

Supplementary information

Haploid embryonic stem cells can be enriched and maintained by simple filtration

Chao Qu^{1,3}, Meng Yan^{1,3}, Suming Yang¹, Lingbo Wang¹, Qi Yin¹, Yuan Liu², Yeguang Chen², Jinsong Li^{1*}

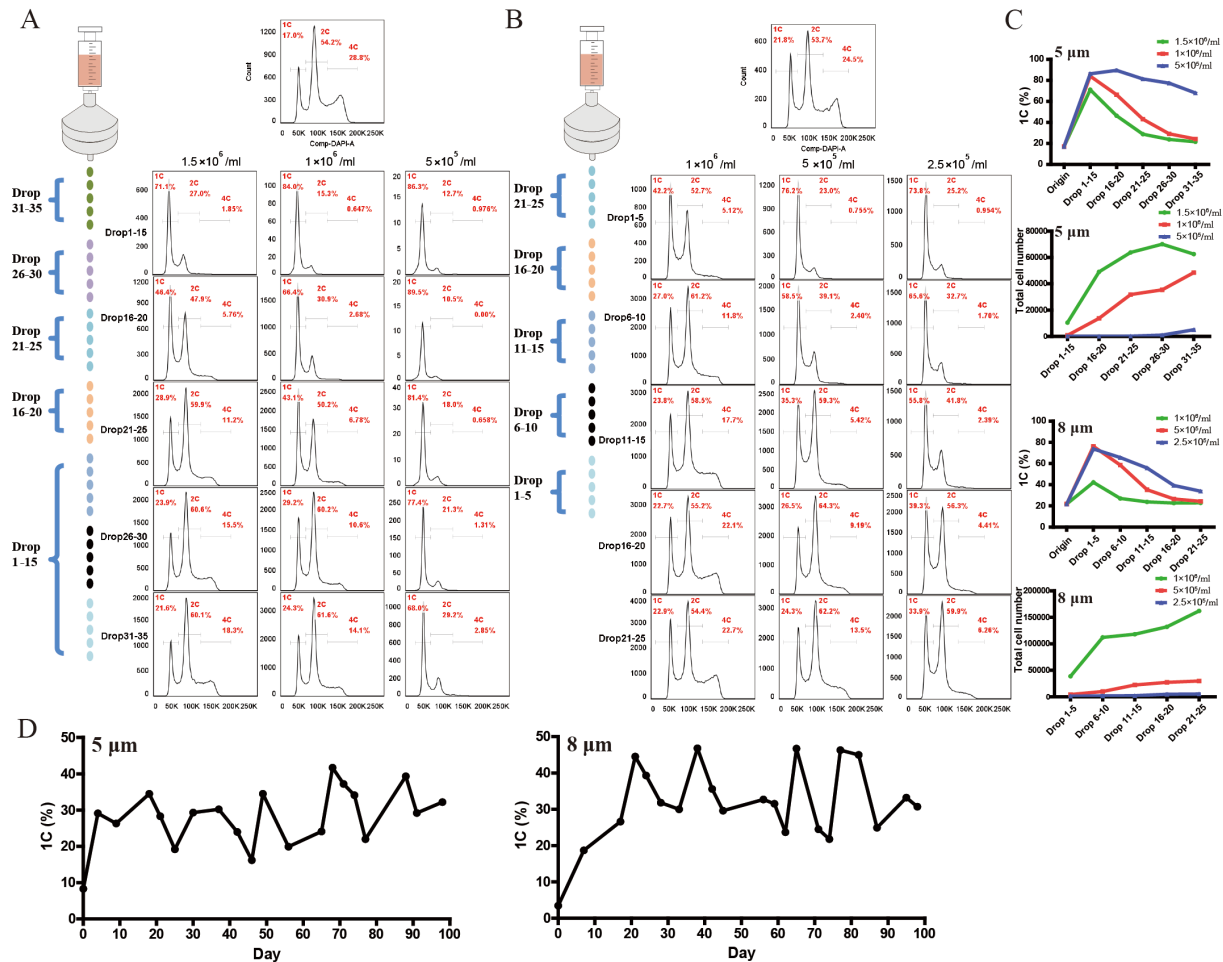


Figure S1. Optimizing the filtration conditions for long-term maintenance of mouse haESCs. (A) Diagram of filtrates collection strategy when using filters with 5 μm membranes (left) and results of 1C, 2C and 4C ratios in filtrates of different drops (right). Cell suspensions with concentrations of $1.5 \times 10^6/\text{ml}$, $1 \times 10^6/\text{ml}$ and $5 \times 10^5/\text{ml}$ were shown here. (B) Diagram of filtrates collection strategy when using filters with 8 μm membranes (left) and results of 1C, 2C and 4C ratios in filtrates of different drops (right). Cell suspensions with concentrations of $1 \times 10^6/\text{ml}$, $5 \times 10^5/\text{ml}$ and $2.5 \times 10^5/\text{ml}$ were shown here. (C) The concentrations of cell suspensions and pore sizes of membranes affected the distribution of 1C ratio and cell numbers in filtered drops. (D) 5 μm and 8 μm pore size membranes.

(D) Filters with 5 μ m (left) and 8 μ m (right) membranes could be used to long-term maintain the haploidy of another cell line, *H19 Δ ^{DMR}-IG Δ ^{DMR}-AGH-OG3*.

Table S1. Summary of gene editing in *H19 Δ ^{DMR}-IG Δ ^{DMR}-AGH* cells enriched by filtration.

Targeted gene	Cell enrichment method	Transfection efficiency (%)	Established cell lines	Cell lines with expected mutation (%)	Cell lines with other mutations (%)	WT cell lines (%)
