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(according to W. Zwerschke's PhD thesis , DKFZ, Heidelberg, Germany, 1997)

## Competent cells: Rubidium Chloride

### Solutions:

RF1 (pH 5.8):

	<b>c</b>	<b>g / mol</b>	<b>g for 500 ml</b>	<b>g for 250 ml</b>
<b>RbCl</b>	100 mM	120.9	6.45	3.23
<b>MnCl<sub>2</sub></b>	50 mM	197.9	4.925	2.46
<b>KAc</b>	30 mM	98.14	1.47	0.735
<b>CaCl<sub>2</sub></b>	10 mM	147	0.74	0.37
<b>glycerol</b>	15 %			

RF2 (pH 6.8):

	<b>c</b>	<b>g / mol</b>	<b>g for 100 ml</b>
<b>MOPS</b>	10 mM	209	0.21
<b>RbCl</b>	10 mM	121	0.12
<b>CaCl<sub>2</sub></b>	75 mM	147	1.1
<b>glycerol</b>	15 %		

Sterilize both by filtration.

- 1 Grow bacteria in 100 ml of 2YT or SOC medium to an  $OD_{600} = 0.5$ .
- 2 Chill on ice for 15 mins.
- 3 Pellet the bacteria for 5 mins. @ 4000 x g, 4 C.
- 4 Resuspend the pellet in 35 ml of ice cold RF1.
- 5 Incubate 15 mins. on ice.
- 6 Spin down as above.
- 7 Resuspend the bacteria in 8 ml of ice cold RF2.
- 8 Incubate 15 mins. on ice.
- 9 Make 40 aliquots of 200  $\mu$ l each, freeze in liquid nitrogen, and store @ -80 C.