particular, segway will partition the input supervision labels into fixed windows of size *res* and use a modified ‘mode’ to choose which label will represent that window during training. This modified ‘mode’ works according to the following rules:

- 1) In general, segway takes the highest-count nonzero label in a given resolution-sized window to be the mode for that window.
- 2) In the case of ties, segway takes the lowest nonzero label.

- 3) Segway takes the mode to be 0 (no label) if and only if all elements of the window are 0.

2.4 Model generation

Segway generates a model (`segway.str`) and initial parameters (`input.master`) appropriate to a dataset using the GMTKL specification language and the GMTK master parameter file format. Both of these are described more fully in the GMTK documentation (cite), and the default structure and starting parameters are described more fully in the Segway article.

The starting parameters are generated using data from the whole genome, which can be quickly found in the Genomedata archive. Even if you are training on a subset of the genome, this information is not used.

You can tell Segway just to generate these files and not to perform any inference using the `--dry-run` option.

Using `--num-instances=starts` will generate multiple copies of the `input.master` file, named `input.0.master`, `input.1.master`, and so on, with different randomly picked initial parameters. Segway training results can be quite dependent on the initial parameters selected, so it is a good idea to try more than one. I usually use `--num-instances=10`.

Using `--mixture-components` will set the number of Gaussian mixture components per label to use in the model (default 1).

Using `--var-floor` will set the variance floor for the model, meaning that if any of the variances of a track falls below this value, then the variance will be floored (prohibited from falling below the floor value). This is by default turned off if not using a mixture of Gaussians; if using a mixture of Gaussians, then it has a default value of $1e-5$.

Warning: You should know the scale of your data and set an appropriate variance floor if the scale is very small.

You may substitute your own `input.master` files but I recommend starting with a Segway-generated template. This will help avoid some common pitfalls. In particular, if you are going to perform training on your model, you must ensure that the `input.master` file retains the same `#ifdef` structure for parameters you wish to train. Otherwise, the values discovered after one round of training will not be used in subsequent rounds, or in the annotate or posterior stages.

You can use the `--num-labels=labels` option to specify the number of segment labels to use in the model (default 2). You can set this to a single number or a range with Python slice notation. For example, `--num-labels=5:20:5` will result in 5, 10, and 15 labels being tried. If you specify `--num-instances=starts`, then there will be *starts* different instances for each of the *labels* labels tried.

The question of finding the right number of labels is a difficult one. Mathematical criteria, such as the Bayesian information criterion, would usually suggest using higher numbers of labels. However, the results are difficult for a human to interpret in this case. This is why we usually use ~25 labels for a segmentation of dozens of input tracks. If you use a small number of input tracks you can probably use a smaller number of labels.

There is an experimental `--num-sublabels=sublabels` option that enables hierarchical segmentation, where each segment label is divided into a number of segment sublabels, each one with its own Gaussian emission parameters. The output segmentation will be defined according to the `--output-label=output_label` option, by default *seg*, which will output by (super) segment label as normal. *subseg* will output in terms of individual sublabels, only printing out the sublabel part, and *full* will print out both the superlabel and the sublabel, separated by a period. For example, a coordinate assigned superlabel 1 and sublabel 0 would display as “1.0”. Using this feature effectively may require manipulation of model parameters.

Segway allows multiple models of the values of a continuous observation tracks using three different probability distributions: a normal distribution (`--distribution=norm`), a normal distribution on asinh-transformed data (`--distribution=asinh_norm`, the default), or a gamma distribution (`--distribution=gamma`). For gamma distributions, Segway generates initial parameters by converting mean μ and variance σ^2 to shape k and scale

theta using the equations $\mu = k\theta$ and $\sigma^2 = k\theta^2$. The ideal methodology for setting gamma parameter values is less well-understood. I recommend the use of `asinh_norm` in most cases.

2.4.1 Virtual Evidence

To use virtual evidence [pearl1988] in Segway, the user specifies a prior probability that a genomic region has a particularly labelled state. In this sense, virtual evidence is a form of semi-supervised learning.

Users supplying virtual evidence while running an entire task together (for example, **train** or **annotate**) will supply the virtual evidence file with `--virtual-evidence`. Users supplying virtual evidence while running steps for a task one at a time will need to specify `--virtual-evidence` during the init step in order to properly generate the input files such as `input.master` and the triangulation file. During **annotate** and **posterior** a file can be supplied with the `--virtual-evidence` option to the init step however it may not be changed during run.

The virtual evidence file supplied to `--virtual-evidence` should be of BED3+2, tab-delimited, format where the 4th column is the label index and the fifth column is the prior. For example,

```
chr1 0 1000 0 0.9
```

will specify a prior probability of 0.9 on label 0 for the region of chr1 from 0 to 1000.

