Simulation notes, Monday 11th April 2016

Steven Court

April 11, 2016

1 Executive summary

- Program now mutates both daughter cells.
- It was hard to match the wavespeed in the simulation to the 1.5 wells h^{-1} Bartek observed in his experiment. I have settled for using diff = 4.0 mm^2 h^{-1} (increased from 1.5) and I have doubled the channel size to 2 mm. This gives a speed of about 1.3 wells h^{-1}. Perhaps chemotaxis is important in the experiment?
- Diffusion / motility can in principle be important for determining number of mutations per well, but for realistic parameter values the differences are indistinguishable from experimental variability.
- The mutation rate is the most important parameter determining number of mutations per well. Suggests including a variable mutation rate is likely important too: I have added a ciprofloxacin-dependent mutation rate (parameter "gama"). Currently it has the form of Eq. 3.
- I get the impression that if we look at the well which has an antibiotic concentration of around the MIC/2, there is not much point sampling DNA from earlier wells as there are usually no mutations present above the 10% cutoff I impose.

2 Fisher wave

Rosalind wondered if the travelling wave really behaves like a Fisher wave in which the speed depends on the diffusion constant D and growth rate μ like $v \sim \sqrt{\mu D}$. Figure 1 shows that this is likely the case.

3 What factors affect expected number of mutations per well?

3.1 Diffusion

Figure 2 shows the effect of D (diff) on the expected number of mutations per well in the case of no antibiotics. We see that for higher values of D the expected number decreases but that the change is not significant with respect to the experimental variability. This decrease is expected if we consider the extreme case in which diffusion is so high that we have 1 well-mixed well. Here the bacteria accumulate the number of mutations M expected in the time it takes to fill 1 well, t_f. In the opposite case, where each well is filled before the subsequent is occupied, then the experiment takes a total time of $N_w \times t_f$ and so we should expect a greater number of mutations to be present. (Naively $N_W \times M$ but this doesn't take into account that we are only including those present with an abundance greater than 10% of the population).

3.2 Mutation rate

Figure 3 shows that the mutation rate has a much stronger effect on the expected number of mutations per well.

4 Dynamics with antibiotics

The dynamics of the population when antibiotics are included is shown in Figure 4. In (a) we see the population reaches it's limit (where the antibiotic concentration a = MIC) and has to wait for a driver mutation to arise. Currently max_drivers=10 and $L = 10000$, so this occurs with probability 1/1000 per mutation event. Are these numbers scaled roughly correctly for *E. coli*? Currently all driver mutations are equal and have the effect of increasing the MIC from 20 ng/ml to 100 ng/ml. I could change this to be more representative of the ciprofloxacin mutational pathway. Fig. 4(b) shows that the population doesn't propogate as a wave due to it

hitting the MIC-barrier. Fig. 4(c) shows the well populations at given times. We see a derease in population until some well in which the population is zero. A driver mutation then occurs (probably in this well or the adjacent well) and causes a peak to appear. The shape on the left never fully recovers since in the time that we were waiting for the driver mutation, the antibiotic was causing cell death in this region and since food is not replenished, the population remains below the maximum possible in a well with no antibiotic. The right-hand side has a dip for the same reason. Letting the simulation continue to run then softens this shape through diffusion.

Recall that cell death is implemented in the death_upon_replication() method, which calculates the probability that a cell dies during a replication event as:

$$d[i] = \left(\frac{a}{\text{MIC}}\right)^2, \tag{1}$$

with the condition that $d \leq 1$. Is this ideal? Ciprofloxacin doesn't kill cells – only prevents them from reproducing. Also, recall that the antibiotic concentration profile is given in well i by:

$$a[i] = \text{min\_antibiotic} + \text{max\_antibiotic}^{\frac{i}{N_w}} \tag{2}$$

where N_w is the number of wells.

