VIROMERS

Obtaining blastocysts:

Allocate four days before beginning viromer experiments to obtaining blastocysts. Superovulate 7-week-old female mice according to protocol (see: *Superovulation* protocol). Perform dissection, flushing, and embryo culture according to protocol (see: "Dissection and flushing" section of *Blastocyst Outgrowth* protocol). Collect blastocysts for viromer experiments at late blastocyst stage after 3 days of culture.

Important notes for obtaining blastocysts:

Place embryos into the incubator AS SOON AS POSSIBLE after collection.

Embryos should not remain in MEM-HEPES collection medium for more than 30 minutes. Embryos in MEM-HEPES should be kept on a heated 37°C microscope stage.

Do not keep embryos out of the incubator for more than 10 minutes.

Use a new micropipette each time you collect embryos after culturing.

Required materials for viromer experiments:

2 µl viromers stock solution

1 µl siRNA (200 µM)

KSOM medium, pre-equilibrated by incubating overnight at 37°C/5% CO_2

(8) 60x15 mm centre-well organ culture dishes (catalog number: 353037)

(4) 0.6 ml microcentrifuge tubes

Viromers:

1. Prepare 1:100 dilution of viromers stock solution. Dilute 2 µl viromers stock solution in 198 µl KSOM medium. Label this Solution A. (Note: Mix all solutions thoroughly but gently by pipetting up-and-down, avoiding air bubbles.)

2. Prepare 10 µM working solution of siRNA. Dilute 1 µl siRNA (200 µM) in 19 µl of KSOM medium. Label this Solution B.

3. Combine 180 µl Solution A with 20 µl Solution B. Final concentration of siRNA is 1 µM.

4. Incubate the A:B mixture for 10 minutes at room temperature.

5. Add the A:B mixture to pre-equilibrated KSOM in the following proportions:

Volume of A:B mixture (µl)	Volume of KSOM (µl)	Total volume (µl)	Final siRNA concentration	Final viromers dilution
80	320	400	200 nM	1:500
40	360	400	100 nM	1:1000
20	380	400	50 nM	1:2000
0	400	400	0 nM	0

6. Transfer the entire volume (400 µl) of each dilution to separate culture dishes.

7. Add 20 late blastocysts to each culture dish.

8. Incubate blastocysts for 4 hours at 37°C in 5% CO_2 in air.

9. After 4 hours, remove the dishes from the incubator and wash blastocysts two times by changing KSOM medium in dish.

10. Transfer each of the four groups of blastocysts to new culture dishes containing fresh pre-equilibrated KSOM medium.

11. Incubate groups of blastocysts overnight at 37°C in 5% CO_2 in air.

12. The next day, collect each group of blastocysts in a 0.6 ml microcentrifuge tube.

13. Assess success of RNAi by using PCR (regular or quantitative PCR) to detect the target mRNA and a control mRNA (e.g. actin).