If running on multiple concatenated segmentations (worlds), the VE file is in BED3+3 format instead, and the world number must be specified for each row in the last column. If this is omitted and a BED3+2 file is submitted instead, the virtual evidence will be applied to all worlds instead. For example, with two worlds,

```
chr1 0 1000 0 0.9 0
```

```
chr1 0 1000 1 0.05 1
```

These examples specify prior probabilities over the region of chr1 from 0 to 1000 for both worlds. A prior probability of 0.9 on label 0 for the first world and a prior probability of 0.05 on label 1 for the second world.

At positions for which some labels are given a prior by the user but other labels not, the remaining probability is uniformly distributed amongst the leftover labels. For example, with 4 labels:

```
chr1 0 1000 0 0.4
```

all labels but label 0 would be given a prior probability of $(1-0.4)/3=0.2$.

2.4.2 Model Customization

You can supply your own custom or modified models to Segway by using the `--structure` option. The model is defined by the syntax that GMTK uses. To learn more about using GMTK to create your own models there the [GMTK documentation](#).

2.4.3 Segment duration model

Hard length constraints

The `--seg-table=file` option allows specification of a *segment table* that specifies minimum and maximum segment lengths for various labels. By default, the minimum segment length is the ruler length and it is set for all labels. Here is an example of a segment table:

```
label len
1:4 200:2200:50
0 200::50
4: 200::50
```

The file is tab-delimited, and the header line with `label` in one column and `len` in another is mandatory. The first column specifies a label or range of labels to which the constraints apply. In this column, a range of label values may be specified using Python slice syntax, so `label 1 : 4` specifies labels 1, 2, and 3. Using `4 :` for a label, as in the last row above, means all labels 4 or higher.

The second column specifies three colon-separate values: the minimum segment length, maximum segment length, and the ruler. In the example above, for labels 1, 2 and 3, segment lengths between 200 and 2200 are allowed, with a 50 bp ruler. If either the minimum or maximum lengths are left unspecified, then no corresponding constraint is applied. If the ruler is left unspecified the default or set value from the `--ruler-scale` option is used.

The ruler is an efficient heuristic that decreases the memory used during inference at the cost of also decreasing the precision with which the segment duration model acts. Essentially, it allows the duration model to switch the behavior of the rest of the model only after a multiple of *scale* bp has passed. Note that the ruler must match all other ruler entries in this file, as well as the option set with `--ruler-scale=scale`. (This may become more free in the future.)

Due to the lack of an epilogue in the model, it is possible to get one segment per sequence that actually does not meet the minimum segment length. This is expected and will be fixed in a future release.

Note that increasing hard minimum or maximum length constraints will greatly increase memory use and run time. You can decrease this performance penalty by increasing ruler size (which makes the precision of the duration model a little coarser), or by using the soft length priors below.

Use these segment lengths along with the supervised learning feature with caution. If you try to create something impossible with your supervision labels, such as defining a 2300-bp region to have label 1, which you have already constrained to have a maximum segment length of 2200, GMTK will produce the dreaded zero clique error and your training run will fail. Don't do this. In practice, due to the imprecision introduced by the 200-bp ruler, a region labeled in the supervision process with label 1 that is only 2000 bp long may also cause the training process to fail with a zero clique error. If this happens either decrease the size of the ruler, increase the size of the maximum segment length, or decrease the size of the supervision region.

Soft length prior

There is also a way to add a soft prior on the length distribution, which will tend to make the expected segment length 100000, but will still allow data that strongly suggests another length control. The default expected segment length of 100000 can't be changed at the moment but will in a future version.

You can control the strength of the prior relative to observed transitions with the `--prior-strength=strength` option. Setting `--prior-strength=1` means there are as many pseudocounts due to the prior as the number of nucleotides in the training regions.

The `--segtransition-weight-scale=scale` option controls the strength of the prior in another way. It controls the strength of the length prior relative to the data from the observed tracks. The default *scale* of 1 gives the soft transition model equal strength to a single data track. Using higher or lower values gives comparatively greater or lesser weight to the probability from the soft length prior, essentially allowing the prior to have more votes in determining where a segment boundary is. The impact of the prior will be a function of both `--segtransition-weight-scale` and `--prior-strength`.

One may effectively use the hard length constraints and soft length priors simultaneously.

2.5 Task selection

Segway will perform either (a) model generation and training or (b) identification separately, so it is possible to train on a subset of the genome and annotate on the whole thing. To train, use:

```
segway train GENOMEDATA TRAINDIR
```

To annotate, run Segway from the same directory you ran the train task above and specify the TRAINDIR from the results of your training:

```
segway annotate GENOMEDATA TRAINDIR IDENTIFYDIR
```

In both cases, replace GENOMEDATA with the Genomedata archive you're using. The use of `--dry-run` will cause Segway to generate appropriate model and observation files but not to actually perform any inference or queue any jobs. This can be useful when troubleshooting a model or task.