5 Variable mutation rate

I have also implemented a ciprofloxacin-dependent mutation rate to see the effect of this. For now I have implemented an arbitrary power law dependence of the form:

$$\gamma = \gamma^{WT}\left[1 + \left(\frac{Xa}{\text{MIC}}\right)^Y\right], \tag{3}$$

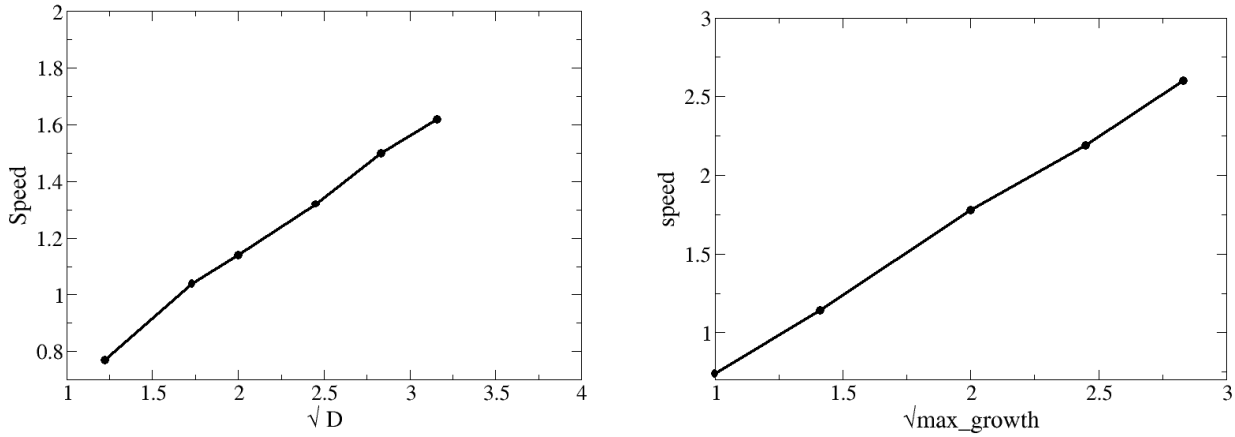
where a is the antibiotic concentration and γ^{WT} is the mutation rate of the wildtype. I choose X and Y to match Bartek's experimental observation that at $a = \text{MIC}/2$, $\gamma \sim 10\gamma^{WT}$ and at $a = \text{MIC}$, $\gamma \sim 100\gamma^{WT}$.

A result of including this ciprofloxacin-dependence that I didn't initially expect is that I also calculate the probability that a given experimental setup reaches the final well in the allowed time (4-days), rather than getting "stuck" at some earlier well having not acquired a driver mutation. I ran 30 experiments for some parameter set with min_antibiotic = 0, max_antibiotic = 50 and saw that 25/30 of the runs reached well number 24. I then increased max_antibiotic to 100 ng/ml and naively expected less runs to reach the final well. Although 27/30 isn't a statistically significant increase, it made me think that by imposing these larger antibiotic concentrations, we are also implicitly increasing the mutation rate, thus increasing the likely-hood of attaining a driver mutation. I.e. in this scenario it may not be obvious or easy to separate the two possible features that (1) a stronger selection gradient is increasing the rate of evolution, as stated in Bartek's and Bob Austin's papers, or (2) the fact that this gradient is actually directly increasing the rate of mutation (and thus evolution). Figure 5 shows an example of the number of mutations per well when I reduce the wildtype γ^{WT} to 1e-3 (as Bartek states in a comment in the code) and use values $X = 15$ and $Y = 2$. I think this is a quite strongly increasing mutation rate, yet we still see no detectable mutations until around well 15.

