Major procedure for detached leaf assay in big experiment

1. 3 weeks before big experiment, prepare isolates growing on new PDA plates
2. 2 weeks before big experiment, growing isolates on peaches
3. Prepare the following items:
4. Autoclave glass wool, make a plug and put into 12 ml syringes, then seal it with invisible tapes (about 100 for big experiment)
5. Autoclave 1 L ddH2O (500 ml each bottle)
6. Autoclave tooth picks (in two small beakers)
7. Autoclave 10 boxes of yellows tips (200 ul)
8. Autoclave 1.7 ml tubes (at least 6 large beakers)
9. Autoclave 3 sets of scissors and forceps
10. Make at least 12 L 70% ethanol
11. Count 100 1 L bottles, weigh 7.5 phytoagar into each bottle and cap the bottle
12. Wash 50 trays and 50 Domes with soap, rinse with deionized water, let them dry on counter
13. Design your experiment (randomize plant genotype and isolate genotype in three replicates), Print the tray labels and cut them in strips, print inoculation maps and stick onto the cabin.
14. Order the following items:
15. 2 bottles of 95% ethanol (4L each)
16. 10 ml pipette (2 bags, 50/each bag)
17. 6 boxes of 1ml sterile tips with filters
18. 100 15 ml- tubes
19. 3 boxes of kimwipes
20. 1 box of cover plate for hemacytometer
21. Two days before big experiment, sort out plants in the order of same genotypes (about 3 hrs to tire my plants and 3 hrs to sort them in order)
22. Items need to borrow
23. Drag one good-wheel cart from CEF-A and use it to transfer plants and autoclave media in Wickson Hall
24. Items to check
25. 50% Grape juice (about 400 ml)
26. Vortex (2), gloves, paper towels
27. Laptop
28. Key to Wickson hall
29. Timer
30. Sharpies
31. At least two sets of 1 ml pipette, 200 ul pipette and 20 ul pipette
32. Racks for 1.7 ml tubes (the more the easier)
33. Two large garbage cans
34. Two carts to put bottles need to wash, one more cart upstairs as working station to put plant on
35. One day before big experiment, prepare the agar flats and it will take at least 6 hours
36. Fill the bottles with 750 ml deionized water, cap the bottle and autoclave all the agars using autoclave machine in both Asmudson Hall and Wickson Hall
37. After putting agar in autoclave machine, Surface sterilize all the trays and domes, let them dry on counter.
38. When autoclave is done, briefly shake the bottle and make the medium even, then pour to the tray, after a while, get rid of any bumbles in the tray agar
39. When the medium is solidified, put tray labels in and cover with the lid
40. Drag plants into lab during day time (for experiment in winter period)
41. The night before the big experiment, collect spores from each isolate and it will take about 12 hours for one person
42. Collecting spores from 98 isolates. DON’T scratch too much of the spores. Once you notice the solution becomes a little bit cloudy, you have enough spores already. If you scratch too much, it will take forever to count spores of one isolate. After centrifuge step, suspend spores in 1-3 ml of grape juice depending on the size of the pellet. It will greatly shorten the following counting step (about 5 hrs)
43. Counting spores (about 3 hrs)
44. Serial dilution to prepare the final inoculum (about 4 hrs)
45. Prepare the detached leaf and put on the agar (about 8 hrs)
46. Inoculate on detached leaf (about 6 hrs)