

Getting started with the E. Coli Whole Cell Model on Sherlock: A Brief Tutorial

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1 Basics

This document is an introduction to running the whole-cell *E. coli* model on the Sherlock computing cluster. In addition to guiding setup and providing a tutorial on modifying the model, it includes a brief primer on whole-cell model fitting in later sections.

First a basic note on file locations. The knowledge base and the code that fits the knowledge base is located in the `wcEcoli/reconstruction/ecoli/` directory. The input information files are in `wcEcoli/reconstruction/ecoli/flat`, and the code that loads it into an object is in `knowledge_base_raw.py`.

Processes are located in `wcEcoli/models/ecoli/processes/`. Each process represents one part of the cell's function, and they are modeled separately in short time steps and then the results of each time step are integrated between modules before initiating the next time step. RNA polymerase elongation is the process used below as an example. Other processes include translation elongation, transcription initiation, and metabolism.

Each process has three components: `initialize` (called only once at the beginning of a simulation), `calculateRequest` (called at the beginning of each timestep), and `evolveState` (called after resources are allocated at each timestep).

In initialize: get needed parameters from the knowledge base, get views of bulk and unique molecules (bulk molecules are indistinguishable from each

other, e.g. inactive RNAP molecules, unique molecules can be distinguished from each other, e.g. active RNAP molecules are each assigned to a location on the genome), create a view so that you can get counts, change counts, and change properties.

In calculateRequest: request the resources that you want for that timestep (don't request all unless you are certain that another process doesn't need this resource as well, don't forget about metabolism).

In evolveState: perform the process, update counts, and update masses (mass must be conserved between steps).

If you want to save new values to be analyzed at the end of the simulation, you must write this information out as a listener. Listeners are programs which record information during the simulation. To add a listener, add the file to `wcEcoli/models/ecoli/listeners/`, and both add an import statement and add the listener to `_listenerClasses` in `wcEcoli/models/ecoli/sim/simulation.py`.

To change the cell's initial conditions, change `wcEcoli/models/ecoli/sim/initial_conditions.py`.

2 Setup

This tutorial assumes you have setup access to the Sherlock cluster. If necessary before following the proceeding steps, do the following setup:

1. Email the research computing staff at `research-computing-support@stanford.edu`, and cc Professor Covert. Request access to the Sherlock cluster, and ask Professor Covert to confirm your request in the email.
2. Follow the setup instructions for logging in to Sherlock on `http://www.stanford.edu/~thorst/computational_resources/sherlock/home/`.
3. Follow all setup instructions in the `wcEcoli/README.md` file (also on the web at `https://github.com/CovertLab/wcEcoli/blob/master/README.md`). In particular, make sure you run

```
cd $HOME/wcEcoli
pyenv local wcEcoli2
```

```
module load wcEcoli/sherlock2
make compile
```

Once done with setup and these basic tutorials on Sherlock and the cluster, return to [here](#) to complete the example.

3 Example: varying trascription rate

3.1 Background

Genes encoding for stable RNA are transcribed at a faster rate in *E. coli*. In the model (prior to this tutorial), all genes were transcribed at the same rate of 39 nt/s. In this tutorial, we will change the model so that genes for rRNA and tRNA are transcribed at 78 nt/s while everything else is still transcribed at 39 nt/s.

Before we start, make sure to complete the setup section (2) if you haven't already. To begin, log in to a sherlock node. Replace 'username' with your username in the command below:

```
ssh username@login.sherlock
```

The code has inevitably evolved since I wrote this tutorial, so let's get on the same page by going back to an older version of the code. However, ensure that you are following the latest iteration of the tutorial instructions (on master), rather than the version that appears with the code you will checkout below. Let's do this tutorial on a new branch. cd into your wcEcoli directory and type the following (you only need to type enough characters of the commit checksum for it to be unique so about 10):

```
git checkout -b tutorialYourName
c7b13cb809637732f993c387ae1c4b11653efdc7
```

This gives you your own copy of the specified commit of the master branch named `tutorialYourName` which you can commit to.