6 Things to do / ideas

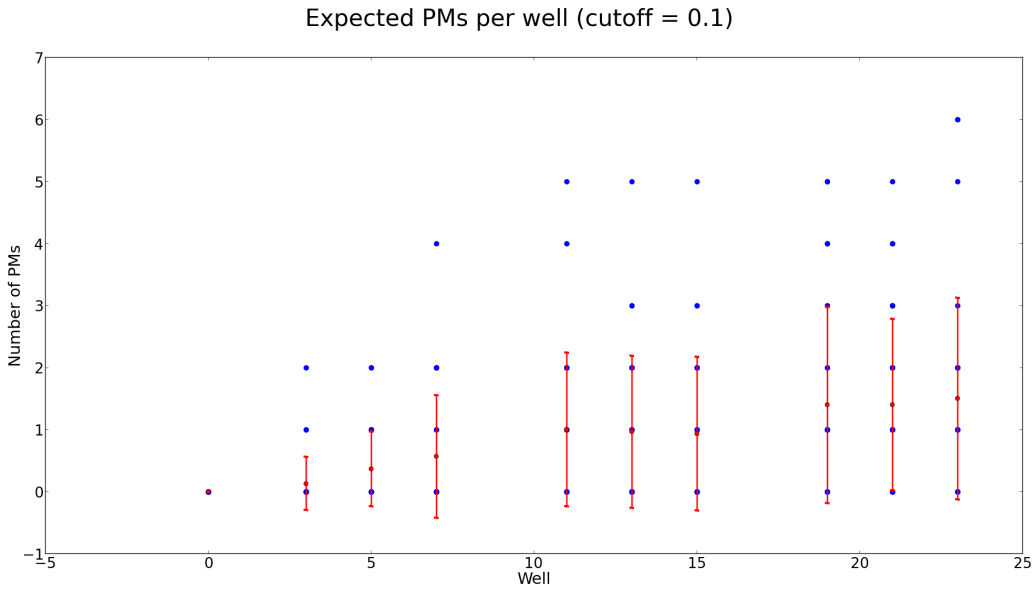
- Track where driver mutations occur? Always arising in the well directly before the well with $a = \text{MIC}$?
- Include different types of driver mutations / benefits for having more than 1?
- Stop antibiotic from killing cells but rather just prevent replication?

7 Figures

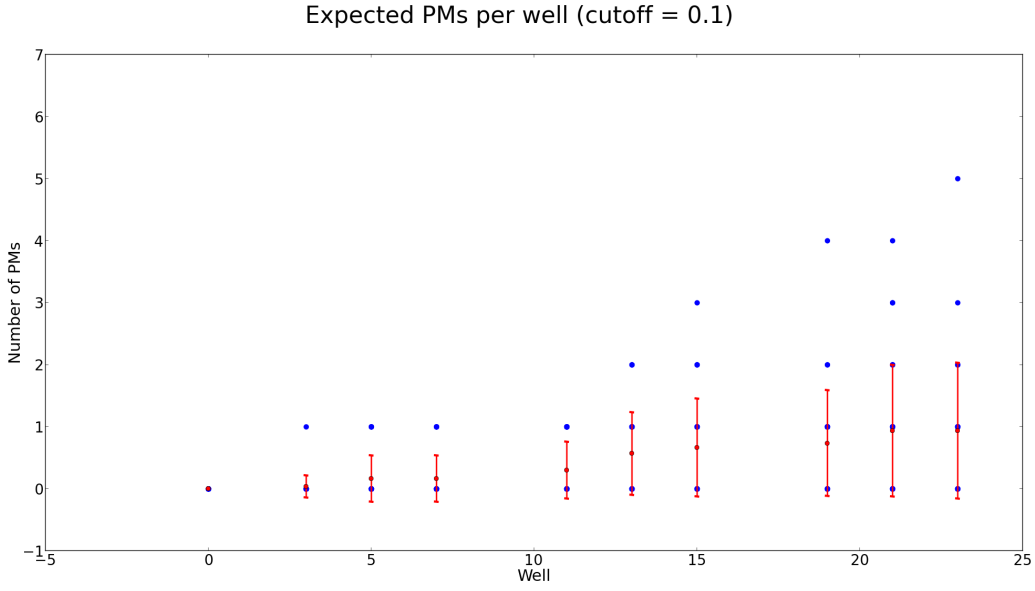


(a) (b)