<i>Iqgap2</i>	FACS	7.69	22	3 (13.64%)	15 (68.18)	4 (18.18)
<i>Iqgap2</i>	5 μ m filtered	6.15	21	4 (19.05)	15 (71.43)	2 (9.52)
<i>Crygc</i>	5 μ m filtered	ND ^a	28	8 (28.57)	12 (42.86)	8 (28.57)
<i>Crygc</i>	8 μ m filtered	ND ^a	29	6 (20.69)	14 (48.28)	9 (31.03)

^a not determined

Table S2. Primer information

Sequence Name	Sequence (5'-3')	Application
<i>Iqgap2</i> sgRNA-1 F	CACCGAGGTCACATCAGATTACATC	Targeting exon 17 of <i>Iqgap2</i>
<i>Iqgap2</i> sgRNA-1 R	AAACGATGTAATCTGATGTGACCTC	
<i>Iqgap2</i> sgRNA-2 F	CACCGACGTCATACTTGCTTGTC	Targeting exon 30 of <i>Iqgap2</i>
<i>Iqgap2</i> sgRNA-2 R	AAACTGACAAGCAAGTATGACGTC	
<i>Iqgap2</i> F	TTGGCTGCTGTTAAATCTGTC	Genotyping of 35kb deletion
<i>Iqgap2</i> R	TCATCTGCTGGTTTGATGGA	
<i>Iqgap2</i> -exon17 F	GCTGTCCCTTTCATTTCCCTAA	Genotyping of mutations in exon 17
<i>Iqgap2</i> -exon17 R	GGTAGTTGATGGTGCTGTCTTC	
<i>Iqgap2</i> -exon30 F	GAGGCCCGTAAATCTCCCA	Genotyping of mutations in exon 30
<i>Iqgap2</i> -exon30 R	GCAGACATGGACCATTAGCC	
<i>Crygc</i> sgRNA F	CACCGTCAAGAGTACCGGCGCTTCC	Targeting <i>Crygc</i>
<i>Crygc</i> sgRNA R	AAACGGAAGCGCCGGTACTCTTGAC	
<i>Crygc</i> /HDRP/1 F	CCCGGGATCCTCTAGAGATTAGGTAGTTGG GAATGGGAGAGTAAT	Amplification of left arm from genome
<i>Crygc</i> /HDRP/1 R	GTCCTGGAAGGCCGGTACTCTTGAGGCC	
<i>Crygc</i> /HDRP/2 F	GAGTACCGGCCTTCCAGGACTGGGGCTCT	Amplification of right arm from genome
<i>Crygc</i> /HDRP/2 R	CATGCCTGCAGGTCGACGATTATCTCTCCAC GGTCTGCTCT	
<i>Crygc</i> F	TCGACCACACTCACCCATTG	Genotyping of mutations of <i>Crygc</i>
<i>Crygc</i> R	ACAGAAGTCAGGGTTTGCCA	

