Simulation notes 5

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1 Expected mutations and strains

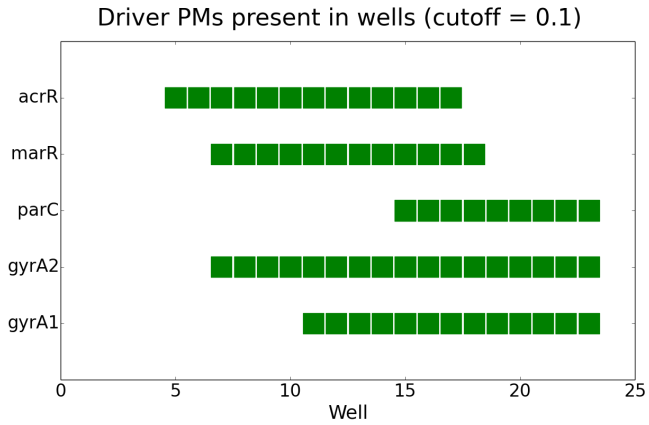
These notes contain various types of plots I generated this week looking at both the expected mutations present in a given well at the end of the experiment, as well as the expected mutant "strains" (as defined in [1]). My idea was that such plots might give drastically different results for different antibiotic concentration profiles and that we might be able to see different mutational pathways being obviously more likely in different environments. I think these plots are interesting, but in reality they require a lot of interpreting. I need to think more carefully of exactly what I want to show. I've not tried to make one, but a "weighted mutational network" showing the likelihood of different evolutionary routes through the space of resistant genomes might be more insightful.

2 The plots

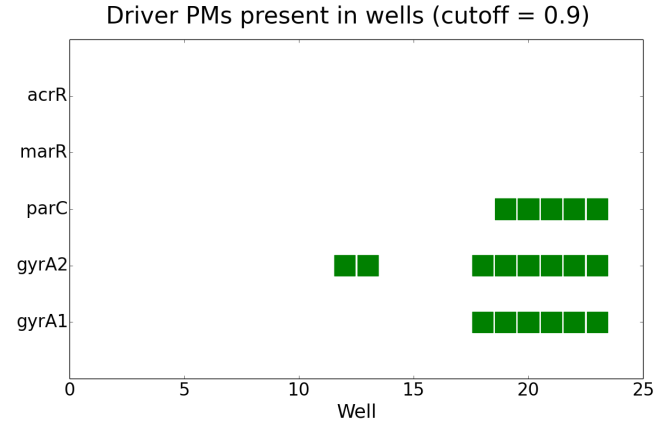
These are the various types of plots I've generated, all made for experiments with a maximum ciprofloxacin concentration of 20,000 ng/ml.

References

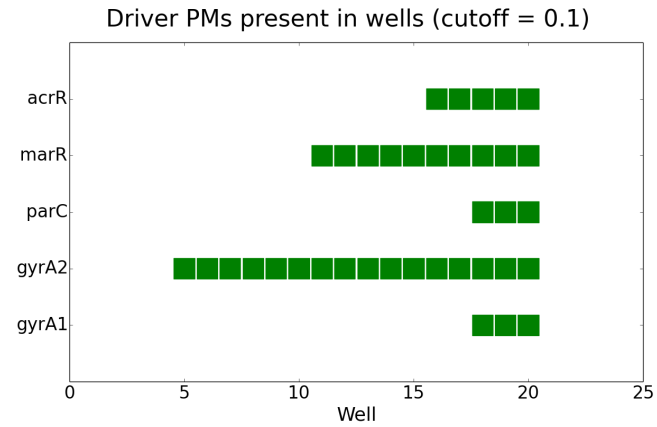
[1] Linda L. Marcusson, Niels Frimodt-Møller, and Diarmaid Hughes. Interplay in the selection of fluoroquinolone resistance and bacterial fitness. *PLoS Pathogens*, 5(8), 2009.