3.2 Running simulations

Now let's try running the unmodified model to make sure it works. First, log in to a compute node, so that the simulation can be run in interactive mode (running in interactive mode on a login node can result in getting booted from the cluster). These nodes are covert-lab specific and it's ok to do more serious computation on them.

```
srun -p mcovert --ntasks-per-node=1 --time=12:00:00 --pty bash -l
```

It is best to run `srun` from your home directory and then `cd` into `wcEcoli` once you are on a compute node. This will load the python virtual environment nicely, which is not necessarily the case if you run `srun` from `wcEcoli` on the login node. Once you are in a compute node, ensure you are in your `wcEcoli` directory.

```
cd $HOME/wcEcoli
```

Once you are logged into the compute node with the python virtual environment loaded, you will see the virtual environment first and the computer number next to your shell cursor like this:

```
(wcEcoli)[sunetid@sh-8-30:~/wcEcoli]$
```

Now prepare the fireworks queue with your simulation task:

```
DESC="Tutorial run, starting code with unmodified parameters."  
python runscripts/fw_queue.py
```

There are several parameters that can be specified in addition to the description (DESC) shown above. Those additional parameters can be explored inside the `/runscripts/fw_queue.py` file. There are several methods to launching jobs via fireworks. Below, we use an interactive launch via `rlaunch`, but jobs can be queued as well, by instead running `qlaunch`. In order to inspect your queue, run `lpad get_fws`. This will list all unclesd past jobs, and their status and id, as well as any queued jobs. Running `lpad reset` will clear this list. Jobs can be filtered by status with the `-s` flag, and if they were not run in interactive mode and you want

to inspect an error message for a fizzled job, the output from each job is stored in the block directories in the top level (`wcEcoli/`). These commands and others related to fireworks are further described in their documentation (<https://pythonhosted.org/FireWorks/introduction.html>).

Now the simulation task is queued up in fireworks and can be launched via:

```
rlaunch rapidfire
```

After about a minute, this should start producing output looking something like this:

```
(wcEcoli)[sunetidt@sh-8-30 ~/wcEcoli]$ rlaunch rapidfire
2016-04-08 14:15:30,917 INFO Hostname/IP lookup (this will take a
    few seconds)
2016-04-08 14:15:32,545 INFO Created new dir
    /home/thorst/wcEcoli/launcher_2016-04-08-21-15-32-542398
2016-04-08 14:15:32,549 INFO Launching Rocket
2016-04-08 14:15:38,572 INFO RUNNING fw_id: 17 in directory:
    /home/thorst/wcEcoli/launcher_2016-04-08-21-15-32-542398
2016-04-08 14:15:38,577 INFO Task started:
    {{wholecell.fireworks.firetasks.initRawData.InitRawDataTask}}.
Fri Apr 8 14:15:38 2016: Instantiating raw_data
Fri Apr 8 14:15:43 2016: Saving raw_data
2016-04-08 14:15:43,868 INFO Task completed:
    {{wholecell.fireworks.firetasks.initRawData.InitRawDataTask}}
2016-04-08 14:15:50,912 INFO Rocket finished
2016-04-08 14:15:51,258 INFO Created new dir
    /home/thorst/wcEcoli/launcher_2016-04-08-21-15-51-256566
2016-04-08 14:15:51,261 INFO Launching Rocket
2016-04-08 14:15:54,788 INFO RUNNING fw_id: 15 in directory:
    /home/thorst/wcEcoli/launcher_2016-04-08-21-15-51-256566
2016-04-08 14:15:54,791 INFO Task started:
    {{wholecell.fireworks.firetasks.fitSimData.FitSimDataTask}}.
Fri Apr 8 14:15:54 2016: Creating/Fitting sim_data (Level 1)
```

Then once the actual simulation starts, you will see:

