**Yeast liquid survival curves (with LOH) assays,**

**Revised for Eppendorf tubes, last updated, 2012 May 19, by H Qin**

**Day 0**

1. Grow yeast overnight in 5ml YPD at 30C.

**Day 1**

1. Next day, restage by 1:1000 dilution to OD <= 0.05. (Typically 15 ul O/N growth to 15ml YPD in flasks)
2. Grow yeast to OD plateau (It takes about 24 hours at 30C for wild isolates of yeast to reach OD >= ~2.0. If the OD is >2.0, do NOT dilute them with fresh media, because we do not want to restart the culture again!!!). Take a small aliquoat with 1:100 dilution, 5 sonication pulses, and check under microscope for budding index. The unbudded cells should be below 80%.
3. Take 2x1ml to two eppendorf tubes. Spind down, decant, and then merge with 1ml water in ONE tube.
4. Wash with water twice (2x1ml)
5. Re-suspend in 1 ml of ddH2O in eppendorf tube (This is the final volume for lifespan assay).
6. Take 10ul sample and add to 990ul water. This is the first serial dilution (100X). For subsequent timpointes, the time when you take 10ul sample is the sampling time.
7. Put the eppendorf tubes to 30C nutator. Notice that the time when you put the tubes into 30C is the starting point of the CLS assay, i.e. time=0.
8. **4 pulses** sonicate at level 2. (Sterilize Sonicator probe by immersing probe in Falcon tube containing 70% ethanol. Sonicate the cells to ensure uniform segregation on level 2, 3 pulses. Use 70% ethanol to rinse and clean the probe between samples to avoid cross containmiation.)
9. For time point zero: Count the cell numbers and budding index under microscope using bright line counting chamber (Usually the 100X dilution should work).
10. Do more serial dilutions by adding 100ul of previous dilution to 900ul water (103X, 104X,105X). The goal is to make 4 cell per microliter.
11. Add 200ul of diluted cells to 150mm plates, aiming for 1000 colonies. (Gupta aim to use 5x104X and 2x105X to catch the time points. For the first time point, 2 dilutions are needed to get good results, for subsequent timepoints, we can refer to the previous plates for the right dilutions.)
12. Incubate the plates at 30C for 2 days and then count them. (Colonies are often visible in one day. For LOH assay, 2 more days at RT can improve the coloration of colonies).

**Day 2, 3, 4, … Take time points**

1. **Repeat steps 6, 7, 8, 10, 11, 12 from Day 1.**

* **Tip:** After viability starts to drop, wash the cells twice with ddH2O, and then re-suspend the cells in exactly the same volume. This wash can avoid re-growth.
* Tip1: If you plate cells on Friday, then plate are best left at 4C and move to 30C on Sunday night or Monday, so that colonies will not over-grow.
* Tip2: Once viability starts to drop, less dilutions are needed to get enough colonies.

**Data analysis and report**

1. When the colonies on plates are darkened enough, take pictures of these plates using DocIt.
2. Count the colonies as white, black, half-blacks, quater-blacks, ¾ blacks, etc.
3. Input the data into a Excel file on Dropbox, calculate viability change by time, frequency of black colonies by time, generate plot.
4. Print figures of viability, black and half-black by time. Staple all figutres, note, counting-log, and put into lab notebook.

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**Yeast liquid survival curves (with LOH) assays,**

**Revised for Eppendorf tubes, last updated, 2012 May 15, by H Qin**

1. Grow yeast overnight in 5ml YPD at 30C.
2. Next day, restage by 1:1000 dilution to OD <= 0.05. (Typically 15 ul O/N growth to 15ml YPD in flasks)
3. Grow yeast to OD plateau (It takes about 24 hours at 30C for wild isolates of yeast to reach OD >= ~2.0. If the OD is >2.0, do NOT dilute them with fresh media, because we do not want to restart the culture again!!!). Take a small aliquoat with 1:100 dilution, 5 sonication pulses, and check under microscope for budding index. The unbudded cells should be below 80%.
4. Take 2x1ml to two eppendorf tubes. Spind down, decant, and then merge with 1ml water in ONE tube.
5. **3 pulses** sonicate at level 2. (Sterilize Sonicator probe by immersing probe in Falcon tube containing 70% ethanol. Sonicate the cells to ensure uniform segregation on level 2, 3 pulses. Use 70% ethanol to rinse and clean the probe between samples to avoid cross containmiation.)
6. Wash with water twice (2x1ml)
7. Re-suspend in 1 ml of ddH2O in eppendorf tube (This is the final volume for lifespan assay).
8. Take 10ul sample and add to 990ul water. This is the first serial dilution (100X). For subsequent timpointes, the time when you take 10ul sample is the sampling time.
9. Put the eppendorf tubes to 30C nutator. Notice that the time when you put the tubes into 30C is the starting point of the CLS assay, i.e. time=0.
10. Pulse sonicate the sampled cells to ensure uniform segregation on level 2, **2 pulses.**
11. For time point zero: Count the cell numbers and budding index under microscope using bright line counting chamber (Usually the 100X dilution should work).
12. Do more serial dilutions by adding 100ul of previous dilution to 900ul water (103X, 104X,105X). The goal is to make 4 cell per microliter.
13. Add 250ul of diluted cells to 150mm plates, aiming for 1000 colonies. (Gupta aim to use 5x104X and 2x105X to catch the time points. For the first time point, 2 dilutions are needed to get good results, for subsequent timepoints, we can refer to the previous plates for the right dilutions.)
14. Put the plates at 30C for 2 days and then count them. (Colonies are often visible in one day).
15. **Optional:** After viability starts to drop, wash the cells twice with ddH2O, and then re-suspend the cells in exactly the same volume. This wash can avoid re-growth.
16. Tip1: If you plate cells on Friday, then plate are best left at 4C and move to 30C on Sunday night or Monday, so that colonies will not over-grow.
17. Tip2: Once viability starts to drop, less dilutions are needed to get enough colonies.

