Supplemental Information

Rapid mitochondrial DNA segregation in primate preimplantation embryos precedes somatic and germline bottleneck

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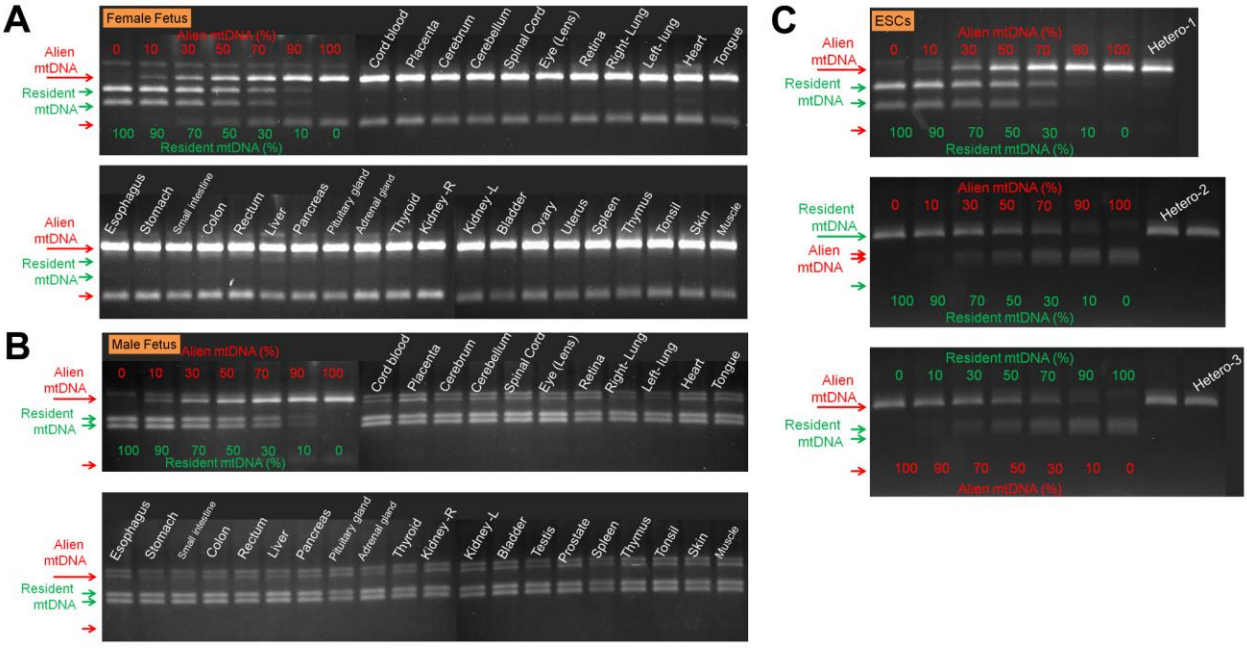

Figure S1. **Restriction fragment length polymorphysm (RFLP) analysis of mtDNA in organs and tissues of heteroplasmic fetuses and ESCs.**

(**A**) MtDNA heteroplasmy detected by RFLP in tissues from the female fetus. Resident mtDNA digested with *KpnI* and alien mtDNA treated with *NheI* produced fragments of 319 bp + 224 bp and 417 bp + 126 bp length, respectively. RFLP analysis showed that the major mitochondrial haplotype in all tissues from this fetus was alien while the resident mtDNA was not detected except in liver and heart. (**B**) Heteroplasmy in tissues from the male fetus. RFLP assay with unique enzymes (*HindIII* for resident mtDNA and *EcoRV* for alien mtDNA) detected presence of both variants in this fetus. However, alien mtDNA bands were noticeably weaker. (**C**) Heteroplasmy in ESC lines produced from heteroplasmic embryos. Two cell lines (Hetero-1 and -3) carried alien mtDNA while Hetero-2 contained resident haplotype. For quantifications, both alien and resident mtDNA were mixed at various ratios (0, 10, 30, 50, 70, 90 and 100%), related to Table 2 .

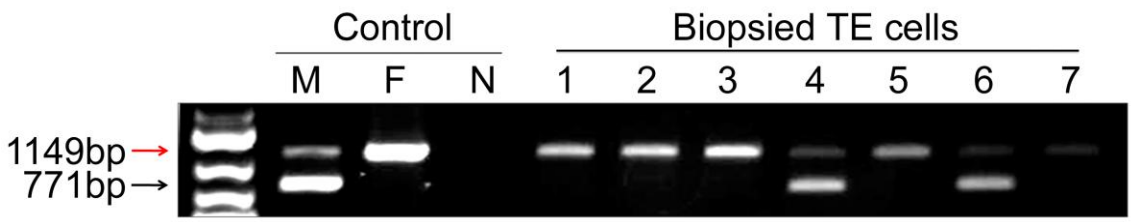

Figure S2. Gender determination in ST blastocysts by TE biopsy and PCR.

DNA was extracted from individually biopsied blastocysts and amplified by PCR. Male DNA samples produced two bands (1149 bp and 771 bp) and female DNA produced only one band (1149 bp). Blood DNA from rhesus monkey males (M) and females (F) was used as controls. For negative control (N) no DNA template was used. Samples in lanes 1, 2, 3, 5 and 7 were females and lanes 4 and 6 were indicated male blastocysts.