Fri Apr 8 14:19:50 2016: Running simulation					
Time (s)	Dry mass	Dry mass	Protein	RNA	Expected
	(fg)	fold change	fold change	fold change	fold change
=====	=====	=====	=====	=====	=====
0	245.46	1.000	1.000	1.000	1.000
0	245.49	1.000	1.000	1.000	1.000
0	245.52	1.000	1.000	1.000	1.000
0	245.54	1.000	1.000	1.000	1.000
0	245.56	1.000	1.000	1.000	1.000
1	245.65	1.001	1.000	1.000	1.000
1	245.74	1.001	1.000	1.000	1.000
2	245.82	1.001	1.001	1.000	1.001
3	245.90	1.002	1.001	1.000	1.001
4	245.98	1.002	1.001	1.000	1.001
5	246.05	1.002	1.001	0.999	1.001
6	246.12	1.003	1.001	0.999	1.001
7	246.18	1.003	1.002	0.999	1.001
8	246.24	1.003	1.002	0.999	1.002
9	246.30	1.003	1.002	0.999	1.002
9	246.37	1.004	1.002	0.999	1.002
10	246.43	1.004	1.002	0.999	1.002
11	246.49	1.004	1.002	1.000	1.002
12	246.55	1.004	1.003	1.000	1.002
13	246.62	1.005	1.003	1.000	1.003
14	246.68	1.005	1.003	1.000	1.003
15	246.74	1.005	1.003	1.000	1.003
16	246.80	1.005	1.003	1.000	1.003
17	246.87	1.006	1.004	1.000	1.003
17	246.93	1.006	1.004	1.000	1.003
18	246.98	1.006	1.004	1.000	1.004
19	247.03	1.006	1.004	1.000	1.004
20	247.07	1.007	1.004	1.000	1.004
21	247.12	1.007	1.004	1.000	1.004
22	247.16	1.007	1.005	1.000	1.004
23	247.21	1.007	1.005	1.001	1.005
24	247.25	1.007	1.005	1.001	1.005

25	247.30	1.007	1.005	1.001	1.005
26	247.34	1.008	1.005	1.001	1.005
26	247.38	1.008	1.005	1.001	1.005
27	247.43	1.008	1.006	1.001	1.005
28	247.47	1.008	1.006	1.001	1.006
29	247.52	1.008	1.006	1.001	1.006
30	247.56	1.009	1.006	1.001	1.006
31	247.60	1.009	1.006	1.002	1.006
32	247.65	1.009	1.007	1.002	1.006
33	247.69	1.009	1.007	1.002	1.006
34	247.73	1.009	1.007	1.002	1.007
34	247.78	1.009	1.007	1.002	1.007
35	247.82	1.010	1.007	1.002	1.007
36	247.86	1.010	1.007	1.002	1.007
37	247.91	1.010	1.008	1.002	1.007
38	247.95	1.010	1.008	1.003	1.007
39	248.00	1.010	1.008	1.003	1.008
40	248.04	1.011	1.008	1.003	1.008

This will take a few minutes to run. At the end you can find the output in the `out/timestamp` directory (timestamp obviously replaced with the actual timestamp of when you started the simulation). Look at the plots in the `/wcEcoli/out/20150623.190922.827654/wildtype_000000/000000/generation_000000/000000/plotOut` directory (replacing the timestamp directory with your own timestamped directory name).

There are several levels to the analysis of any simulation, represented by the various directories. The top level directory `/wildtype_000000` contains analyses at the variant level, indicating the environmental conditions (for instance, you could compare basal vs. anaerobic at this scale). Inside that directory, every cohort will have its own folder, labeled by the seed number (in this case `/000000` is the only seed). This level allows for multigeneration analysis, and contains data from all generations within a seed. For each seed, each generation is available in the `/000000/generation_000000` directory. Finally, after looking inside the generation folder, there will be additional

folders for each seed, however many were specified in the initial run, that contains single cell level data for that seed within that generation. Those folders are labeled by the seed number, within the `generation_000000` directory, so the single cell data in this instance is `/000000` within the generation folder.

3.3 Add a new parameter to the model

First, let's add a new elongation rate to the knowledge base. In `reconstruction/ecoli/flat` is a tsv called `growthRateDependentParameters.tsv`. Add a new column for the fast rna polymerase rate (estimate that it is double the normal rate). The normal rate at each growth rate is stored in the column `"rnaPolymeraseElongationRate"`. This command will generate the file with the new column appended, or edit the file in a different way if desired. If you are copying and pasting, some of the characters may not translate as expected, and so it is encouraged to type out commands yourself, both to avoid this issue and better understand their purpose.

```
awk '{if(NR==1){print $0 "\t" "rnaPolymeraseElongationRateFast
(units.nt/units.s)"}else{print $0 "\t" ($9*2)}}'
growthRateDependentParameters.tsv > temp && mv temp
growthRateDependentParameters.tsv
```

Alternatively, you could open the `.tsv` file in a spreadsheet editor and add the new column by hand.