(a) Experiment 92

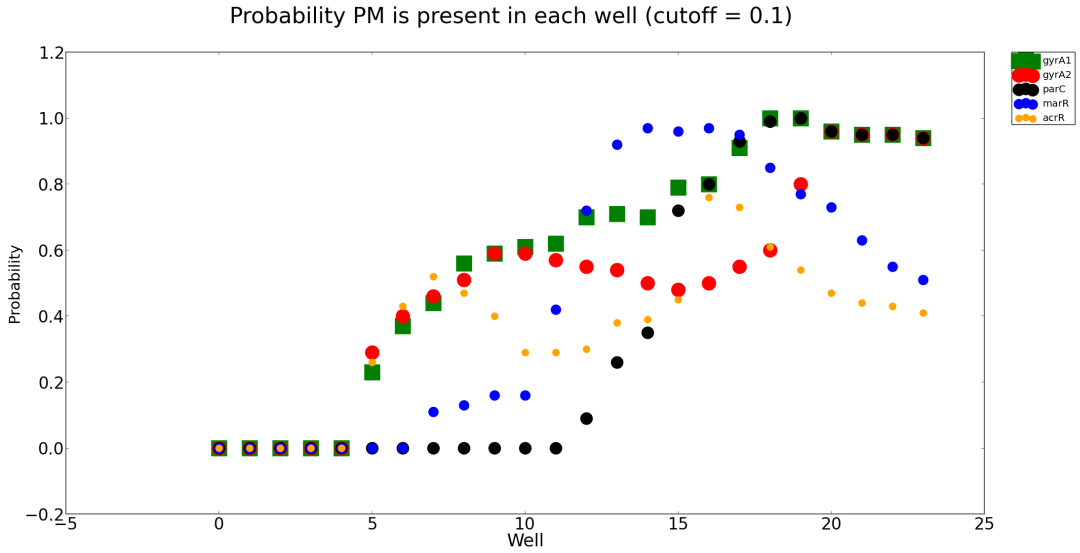


(b) Experiment 92

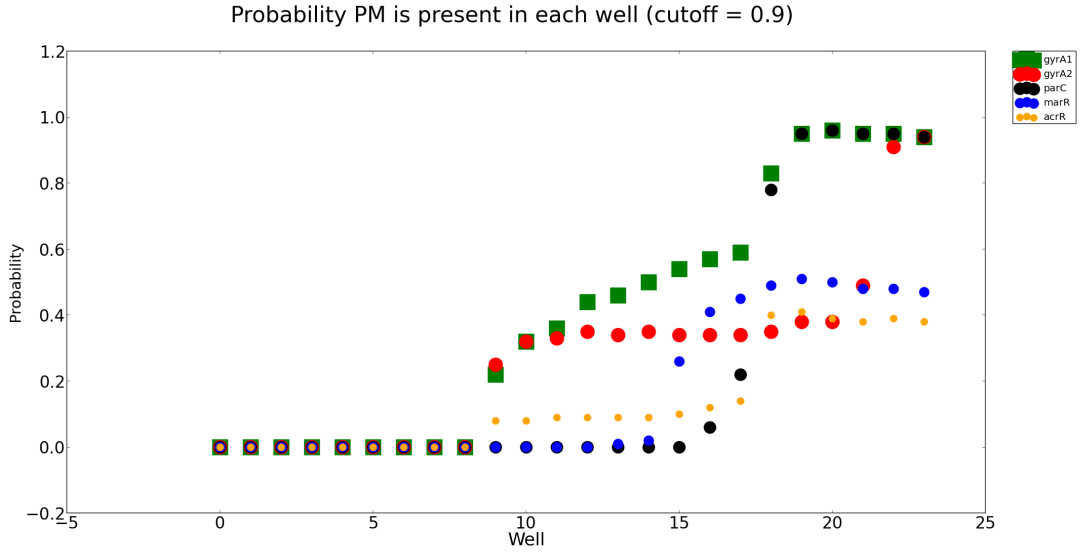


(c) Experiment 44

Figure 1: These plots are for individual experiments and show which driver mutations are present above some cutoff abundance in each well at the end of the experiment (3 days). (a) We see that acrR and marR mutations are present, and above the experimentally detectable threshold of 10% in the intermediate wells but are ultimately lost since they were not part of the genome that successfully mutated in a later well. From the data in [1] we know that to be viable at 20,000 ng/ml of ciprofloxacin the genome must contain all 3 of the gyrA1, gyrA2 and parC mutations in the final well. (b) Shows the plot produced from the same experiment as in (a) but for a cutoff abundance of 0.9. It thus shows that the 3 mutations are all probably fixed in the final well population (we already *know* they have to be). It also shows that the gyrA2 mutation did not arise and persit from very early on, but arose at least twice. (c) Shows an example of an experiment which failed to reach the final well. Since we know that a genome with all 3 of gyrA1, gyrA2 and parC is viable right up to the final well, we know that these 5 mutations, all experimentally detectable, must be split between different genomes. (Hence these plots are interesting from the point of view of sampling DNA randomly from wells but not individual clones since they tell us little about which strains are actually present).

