Aliquoting and Testing of Soluble CRE Recombinase Enzyme

1.0 Equipment

1.1 Embryo handling device

1.2 Incubator (37°C, 5% CO_2)

1.3 Safety glasses

1.4 Cyclone Vortex Mixer

1.5 Ice bucket

1.6 Gilson pipettes (P20, P200)

1.7 Falcon tube rack

1.8 Powerpette

1.9 Countdown timer

1.10 Microscope

1.11 Heated stage

1.12 96-well cold block (PCR cooler)

1.13 Empty 200µl tip rack box

2.0 Supplies

2.1 Cre Recombinase enzyme, TAT-Cre

2.2 DMEM

2.3 KSOM

2.4 Silicone fluid

2.5 hTF

2.6 Embryo handling device tips

2.7 10ml serological pipettes

2.8 Ice

2.9 Autoclaved 0.5ml PCR tubes

2.10 Embryo handling device

2.11 1.5ml Eppendorf tube

2.12 14ml falcon tube

2.13 200µl pipette tips

2.14 20µl pipette tips

2.15 35:3004 embryo culture dishes

2.16 Silicone fluid

2.17 Marker Pen

3.0 Procedure

3.1 General Information

3.1.1 The Cre recominase is shipped on dry ice.

3.1.2 The vials of Cre recombinase can then be stored in a -80°C freezer.

3.1.3 The Cre recombinase stock solution is prepared and shipped at varying concentrations. Refer to the Cre dilution table in Appendix 1 when preparing working concentrations from the stock solution.

3.1.4 It is important to keep the Cre enzyme cold, on wet ice, whilst being transported between rooms or when in use on the bench.

3.1.5 Aim to use a homozygous embryo stock to test the efficacy of varying CRE concentrations.

3.1.6 The allele conversion from tm1a to tm1b can be determined via blastocyst genotyping.

3.1.7 Prepare embryo culture dishes with KSOM media. Quantity of culture dishes required to be determined by the number of Cre concentrations to be tested.

3.1.8 Aim to use 30-40 embryos per Cre test culture dish.

3.1.9 Thaw embryos, then pool them altogether and place into a KSOM culture dish and incubate until ready to use.

3.2 Aliquoting the Cre recombinase

3.2.1 Fill an ice bucket with ice and take to the -80°C freezer. Remove Cre vials from the -80°C freezer and place into the wet ice.

3.2.2 In the lab, stand a 1.5ml Eppendorf in the wet ice. Using a 200µl pipette, gently pipette the Cre enzyme up and down inside the container vial, 3-4 times. Aspirate all the Cre recombinase solution and expel into the 1.5ml Eppendorf tube. Repeat this step for all vials **PROVIDED THEY ARE THE SAME CONCENTRATION.** Do not mix concentrations of Cre.

3.2.3 Fill the 96-well cold block (PCR cooler), kept in the -80^{0}C freezer, with empty autoclaved 0.5ml PCR tubes.

3.2.4 Once the contents from all vials of the same concentration have been placed in the 1.5ml Eppendorf, vortex the pooled Cre for 5 seconds and place the Eppendorf back into the ice to maintain temperature.

3.2.5 Pipette 5µl aliquots of the pooled Cre enzyme into each 0.5ml PCR tube. Keep the tubes in the cold block during this process.

3.2.6 Take the cold block holding the 5µl aliquots to the -80°C freezer. Working in the freezer, transfer the 0.5ml PCR tubes into an empty 200µl tip rack box.

3.2.7 Label the lid of the tip rack box with 'Cre recombinase', 'aliquoted date', 'concentration (mg)' and 'lot. no.'.

3.3 Testing the Cre concentrations

3.3.1 Prepare 3x Cre treatment dishes of varying concentrations (0.6µM, 1.2µM & 1.8µM) per concentration of Cre (e.g 4.5mg, 10mg, 15mg).

Refer to the Cre dilution table in Appendix 1 to ascertain how to prepare these working concentrations from the aliquoted Cre solution.

3.3.2 Label each dish; 'Cre', original concentration of Cre (e.g 15mg), diluted concentration (e.g. 1.2uM), strain of embryos and the date.

Ensure the DMEM media is used within the expiry date and within 3 months of opening.

3.3.3 Place the Cre treatment dishes in the incubator for 20mins to equilibrate, then transfer the embryos from the KSOM culture dish to the Cre dishes in order to perform the Cre treatment. Ensure each dish has approx. the same quantity of embryos.

3.3.4 Once the Cre treatment is completed, transfer embryos to a corresponding KSOM culture dish prepared in step 3.8 for extended culture.

3.3.5 Label each dish; 'KSOM', Cre original concentration (e.g 15mg), diluted concentration (e.g. 1.2µM), strain of embryos and the date.

3.3.6 Once the embryos have been cultured over 3-4 days and have reached the morula/blastocyst stage of development, prepare a 96-well cell culture plate for blastocyst genotyping.

3.3.7 Once the cell culture plate has equilibrated in the incubator, plate embryos from each experimental group according.

3.3.8 After 7 days of incubation in 37°C and 5% CO_2, prepare the cell culture plate for genotyping.

3.3.9 Send the plate out for genotyping.

3.3.10 A positive genotype confirmation for each working Cre concentration will verify its efficacy to carry out site-specific recombination events and a particular working concentration of Cre can be determined for use in future Cre treatment.

4.0 Appendix 1

15mg/ml Cre Dilution Table		
Required Concentration	Cre Vol.	DMEM Vol.
0.3µM	1µl	1936µl
0.6µM	1µl	581µl
1.2µM	2µl	580µl
1.8µM	3µl	579µl
2.4µM	4µl	576µl
3µM	5µl	575µl

10mg/ml Cre Dilution Table		
Required Concentration	Cre Vol.	DMEM Vol.
0.3µM	1µl	774µl
0.6µM	2µl	773µl
1.2µM	4µl	772µl
1.8µM	4µl	512µl
2.4µM	4µl	384µl
3µM	4µl	306µl

6mg/ml Cre Dilution Table		
Required Concentration	Cre Vol.	DMEM Vol.
0.3µM	1µl	463µl
0.6µM	2µl	463µl
1.2µM	4µl	464µl
1.8µM	4µl	309µl
2.4µM	4µl	232µl

4.5mg/ml Cre Dilution Table		
Required Concentration	Cre Vol.	DMEM Vol.
0.3µM	3µl	1044µl
0.6µM	3µl	520µl
1.2µM	3µl	259µl
1.8µM	6µl	343µl
2.4µM	6µl	256µl
3µM	6µl	203µl

2mg/ml Cre Dilution Table		
Required Concentration	Cre Vol.	DMEM Vol.
0.3µM	3µl	462µl
0.6µM	6µl	459µl
1.2µM	9µl	340µl
1.8µM	12µl	298µl
2.4µM	12µl	221µl
3µM	12µl	174µl