To check that the parameter was added, examine the knowledge base object in `ipython` after switching back to the `wcEcoli` directory.

```
ipython
import reconstruction.ecoli.knowledge_base_raw as kbr
kb=kbr.KnowledgeBaseEcoli()
kb.growthRateDependentParameters[0]
```

You should see the `'rnaPolymeraseElongationRateFast': 78 [nucleotide/s]` entry in the resulting dictionary.

3.4 Create a new process

Let's split the transcript elongation process into a slow process and a fast process. Go to `models/ecoli/processes/` :

```
cd models/ecoli/processes/

cp transcript_elongation.py transcript_elongation_fast.py

mv transcript_elongation.py transcript_elongation_slow.py
```

Now we need to add these processes to the simulation, so that they actually get called. Let's go back to the `wcEcoli` directory. Open `models/ecoli/sim/simulation.py` and change line 17 to read:

```
from models.ecoli.processes.transcript_elongation_slow import
    TranscriptElongationSlow
```

Then add a line just under this:

```
from models.ecoli.processes.transcript_elongation_fast import
    TranscriptElongationFast
```

Under process classes, add `TranscriptElongationFast` and change `TranscriptElongation` to `TranscriptElongationSlow`. `_processClasses` should now look like this:

```
_processClasses = (
    Metabolism,
    RnaDegradation,
    TranscriptInitiation,
    TranscriptElongationSlow,
    TranscriptElongationFast,
    PolypeptideInitiation,
    PolypeptideElongation,
    ReplicationElongation,
    ProteinDegradation,
    Complexation,
    ChromosomeFormation,
```

```
Equilibrium,  
)
```

3.5 Change the model output

Now let's change the listeners to record output from these new processes. We write data to listeners, and the listener is responsible for recording the data to the file, as well as transforming it as necessary (calculating averages, for example). The transcription submodels write to three listeners: `GrowthLimits`, `RnapData`, and `TranscriptElongationListener`. We need to make several changes in order to correctly log transcription data. The fast and slow transcription submodels need to both write to the same listener attributes, so we need to set them up to add data together, rather than overwriting one another's output. You will notice two functions at the end of each listener file, `tableCreate(...)` and `tableAppend(...)`. For each listener, `tableCreate(...)` sets values that will not change throughout the simulation, such as units, while `tableAppend(...)`, which we are editing here, will keep track of the values that are updated at each timestep.

First, we need to update the listeners to zero out the relevant attributes each time step, so that we can add to them from each model. Open `models/ecoli/listeners/growth_limits.py` and add the following lines to the end of `tableAppend(...)`, after line 99:

```
self.ntpRequestSize = np.zeros(len(self.ntpIds), np.float64)  
self.ntpAllocated = np.zeros(len(self.ntpIds), np.float64)  
self.ntpUsed = np.zeros(len(self.ntpIds), np.float64)
```

We need to make two changes to `models/ecoli/listeners/rnap_data.py`. At line 73, update the lines that read:

```
self.ntpCountInSequence = np.zeros(21, np.int64)  
self.ntpCounts = np.zeros(21, np.int64)
```

To read:

```
self.ntpCountInSequence = np.zeros(0, np.int64)
```

```
self.ntpCounts = np.zeros(0, np.int64)
```

This initializes some arrays we'll be writing to to be completely empty, which allows us to append to them without having a bunch of leading data we don't want. Next, we reset the relevant attributes as in `GrowthLimits`. Add the following to the bottom of `tableAppend(...)`, after line 117:

```
self.rnapStalls = np.zeros(0, np.int64)
self.ntpCountInSequence = np.zeros(0, np.int64)
self.ntpCounts = np.zeros(0, np.int64)
self.expectedElongations = 0
self.actualElongations = 0
self.didTerminate = 0
self.terminationLoss = 0
```

Finally, we need to update `models/ecoli/listeners/transcript_elongation_listener.py`. Add to the bottom of `tableAppend(...)`, after line 43:

```
self.countRnaSynthesized =
    np.zeros(self.countRnaSynthesized.shape,
             self.countRnaSynthesized.dtype)
```

3.6 Modify a process

Now let's modify the new processes. Open `models/ecoli/processes/transcript_elongation_slow.py` and change all references of `TranscriptElongation` to `TranscriptElongationSlow`.