2.6 Train task

Most users will generate the model at training time, but to specify your own model there are the `--structure=filename` and `--input-master=filename` options. You can simultaneously run multiple *instances* of EM training in parallel, specified with the `--instances=instances` option. Each instance consists of a number of rounds, which are broken down into individual tasks for each training region. The results from each region for a particular instance and round are combined in a quick *bundle* task. It results in the generation of a parameter file like `params.3.params.18` where 3 is the instance index and 18 is the round index. Training for a particular instance continues until at least one of these criteria is met:

- the likelihood from one round is only a small improvement from the previous round; or
- 100 rounds have completed.

Specifically, the “small improvement” is defined in terms of the current likelihood L_n and the log likelihood from the previous round L_{n-1} , such that training continues while

$$\left| \frac{\log L_n - \log L_{n-1}}{\log L_{n-1}} \right| \geq 10^{-5}.$$

This constant will likely become an option in a future version of Segway.

As EM training produces diminishing returns over time, it is likely that one can obtain acceptably trained parameters well before these criteria are met. Training can be a time-consuming process. You may wish to train only on a subset of your data, as described in [Positions](#).

When all instances are complete, Segway picks the parameter set with the best likelihood and copies it to `params.params`.

There are two different modes of training available, unsupervised and semisupervised.

2.6.1 Unsupervised training

By default, Segway trains in unsupervised mode, which is a form of clustering. In this mode, it tries to find recurring patterns suggested by the data without any additional preconceptions of which regions should be tied together.

2.6.2 Semisupervised training

Using the `--semisupervised=file` option, one can specify a BED file as a list of regions used as supervision labels. The *name* field of the BED File specifies a label to be enforced during training. For example, with the line:

```
chr3    400    800    2
```

one can enforce that these positions will have label 2. You might do this if you had specific reason to believe that these regions were enhancers and wanted to find similar patterns in your data tracks. Using smaller labels first (such as 0) is probably better. Supervision labels are not enforced during the annotate task, and therefore cannot be specified during annotate.

You can also choose to specify a soft assignment for the supervision label. For example, with the line:

```
chr3    400    800    0:5
```

one can enforce that these positions will have a label in the range of [0,5). In other words, the label will be restricted to one of {0, 1, 2, 3, 4}. You may want to do this if you know the appearance of the patterns in the regions but you believe they might belong to more than one label. For soft assignment currently we only support a fixed size of the range of labels. For example, you may specify 0:5 and 3:8 in a single supervision label BED file, but you can't specify 0:5 (range size 5) and 6:8 (range size 2).

To simulate fully supervised training, simply supply supervision labels for the entire training region.

None of the supervision labels can overlap with each other. You should combine any overlapping labels before specifying them to Segway.

It is also possible for nonoverlapping labels to violate the ruler constraints set by Segway for GMTK. This happens when your supervision labels specify a transition that doesn't fall on a ruler boundary. For example, if your supervision labels are directly adjacent, such as:

```
chr1    10     20     0:2
chr1    20     30     2:4
```

and your ruler is set so it won't allow a transition to occur on 20 then your jobs will terminate with a 'zero clique' error. To resolve this, either avoid having directly adjacent supervision labels or, if possible, set `-ruler-scale=1` and run Segway again.

2.6.3 General options

The `--dont-train=file` option specifies a file with a newline-delimited list of parameters not to train. By default, this includes the `dpmf_always`, `start_seg`, and all GMTK DeterministicCPT parameters. You are unlikely to use this unless you are generating your own models manually.

Seeding

Segway can be forced to run with a specified random number generator seed by setting the `SEGWAY_RAND_SEED` environment variable. This is an optional for Segway and is primarily useful for reproducing results future results or debugging . For example, if you are using bash as your shell you can run:

```
SEGWAY_RAND_SEED=1498730685
```

To set the random number generator seed to the number 1498730685. If you decide to seed the random number generator, it is recommended to pick a number unique for your own usage.

2.6.4 Recovery

Since training can take a long time, this increases the probability that external factors such as a system failure will cause a training run to fail before completion. You can use the `--recover=dirname` option to specify a previous work directory you're recovering from.

2.7 Annotate task

The **annotate** mode of Segway uses the Viterbi algorithm to decode the most likely path of segments, given data and a set of parameters, which can come from the **train** task. Annotate runs considerably more quickly than training. While the underlying inference task is very similar, it must be completed on each region of interest only once rather than hundreds of times as in training.

You must run `annotate` from the same directory from where the `train` task was run. You can either manually set individual input master, parameter, and structure files, or implicitly use the files generated by the **train** task completed in `traindir`, and referenced in `traindir/train.tab`. If you are using training data from an old version of Segway, you must either create a `train.tab` file or specify the parameters manually, using `--structure`, `--input-master`, and `--trainable-params`.

