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## Transformation "Ultra-Competent" *E. coli* (Inoue Method)

Inoue, H., H. Nojima, and H. Okayama. 1990. High efficiency transformation of *Escherichia coli* with plasmids. *Gene* **96**:23-28. [Citation Abstract](#)

### Procedure

1. Inoculate from an overnight grown in LB.
2. Grow in 250 ml "[SOB](#)" at 18C until OD<sub>600</sub> = 0.6.
2. On ice for 10 minutes.
3. Spin at 2500 x g (5000 rpm in a Sorvall GSA or 3000 rpm in a Beckman J-6B centrifuge) for 10 min. at 4C.
4. Resuspend cells gently in 80 ml of ice cold "[TB](#)". 5. On ice for 10 minutes.
6. Spin at 2500 x g (5000 rpm in a Sorvall GSA, 5500 rpm in a Sorvall SS-34, or 3000 rpm in a Beckman J-6B centrifuge) for 10 min. at 4C.
7. Resuspend cells gently in 20 ml of ice cold "TB".
8. Add DMSO to a final concentration of 7%.
9. Place on ice for 10 minutes.
10. Aliquot into 1-2 ml and freeze in liquid nitrogen.
11. Store in liquid nitrogen.

### SOB Medium and TB (Transformation Buffer)

<b>SOB</b>	2% (w/v) bacto tryptone	<b>TB</b>	
	0.5% (w/v) yeast extract		10 mM Pipes
	10 mM NaCl		55 mM MnCl <sub>2</sub>
	2.5 mM KCl		15 mM CaCl <sub>2</sub>
	10 mM MgCl <sub>2</sub>		250 mM KCl
	10 mM MgSO <sub>4</sub>		<i>adjust pH to 6.7 with 5N KOH prior to adding the MnCl<sub>2</sub></i> thanks to Markus Schneemann for the tip!
	<i>pH 6.7 - 7.0</i>		

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