Next create a Boolean vector that is True at the indices of transcripts that should be elongated at the slow rate and False at the indices of transcripts that should be elongated at the fast rate. In the initialize function add the following line (after line 73):

```
self.slowRnaBool =
    ~(sim_data.process.transcription.rnaData["isRRna5S"] |
      sim_data.process.transcription.rnaData["isRRna16S"] |
```

```
sim_data.process.transcription.rnaData["isRRna23S"] |  
sim_data.process.transcription.rnaData["isTRna"])
```

isRRna5S, isRRna16S, isRRna23S, and isTRna are properties of `rnaData`, which is found in the simulation data set (`sim_data`). These are vectors of Boolean values. The tilde inverts the boolean value, so this creates a vector that is False for all 5S rRNA, 16S rRNA, 23S rRNA, and tRNA, and True for everything else. Now let's change the view onto the active RNA polymerases to a view onto only the active RNA polymerases that are on transcripts that should be elongated at the slow rate. Change the definition of `self.activeRnaPolys` (line 79) to read:

```
self.activeRnaPolys = self.uniqueMoleculesView('activeRnaPoly',  
rnaIndex = ("in", np.where(self.slowRnaBool)[0]))
```

Next, we need to update our writes to the listeners to increment or append, rather than overwrite, the attributes that are updated. First, add a new import at the top of the file (after line 25):

```
from wholecell.listeners.listener import WriteMethod
```

Second, we need to change our calls to `writeToListener(...)` to either increment (in the case of numeric attributes), or append (in the case of arrays), rather than overwriting. Modify all of the `writeToListener(...)` calls (except for `ntpPoolSize`) since this is one value that both slow and fast elongation see, to have a new parameter at the end: `WriteMethod.increment`, except for the writes to `rnapStalls`, `ntpCountInSequence`, and `ntpCounts`, which instead should have `WriteMethod.append`. Overall, the lines should look like the following (although in the file they are scattered around a bit):

```
self.writeToListener("GrowthLimits", "ntpRequestSize",  
maxFractionalReactionLimit * sequenceComposition,  
WriteMethod.increment)  
  
self.writeToListener("GrowthLimits", "ntpAllocated",  
self.ntps.counts(), WriteMethod.increment)
```

```

self.writeToListener("TranscriptElongationListener",
    "countRnaSynthesized", terminatedRnas, WriteMethod.increment)

self.writeToListener("GrowthLimits", "ntpUsed", ntpsUsed,
    WriteMethod.increment)
self.writeToListener("RnapData", "rnapStalls", rnapStalls,
    WriteMethod.append)
self.writeToListener("RnapData", "ntpCountInSequence",
    ntpCountInSequence, WriteMethod.append)
self.writeToListener("RnapData", "ntpCounts", ntpCounts,
    WriteMethod.append)
self.writeToListener("RnapData", "expectedElongations",
    expectedElongations.sum(), WriteMethod.increment)
self.writeToListener("RnapData", "actualElongations",
    sequenceElongations.sum(), WriteMethod.increment)
self.writeToListener("RnapData", "didTerminate",
    didTerminate.sum(), WriteMethod.increment)
self.writeToListener("RnapData", "terminationLoss",
    (terminalLengths - transcriptLengths)[didTerminate].sum(),
    WriteMethod.increment)

```

This is all we need to change in this file. Now let's open the fast transcript elongation file `models/ecoli/processes/transcript_elongation_fast.py`. Similarly to what we did in the slow elongation file, let's change all references of `TranscriptElongation` to `TranscriptElongationFast`.

Next, let's change the elongation rate. Change line 60 to read:

```

self.rnapElngRate =
    sim_data.growthRateParameters.rnaPolymeraseElongationRateFast.
    asNumber(units.nt / units.s)

```

Now, let's create the opposite of the Boolean vector that we created in `transcript_elongation_slow.py`. In the `initialize` function add the following line (after line 74):

```

self.fastRnaBool =
    (sim_data.process.transcription.rnaData["isRRna5S"] |
    sim_data.process.transcription.rnaData["isRRna16S"] |
    sim_data.process.transcription.rnaData["isRRna23S"] |

```

```
sim_data.process.transcription.rnaData["isTRna"])
```

Now let's change the view onto the active rna polymerases to a view onto only the active RNA polymerases that are on transcripts that should be elongated at the fast rate (line 80):