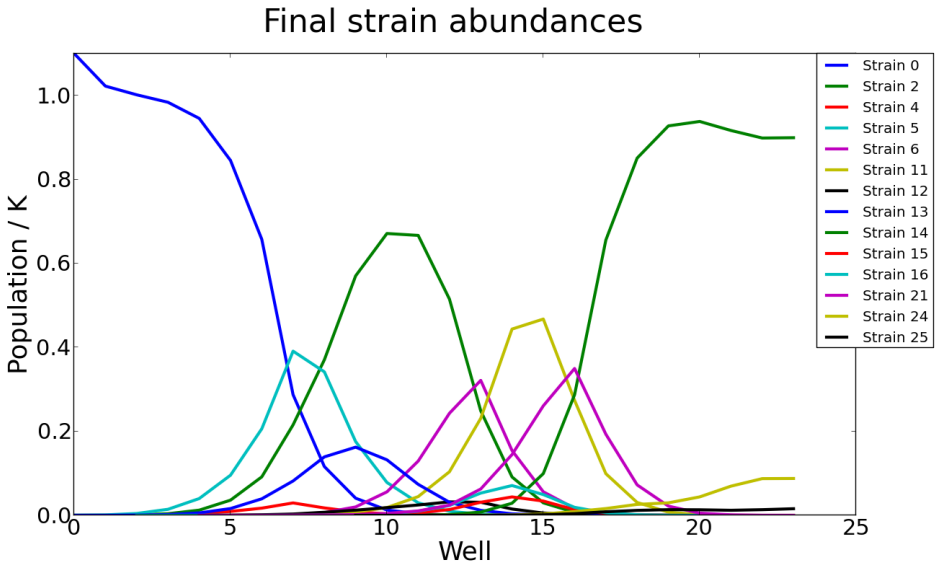


(a)

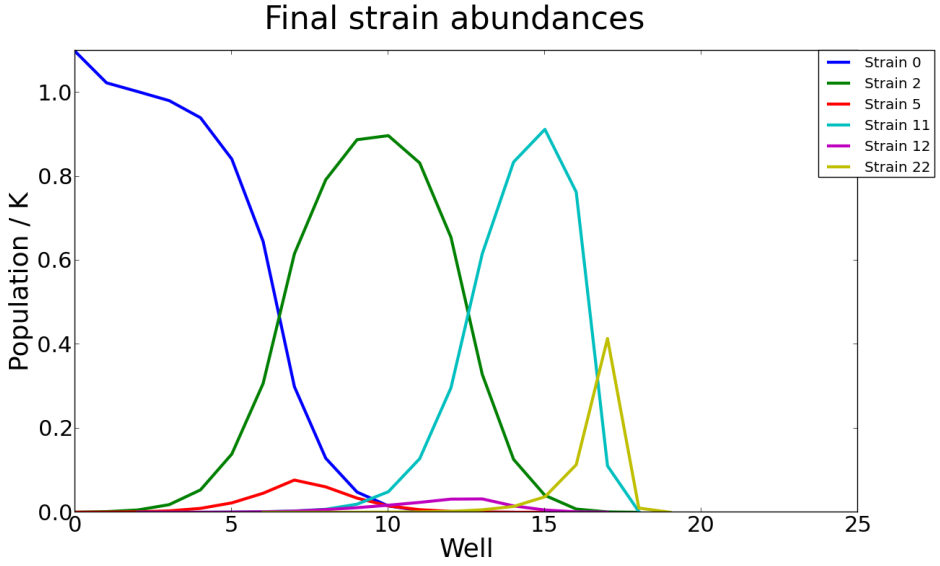


(b)

Figure 2: Here I show the probability (calculated from 100 experiments) that each of the 5 driver mutations are present above some cutoff abundance in each well. These plots are for the same set of 100 experiments with a maximum ciprofloxacin of 20,000 ng/ml and a cutoff of (a) 0.1 and (b) 0.9. Each driver mutation is represented by a different shape and colour. An interesting feature is the non-monotonicity arising from the complicated mutational pathways through a genome space where each genotype has a different MIC and fitness. For example, acrR (yellow) in plot (a) has two maximums and marR (blue) is very likely to be detected in wells 13–17 but becomes increasingly less likely to be found in earlier or later wells. Consider plot (b). If we look at the data in [1] we see that to reach the final well, all 3 of gyrA1, gyrA2 and parC must be present in the genome, hence these 3 symbols coincide in the final well. However, to be viable in the second-final well there is another genotype which lacks the gyrA2 mutation, explaining why the red circle (gyrA2) sits slightly below gyrA1 and parC here.