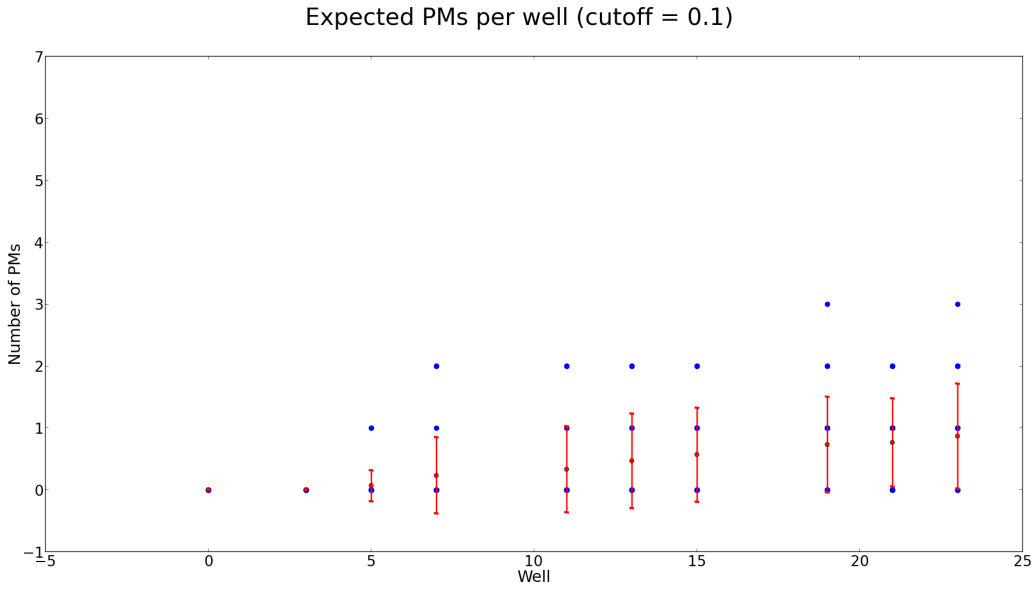
Figure 1: Population wave is Fisher-like. (a) Shows the speed of the wave v versus $\sqrt{D}$ where D is the diffusion constant (parameter diff). (b) Shows v versus $\sqrt{\mu}$ where μ is the parameter max_growth_rate. For a true Fisher wave we expect $v \sim \sqrt{\mu D}$.


Expected PMs per well (cutoff = 0.1)
Number of PMs
Well
7
6
5
4
3
2
1
0
−1
−5
0
5
10
15
20
25

