

## **Supplementary tables**

### **Mapping of a responsible region for sex reversal upstream of *Sox9* by production of mice with serial deletion in a genomic locus**

Yuya Ogawa<sup>#, 1</sup>, Miho Terao<sup>#, 1</sup>, Satoshi Hara<sup>#, 1</sup>, Moe Tamano<sup>1</sup>, Haruka Okayasu<sup>1</sup>, Tomoko Kato<sup>§,1</sup> and Shuji Takada<sup>\*,1</sup>

<sup>1</sup> Department of Systems BioMedicine, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan

<sup>#</sup>These authors contributed equally to this work.

<sup>§</sup>Present address: Tokyo Metropolitan Institute of Medical Science, Regenerative Medicine Project, Tokyo 156-8506, Japan

\*Corresponding author: Shuji Takada (takada-s@ncchd.go.jp, 2-10-1 Okura, Setagaya, Tokyo 157-8535, Japan, Tel & Fax: +81-3-3417-2498).

Table S1. Genotypes of F<sub>0</sub> mice obtained by injection of sgRNA1/2/3/4/5/6.

Mouse ID	Karyotype	Phenotypic sex	Indel												Deletion							
			F1/R1	F2/R2	F3/R3	F4/R4	F5/R5	F6/R6	F1/R2	F1/R3	F1/R4	F1/R5	F1/R6	F2/R3	F2/R4	F2/R5	F2/R6	F3/R4	F3/R5	F3/R6	F4/R5	F4/R6
#1	XX	Female	WT	indel	ND	ND	indel	WT	WT	WT	WT	WT	WT	WT	O							
#2	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#3	XY	Male	WT	indel	ND	ND	indel	WT	WT	WT	WT	WT	WT	WT	O							
#4	XY	Male	WT	indel	ND	ND	indel	WT	WT	WT	WT	WT	WT	WT	O							
#5	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#6	XY	Male	WT	ND	ND	ND	ND	ND	ND	ND	O											
#7	XY	Male	WT	indel	ND	ND	ND	ND	ND	ND	ND	O										
#8	XX	Female	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#9	XY	Male	WT	ND	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#10	XY	Male	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#11	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#12	XY	Male	WT	ND	indel	WT	WT	WT	WT	WT	WT	WT	O									
#13	XY	Male	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#14*	XX	Female	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#15	XY	Male	WT	indel	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#16	XY	Male	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#17	XX	Female	WT	indel	ND	WT	WT	WT	WT	WT	WT	WT	O									
#18	XY	Male	WT	indel	ND	WT	WT	WT	WT	WT	WT	WT	O									
#19	XX	Female	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#20	XX	Female	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#21	XY	Male	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#22	XX	Female	WT	indel	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#23	XY	Male	WT	indel	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#24	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#25	XX	Female	WT	indel	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#26	XY	Male	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#27	XX	Female	WT	ND	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#28	XY	Male	WT	indel	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#29	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#30	XX	Female	WT	ND	indel	WT	WT	WT	WT	WT	WT	WT	O									
#31	XY	Male	WT	indel	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#32	XY	Male	WT	indel	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#33	XX	Female	WT	indel	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#34	XY	Male	WT	ND	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#35	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#36	XX	Female	WT	indel	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							
#37	XY	Male	WT	indel	ND	indel	WT	WT	WT	WT	WT	WT	WT	O								
#38	XX	Female	WT	indel	WT	indel	WT	WT	WT	WT	WT	WT	WT	O								
#39	XX	Female	WT	indel	indel	WT	WT	WT	WT	WT	WT	WT	O									
#40	XX	Female	WT	indel	ND	ND	ND	WT	WT	WT	WT	WT	WT	WT	O							

Columns below 'Indel' and 'Deletion' show pairs of PCR primers used for genotyping.

Parentheses designate deleted regions.

WT: no mutation observed; indel: indel was observed; ND: not detected by PCR amplification; o: PCR product was identified

\*PCR amplicon was not observed by any combinations of genotyping primers.

**Table S2. Genotypes of F<sub>0</sub> mice obtained by injection of sgRNA2/6/7.**

Mouse ID	Karyotype	Phenotypic sex	Indel			Deletion		
			F1/R1	F2/R2	F6/R6	F1/R2 (Δ7-2)	F2/R6 (Δ2-6)	F1/R6 (Δ7-6)
#41	XY	Male	indel	indel	indel	○		
#42	XY	Male	indel	indel	indel		○	○
#43*	XX	Female	ND	ND	ND			
#44	XX	Female	ND	ND	indel	○		
#45	XX	Female	indel	indel	indel			○
#46	XY	Female	ND	ND	indel	○		○
#47	XY	Male	indel	indel	indel			
#48	XY	Female	ND	ND	indel	○		○
#49	XX	Female	indel	ND	ND	○		○
#50	XY	Male	indel	indel	indel			
#51	XY	Male	indel	indel	indel			○
#52	XX	Female	ND	indel	indel			
#53	XX	Female	indel	ND	ND		○	
#54	XY	Female	ND	ND	ND			○
#55	XY	Male	indel	indel	indel			
#56	XY	Male	ND	indel	indel			
#57	XY	Male	ND	ND	indel	○		

Columns below 'Indel' and 'Deletion' show pairs of PCR primers used for genotyping.

Parentheses designate deleted regions.

WT: no mutation observed; indel: indel was observed; ND: not detected by PCR amplification; ○: PCR product was identified; Blank: PCR product was not identified

Mice exhibiting XY sex reversal are highlighted in yellow.

\*PCR amplicon was not observed by any combinations of genotyping primers.

**Table S3. Germline transmission of serial deletion alleles.**

sgRNA combination	F <sub>0</sub> mice	Genotype of F <sub>0</sub>	No. of F <sub>1</sub> genotyped	No. of F <sub>1</sub> mouse	
				WT	Deletion*
sgRNA1/2/3/4/5/6	#6	Δ2-3/Δ4-6	7	4	<b>1 (Δ2-3)</b> , 2 (Δ2-3; Δ4-6)
	#9	Δ2-3/Δ3-6/Δ5-6	8	0	<b>3 (Δ3-6)</b> , 2 (Δ2-3), 3 (Δ2-3; Δ3-6)
	#16	Δ2-6/Δ2-4	9	1	<b>2 (Δ2-4)</b> , 5 (Δ2-6)
	#21	Δ2-5	7	6	<b>1 (Δ2-5)</b>
	#25	Δ4-6	5	3	<b>2 (Δ4-6)</b>
	#26**	Δ2-5	9	0	2 (Δ2-5), <b>7 (Δ5-6)</b>
	#33	Δ2-3/Δ4-6	3	2	1 (Δ2-3)
	#34	Δ2-4	14	11	3 (Δ2-4)
	#35	Δ5-6	7	5	2 (Δ5-6)
sgRNA2/6/7	#37**	Δ3-4	11	5	6 (Δ2-5)
	#45	Δ7-6	9	5	<b>4 (Δ7-6)</b>
	#57	Δ7-2	10	6	<b>4 (Δ7-2)</b>

\*Established deletion lines are shown in bold.

\*\*Deletion patterns observed at F<sub>1</sub> generation was not identified in F<sub>0</sub> mice. #26 and #37 are considered as mosaic individuals.