# Generation of mFwe knockout n2a cells from Yao CK et al. (2017)

**Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu**

## Abstract

This protocol describes the generation of mFwe Knockout n2a cells. It is from 'Flower $Ca^{2+}$ channel in CME and ADBE' of Yao CK et al.

Please see the manuscript for the full method details.

**Citation:** Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu Generation of mFwe knockout n2a cells from Yao CK et al. (2017). **protocols.io**
dx.doi.org/10.17504/protocols.io.hgwb3xe
**Published:** 04 Apr 2017

## Materials

pSpCas9(BB)-m/ratFwe-gRNA-2A-GFP plasmid by Lopofectamine 3000 by Thermo Fisher Scientific

- Trypsin by Contributed by users
- 10% FBS/1XHBSS solution by Contributed by users

## Protocol

### Step 1.

Transfect 3 x $10^5$ n2a cells cultured on 6-well plate with 2 µg of pSpCas9(BB)-m/ratFwe-gRNA-2A-GFP plasmid by Lopofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's instructions.

AMOUNT
2 µl Additional info:

### Step 2.

Culture the cells for three days.

DURATION
12:00:00

### Step 3.

Detach the cells by trypsin treatment.

**Step 4.**

Add 10% FBS/1XHBSS solution to stop trypsin activity.

**Step 5.**

Collect the cells by centrifugation.

**Step 6.**

Resuspend the cells in 10% FBS/1XHBSS solution.

**Step 7.**

Adjust the cell density to 1 x $10^6$ per ml.

**Step 8.**

Filter the cells through 40 µm-Teflon mesh to eliminate aggregation.

**Step 9.**

Sort GFP-positive cells out using flow cytometry, and plate the cells in 96-well plate filled with 10%FBS/MEM solution in which each well approximately includes one cell.

**Step 10.**

After three-week culture, subject survival single cell-driven colonies to immunostaining and immunoblotting for mFwe2 to verify the knockout of mFwe2.

NOTES

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One of confirmed mFwe knockout n2a cell lines was used in S9A Fig.