(a) $D = 1.5$

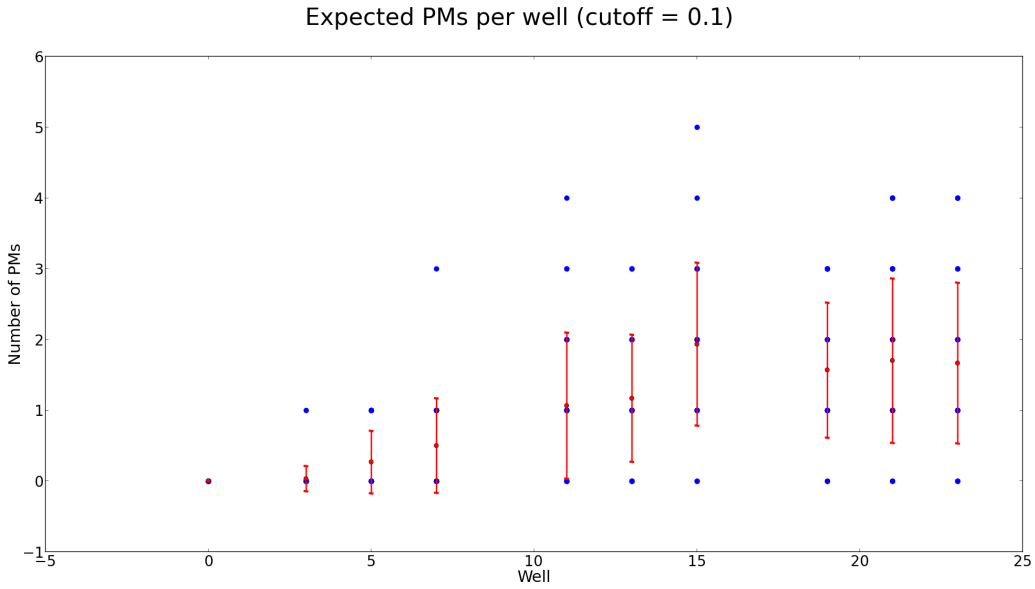

Expected PMs per well (cutoff = 0.1)
Number of PMs
Well
7
6
5
4
3
2
1
0
−1
−5
0
5
10
15
20
25

(b) $D = 6.0$

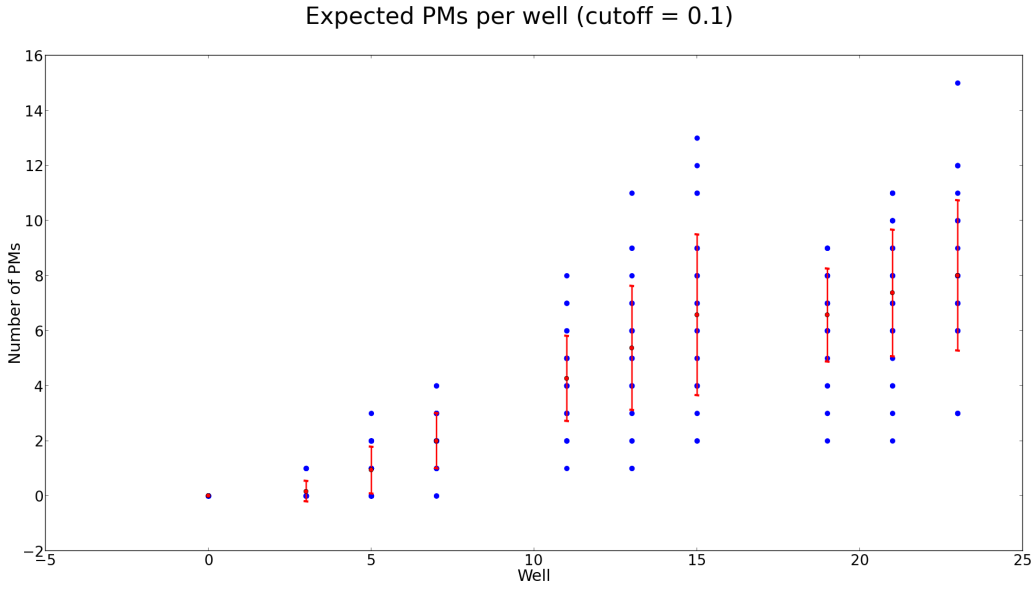


(c) $D = 12.0$

Figure 2: Expected number of mutations per well, with no antibiotics, for diffusion constants of $D = 1.5$, 6.0 and 12.0. Other parameters are: food_0=1e5, γ =1e-2, max growth $\mu = 2.0$, size of channel = 1 mm. We see that although D is increased by an order f magnitude the expected number of mutations per well only decreases slightly and this decrease will not be distinguishable from between-experiment variability. I have increased D from 1.5 to 12.0, but what about decreasing it? It might have a larger effect since maybe we are already in a regime of high diffusion; i.e. decreasing cell motility might dramatically increase number of mutations?