Table S1. Heteroplasmy in blastomeres of monkey 2-cell embryos, related to Figure 2A

Embryo	Alien mtDNA (%)		Mean (%)	S.D.	CV (%)	Range
	Blastomere 1	Blastomere 2				
1	47	48	47.5	0.7	1.5	1
2	59	60	59.5	0.7	1.2	1
3	50	51	50.5	0.7	1.4	1
4	56	54	55	1.4	2.6	2
5	61	56	58.5	3.5	6.0	5
6	40	47	43.5	4.9	11.4	7
7	45	55	50	7.1	14.1	10
8	41	54	47.5	9.2	19.4	13
9	72	87	79.5	10.6	13.3	15
10	57	75	66	12.7	19.3	18
11	63	40	51.5	16.3	31.6	23
12	46	73	59.5	19.1	32.1	27
13	57	28	42.5	20.5	48.2	29
14	36	70	53	24.0	45.4	34
		Mean	54.6	9.4	17.7	13.3
		S.D.	9.7			11.4
		CV (%)	17.9			

S.D., standard deviation; CV, coefficient of variation; Range, differences between maximum and minimum values in daughter blastomeres.

Table S2. Heteroplasmy in blastomeres of monkey 4-cell embryos, related to Figure 2B

Embryo	Alien mtDNA (%) in blastomere				Mean	S.D.	CV (%)	Range
	1	2	3	4				
1	52	55	55	57	54.8	2.1	3.8	5
2	53	54	55	59	55.3	2.6	4.8	6
3	46	49	50	52	49.3	2.5	5.1	6
4	53	58	59	64	58.5	4.5	7.7	11
5	31	40	28	35	33.5	5.2	15.5	12
6	62	69	73	75	69.8	5.7	8.2	13
7	67	58	66	71	65.5	5.4	8.3	13
8	57	59	65	72	63.3	6.8	10.7	15
9	48	50	59	65	55.5	7.9	14.3	17
10	34	46	49	53	45.5	8.2	18.0	19
11	44	58	60	66	57.0	9.3	16.3	22
12	43	43	64	66	54.0	12.7	23.6	23
13	41	43	64	65	53.3	13.0	24.5	24
14	25	42	50	50	41.8	11.8	28.2	25
15	27	29	36	54	36.5	12.3	33.7	27
16	41	44	48	70	50.8	13.1	25.9	29
17	55	69	72	88	71.0	13.5	19.1	33
18	43	54	55	78	57.5	14.7	25.6	35
19	40	56	71	76	60.8	16.2	26.7	36
20	13	24	28	49	28.5	15.1	52.9	36
21	45	47	67	85	61.0	18.8	30.9	40
22	21	32	33	67	38.3	19.9	52.1	46
23	3	23	35	52	28.3	20.6	73.0	49
24	12	31	34	77	38.5	27.5	71.3	65
				Mean	51.2	11.2	25.0	25.3
				S.D.	12.3			15.1
				CV (%)	24.1			

S.D., standard deviation; CV, coefficient of variation; Range, differences between maximum and minimum values in daughter blastomeres.

Table S3. Heteroplasmy in ICM and TE of monkey blastocysts, related to Figure 2F

Embryo	Alien mtDNA (%)		Mean	S.D.	CV (%)	Range
	ICM	TE				
1	81	58	69.5	16.3	23.4	23
2	73	66	69.5	4.9	7.1	7
3	66	52	59	9.9	16.8	14
4	63	56	59.5	4.9	8.3	7
5	63	59	61	2.8	4.6	4
6	62	43	52.5	13.4	25.6	19
7	60	56	58	2.8	4.9	4
8	58	60	59	1.4	2.4	2
9	58	68	63	7.1	11.2	10
10	57	51	54	4.2	7.9	6
11	56	68	62	8.5	13.7	12
12	52	83	67.5	21.9	32.5	31
13	51	53	52	1.4	2.7	2
14	50	53	51.5	2.1	4.1	3
15	46	58	52	8.5	16.3	12
16	44	58	51	9.9	19.4	14
17	42	62	52	14.1	27.2	20
18	40	48	44	5.7	12.9	8
Mean	56.8	58.4	57.6	7.8	13.4	11.0
S.D.	10.7	9.0	7.1			8.0
CV (%)	18.8	15.4	7.1			

S.D., standard deviation; CV, coefficient of variation; Range, differences between ICM and TE values.

Table S4. Heteroplasmy in individual cells of Hetero-1 ESC line.

	Alien mtDNA (%)
Clone-1	90.7
Clone-2	92.9
Clone-3	92.9
Clone-4	100
Clone-5	100
Clone-6	100
Clone-7	100
Clone-8	100
Clone-9	100
Mean	97.4%

Table S5. mtDNA carryover in organs and tissues of ST offspring

Organ	Fetus #1 (%)	Fetus #2 (%)
Cerebrum	ND	0.19
Cerebellum	ND	ND
Heart	ND	0.48
Lung	ND	ND
Blood	ND	0.13
Stomach	ND	ND
Small intestine	ND	ND
Colon	ND	ND
Liver	ND	ND
Pancreas	ND	ND
Adrenal gland	ND	0.2
Thyroid	ND	ND
Kidney-right	ND	ND
Kidney-left	ND	ND
Bladder	ND	ND
Uterus	ND	ND
Spleen	ND	ND
Thymus	ND	ND
Skin	ND	ND
Skeletal Muscle	ND	ND

ND, not detectable