Title page

The full manuscript title:

Production of the medaka derived from vitrified whole testes by germ cell

transplantation

Author: Shinsuke Seki^{1,2}, Kazunari Kusano², Seungki Lee², Yoshiko Iwasaki², Masaru

Yagisawa², Mariko Ishida², Tadashi Hiratsuka², Takao Sasado³, Kiyoshi Naruse³, Goro

Yoshizaki².

¹ Bioscience Education and Research Support Center, Akita University, 1-1-1 Hondo, Akita,

Akita 010-8543, Japan

² Department of Marine Bioscience, Tokyo University of Marine Science and Tenchology,

4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

³ Laboratory of Bioresources, National Institute for Basic Biology, 38 Saigo-naka,

Myodaiji-cho, Okazaki, Aichi 444-8585, Japan

The full author list:

1. (Correspondence author)

First / last name: Shinsuke Seki

Affiliation: Bioscience Education and Research Support Center, Akita University, 1-1-1

Hondo, Akita, Akita 010-8543, Japan.

E-mail address: sseki@gipc.akita-u.ac.jp

Phone number: +81-18-884-6195

2. First / last name: Kazunari Kusano

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and

Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: reuben.k4649@gmail.com

3. First / last name: Seungki Lee

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: skmetany@gmail.com

4. First / last name: Yoshiko lwasaki

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: yoshiko0107@hotmail.com

5. First / last name: Masaru Yagisawa

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: masaru.meets.snow@gmail.com

6. First / last name: Mariko Ishida

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: blackrose.jyorokua@spice.ocn.ne.jp

7. First / last name: Tadashi Hiratsuka

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: tanago th 1018@yahoo.co.jp

8. First / last name: Takao Sasado

Affiliation: Laboratory of Bioresources, National Institute for Basic Biology, 38 Saigo-naka, Myodaiji-cho, Okazaki, Aichi 444-8585, Japan

E-mail address: tsasado@gmail.com

9. First / last name: Kiyoshi Naruse

Affiliation: Laboratory of Bioresources, National Institute for Basic Biology, 38 Saigo-naka, Myodaiji-cho, Okazaki, Aichi 444-8585, Japan

E-mail address: naruse@nibb.ac.jp

10. First / last name: Goro Yoshizaki

Affiliation: Department of Marine Bioscience, Tokyo University of Marine Science and

Tenchology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

E-mail address: ymiura0@kaiyodai.ac.jp

Correspondence and requests for materials should be addressed to Shinsuke Seki (email: sseki@gipc.akita-u.ac.jp)

Supplementary Onine Material

Tables S1 and S2

Supplementary Table S1

Efficiency of making triploid recipients and spermatogonia transplantation of donor-derived offspring in each experimental group

Donor olvas-GFP, orange		Recipient WT, black	No. of recipient	No. of recipient	No. of Triploid	GT of Triploid	
Group	Vitrification of testis	Heat shock	recipient	survived	recipient	recipient	
1	No	Yes	10	6/10 (60.0%)	5/6 (83.3%)	5/5 (100.0%)	
2	No	Yes	54	30/54 (55.6%)	1330 (43.3%)	8/13 (61.5%)	
3	No	Yes	41	24/41 (58.5%)	16/24 (66.7%)	13/16 (81.3%)	
4	No	Yes	36	24/36 (66.7%)	12/24 (50.0%)	9/12 (75.0%)	
5	Yes	Yes	20	13/20 (65.0%)	8/13 (61.5%)	7/8 (87.5%)	
6	Yes	Yes	44	29/44 (65.9%)	16/29 (55.2%)	11/16 (688%)	
7	Yes	Yes	47	24/47 (51.1%)	13/24 (54.2%)	10/13 (76.9%)	
8	Yes	Yes	50	29/50 (58.0%)	14/29 (48.3%)	10/29 (71.4%)	

Supplementary Table S2

Efficiency of making triploid recipients and spermatogonia transplantation and appearance rate of donor-derived offspring among F1 generation

Donor			Recipient				No. of	No. of	GT of	Phenotype of F1	
Group	Body color	Vitrification of testis	Group	Body color	Heat shock	N	recipient survived	Triploid recipient	Triploid recipient	Donor- derived	Recipient- derived
-	-	-	Wild type	Black	No	5	26/97 (36.6 ± 16.0%)	-	-	-	-
-	-	-	Wild type	Black	Yes	8	51/152 (35.7 ± 15.9%)	28/51 (56.8 ± 29.4%)	-	-	-
-	-	-	olvas-GFP transgenic	Orange	Yes	5	55/165 (38.5 ± 30.6%)	40/50 (75.4 ± 14.9%)	-	-	-
olvas-GFP transgenic	Orange	No	Wild type	Black	Yes	4	86/166 (49.8 ± 10.6%)	47/86 (58.7 ± 7.3%)	35/47 (63.6 ±17.1%)	-	-
olvas-GFP transgenic	Orange	Yes	Wild type	Black	Yes	4	115/192 (60.9 ± 2.8%)	62/115 (54.8 ± 2.1%)	43/62 (70.0 ± 6.9%)	1169 (100%)	0 (0%)
Inbred line (Kaga)	Black	Yes	olvas-GFP transgenic	Orange	Yes	2	46/100 (45.7 ± 3.4%)	35/46 (77.3 ± 6.9%)	20/35 (58.6 ± 23.3%)	50 (100%)	0 (0%)
Endangered wild population (Tokyo medaka)		Yes	o <i>lvas-GFP</i> transgenic	Orange	Yes	3	28/148 (20.0 ± 6.2%)	9/20 (37.8 ± 21.1%)	6/9 (63.9 ± 12.7%)	50 (100%)	0 (0%)
Line has low fertility (wnt4b -/-)	Orange	Yes	Wild type	Black	Yes	3	41/155 (29.2 ± 1.7%)	25/41 (61.4 ± 3.4%)	21/25 (83.3 ± 11.0%)	259 (100%)	0 (0%)