The `--bed=bedfile` option specifies where the segmentation should go. If *bedfile* ends in `.gz`, then Segway uses gzip compression. The default is `segway.bed.gz` in the working directory.

You can load the generated BED files into a genome browser. Because the files can be large, I recommend placing them on your web site and supplying the URL to the genome browser rather than uploading the file directly. When using the UCSC genome browser, the `bigBed` utility may be helpful in speeding access to parts of a segmentation.

The output is in BED format (<http://genome.ucsc.edu/FAQ/FAQformat.html#format1>), and includes columns for chromosome, start, and end (in zero-based, half-open format), and the label. Other columns to the right are used for display purposes, such as coloring the browser display, and can be safely ignored for further processing. We use colors from ColorBrewer (<http://colorbrewer2.org/>).

2.7.1 Recovery

The `--recover=dirname` allows recovery from an interrupted `annotate` task. Segway will requeue jobs that never completed before, skipping any windows that have already completed.

2.7.2 Creating layered output

Segway produces BED files as output with the segment label in the name field. While this is the most sensible way of interchanging the segmentation with other programs, it can be difficult to visualize. To solve this problem, Segway will also produce a *layered* BED file with rows for each possible Segment label and thick boxes at the location of each label. This is what we show in the screenshot figure of the Segway article. This is much easier to see at low levels of magnification. The layers are also labeled, removing the need to distinguish them exclusively by color. While Segway automatically creates these files at the end of an annotate task, you can also use **segway-layer** with a standard BED segmentation to repeat the layering process, which you may want to do if you want to add mnemonic labels instead of the initial integers used as labels. **segway-layer** supports the use of standard input and output by using `-` as a filename, following a common Unix convention.

The mnemonic files used by Segway and Segtools have a simple format. They are tab-delimited files with a header that has the following columns: `old`, `new`, and `description`. The `old` column specifies the original label in the BED file, which is always produced as an integer by Segway. The `new` column allows the specification of a short alphanumeric mnemonic for the label. The `description` column is unused by **segway-layer**, but you can use it to add helpful annotations for humans examining the list of labels, or to save label mnemonics you used previously. The row order of the mnemonic file matters, as the layers will be laid down in a similar order. Mnemonics sharing the same alphabetical prefix (for example, `A0` and `A1`) or characters before a period (for example, `0.0` and `0.1`) will be rendered with the same color.

segtools-gmtk-parameters in the Segtools package can automatically annotate an initial hierarchical labeling of segmentation parameters. This can be very useful as a first approximation of assigning meaning to segment labels.

A simple mnemonic file appears below:

old	new	description
0	TSS	transcription start site
2	GE	gene end
1	D	dead zone

2.8 Posterior task

The **posterior** inference task of Segway estimates for each position of interest the probability that the model has a particular resolution-sized section given the data. The posterior output does not fall on segment boundaries since segment duration is itself probabilistically modeled. This information is delivered in a series of numbered BED files, one for each segment label. In hierarchical segmentation mode, setting the `-output-label` option to `full` or `subseg` will cause segway to produce a wiggle file for each sublabel instead, identified using the label and the sublabel in the file name before the file extension. For example, the bedGraph file for label 0, and sublabel 1 would be called `posterior0.1.bedGraph`. The individual values will vary from 0 to 100, showing the percentage probability at each position for the label in that file. In most positions, the value will be 0 or 100, and substantially reproduce the Viterbi path determined from the **annotate** task. The **posterior** task uses the same options for specifying a model and parameters as **annotate**.

Posterior results can be useful in determining regions of ambiguous labeling or in diagnosing new models. The mostly binary nature of the posterior assignments is a consequence of the design of the default Segway model, and it is possible to design a model that does not have this feature. Doing so is left as an exercise to the reader.

You may find you need to convert the bedGraph files to bigWig format first to allow small portions to be uploaded to a genome browser piecewise.

2.8.1 Recovery

Recovery is not yet supported for the posterior task.

2.9 Modular interface

Segway additionally supports running the tasks in a more modular manner. Each task is subdivided into 3 common steps:

init: Generates all input files, but does not submit any jobs to GMTK (besides triangulate). At the end of this step the `input.master`, `segway.str`, auxiliary files, tri files, and the `window.bed` will all be generated.

run: Submits jobs to GMTK. Produces params and viterbi files.

finish: Selects the best training and identify instances and uses these to generate the output files.

run-round: This step is specific to training. It will run a single round of training and then stop, allowing a user to view results and modify files.

