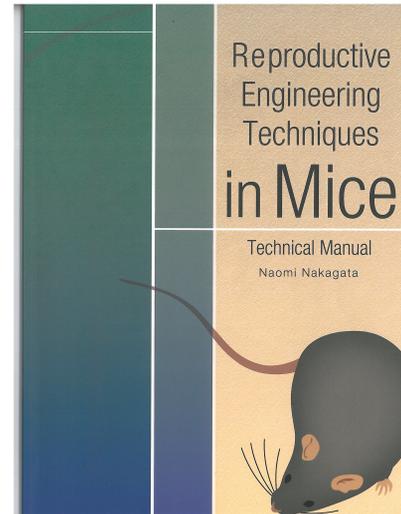




# Reproductive Engineering Techniques in Mice



Technical Manual

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# Preface

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In recent years, the number of genetically engineered mice being produced has increased dramatically. Moreover, the rapid progress in the development of genome-editing (TALEN and CRISPR/Cas9) techniques for molecular biology research has been remarkable, so much so that a genetically engineered mouse strain can be produced easily in a few months. Production has been supported by reproductive engineering techniques such as *in vitro* fertilization, embryo and sperm cryopreservation, and embryo transfer techniques. Such techniques have become invaluable peripheral technologies, and their use is expanding rapidly.

This rapid expansion has led to the publication of many technical manuals relating to reproductive engineering techniques in mice hereto. However, mouse reproductive engineering techniques mainly involve delicate operations under a stereoscopic microscope, which means that a sufficiently detailed technical manual has not yet been published.

With that in mind, in this book we aim to create a mouse reproductive engineering technique manual that can be easily understood by anyone. We included a generous number of diagrams, photographs and videos in our manual, and explained each step of each reproductive engineering technique as clearly and thoroughly as we could. We sincerely hope that our manual will become the definitive guide for students, technicians, researchers and other people wishing to study mouse reproductive engineering techniques.

Naomi Nakagata

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\*  Please see page 90 for details.

## 8-1 Storage of Media and Solutions in Ampoules Under Nitrogen Gas

### Materials and Equipment

1. Twin jet ampoule sealer (Adelphi Manufacturing, West Sussex, UK)
2. Ampoule (sterilized via hot air sterilization (180°C, 3 hours))
3. Medium
4. Syringe and 18 gauge needle
5. Forceps
6. Nitrogen gas

### Procedures

#### Cleaning and Sterilizing Glass Ampoules

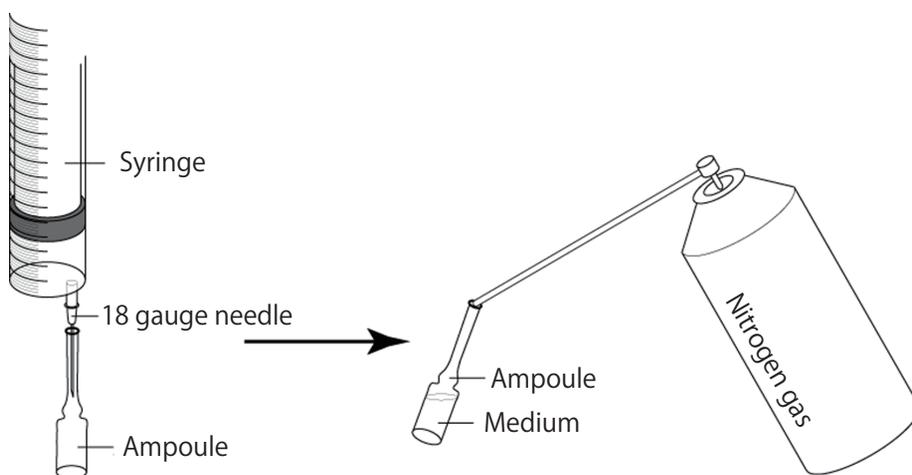
1. Rinse the glass ampoules one time using tap water.
2. Rinse the ampoules 2 times using distilled water.
3. Heat sterilize the ampoules at 180°C for at least 3 hours.

