Measuring UV-induced Mutagenesis at the *CAN1* Locus in *Saccharomyces cerevisiae*

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[Abstract] There are several methods to measure the capacity of yeast cell to respond to environmental impacts on their genome by mutating it. One frequently used method involves the detection of forward mutations in the *CAN1* gene. The *CAN1* gene encodes for an arginine permease that is responsible for the uptake of arginine and it can also transport the toxic analog of arginine, canavanine (Whelan *et al.*, 1979). When *CAN1* cells are grown on a media containing canavanine but lacking arginine, the cells die because of the uptake of the toxic canavanine. However, if a mutation in the *CAN1* gene inactivates the permease, that cell survives and forms a colony on the plate.

The following protocol describes the measurement of UV-induced mutagenesis at the *CAN1* locus.

Materials and Reagents

1. Strains that have the wild type *CAN1* gene (*e.g.*, BY4741, EMY747)
2. Yeast nitrogen base [w/o amino acids and w $(NH_4)_2SO_4$] (Difco)
3. Adenine (Sigma-Aldrich)
4. Arginine (Sigma-Aldrich)
5. Canavanine (Sigma-Aldrich)
6. Histidine (Sigma-Aldrich)
7. Isoleucine (Sigma-Aldrich)
8. Leucine (Sigma-Aldrich)
9. Lysine (Sigma-Aldrich)
10. Methionine (Sigma-Aldrich)
11. Phenylalanine (Sigma-Aldrich)
12. Tryptophan (Sigma-Aldrich)
13. Tyrosine (Sigma-Aldrich)
14. Uracil (Sigma-Aldrich)
15. Valine (Sigma-Aldrich)
16. Yeast extract

17. Pepton
18. D-glucose
19. Bacto agar
20. Yeast extract-pepton-D-glucose (YPD) media (see Recipes) (or other media needed for the strain in use)
21. Synthetic complete media (SC) plates (see Recipes)
22. SC-arginine plates containing canavanine (+can) (see Recipes)

Equipment

1. 30 °C incubator-shaker (180-200 round per minute)
2. 30 °C incubator
3. Glass slide
4. Centrifuge
5. Microscope
6. Vortex
7. UV-irradiation machine
8. Box
9. Spreader
10. Culture tube
11. Waterbath sonicator
12. Hemocytometer (Burker counting chamber)

Procedure

1. By inoculating a single colony from a fresh plate, grow up 10 ml overnight cultures in YPD (one reference, or wild type strain, and the strains to be examined).
2. Put the culture tube into a waterbath sonicator and sonicate the cells at room temperature for 3 min to disrupt clumps (40 Hz). Check for the presence of clumps under the microscope.
3. Make 200x dilutions of each strain in water (5 µl cell culture into 995 µl water) and put 10 µl onto a Burker-chamber.
4. Count the cells under the microscope in a big square of the Burker-chamber (bordered by three lines) (Figure 1). 1 cell in a big square means 1 x 10^4 cells/ml.
5. Calculate the density of the original cultures (multiply the counted cell number by 200).
6. Make 10x serial dilutions of each strain, starting from 10^8 to 10^3 cells/ml. If necessary concentrate cells to get 10^8 cells/ml. When calculating the volume of a given dilution step,

take into consideration how many plates you will be plating from that dilution (see steps 8 and 9 below).

7. Label the SC and the +can plates with the strain names and the UV doses you want to apply. For each UV dose, including zero, label 2 SC and 2 +can plates for each strain. The SC plates will be used to calculate survival at different UV doses, the +can plates will be used to calculate mutagenesis at different UV doses.
8. Plate 200 µl on the control, 0 J/m^2 SC plates from the 10^3 cells/ml dilution. For wild type, EMY747 or BY4741 cells that are quite resistant to UV showing 20% survival at 80 J/m^2, for up to that dose the 10^3 cells/ml dilution should be used for plating. In case of more sensitive strains the expected survival rate should be taken into consideration when determining which dilution to use for plating for given UV doses.
9. Plate 200 µl cells on the +can plates from the 10^8 cells/ml dilutions.
10. Wait till plates absorb the moisture, then irradiate the plates without lids, with the required UV doses. Make sure the irradiated plates are not exposed to white light after irradiation (work with yellow light on), and they are placed right away in a box that shields them from light and put in the 30 °C incubator.
11. Incubate the plates until colonies grow to 2-3 mm in diameter. For SC plates it takes usually 2-3 days, for +can plates it takes up to 5 days (check them under yellow light).
12. Count the colonies on each plate.
13. Calculate the percentage of survival on SC plates. Divide the average number of colonies of the two 0 J/m^2 plates with the number of cells that were plated on one plate and multiply it by 100. That gives the percentage of cells that survived plating.
14. Calculate the survival at each UV doses. Multiply the average colony number of the two parallel plates with the plating survival percentage calculated from 0 J/m^2 plates (see step 13 above), and divide it by the number of cells that was plated. (Take into consideration the actual volumes you plated at different doses: 100 µl, or 200 µl!)
15. Calculate mutagenesis from +can plates. Multiply with the survival percentage the number of cells plated on a plate at the given UV dose. Calculate the average number of colonies from the two parallel plates of the same UV dose. That gives you the number of mutants/plated cells. Based on that calculate how many mutants would be in 10^6 cells, because mutagenesis data usually corresponds to 10^6 cells. (Take into consideration the actual volumes you plated at different doses: 100 µl, or 200 µl!)