The desired step may be selected by adding it to the task with a hyphen separating the two, in the form `<task>-<step>`. For example, to run **init** for the **train** step, a user would call `segway train-init GENOMEDATA TRAINDIR`

2.10 Python interface

I have designed Segway such that eventually one may call different components directly from within Python.

You can then call the appropriate module through its `main()` function with the same arguments you would use at the command line. For example:

```
from segway import run
GENOMEDATA_DIRNAME = "genomedata"
run.main(["train", "--random-starts=3", GENOMEDATA_DIRNAME])
```

All other interfaces (the ones that do not use a `main()` function) to Segway code are undocumented and should not be used. If you do use them, know that the API may change at any time without notice.

2.11 Command-line usage summary

All programs in the Segway distribution will report a brief synopsis of expected arguments and options when the `--help` option is specified and version information when `--version` is specified.

usage: `segway [global_args] COMMAND [args]...`

Segmentation and automated genome annotation.

optional arguments:

-h, --help	show this help message and exit
--version	show program's version number and exit

list of commands:

train create a model with learned parameters - train-init prepare initial models for parallel training - train-run train initial models to completion criterion, in parallel - train-run-round train models for one round, in parallel - train-finish select best model and prepare for *annotate*

annotate label a genome using a model - annotate-init prepare for parallel annotation - annotate-run annotate the genome, in parallel - annotate-finish concatenate parallel annotation results

posterior infer posterior probabilities of each label across a genome - posterior-init prepare for parallel posterior inference - posterior-run infer posterior probabilities, in parallel - posterior-finish concatenate parallel posterior inference results

Use *segway COMMAND -help* for help specific to command COMMAND.

Technical variables:

- m PROGRESSION, --mem-usage PROGRESSION** try each float in PROGRESSION as the number of gibibytes of memory to allocate in turn (default 2,3,4,6,8,10,12,14,15)
- S SIZE, --split-sequences SIZE** split up sequences that are larger than SIZE bp (default 2000000)
- v NUM, --verbosity NUM** show messages with verbosity NUM (default 0)
- cluster-opt OPT** specify an option to be passed to the cluster manager

Flags:

- n, --dry-run** write all files, but do not run any executables

Citation: Hoffman MM, Buske OJ, Wang J, Weng Z, Bilmes J, Noble WS. 2012. Unsupervised pattern discovery in human chromatin structure through genomic segmentation. Nat Methods 9:473-476. <https://doi.org/10.1038/nmeth.1937>

2.11.1 Utilities

Usage: *segway-layer* [OPTION]... [INFILE] [OUTFILE]

Options:

- version** show program's version number and exit
- h, --help** show this help message and exit
- b FILE, --bigBed=FILE** specify bigBed output file
- m FILE, --mnemonic-file=FILE** specify tab-delimited input file with mnemonic replacement identifiers for segment labels
- no-recolor** don't recolor labels
- s <ATTR VALUE>, --track-line-set=<ATTR VALUE>** set ATTR to VALUE in track line

Usage: *segway-winner* [OPTION]... DIR

Options:

- version** show program's version number and exit
- h, --help** show this help message and exit
- i, --input-master** print input master file name
- p, --params** print parameters file name
- c, --copy** copy files to final winning file locations

--clobber overwrite existing files

2.12 Environment Variables

SEGWAY_CLUSTER

Forces segway to use a specific cluster environment. Setting this to 'local' forces segway to use run locally and use no cluster environment.

SEGWAY_NUM_LOCAL_JOBS

Sets the maximum number of jobs when running locally.

SEGWAY_RAND_SEED

Sets the seed for the random number generator. This is useful for reproducing results.

2.13 Running Segway for large jobs

It is highly recommended that a terminal mutliplexer, such as [tmux](#) or [GNU screen](#), is used to manage your terminal sessions running Segway. Using either of these programs allows you to create a session to run longer segway jobs that you can safely detach from without losing your work. For more information, see their respective documentation.

2.14 Helpful commands

Here are some short bash scripts or one-liners that are useful:

There used to be a recipe here to continue Segway from an interrupted training run, but this has been replaced by the `-old-directory` option.

Make a tarball of parameters and models from various directories:

```
(for DIR in traindir1 traindir2; do
echo $DIR/{auxiliary,params/input.master,params/params.params,segway.str,
↪triangulation}
done) | xargs tar zcvf training.params.tar.gz
```

Rsync parameters from `$REMOTEDIR` on `$REMOTEHOST` to `$LOCALDIR`:

```
rsync -rtvz --exclude output --exclude posterior --exclude viterbi \
--exclude observations --exclude "*.observations" --exclude accumulators \
$REMOTEHOST:$REMOTEDIR $LOCALDIR
```

Print all last likelihoods:

```
for X in likelihood.*.tab; \
do dc -e "8 k $(tail -n 2 $X | cut -f 1 | xargs echo | sed -e 's/--/g') \
sc sl ll lc - ll / p"; \
done
```

Recover as much as possible from an incomplete identification run without completing it (which can be done with `-old-directory`). Note that this does not combine adjacent lines of same segment. BEDTools might be able to do this for you. You will have to create your own header.txt with appropriate track lines.