#### Ignition

1. Open the gas cock and ignite the twin jet ampoule sealer.
2. Adjust the flames of the twin jet ampoule sealer so that the flame burns blue.

#### Sealing Ampoules

1. Add an appropriate amount of medium to each ampoule.
2. Introduce nitrogen gas into one of the ampoules and immediately seal the tip of the ampoule using the flames of the twin jet ampoule sealer. Repeat for all the remaining ampoules.



[Filling Ampoules with Medium] No. 20-01 

## 8-2 Table of Media Composition

### mHTF

#### Composition of mHTF

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	593.8	Sigma	S 5886
KCl	35.0	Sigma	P 5405
MgSO <sub>4</sub> • 7H <sub>2</sub> O	4.9	Sigma	M 2773
KH <sub>2</sub> PO <sub>4</sub>	5.4	Sigma	P 5655
CaCl <sub>2</sub>	57.0	Sigma	C 5670
NaHCO <sub>3</sub>	210.0	Sigma	S 5761
Glucose	50.0	Sigma	G 6152
Na-lactate**	0.34 mL	Sigma	L 7900
Na-Pyruvate	3.7	Sigma	P 4562
Penicillin G	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
<b>BSA</b> (Albumin, Bovine serum Fraction V Fatty Acid-Free)	<b>400</b>	<b>MERCK/ CALBIOCEM</b>	<b>126575</b>
<b>0.5% phenol red</b>	<b>0.04 mL</b>	<b>Sigma</b>	<b>P 0290</b>

\*Water for embryo transfer; Sigma W1503

\*\*Assay; 70%

mHTF is enclosed in brown ampoules and stored at 4°C.

### References

1. Kito S., Hayao T., Noguchi-Kawasaki Y., Ohta Y., Hideki U., and Tateno S. 2004. Improved *in vitro* fertilization and development by use of modified human tubal fluid and applicability of pronucleate embryos for cryopreservation by rapid freezing in inbred mice. *Comp. Med.* 54(5): 564-570.

## Hyaluronidase

### Composition of Hyaluronidase

Prepare 1% solution stock as indicated below. Filter-sterilize and store in 100  $\mu\text{L}$  aliquots at  $-20^{\circ}\text{C}$ . Before use, dilute the stock by 10 times. ex.) Add 20  $\mu\text{L}$  stock solution (1%) to a 200  $\mu\text{L}$  drop of mHTF containing oocytes to make a diluted solution of about 0.1%.

Reagent	mg*	Vendor	Catalog Number
Hyaluronidase	10	Sigma	H 3506

\*mg/mL in mHTF

**0.3 M Sucrose (BSA -)****Composition of 0.3 M sucrose (BSA-)**

Reagent	mg*	Vendor	Catalog Number
Sucrose	2053.8	Sigma	S 1888

\*mg/20 mL in PB1

**Composition of PB1(BSA-)**

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277

\*Water for embryo transfer; Sigma W1503

0.3 M Sucrose (BSA-) is enclosed in brown ampoules and stored at 4°C.

**0.3 M Sucrose (BSA +)****Composition of 0.3 M sucrose (BSA+)**

Reagent	mg*	Vendor	Catalog Number
Sucrose	2053.8	Sigma	S 1888

\*mg/20 mL in PB1

**Composition of PB1(BSA+)**

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503

0.3 M Sucrose (BSA+) is enclosed in brown ampoules and stored at 4°C.

**KSOM/AA****Composition of KSOM/AA**

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	555.0	Sigma	S 5886
KCl	18.5	Sigma	P 5405
KH <sub>2</sub> PO <sub>4</sub>	4.75	Sigma	P 5655
MgSO <sub>4</sub> · 7H <sub>2</sub> O	4.95	Sigma	M 2773
CaCl <sub>2</sub> · 2H <sub>2</sub> O	25.0	Sigma	C 7902
NaHCO <sub>3</sub>	210.0	Sigma	S 5761
Glucose	3.6	Sigma	G 6152
Na-Pyruvate	2.2	Sigma	P 4562
DL-Lactic Acid sodium salt	0.174 mL	Sigma	L 1375
10 mM EDTA	100 µL	Sigma	E 6635
Streptomycin	5.0	Sigma	S 9137
Penicillin	6.3	Sigma	P 7794
0.5% phenol red	0.1 mL	Sigma	P 0290
L-Glutamine	14.6	Sigma	G 8540
MEM Essential Amino Acids solution	1.0 mL	GIBCO	11130-051
MEM Non-essential Amino acid solution	0.5 mL	Sigma	M 7145
BSA	100.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503

KSOM/AA is enclosed in brown ampoules and stored at 4°C.