(a) Experiment 92



(b) Experiment 44

Figure 3: These plots each correspond to single experiments and show the abundance of specific strains (resistant genotypes) across all wells at the end of the experiment. These plots can show very nice peaks where certain strains are dominating, and it is sometimes possible, but definitely not always, to infer the actual "mutational pathway" from them. In total there are 32 "strains" (28 strains included in [1], the 3 that were not, plus the WT which is strain 0). I need to write these as something like "01001" rather than "Strain 12", but one needs to keep referring back to [1] to understand these plots in any case. Plot (a) corresponds to the same experiment in Fig. 1a and (b) corresponds to Fig. 1c, i.e. the experiment that didn't reach the final well.

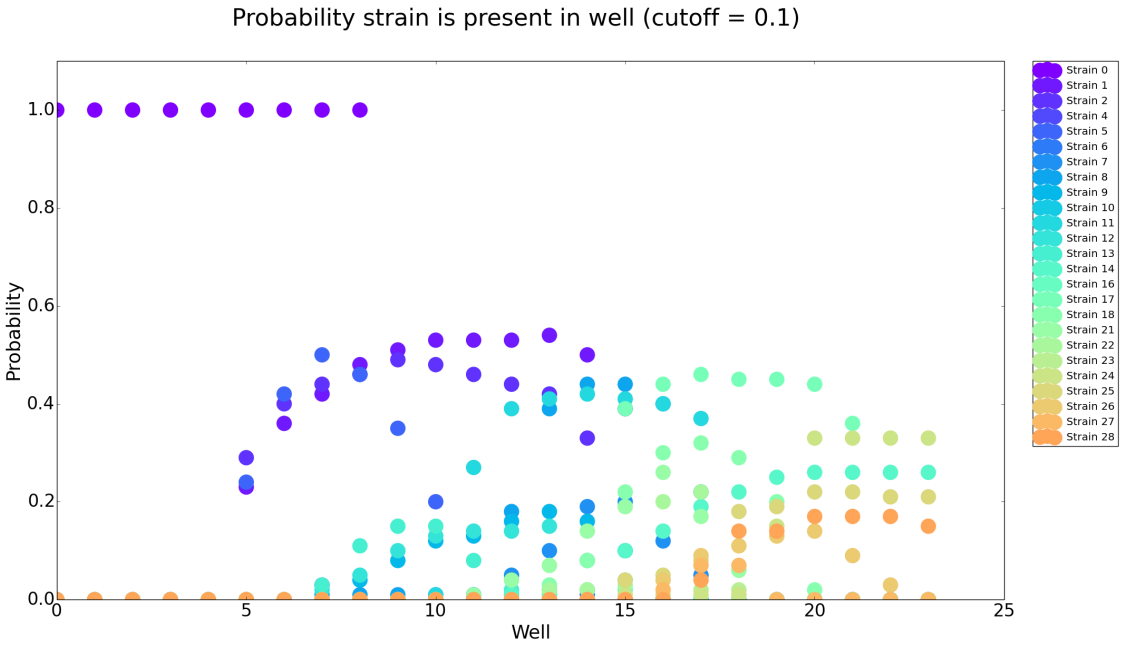


Figure 4: This plot did not turn out quite as nice as I had hoped. It shows the probability (calculated from 100 experiments) that for a given well each strain is above a certain abundance. I hoped this plot would be easy to compare when made for different antibiotic profiles and might produce something interesting. Maybe if I choose colours and shapes more carefully, and increase the cutoff I will have plots that are more easily comparable. In any case, I think there is still one interesting observation. Again, at 20,000 ng/ml of ciprofloxacin, only 4 strains are actually viable: strains 14, 24, 25 and 28 (the 4 colours seen in well 23). All of these strains have the same MIC but strain 14 is objectively the "best", having the highest fitness. What I think is nice is that strain 14 is not the most likely to be present in the final well: due to the interaction of the environment and the complicated genotype-fitness landscape, an inferior genotype is more likely to emerge. Initially I thought that if we extended this maximum ciprofloxacin concentration as a plateau for 5 more wells, the global optimal might emerge, but since strain 14 does not contain either marR or acrR, and the other 3 viable genotypes do, the fact that we do not allow back-mutations in our simulations will prevent this from happening. If a sub-optimal genotype fixes in this final well, strain 14 will never emerge.