TECHNICAL MATTERS

3.1 Working files

Segway must create a number of working files in order to accomplish its tasks, and it does this in the directory specified by the required *workdir* argument. When running training *workdir* is *TRAINDIR*; when running identification *workdir* is *IDENTIFYDIR*.

The observation files can be quite large, taking up 8 bytes per track per position and cannot be compressed. As a result they are written out to a temporary directory on an as-needed basis. This is because otherwise they could take terabytes for identifying on the whole human genome with dozens of tracks.

You will find a full description of all the working files in the *Workdir files* section.

3.2 Temporary files

The **identify** and **posterior** tasks create temporary observation files in directories indicated by the Python *tempfile.gettempdir()* function, which searches for an appropriate directory as described in the documentation for *tempfile.tempdir* <<https://docs.python.org/2/library/tempfile.html#tempfile.tempdir>>. If you need to specify that temporary files go into a particular directory, set the *TMPDIR* environment variable. It is highly recommended that you ensure that your temporary directory does not reside on a slow storage medium such as a NFS filesystem. Since many temporary files are created and deleted this can significantly impact performance.

3.3 Distributed computing

Segway can currently perform the training, identification, and posterior tasks only using a cluster controllable with the DRMAA interface. We have only tested it against Sun Grid Engine and Platform LSF, but it should be possible to work with other DRMAA-compatible distributed computing systems, such as PBS Pro, PBS/TORQUE, Condor, or GridWay. If you are interested in using one of these systems, please open an issue on Github <<https://github.com/hoffmangroup/segway/issues/new>> to correct all the fine details. A standalone option exists when you set the *SEGWAY_CLUSTER* environment variable to *local*. Try installing the free Open Grid Scheduler on your workstation if you want to run Segway without a full clustering system.

The `--cluster-opt` option allows the specification of native options to your clustering system — those options you might pass to `qsub` (SGE) or `bsub` (LSF).

3.4 Memory usage

Inference on complex models or long sequences can be memory-intensive. In order to work efficiently when it is not always easy to predict memory use in advance, Segway controls the memory use of its subtasks on a cluster with a trial-and-error approach. It will submit jobs to your clustering system specifying the amount of memory they are expected to take up. Your clustering system should allocate these jobs such that the amount of memory on one host is not overcommitted. If a job takes up more memory than allocated, then it will be killed and restarted with a larger amount of memory allocated, along the progression specified in gibibytes by `--mem-usage=progression`. The default *progression* is 2,3,4,6,8,10,12,14,15.

We usually train on regions of no more than 2,000,000 frames, where a single frame contains the number of nucleotides set by the `--resolution` option. If you use more than that many, GMTK might run out of dynamic range. This manifests itself as a “zero clique error.” Identify mode rescales probabilities at every frame so that this is not a problem. However, you will probably want to split the input sequences somewhat because larger sequences make more difficult work units (greater memory and run time costs) and thereby impede efficient parallelization. The `--split-sequences=size` option will split up sequences into windows with *size* base pairs each. The default *size* is 2,000,000. Decreasing to 500,000 will greatly improve speed at the cost of more artefacts at split boundaries.

3.5 Reporting

Segway produces a number of logs of its activity during tasks, which can be useful for analyzing its performance or troubleshooting. These are all in the `workdir/log` directory.

3.5.1 Shell scripts

Segway produces three shell scripts in the log directory that you can use to replay its subtasks at different levels of abstractions. The top-level `segway.sh` records the command line used to run Segway. The `run.sh` script gives you the GMTK commands called by Segway. A small number of these are still produced when `--dry-run` is specified. The `details.sh` script contains the exact commands dispatched by Segway, including wrapper commands that monitor memory usage, create and delete local temporary files with observation data, and convert GMTK’s output to BED, among other things.

Segway also writes a `cmdline` directory in both the `traindir` and `identifydir`. Each instance has its own folder, and for each job segway queues, a shell script (with the job’s name) is written containing the GMTK command of the queued job.

For example, `traindir/cmdline/0/emt0.0.0.uuid.sh` is the shell script containing the GMTK commands and arguments for job `emt0.0.0.uuid` in instance 0 of training.

3.5.2 Summary reports

The `jobs.*.tab` file contains a tab-delimited file with each job Segway dispatched for this instance in a different row, reporting on job identifier (`jobid`), job name (`jobname`), GMTK program (`prog`), number of segment labels (`num_segs`), number of frames (`num_frames`), maximum memory usage (`maxvmem`), CPU time (`cpu`) and exit/error status (`exit_status`). Jobs are written as they are completed. The exit status is useful for determining whether the job succeeded (status 0) or failed (any other value, which is sometimes numeric, and sometimes text, depending on the clustering system used).

