

## Sperm swim-out

(reagents and buffers listed at end of protocol)

### Protocol

1. Prepare Donner medium.
2. Set up for swim-out and sperm prep:
  1. 6-cm culture dishes with 5 ml Donner's medium each (1 dish per mouse)
  2. squares of parafilm (1 per mouse)
  3. Beaker of ddH<sub>2</sub>O and dissection tools
3. Sac mouse (or mice). Remove testes and epididymes.
  1. Put 1 testis in Bouin's fixative for histology
  2. Snap freeze the other testis in liquid nitrogen
  3. Both epididymes in PBS on ice (in 3-cm culture dish)
4. Separate caput, corpus, and cauda epididymis. Snap freeze both caputs. Discard corpus.
5. Move caudas to parafilm (if doing multiple mice, do one mouse at a time on separate pieces of parafilm). Cut 3-5 slices in each cauda and transfer to a petri dish containing Donner's medium, rinsing instruments between each mouse.
6. Swirl dishes a few times and place at 37C, shaking lightly (or come by and swirl ~ every 10 min). Incubate at 37C for 1 hr.
7. Filter sperm solutions through a 40 µm strainer into a 50 ml Falcon. Can pool sperm from different mice at this point.
8. Spin 2500xg, 8 min, 4C.
9. Remove supernatant, resuspend in 1 ml cold sterile PBS, transfer to a 2 ml eppendorf.
10. Wash out the Falcon with another 500-1000 µl cold sterile PBS and add the wash to the 2 ml eppendorf.
11. Spin 2500xg, 8 min, 4C.
12. Remove supernatant. Resuspend in 1 ml cold sterile PBS. \*If bloody, use 0.45% NaCl instead and repeat washing until blood is gone. Usually needs 1x wash in 0.45% NaCl. Resuspend in 1 ml PBS.
13. Prepare 2 separate 1:20 dilutions in ddH<sub>2</sub>O for counting. Count on hemocytometer. For a 1:20 dilution,  $C = (\text{total sperm \#}) / (\text{total row \# counted}) \times 10^6 \text{ sperm/ml}$ .
14. Spin 2500xg, 8 min, 4C. Resuspend in required volume of PBS.

## **Solutions and reagents**

0.45% NaCl in ddH<sub>2</sub>O

### Donner stock:

135 mM NaCl	13.5 ml, 5M stock
5 mM KCl	2.5 ml, 1M stock
1 mM MgSO <sub>4</sub>	500 ul, 1M stock
2 mM CaCl <sub>2</sub>	1 ml, 1M stock
30 mM HEPES	15 ml, 1M stock (pH 7.5)
	467.5 ml H <sub>2</sub> O

### Donner medium (make fresh):

(for 50 ml)

2.65 ml 60% lactate syrup
50 ul 1M sodium pyruvate
1 g BSA
1.25 ml 1M NaHCO <sub>3</sub>
46.05 ml Donner stock

## **References**

Siklenka K, Erkek S, Godmann M, et al. Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science*. 2015;350(6261):aab2006.

Lesch BJ, Tothova Z, Morgan EA, et al. Intergenerational epigenetic inheritance of cancer susceptibility in mammals. *Elife*. 2019;8:e39380. Published 2019 Apr 9.