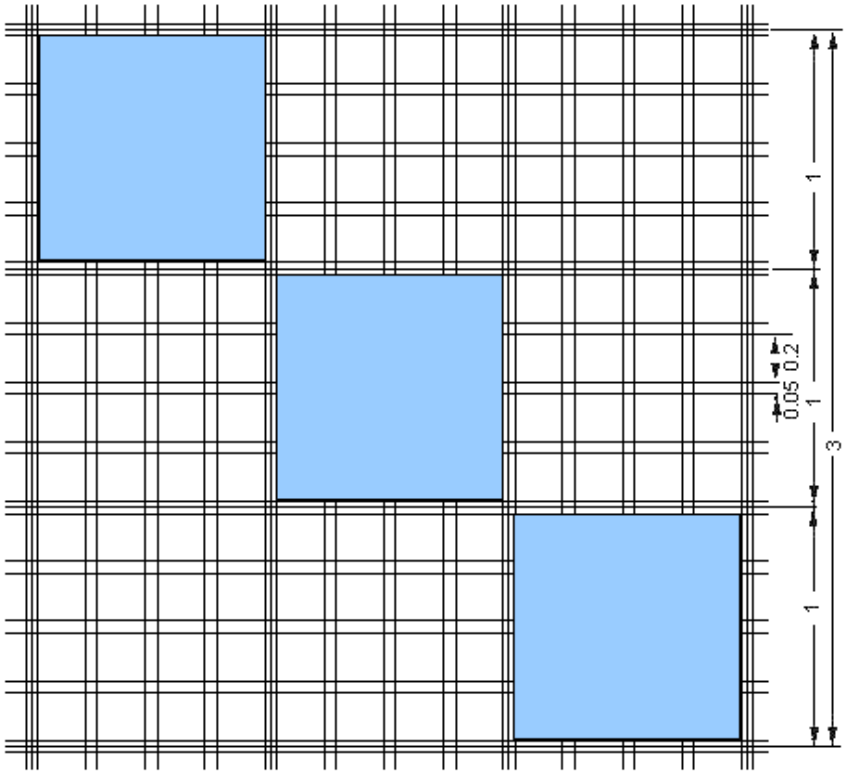

Figure 1. Burker chamber. One cell in one blue area means 1 x 10^4 cells/ml. (Source: http://openwetware.org/wiki/IGEM:University_of_Debrecen:_transfection)

Representative data

1. Since the result of this experiment depends on the number of inactivating mutations in the *CAN1* gene inflicted upon by UV, the number of mutants can vary. Because of that average numbers should be calculated based on 3-5 experiments.

Recipes

1. YPD liquid
   1% yeast extract
   2% pepton
   2% D-glucose
2. SC plates
   Bacto-agar: 16.6 g/L
   D-glucose: 20 g/L
   12 media mix: 7.2 g/L

12 media mix

Yeast nitrogen base [w/o amino acids and w $(NH_4)_2SO_4$]	400 g
Adenine	1.8 g
Arginine	1.2 g
Histidine	1.2 g
Isoleucine	1.8 g
Leucine	1.8 g
Lysine	1.8 g
Methionine	1.2 g
Phenylalanine	3.0 g
Tryptophan	1.2 g
Tyrosine	1.8 g
Uracil	1.2 g
Valine	9.0 g

3. SC -arginine plates containing canavanine

   Same as the SC plates, but the media mix contains 3.6 g canavanine instead of arginine

Acknowledgments

We used this protocol in our work (Daraba *et al.*, 2014). Funding support: Wellcome Trust, 070247/Z/03/A.

References

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2. Whelan, W. L., Gocke, E. and Manney, T. R. (1979). The CAN1 locus of *Saccharomyces cerevisiae*: fine-structure analysis and forward mutation rates. *Genetics* 91(1): 35-51.