The `likelihood.*.tab` files each track the progression of likelihood during a single instance of EM training. The file has a single column, one for each round of training, which contains the log likelihood. More positive values are better.

3.5.3 GMTK reports

The `jt_info.txt` and `jt_info.posterior.txt` files describe how GMTK builds a junction tree. It is of interest primarily during GMTK troubleshooting. You are unlikely to use it.

3.5.4 Task output

The `output` directory contains the output of the actual GMTK commands run by Segway. The `o` directory contains standard output and the `e` directory contains standard error. If a job fails and repeats, the output from the new job is appended to the old. The `--verbosity=verbosity` option controls how much diagnostic information that GMTK writes into these files. The default and minimum value is 0. Raise this value for more information, and see the GMTK documentation for a description of various levels of verbosity. Setting `verbosity=30` can be particularly helpful in diagnosing model problems. Keep in mind that very high values (above 60) will produce tons of output===maybe terabytes.

Warning: Running Segway in identify mode with non-zero verbosity is currently not supported and may result in errors.

3.6 Performance

Some factors that affect compute time and memory requirements:

- the length of the longest region you are training or identifying on
- the number of tracks
- the number of labels

The longest region forms a bottleneck during training because Segway cannot start the next round of training before all regions in the previous round are done. So if you specify three regions, one of which is 10 Mbp long, and the other are 100 kbp, the 10 Mbp region is going to be a limiting factor. You can use `--split-sequences` (see above) to put an upper bound on region size.

3.7 Names used by Segway

3.7.1 Workdir files

Segway expects to be able to create many of these files anew. To avoid data loss, by default, it will quit if they already exist. If you use the `--clobber` option, Segway will overwrite the whole workdir instead.

Filename	Description
<code>accumulators/</code>	intermediate files used to pass E-step results to the M-step of EM training
<code>→ acc.*.bin</code>	accumulator for a particular instance and region (reused each round)
<code>auxiliary/</code>	miscellaneous model files
<code>→ dont_train.list</code>	defines list of hidden random variables that are not trained
<code>→ segway.inc</code>	C preprocessor (<code>cpp</code>) include file used in structure
<code>cmdline</code>	shell scripts containing commandlines/arguments of individual GMTK jobs
<code>cmdline/0,1,.../</code>	GMTK job shell scripts for a particular training instance(0,1,...)

continues on next page

Table 1 – continued from previous page

Filename	Description
cmdline/identify/	GMTK job shell scripts for identification
intermediate	files containing best training filenames per instance
→ train_result.*.tab	per instance information containing the filenames of the params resulting in the best likelihood
likelihood/	GMTK’s report of the log likelihood for the most recent M-step of EM training
→ likelihood.*.ll	contains text of the last log likelihood value for an instance. Segway uses this to decide when to stop training
→ validation.output.*.ll	contains text of the last validation GMTK output for an instance
→ validation.output.winner.*.ll	contains text of the current best validation GMTK output for an instance
→ validation.sum.*.ll	contains text of the last validation set log likelihood for an instance
→ validation.sum.winner.*.ll	contains text of the current best validation set log likelihood for an instance
log/	diagnostic information
→ details.sh	script file that includes the exact command-lines queued by Segway, with wrapper scripts
→ jobs.*.tab	tab-delimited summary of jobs queued per instance, including resource informatoin and exit status
→ jt_info.txt	log file used by GMTK when creating a junction tree
→ jt_info.posterior.txt	log file used by GMTK when creating a junction tree in posterior mode
→ likelihood.*.tab	tab-delimited summary of log likelihood by training instance; can be used to examine how fast training converges
→ validation.sum.*.tab	tab-delimited summary of full validation set log likelihood by training instance
→ validation.output.*.tab	tab-delimited summary of validation log likelihood for each window in the validation set by training instance
→ run.sh	list of commands run by Segway, not including wrappers that create and clean up temporary files such as observations used during identification
→ segway.sh	reports the command-line used to run Segway itself
→ *.*.float32	continuous data for a particular region
→ *.*.int	indicator data (present/absent) for a particular region
→ float32.list	list of continuous data files
→ int.list	list of indicator data files
output/	diagnostic output of individual GMTK jobs
output/e/	stderr
output/e/0,1,...	stderr for a particular training instance (0, 1, ...)
output/e/identify	stderr for identification
output/o/	stdout
params/	generated and trained parameters for a given instance
→ input.*.master	generated hyperparameters and starting parameters
→ input.master	best set of hyperparameters and starting parameters
→ params.*.params.*	trained parameters for a given instance and round
→ params.*.params	final trained parameters for a given instance
→ params.params	best final set of trained parameters
segway.bed.gz	segmentation in BED format
segway.str	dynamic Bayesian network structure
train.tab	important file locations and hyperparameters used in training, to be passed to identify
triangulation/	triangulation files used for DBN interface
→ segway.str.*.*.trifile	triangulation file
viterbi/	intermediate BED files created during distributed Viterbi decoding, which get merged into segway.bed.gz
window.bed	a BED file containing chromosome regions and the indices Segway assigns to them