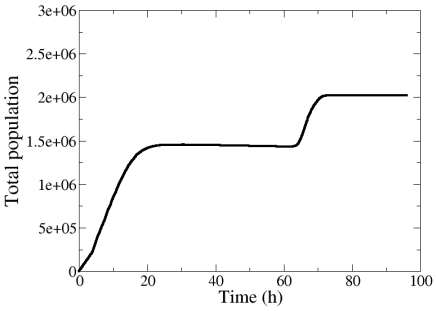


(a) γ =1e-2

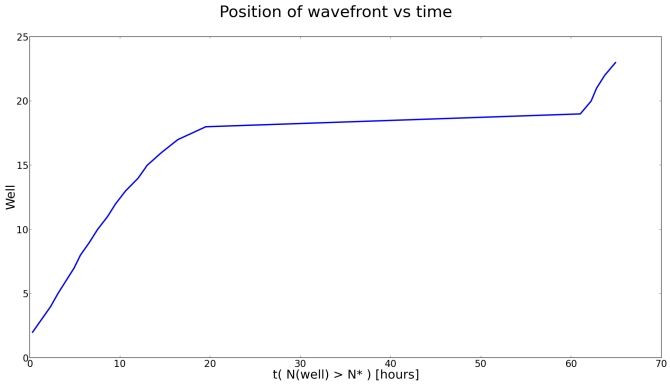


(b) γ =5e-2

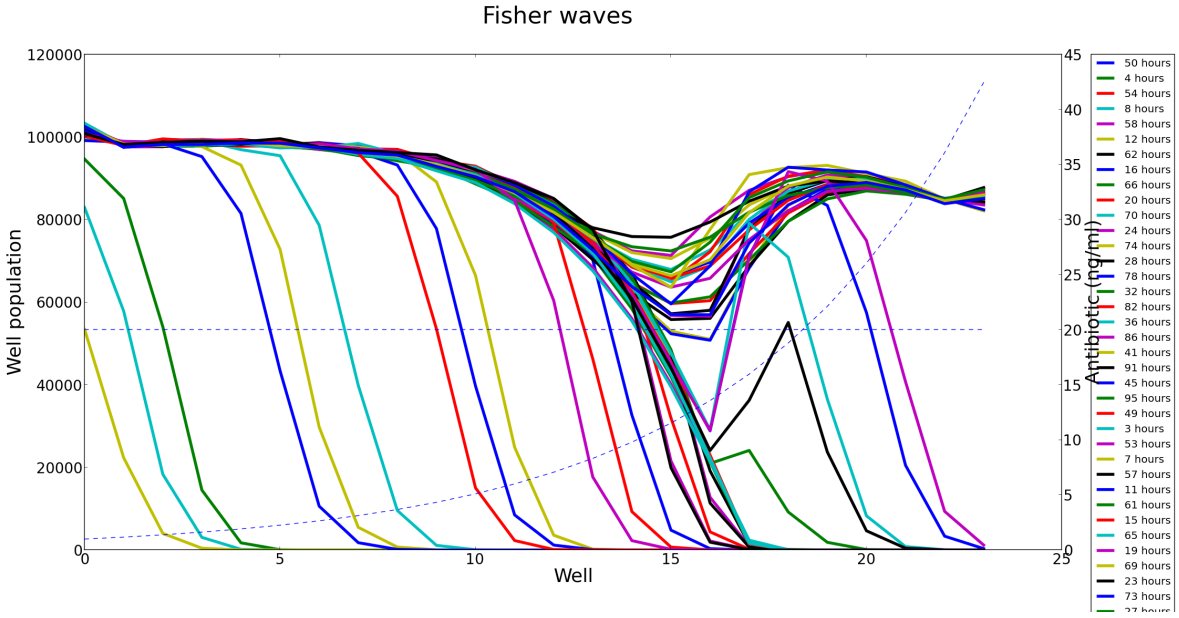
Figure 3: We see that changing the mutation rate has a much stronger effect on the expected number of mutations per well compared to the diffusion constant (Figure 2). This suggests correctly incorporating the ciprofloxacin-dependence on mutation rate is probably quite important. From (a) to (b), mutation rate is only increased 5-fold. Parameters: $D = 1.5$, $\mu = 2.0$, food$_0$=1e5. Antibiotic was present here, with min_antibiotic = 0, max_antibiotic = 50.