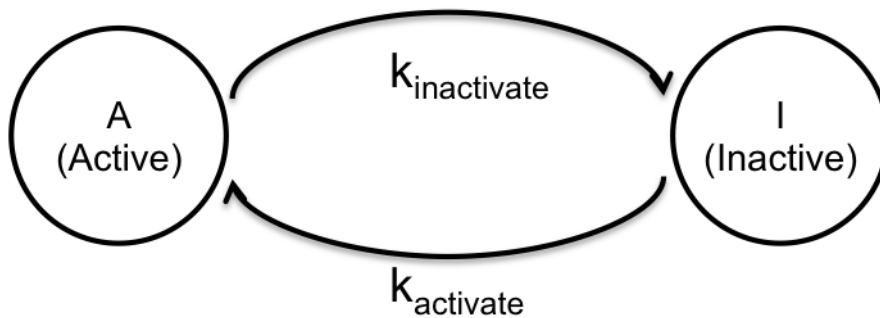
```
self.activeRnaPolys = self.uniqueMoleculesView('activeRnaPoly',  
    rnaIndex = ("in", np.where(self.fastRnaBool)[0]))
```

Also, we need to update our writes to the listeners as before. First, add a new import at the top of the file (after line 25):

```
from wholecell.listeners.listener import WriteMethod
```

Finally, update the `writeToListener(...)` lines to match the changes you made in `TranscriptElongationSlow`, above. Now data from both submodels will be recorded in the whole cell output.

Now let's change the calculation of the RNA polymerase activation rate. In the model, an RNA polymerase can either be active or inactive:



To calculate the rate of activation, k_{activate} , we write an equation for the rate of change of the number of RNAP active at steady state:

$$\frac{dA}{dt} = k_{\text{activate}}I - k_{\text{inactivate}}A = 0$$

This gives us that

$$k_{activate} = k_{inactivate} \frac{A}{I}$$

We know from experiments the fraction of RNA polymerases that are active, f_{active} :

$$f_{active} = \frac{A}{A + I}$$

Using that

$$f_{active} = 1 - f_{inactive}$$

We can rearrange and solve for the rate of activation:

$$k_{activate} = k_{inactivate} \left(\frac{f_{active}}{1 - f_{active}} \right)$$

We can calculate the rate of inactivation from the elongation rate, the transcript lengths, and the synthesis probabilities:

$$k_{inactivate} = \frac{k_{elong}}{L_i} \cdot \frac{1}{p_{synth}}$$

We want to change the elongation rate to be a vector, rather than a constant:

$$k_{inactivate} = \frac{k_{elong,i}}{L_i} \cdot \frac{1}{p_{synth}}$$

Now let's do this in the code. In `models/ecoli/processes/transcript_initiation.py`, first add some lines to the `initialize` function so we can access data in another function. After line 56, add the following:

```
self.rnaPolymeraseElongationRateFast =
    sim_data.growthRateParameters.rnaPolymeraseElongationRateFast
```

Below this after line 69, add:

```
self.is_tRNA = sim_data.process.transcription.rnaData['isTRna']
```

Next, add a line to pass the fast elongation rate to the function we would like to change. On line 80, change the function call to look like the following:

```
self.activationProb = self._calculateActivationProb(  
    self.fracActiveRnap,  
    self.rnaLengths,  
    self.rnaPolymeraseElongationRate,  
    self.rnaPolymeraseElongationRateFast,  
    self.rnaSynthProb,  
)
```

Now, we need to make some changes to the `_calculateActivationProb` function (on line 145). Change the function definition to the following:

```
def _calculateActivationProb(self, fracActiveRnap, rnaLengths,  
    rnaPolymeraseElongationRate, rnaPolymeraseElongationRateFast,  
    synthProb):
```

The following lines should be adding at the top of the function. The tilde operator inverts the booleans.

```
fastRnaBool = self.is_5SrRNA | self.is_16SrRNA | self.is_23SrRNA |  
    self.is_tRNA  
slowRnaBool = ~fastRnaBool
```

Now, add a line below this for a vector of the elongation rate for each RNA strand:

```
elngRateVector = rnaPolymeraseElongationRate * slowRnaBool +  
    rnaPolymeraseElongationRateFast * fastRnaBool
```