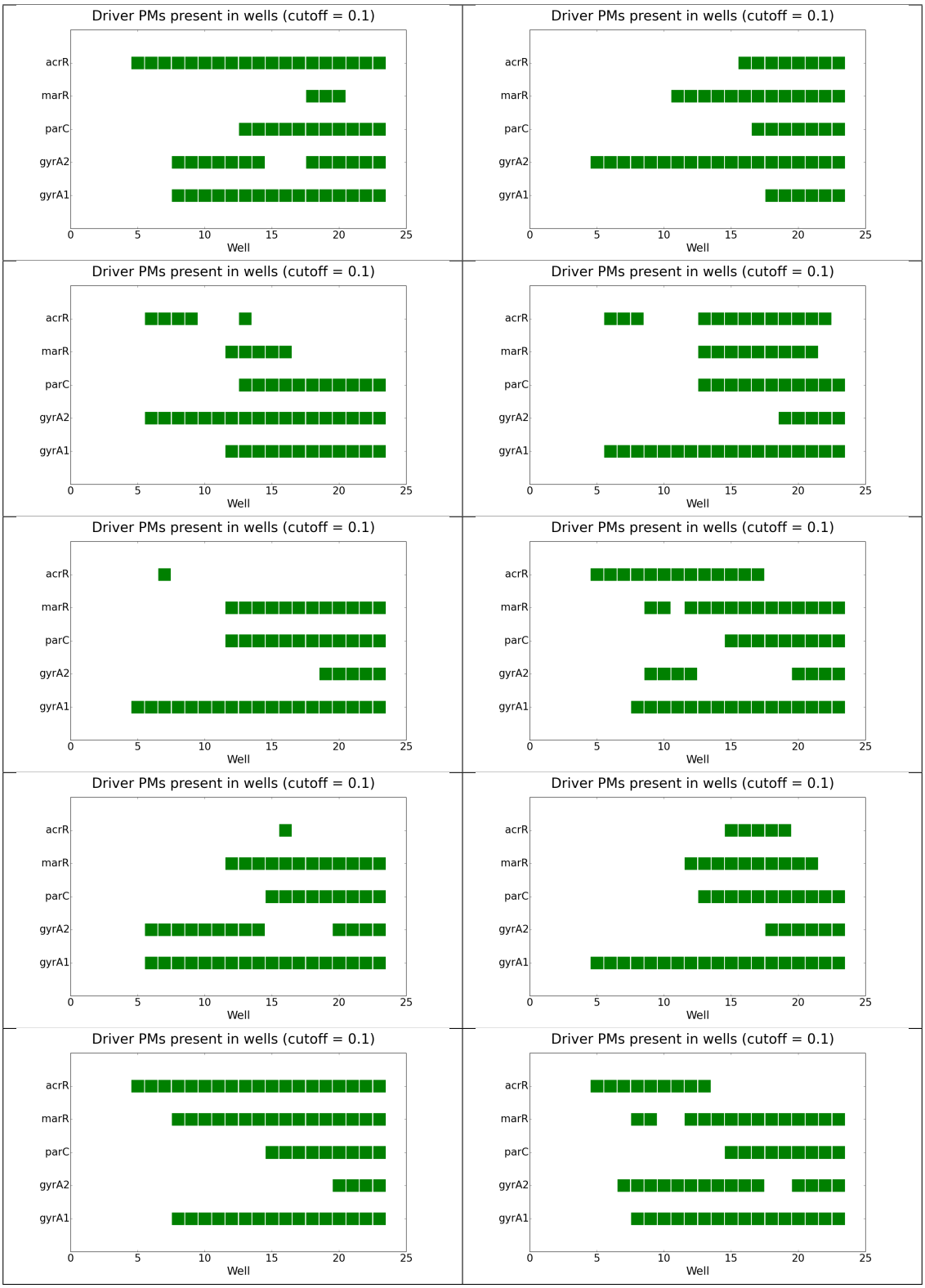


Figure 5: Random selection of experiments; plots as in Fig. 1 with cutoff = 0.1, i.e. experimentally detectable.

