Yeast Transformation (high efficiency)

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This more involved method gives higher transformation efficiency than the semi rapid method. Use DMSO if transforming a library to yeast and/or transforming a mutant strain which transforms poorly using the LiOAc method.

1. Inoculate 5 mls YPD with a single yeast colony. Grow O/N at 30 degrees C.

2. Add 0.5 mls culture to 4.5 mls fresh media, check A660. Add suitable amount of cells to 60 mls fresh media to give A660 = 0.2 (2 x 106 cells/ml). Grow to A660 = 1.0 (2 x 107 cells/ml), takes approximately 5 hours.

3. Spin down 50 mls cells, wash in 10 mls sterile water, re centrifuge, resuspend in 1 ml sterile water. Transfer to 1.5 ml sterile microfuge tube, spin down, resuspend in 1 ml sterile TE / LiOAc (made fresh from 10X TE [0.1M Tris-HCl, 0.01M EDTA, pH 7.5] and 10X LiOAc [1M LiOAc pH 7.5, adjusted with diluted acetic acid]). Spin down, resuspend in 0.25 mls TE / LiOAc (4 x 109 cells/ml).

4. Mix 50 ul yeast cells with transforming DNA and 5 ul single stranded carrier DNA (10 mg/ml, boiled and quick chilled on ice) in a 1.5 ml microfuge tube.

5. Add 300 ul sterile PEG (40% PEG 4000, 1X TE, 1X LiOAc, made fresh from sterile 50% PEG 4000, 10X TE, and 10X LiOAc). Mix thoroughly.

6. Incubate at 30 degrees C for 60 minutes with occasional gentle shaking.

7. Add 40 ul DMSO, mix thoroughly. (This increases transformation 5-10 fold.)

8. Heat shock at 42 degrees C for 15 minutes.

9. Microfuge 10 seconds, remove supernatant. Resuspend in 1 ml 1X TE. Microfuge 10 sec. Resuspend in 1 ml 1X TE. Plate 200 ul on selective media.