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**Yeast liquid survival curves (with LOH) assays, last updated, 2012 May 15, by H Qin**

1. Grow yeast overnight in 5ml YPD at 30C.
2. Next day, restage by 1:1000 dilution to OD <= 0.05. (Typically 15 ul O/N growth to 15ml YPD in flasks)
3. Grow yeast to OD plateau (It takes about 24 hours at 30C for wild isolates of yeast to reach OD >= ~2.0). Take a small aliquoat with 1:100 dilution, 5 sonication pulses, and check under microscope for budding index. The unbudded cells should be below 80%.
4. Take 10 ml of cells, which is twice of the final water volume and centrifuge at 10-15 C/ 1000 rpm for 5 minutes. (This can be done in 15ml blue Falcon tubes).
5. Spin cells down in 15 ml Falcon tubes. Add equal volume of ddH2O.
6. Sonication in 15ml Falcon tubes for 5 minutes in a water-bath sonicator (Fisher F20D). All sonications should be done with plastic tubes, because debris fall off from glass tubes during sonication). ( OLD: Sterilize Sonicator probe by immersing probe in Falcon tube containing 70% ethanol. Sonicate the cells to ensure uniform segregation on level 2, 3 pulses. Use 70% ethanol to rinse and clean the probe between samples to avoid cross containmiation.)
7. Spin cells down in 15 ml Falcon tubes. Wash with ddH2O 2 times.
8. Re-suspend in glass tubes with 5 ml of ddH2O (This is the final volume for lifespan assay). Put these tubes to 30C shaker. Use a rubber band to avoid clashing of tubes. Notice that the time when you put the tubes into 30C is the starting point of the CLS assay, i.e. time=0.
9. Take 20ul sample and add to 180ul water. This is the first serial dilution (10X). For subsequent timpointes, the time when you take 20ul sample is the sampling time.
10. Sonicate the cells to ensure uniform segregation on level 2, 2 pulses.
11. Do more serial dilutions by adding 100ul of previous dilution to 900ul water (102X, 103X, 104X,105X).
12. Optional: Count the cell numbers under microscope using bright line counting chamber (Usually the 100X dilution should work).
13. Add 100ul of each dilution to 100mm YPD or MLA plates (200-250ul to 150mm plates). (We aim to use 104X to catch the time points. But, we also plate 103X and 105X dilutions on plates to make sure CFUs are countable. For the first time point, 3 dilutions are needed to get good results, for subsequent timepoints, we can refer to the previous plates for the right dilutions.)
14. Put the plates at 30C for 2 days and then count them. (Colonies are often visible in one day).
15. Optional: After viability starts to drop, wash the cells twice with ddH2O, and then re-suspend the cells in exactly the same volume. This wash can avoid re-growth.
16. Tip1: If you plate cells on Friday, then plate are best left at 4C and move to 30C on Sunday night or Monday, so that colonies will not over-grow.
17. Tip2: Once viability starts to drop, less dilutions are needed to get enough colonies.

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Old 2006 version From University of Rochester

1. Grow yeast overnight in 5ml, 30C.
2. Next day, restage to OD=0.01 (Meng add 20ul overnight grown yeast to 10ml YPD. This is about 2OD x 20ul/10ml=0.004OD~ 0.01OD )
3. Grow exactly 24 hours at 30C for wild isolates of yeast, but 48 hours for BY4743. When examined under microscope, 80% of the cells should be unbudded.
4. Spin cells down. Wash with water 3 times.
5. Re-suspend in 1/2 volume of water.
6. Taking time points: Meng often did 1:100 dilution, which gives 30-60 x16x10^4 cells/ml. He then does 300-700 dilution, and put 3000 cells/200ul to 150mm plates.

Cells are chilled on ice for 20 minutes, then sonicated for 30’’.