3.7.2 Job names

In order to watch Segway's progress on your cluster, it is helpful to understand how it names jobs. A job name for the training task might look like this:

```
emt0.1.34.trainidir.ed03201cea2047399d4cbcc4b62f9827
```

In this example, `emt` means expectation maximization training, the `0` means instance `0`, the `1` means round `1`, and the `34` means window `34`. The name of the training directory is `trainidir`, and `ed03201cea2047399d4cbcc4b62f9827` is a universally unique identifier for this particular Segway run. This can be useful if you want to manage all of your jobs on your clustering system with wildcard specification. On SGE you can delete all the jobs from this run with:

```
qdel "*.ed03201cea2047399d4cbcc4b62f9827"
```

On LSF, use:

```
bkill -J "*.ed03201cea2047399d4cbcc4b62f9827"
```

Jobs created in the identify (`vit`) or posterior (`jt`) task are named similarly:

```
vit34.identifydir.4f32630d53724f08b34a8fc58793307d  
jt34.identifydir.4f32630d53724f08b34a8fc58793307d
```

Of course, there are no instances or rounds for the identify task, so only the sequence index is reported.

3.7.3 Tracks

Tracks are named according to their name in the Genomedata archive. For GMTK internal use, periods are converted to underscores.

FREQUENTLY ASKED QUESTIONS

4.1 How do I troubleshoot errors that occur in the training or identification process?

See *Troubleshooting* and *Task output*.

4.2 How do I make segments longer?

There are several ways to do this:

- Soft weight constraints:
 - `--segtransition-weight-scale` will increase the strength of the soft length prior for longer segments. `--prior-strength` also does this but empirically seems to have less of an effect than `--segtransition-weight-scale`
- Hard weight constraints
 - The `--seg-table` option will allow you to specify a hard minimum segment length, as described [here](#).
 - Downsampling resolution

See *Segment duration model* for model related methods.

4.3 How can I make Segway go faster?

- Train on smaller portion of the genome. Use the `--include-coords` option and supply a BED file.
- Splitting up into smaller subsequences by reducing `--split-sequences` can also help.

TROUBLESHOOTING

When Segway reports an error, it usually means that dispatched GMTK jobs failed somehow. Look in `log/jobs.tab` to see all the jobs and whether they reported an error in the form of nonzero exit status in the last column. If the job had a “75” exit status (or an “EX_TEMPFAIL”) the job simply ran out of memory. If this was the last job with this error, increase the allowed memory in your `-mem-usage` option. Otherwise if the job had a non-zero exit status and it wasn’t due to out of memory issues, look in `output/e/instance/jobname` to find the cause of the underlying error. See [Task output](#) for more output information.

Are your bundle jobs failing? This might be because an accumulator file (written by individual job) is corrupted or truncated. This can happen when you run out of disk space.

If it is not immediately apparent why a job is failing, it is probably useful to look in `log/details.sh` to find the command line that Segway uses to call GMTK. Try running that to see if it gives you any clues. You may want to switch the GMTK option `-verbosity 0` to `-verbosity 30` to get more information.

An error like

```
ERROR: discrete observed random variable 'presence_dnase', frame 0, line 23, specifies a feature element
14:14 that is out of discrete range ([23:45] inclusive) of observation matrix
```

probably indicates that you are incorrectly mixing and matching `train.tab` files and `segway.str` files from different training runs.

If you are unable to resolve your issue on your own, consider inquiring on the mailing list segway-users@uw.edu listed on the [Support](#) page.

SUPPORT

For support of Segway, please write to the <segway-users@uw.edu> mailing list, rather than writing the authors directly. Using the mailing list will get your question answered more quickly. It also allows us to pool knowledge and minimize redundant inquiries. You can subscribe here:

<https://listserv.utoronto.ca/cgi-bin/wa?A0=segway-l>

Specifically, if you want to report a bug or request a feature, please do so using the Segway issue tracker at:

<https://github.com/hoffmangroup/segway/issues>

If you do not want to read discussions about other people's use of Segway, but would like to hear about new releases and other important information, please subscribe to <segway-announce@uw.edu> by visiting this web page:

<https://listserv.utoronto.ca/cgi-bin/wa?A0=segway-announce-l>

Announcements of this nature are sent to both `segway-users` and `segway-announce`.

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