Now change the next line to reflect the new rate vector:

```
expectedTranscriptionTime = 1. / elngRateVector * rnaLengths
```

Now we can run a simulation and rRNA and tRNA would be transcribed at the new fast rate, while everything else was transcribed at the same slow rate before. Follow these steps to run the new simulation code:

Queue up the tasks in fireworks:

```
DESC="Tutorial run, adding different RNA polymerization rate for  
r- and t- RNAs." python runscripts/fw_queue.py
```

Run the tasks in the queue until finished:

```
rlaunch rapidfire
```

To check for the success of your simulation, open the `wcEcoli/out/` folder, and the folder timestamped to the simulation just run, then click through to the output plots for the single seed we ran within a single generation. Specifically, look at the `massFractionSummary` plot. Does everything roughly double in one cell cycle? If not, why might that be?

3.7 Changing the fitting

Even though we've changed the model, the simulation will be initialized the same way as before. This is a problem because before running a simulation, we need to fit several parameters such as the initial counts of RNA polymerases. Because some things are now being transcribed at a faster rate, we should need fewer RNA polymerases to make the same number of transcripts. Additionally, if some things are being transcribed more quickly, then RNA polymerases will be inactivating more quickly. In order to maintain a constant ratio of active to inactive RNA polymerases (experimentally shown to be around 20%), the activation rate must also increase now.

We calculate the number of RNA polymerases that we need initially by writing an equation for the rate of change of some RNA R_i (rate of synthesis - rate of degradation = rate of dilution due to growth):

$$\frac{dR_i}{dt} = \frac{k_{elong}}{L_i} P_i(t) - \frac{\ln(2)}{h_i} R_i(t) = \frac{\ln(2)}{\tau_d} R_i(t)$$

L_i is the length of transcript i ; P_i is the number of RNA polymerases actively transcribing R_i at time t ; h_i is the half life of R_i ; τ_d is the length of the cell cycle; k_{elong} is the transcript elongation rate. Right now this is a constant value for all transcripts. We want to change this to be different for rRNA and tRNA. Let's change this in the code. Open the file `reconstruction/ecoli/fit_sim_data_1.py` and go to the `setRNAPCountsConstrainedByPhysiology` function. This is what we need to change. Let's add the Boolean vectors for fast and slow transcripts. Right at the beginning of this function definition (after line 473), add these two lines:

```
fastRnaBool = (sim_data.process.transcription.rnaData["isRRna5S"]
               | sim_data.process.transcription.rnaData["isRRna16S"] |
               sim_data.process.transcription.rnaData["isRRna23S"] |
               sim_data.process.transcription.rnaData["isTRna"])
slowRnaBool = ~fastRnaBool
```

Let's break up the calculation of `nActiveRnapNeeded` into `nActiveRnapNeededForFast` and `nActiveRnapNeededForSlow`. Change the `nActiveRnapNeeded` calculation on line 507 to read:

```
nActiveRnapNeededForSlow =
    calculateMinPolymerizingEnzymeByProductDistributionRNA(
        rnaLengths[slowRnaBool],
        sim_data.growthRateParameters.rnaPolymeraseElongationRate,
        rnaLossRate[slowRnaBool])
```

Add a line under this for the calculation for the fast transcripts:

```
nActiveRnapNeededForFast =
    calculateMinPolymerizingEnzymeByProductDistributionRNA(
        rnaLengths[fastRnaBool],
        sim_data.growthRateParameters.rnaPolymeraseElongationRateFast,
        rnaLossRate[fastRnaBool])
```

Below this, add a line to calculate the total number of RNA polymerases needed:

```
nActiveRnapNeeded = nActiveRnapNeededForFast +
                    nActiveRnapNeededForSlow
```

Now the fitting of the initial number of RNA polymerases and the activation rate is fixed. We can run a simulation and the model will reflect changes to both the processes and fitting for rRNA and tRNA to be transcribed at the new fast rate. Queue up the tasks in fireworks:

```
DESC="Tutorial run, updated model fitting." python  
runscripts/fw_queue.py
```

Run the tasks in the queue until finished:

```
rlaunch rapidfire
```

Check the output to make sure that the RNA mass fractions more closely double over the cell cycle.