



Supplementary Fig. 1. Feasibility of DTS and PTS cryomedia on post-thaw recovery and viability of primordial germ cells (PGCs). (A) Morphology of a PGC line (W4) without cryopreservation (unfrozen control; left) and after being frozen in DTS (middle) or PTS (bottom) cryomedia. Black arrowheads indicate ruptured PGCs. Scale bars indicate 25 μm . (B–D) Recovery rates of total cells, including (B) dead cells and (C) viable cells, and (D) the viability of recovered cells after the cryopreservation of a PGC line (W4) in CELLBANKER[®] 1 (CB1), DTS, or PTS. Significance was calculated vs. CB1 ([†] $P > 0.05$) using the Wilcoxon test. (E–J) The viability and recovery rates of three PGC lines (W4, W19, W2) and freshly isolated PGCs from blood (naive PGCs) after cryopreservation in DTS or PTS cryomedia were analyzed. Recovery rates of total cells, including (E and H) dead cells and (F and I) viable cells after thawing. (D and G) Viability of cells recovered after thawing. Significance was calculated vs. W4 in each cryomedia (DTS: [#] $P > 0.05$, PTS: * $P > 0.05$ and [§] $P < 0.05$) using the Wilcoxon test. Values in (B)–(G) are presented as mean \pm SEM.

A**B**

Recipient ID	Cryomedia	PGC lines	No. of white chicks (Donor-derived)	No. of black chicks (Recipient-derived)	Proportion of donor- derived offspring (%)
R404	DTS	W4	122	22	84.7
R420	DTS	W4	2	155	1.3
R425	DTS	W4	34	88	27.9
R429	DTS	W4	21	102	17.1
R442	DTS	W4	21	111	15.9
R443	DTS	W4	8	121	6.2
R446	DTS	W4	1	38	2.6
R410	DTS	W19	64	31	67.4
R411	DTS	W19	85	41	67.5
R412	DTS	W19	65	110	37.1
R422	DTS	W19	66	44	60.0
R426	DTS	W19	5	2	71.4
R428	DTS	W19	79	36	68.7
R435	PTS	W4	26	92	22.0
R457	PTS	W4	89	12	88.1
R458	PTS	W4	5	5	50.0
R432	PTS	W19	36	100	26.5
R440	PTS	W19	95	16	85.6
R441	PTS	W19	4	103	3.7
R445	PTS	W19	6	100	5.7
R447	PTS	W19	6	108	5.3
R460	PTS	W19	13	51	20.3
R463	PTS	W19	104	27	79.4

Supplementary Fig. 2. Germline transmission of cultured PGCs after freeze-thaw. (A) Phenotypes of offspring from male BPR recipients transplanted with frozen-thawed PGCs of WL by mating with normal female BPR. (right) White offspring derived from frozen-thawed WL PGCs and (left) black offspring derived from recipient BPR PGCs. (B) Percentage of male BPR recipients producing donor-derived offspring.