Evolutionary Timeline Extension by Dr. Alex Tobias

Students should take, say, 10 microliters from the most recent culture, and use that to inoculate about 1 ml of fresh medium to start a new "subculture." All of these serial passage cultures should contain no antibiotic, or have one set contain antibiotic and the other set not. That would be best actually.

Around 1 day later for each of these cultures, they should spread around 20 microliters on an agar plate - again, one set with antibiotics and one set without, if possible. If you only can do one set, definitely do without antibiotics. If you have lots of plates, have them plate different volumes, like 20, 50, 100 microliters.

When the plates are incubated and the colonies have grown - between 30 and 37 degC is good for E. coli, the students should count the number of fluorescent and non-fluorescent colonies with the black light used as illumination to show the fluorescent ones.

In each successive passing cycle, the percent of fluorescent colonies should decrease because of plasmid loss due to lack of antibiotic selection pressure. This can be compared with the antibiotic set, if that is run too.

These data can be plotted against time or # of cycles to determine the slope or rate of plasmid loss, and also the point at which complete loss from the population occurs or is predicted to occur.