**Optimized procedure for spore collection**

It’s optimized for two people (A and B) working together

1. Divide 98 isolates into three groups: 26-36-36 or 30-36-32 due to the capacity of the centrifuge for the spinning down step (Maximum 36 samples each round)
2. One person can start early for the spore collection of 26 isolates or two can start at same time for the first round of 30 isolates.
3. When the samples are ready for the first round of centrifugation, A continues to do the spore collection from plates. B does the centrifugation. During the waiting time, B labels 1.7 ml tubes to transfer spores (two tubes for each isolate, one for diluting spores, one for quantification).
4. After centrifugation, B pours the supernatant into the waste bucket and re-suspends the spore pellet in grape juice. Add 1-3 ml grape juice based on the size of the spore pellet. Transfer the spores to newly labeled 1.7 ml tubes for spore dilution and keep about 50ul spore solution in the tube for quantification using the same tip. When all isolates of the first round are ready, put the set for dilution on ice and take the set for quantification to lab.
5. B does the quantification using the hemacytometer. Count spores of five squares and record the number on laptop. Using the formula, get different volume of each isolate for the 100X dilution. Then do the 10X and 1X dilution by adding 50 ul spore solution to 450 ul of grape juice.
6. When B does the dilution, check the progress of A. If 36 isolates are ready, put them in centrifuge. When B finishes the dilution of first round of isolates, second round is ready to re-suspend in grape juice.
7. A continues to collect spores of the third set. B repeats the above steps, re-suspending spores in 1-3 ml of grape juice. Put the second set of tubes for dilution on ice. Then do the quantification.
8. When A finishes the third set of collecting spores, A puts them in centrifuge and has a brief break until B finishes the counting of second set.
9. A starts doing the spore dilution of the second set; B re-suspends spores in 1-3 ml of grape juice, transfers to new tubes and starts counting of the third set.
10. When B finishes counting of the third set, B will join A for the spore dilution.
11. When all work is done, autoclave the garbage.
12. Divide 98 isolates into three groups: 26-36-36 or 30-36-32 due to the capacity of the centrifuge for the spinning down step (Maximum 36 samples each round)

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| Person A | Person B |
| \*\*\* Can start early for the spore collection of 26 isolates or both can start at same time for the first round of 30 isolates.   1. When the samples are ready for the first round of centrifugation, A continues to do the spore collection from plates. 2. A completes first 36 isolates. 3. A continues to collect spores of the third set. 4. When A finishes the third set of collecting spores, A puts them in centrifuge and has a brief break until B finishes the counting of second set. 5. A starts doing the spore dilution of the second set. 6. When B finishes counting of the third set, B will join A for the spore dilution. | 1. When the samples are ready for the first round of centrifugation, B does the centrifugation. During the waiting time, B labels 1.7 ml tubes to transfer spores (two tubes for each isolate, one for diluting spores, one for quantification). 2. After centrifugation, B pours the supernatant into the waste bucket and re-suspends the spore pellet in grape juice. Add 1-3 ml grape juice based on the size of the spore pellet. Transfer the spores to newly labeled 1.7 ml tubes for spore dilution and keep about 50ul spore solution in the tube for quantification using the same tip. When all isolates of the first round are ready, put the set for dilution on ice and take the set for quantification to lab. 3. B does the quantification using the hemacytometer. Count spores of five squares and record the number on laptop. Using the formula, get different volume of each isolate for the 100X dilution. Then do the 10X and 1X dilution by adding 50 ul spore solution to 450 ul of grape juice. 4. When B does the dilution, check the progress of A. If 36 isolates are ready, put them in centrifuge. When B finishes the dilution of first round of isolates, second round is ready to re-suspend in grape juice. 5. B repeats the above steps, re-suspending spores in 1-3 ml of grape juice. Put the second set of tubes for dilution on ice. Then do the quantification. 6. B finishes the counting of second set. 7. B re-suspends spores in 1-3 ml of grape juice, transfers to new tubes and starts counting of the third set. 8. When B finishes counting of the third set, B will join A for the spore dilution. |

1. When all work is done, autoclave the garbage.