Supplementary Guide for:

Genetically tractable embryonic cell lines from sea urchins

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Filename: Supplementary Information
Description: This file contains Supplementary Methods and Supplementary Figures 1-11.

Filename: Supplementary Table 1
Description: Growth and viability of embryonic cells derived from *L. variegatus* blastula from the first mating pair in different growth media (M1, SOM, and UM with different concentrations of FBS), percentage of red cells, and growth of size fractionated cultures that separated embryoid bodies from individual cells at day 152 in culture.

Filename: Supplementary Table 2
Description: Single-cell RNA sequencing of *L. variegatus* embryonic spheroids in Urchin Media (UM) with 3% FBS. The "Marker Gene Expression" tab shows the average expression of each gene in each cluster. The "All Clusters Diff Expression" tab shows the differential gene expression in each cluster compared to all of the other clusters (log2 fold change).

Filename: Supplementary Table 3
Description: Genetic validation of *L. variegatus* embryonic cell cultures from the first and second mating pairs, *L. variegatus* ovary cell cultures, and *S. purpuratus* embryonic cell cultures identified by 18S targeted amplicon sequencing.

Filename: Supplementary Table 4
Description: Growth and viability of embryonic cells derived from *L. variegatus* blastula from the second mating pair in UM with 3%, 5%, 10%, 15% FBS, and percentage of red cells.

Filename: Supplementary Table 5
Description: Cryopreservation of *L. variegatus* cultured cells using a variety of different cryopreservation conditions (slow freezing, vitrification, and different cryoprotective agents).

Filename: Supplementary Table 6
Description: Single-cell RNA sequencing of *L. variegatus* embryonic cultures in UM with 5%, 10%, 15% FBS. The "Marker Gene Expression" tab shows the average expression of each gene in each cluster. The "All Clusters Diff Expression" tab shows the differential gene expression in each cluster compared to all of the other clusters (log2 fold change).

Filename: Supplementary Table 7
Description: Transcriptional changes in the *L. variegatus* embryonic cell cultures assessed by bulk RNA sequencing on Day 20, 182, 313, 445, and 738 in UM with 5%, 10%, 15% FBS. Transcripts per million (TPM) for all genes with detectable expression, and TPM for selected marker genes.

Filename: Supplementary Table 8
Description: Transfection conditions tested with *L. variegatus* embryonic cell cultures. Lipofectamine, PEI, electroporation, and nucleofection were tested for their ability to transfect *L. variegatus* embryonic cell lines using transcribed mRNA encoding red fluorescence protein (RFP), a plasmid encoding cerulean fluorescent protein (CFP), and fluorescent-labeled dextran (tetramethylrhodamine dextran) as reporters.

Filename: Supplementary Table 9
Description: Codon usage analysis of the *L. variegatus* exome used to create a codon-optimized dual reporter lentiviral vector.

Filename: Supplementary Table 10
Description: Growth and viability of *L. variegatus* adult cell cultures derived from ovary (n=7), Aristotle's lantern muscle (n=4), coelomocytes (n=3), radial nerve cord (n=2), tube feet (n=1), spines (n=1).

Filename: Supplementary Table 11
Description: Growth and viability of embryonic cells derived from *S. purpuratus* gastrula, cultured in Urchin Media (UM) with different concentrations of FBS (5%, 10% or 15%) at 17℃. Growth and viability of *S. purpuratus* embryonic cells cultured at room temperature (22℃) after Day 51 in culture.

Filename: Supplementary Video 1
Description: *L variegatus* embryoid body contracting at Day 47 in culture.

Filename: Supplementary Video 2
Description: *L variegatus* embryoid body contracting at Day 106 in culture.

Filename: Supplementary Video 3
Description: *L. variegatus* embryoid body contracting at Day 422 in culture.

Filename: Supplementary Video 4
Description: *L. variegatus* attached embryonic cells contracting at Day 32 in culture.

Filename: Supplementary Video 5
Description: *L variegatus* embryoid body swimming aided by cilia at Day 52 in culture.

Filename: Supplementary Video 6
Description: *L variegatus* embryoid body swimming aided by cilia at Day 78 in culture.

Filename: Supplementary Video 7
Description: *S. purpuratus* spheroid with active cilia on the surface at Day 47 in culture.

Filename: Supplementary Video 8
Description: *S. purpuratus* embryoid body contracting at Day 12 in culture.