Preparation of reagents

SGM17 hypertonic medium:

Add 17.1g sucrose and 1.2g glycine into 100ml GM17 fluid medium, autoclave and save at 4C°.

SGM17 recovery medium (SGM17Mc):

Add 2 mol/L $MgCl_2$ 100 μL and 0.5 mol/L $CaCl_2$ 40 μL into 10ml sterile SGM17 medium before use.

Washing buffer/ electroporation buffer (EB):

Add 17.1g sucrose and 10mL glycerol and then add water to 100ml.

Preparation of competent state of *L.Lactis* MG1363:

Inoculate MG1363 into GM17 liquid medium and cultivate overnight.

Inoculate 5% MG1363 solution into SGM17 medium and cultivate until OD=0.8.

Keep the bacterial in ice for 10 minutes, and then centrifuge at 4C°, 4000rpm for 15 min, discard the supernatant.

Wash the bacterial with equal volume, 1/2 volume and 1/4 volume of the ice cold EB for 3 times

and resuspend the bacterial with 1/100 volume of the ice cold EB. For each time of washing, the bacterial is centrifuged at 4 C°, 4000rpm for 15 min, then the supernatant is discarded and the bacterial is resuspended by blowing and suction with EB.

Keep the bacterial at -80 C° for future use.

Electroporation of *L.Lactis* MG1363:

Add 2 µl 50ng/µl pMG36e into 50 µl MG1363 competent bacterial, keep on ice for 10 min. Electroporate at 400Ω 2kV with ice cold 2mm electroporation cuvettes.

Immediately add 950 µl SGM17Mc, keep on ice for 10 minutes, recover at 30C° for 3 hours.

Centrifuge the bacterial solution at 4000rpm for 15 min, resuspend with 500µl SGM17Mc and spread 200 µl of the bacterial onto the GM17 plate with 10 ng/ml erythromycin for 1~2 days.