(a) Total population of all wells vs time.



(b) Propogation of wavefront; no longer a steady wave.



(c) "Fisher" wave profile is destroyed

Figure 4: Dynamics of population when antibiotic is present. (a) Population can't progress beyond well with antibiotic concentration a = MIC until a driver mutation is acquired. (b) Wavefront no-longer propogates as Fisher wave. (c) Well populations at various times (throughout 30 experiments). Blue-dashed curve shows antibiotic concentration profile across wells; horizontal dashed line shows MIC of WT (20 ng/ml). See text for explanation. Note that the antibiotic reaches the MIC value of 20 ng/ml only at well 19. Hence when a driver mutation arises it may not have much opportunity to "carry" other passenger mutations to fixation. Also, Bartek has said the MIC is 30 ng/ml in his data set, which isn't reached until well 21 (when max_antibiotic = 50 ng/ml). Increasing this profile will increase the chances that drivers can carry other mutations, and will also increase the mutation rate if we include this antibiotic concentration dependence. The form of Eq.2 means that if I increase max_antibiotic to 100 ng/ml, an MIC of 20 ng/ml is reached at well 16, or an MIC of 30 ng/ml is reached at well 18. Perhaps we could see more mutations by having a steeper initial concetration increase?

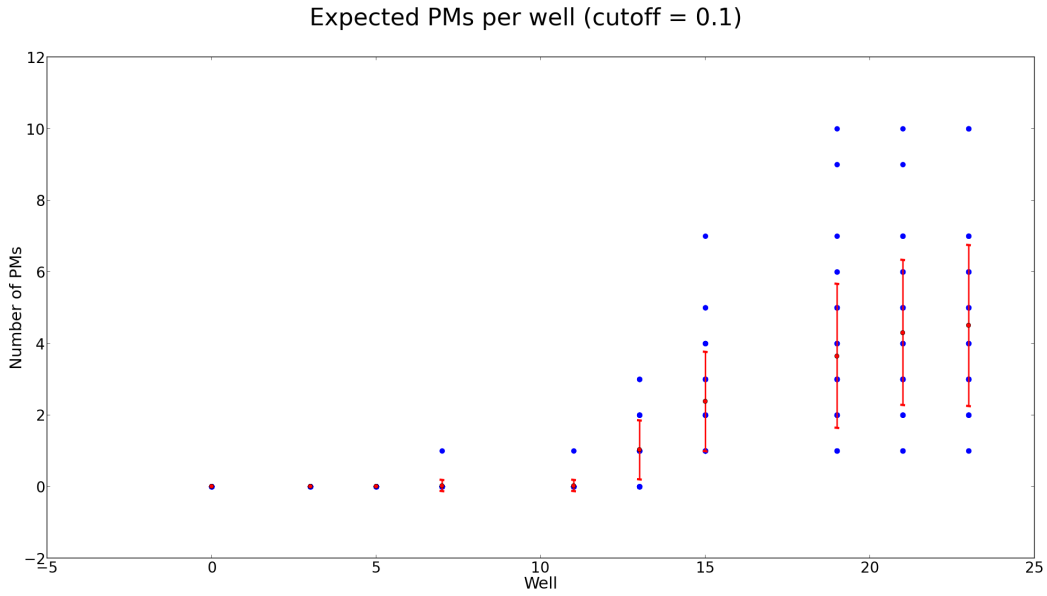


Figure 5: Varying mutation rate with antibiotic concentration. Set γ^{WT}=1e-3 and used $X = 15$, $Y = 2$ in Eq. 3. Even with this strong increase in mutation rate, we observe no detectable mutations until around well 15 (which is close to $a = \text{MIC}/2$). $D = 4.0$, channel size = 2 mm.