

Mutagenesis Protocol

Cells: JS200. Make sure they are still temperature-sensitive after you grow them in LB at 30 C. They are tet resistant. See protocols in book chapter (attached) for detailed protocols on how these cells need to be grown and how to make them competent. Since I wrote the book chapter we do our temperature-sensitivity assays in 2XYT plates, because LB sometimes doesn't give consistent results.

To speed up your experiments I also included JS200 cells transformed with pHSG-WT Pol I and pHSG-EP Pol I. These cells are ready to be transformed with your target plasmid.

Plasmids (2 microL each):	Resistance (Microg/microL)	Conc.
Pol I plasmids		
pHSG-WT Pol I	chloramphenicol	0.9
pHSG-EP Pol I	chloramphenicol	0.03
Reporters		
pLA230	carbenicillin	0.3
pGFP UV	carbenicillin	0.5

Find the sequence files of pHSG- Pol I, pLA230 and pGFP UV attached as strider files.

pHSG plasmids are not maintained well in cells expressing F' (such as XL-1 blue). We use JS200 for plasmid amplification and purification purposes.

To generate a library *in vivo*:

1) Transformation of your target plasmid: transform the ColE1 plasmid encoding your target sequence into JS200 cells carrying the EP pol I plasmid (i.e. The two plasmids are transformed sequentially, and in both cases cells are kept at 30C). As a reversion reporter for mutagenesis you can use pLA230 as described in our PNAS article. pLA230 transformants are simply plated on carbenicillin plates to score for ochre revertants. We have also been performing forward mutation assays, using the plasmid pGFPuv as a reporter (clontech). In this case, at the end of the mutagenesis experiment the GFP transformants need to be miniprep'd; the DNA is transformed into BL21 cells (for some reason GFP fluorescence is brighter in this strain); mutagenesis is scored in the form of cells with decreased fluorescence.

2) Mutagenesis: take your transformants that have been grown at 30 and dilute them by a factor of 10^5 into 2XYT media. Grow this culture at 37 to saturation. After one round of *in vivo* mutagenesis, typically we get ~10% GFP colonies with decreased fluorescence. See recent data on frequency, spectrum and distribution of mutations in the attached document. Note that we don't see a decrease in the frequency of mutations as we move away from the plasmid origin of replication. The data obtained with the different reporters that was published in the PNAS article is therefore likely artifactual.

3) Iteration: For reasons we don't understand mutagenesis is not continuous in culture, i.e. mutagenesis does not continue to increase. If you just keep passing your cells in 2XYT at 37. For additional mutagenesis you need to retransform your library into

fresh JS200 cells expressing error-prone Pol I. Wash the transformants from the plate with ~4 ml LB, dilute this thick broth to ~OD=3. Pass a $1:10^3$ dilution of these cells into 2XYT media and mutagenize as described above. After two rounds of mutagenesis, we got 30% colonies with decreased fluorescence and found by sequencing ~1 mutation in 1200 bp. (see attached detailed protocol)

Good luck with your experiments, please feel free to contact me if you have any questions/concerns. I'd be happy to assist you to design your experiments to optimize the performance of our mutagenesis system.

Best regards

Manel Camps

1. Pick glycerol stocks of JS200 cells (w/ WT or EP PolI) into LB + CM (30 µg/ml)
2. 30°C o/n – no shaking
3. Shake @ 30°C for 2 hr

Quick Comp Cells (steps 3-8 all done at 4°C)

4. Transfer 1.5 ml to a microcentrifuge tube, cool to 4°C
5. Spin down at 13000 rpm for 1 min
6. Remove supernatant, resuspend in 1 ml 10% glycerol
7. Repeat 4 & 5 2x
8. Spin down at 13000 rpm for 1 min
9. Remove supernatant, resuspend in 100 µl 10% glycerol

10. Transform 1 µl of target plasmid in 40 µl quick comp cells
11. Recover for 1 hr @ 30°C in 1 ml SOC
12. Plate on LB + CM + Carb (or other appropriate antibiotic)
13. Incubate @ 30°C o/n
14. Pick colonies into 5 ml of LB + CM + Carb
15. 30°C o/n – no shaking
16. Shake @ 30°C for 2 hr
17. Take 2 µl and inoculate 5 ml of 2XYT + CM + Carb

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18. 37°C for 15 min – no shaking
19. Shake @ 37°C o/n

20. Miniprep 1.5 ml of culture
21. Transform 1 µl of DNA prep into 40 µl quick comp cells
22. Recover for 1 hr @ 30°C in 1 ml SOC
23. Plate on LB + CM + Carb
24. Incubate @ 30°C o/n
25. Collect colonies with 3 ml, then 1ml LB + CM + Carb
26. Dilute to OD₆₀₀ 3.0
27. Inoculate 5 ml 2XYT + CM + Carb with 2 µl of diluted colony cocktail
28. Return to step 18 for next round of mutagenesis