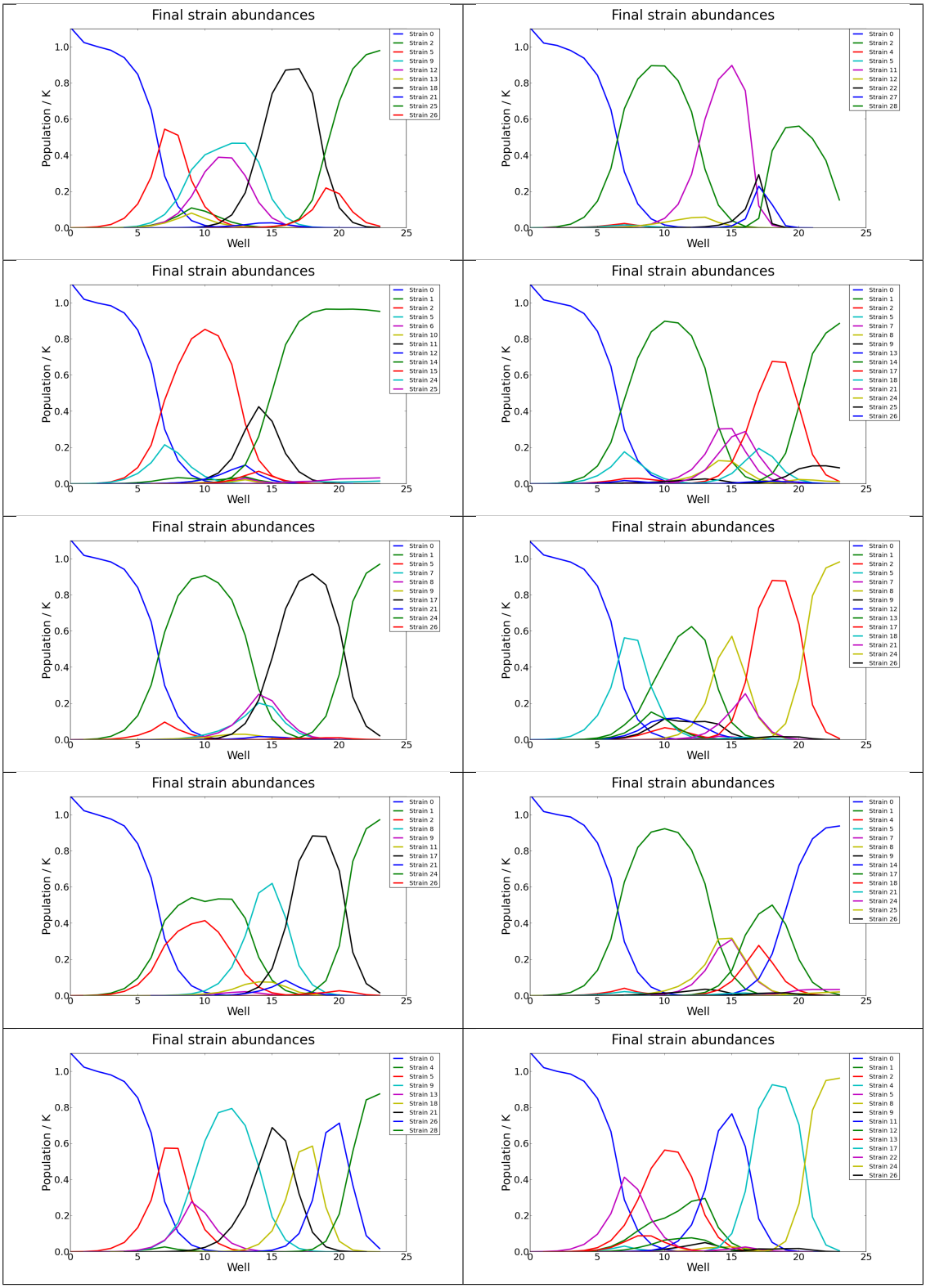


Figure 6: Plots as in Fig. 3 with panels here corresponding to the same panels (experiments) in Fig. 5. I have noticed a few times that some independent experiments have produced almost identical plots. (Though maybe this is expected since there is obviously a finite number of mutational pathways).