## References

1. Lawitts J. A., and Biggers J. D. 1993. Culture of preimplantation embryos. *Methods Enzymol.* 225:153-164.

## 0.8 M Sucrose

### Composition of 0.8 M sucrose

Reagent	mg*	Vendor	Catalog Number
Sucrose	5476.8	Sigma	S 1888

\*mg/20 mL in PB1

### Composition of PB1

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503

0.8 M Sucrose is enclosed in brown ampoules and stored at 4°C.

**PB1****Composition of PB1**

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503  
PB1 is enclosed in brown ampoules and stored at 4°C.

## 1 M DMSO

## Composition of 1 M DMSO

Reagent	mL*	Vendor	Catalog Number
DMSO	1.56	Sigma	D 2650
PB1	18.44	-	-

\*Final volume: 20 mL

## Composition of PB1

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503

1 M DMSO is enclosed in brown ampoules and stored at 4°C.

## DAP213

## Method of preparing DAP213

1. Solution A and Solution B are first prepared and are each fully dissolved.
2. Equal volumes of A and B are then mixed to form DAP213.

## Solution A

Reagent	mL*	Vendor	Catalog Number
PB1	2.3088	-	-
DMSO	3.1252	Sigma	D 2650
Propylene glycol (PG)	4.556	Sigma	134368

## Caution

The solution may become cloudy when DMSO is added.

## Solution B

Reagent	mg*	Vendor	Catalog Number
Acetamide (AA)	1181.4	Sigma	A 0500

\*mg/10 mL in PB1

## Composition of PB1

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503  
DAP213 is enclosed in brown ampoules and stored at 4°C.

## 0.25 M Sucrose

### Composition of 0.25 M sucrose

Reagent	mg*	Vendor	Catalog Number
Sucrose	1711.5	Sigma	S 1888

\*mg/20 mL in PB1

### Composition of PB1

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	800.0	Sigma	S 5886
KCl	20.0	Sigma	P 5405
CaCl <sub>2</sub>	12.0	Sigma	C 5670
KH <sub>2</sub> PO <sub>4</sub>	20.0	Sigma	P 5655
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10.0	Sigma	M 2393
Na <sub>2</sub> HPO <sub>4</sub>	115.0	Sigma	S 5136
Na-Pyruvate	3.6	Sigma	P 4562
Glucose	100.0	Sigma	G 6152
Penicillin	7.5	Sigma	P 7794
Streptomycin	5.0	Sigma	S 1277
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503

0.25 M Sucrose is enclosed in brown ampoules and stored at 4°C.

**mWM****Composition of mWM**

Reagent	mg/100 mL*	Vendor	Catalog Number
NaCl	640.0	Sigma	S 5886
KCl	35.6	Sigma	P 5405
KH <sub>2</sub> PO <sub>4</sub>	16.2	Sigma	P 5655
MgSO <sub>4</sub> • 7H <sub>2</sub> O	29.4	Sigma	M 7774
NaHCO <sub>3</sub>	190.0	Sigma	S 5761
Glucose	100.0	Sigma	G 6152
Na-Pyruvate	2.5	Sigma	P 4562
Ca-lactate pentahydrate	46.0	Sigma	C 8356
Streptomycin	5.0	Sigma	S 1277
Penicillin G	7.5	Sigma	P 7794
0.5% phenol red	0.2 mL	Sigma	P 0290
20 mM 2-ME	10.0 µL	Sigma	M 7522
100 mM EDTA	50.0 µL	Sigma	E 6635
BSA	300.0	Sigma	A 4378

\*Water for embryo transfer; Sigma W1503  
mWM is enclosed in brown ampoules and stored at 4°C.