University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	Preparing Competent Cells (DH5α *Escherichia coli* cells)		
	Version:	1		
	Description:	How to make competent DH5α *E. coli* cells		
	Trigger:	Use this procedure when competent cells are required so that they may be used for transformation.		
Last updated	Name:	Elizabeth Brookes	Date:	16.07.14
You will need	Time:	2 days		
	PPE:	Gloves Lab coat		
	Equipment:	pH meter Autoclave Incubator NanoDrop Ice Bath GSA Bottle Centrifuge Autoclaved Microtubes		
	Materials:	Super Optimal Broth (SOB Medium): o 525mL Milli-Q water o 10g bacto-tryptone (LP0042) o 2.5g bacto-yeast extract (LP0021) o 0.25g NaCl o 0.93g KCl o 5M NaOH solution Liquid Broth (LB) *For instructions on how to make liquid broth see 'A2 Preparation of Liquid Broth Media Protocol'* LB Agar Plates *For instructions on how to make LB agar plates see 'A3 Making Agar Plates Protocol'* 2mL of 2M $MgCl_2$ Transformation Buffer: o 0.6g HEPES o 0.55g $CaCl_2.2H_2O$ o 4.66g KCl o Milli-Q Water o 2.72g $MnCl_2.4H_2O$ Dimethyl Sulfoxide (DMSO) Liquid Nitrogen or Dry Ice		
Super Optimal Broth (SOB) Preparation				
Step 1	In a conical flask containing 475mL of Milli-Q water add the following: o 10g bacto-tryptone (LP0042) o 2.5g bacto-yeast extract (LP0021) o 0.25g NaCl			

Step 2	In a separate beaker containing 50mL of Milli-Q water add 0.93g of KCl to make a 250M KCl solution.
Step 3	Mix both solutions thoroughly until completely dissolved.
Step 4	Take 5mL of the 250M KCl solution and add it to the first solution in the conical flask. *Note: The remaining KCl solution can be stored in the bar fridge.*
Step 5	Adjust the pH of the mixed solution using a pH meter to pH 7 by adding small volumes of 5M NaOH solution. *Note: For instructions on how to use the pH meter see 'G1 Using the PH Meter'*
Step 6	Make the final volume of the SOB solution in the conical flask up to 500mL using Milli-Q water.
Step 7	Autoclave the SOB solution *Note: For instructions on how to use the autoclave see 'G3 Autoclave Protocol' and use the wet settings.*
Plating the DH5α *E. coli* Cells	
Step 8	Plate the DH5α *E. coli* cells onto LB agar plates using a streaking technique and incubate them overnight at 37℃ (for approximately 16 hours).
Colony Selection and Culture	
Step 9	Add 125μL of 2M $MgCl_2$ solution to 25mL of the SOB medium in a newly autoclaved conical flask.
Step 10	Select 2 colonies from the plated DH5α *E. coli* cells using pipette tips and add them (with the tip) to the new SOB mixture. Select colonies that are completely separate from all other colonies. *Note: The plates containing* the DH5α *E. coli* cell colonies can be stored in the refrigerator at 4℃.
Step 11	Place the SOB mixture with selected colonies in the shaking incubator at 37℃ and 200rpm for approximately 30 minutes. *Note: While waiting it is advisable to make up Transformation Buffer if it is not already prepared. See below for instructions on how to make Transformation Buffer.*
Step 12	Increase the volume of the colony and SOB mixture to 250mL by adding 225mL of SOB and 1.1mL of 2M $MgCl_2$ solution.
Step 13	Return the colony and SOB mixture to the shaking incubator at 37℃ and 200rpm.
Step 14	Measure the optical density of the culture using the NanoDrop spectrophotometer at appropriate time intervals (approximately every 10 minutes). *Note: For more detailed instructions on this procedure and how to operate the NanoDrop see 'A4 Measuring OD Protocol'.*
Step 15	Once the absorbance is close to 0.6, remove the culture from the shaking incubator and place it in an ice bath for 10 minutes.
Step 16	Centrifuge the cells in an autoclaved GSA bottle at 4000rpm at 4℃ for 10 minutes. When this is completed discard the supernatant. This should leave a gel-like solid (pellet) at the bottom of the bottle.
Step 17	Resuspend the pellet in 80mL of ice-cold transformation buffer and place on an ice bath for 10 minutes.
Step 18	Centrifuge the resuspended pellet at 4000rpm at 4℃ for 10 minutes and once again, discard the supernatant.
Step 19	Gently resuspend the pellet in 20mL of transformation buffer and add in dimethyl sulfoxide (DMSO) to a final concentration of 7%. Place the mixture on an ice bath for 10 minutes.
Step 20	Aliquot approximately 200μL volumes of the mixture into autoclaved microtubes and

	immediately drop them in liquid nitrogen or dry ice (if available). The now competent DH5α *E. coli* cells can by stored in the -80℃ freezer.
Transformation Buffer	
Step 1	Combine the following substances to a clean beaker: o 0.6g HEPES o 055g $CaCl_2.2H_2O$ o 4.66g KCl
Step 2	Make the final volume up to 250mL using Mill-Q water.
Step 3	Adjust the pH of the buffer to 7 using the pH meter and acid or base.
Step 4	After adjusting the pH, add $MnCl_2.4H_2O$. Filter and store the buffer in the cold room at 4℃.
Version history	The original version is an edited form of the procedure performed by Gayle and Sean on 07.01.14.