

Title: The mosaic (MSC) mutants of cucumber: a method to produce knock-downs of mitochondrial transcripts

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Table S1 Low-coverage regions in the mt DNA of the mitochondrial mutant MSC3, 12, and 16 as compared to the progenitor, wild-type inbred B.

Mutant Line	Region (bp)		Size (bp)	Chromosome*	Annotation
	Start	End			
MSC3	392,242	441,105	48,863	MC	<i>nad5ex4</i> (NADH dehydrogenase subunit 5 C-terminus); <i>trnW-CCA</i> (tRNA-Trp); <i>atp4</i> (ATPase subunit 4) and chloroplast-like DNA
	585,075	636,619	51,544	MC	
	1	31,162	31,161	83.8 kb	
	Total		131,568	7.8%	
MSC12	149,606	161,705	12,099	MC	<i>rps7</i> (ribosomal protein S7) JLV5-Del (Lilly <i>et al.</i> 2001) was identified in MSC16)
	195,676	267,406	71,730	MC	
	347,550	362,669	15,119	MC	
	567,690	568,518	828	MC	
	572,554	622,942	50,388	MC	
	911,926	917,574	5,648	MC	
	22,564	25,406	2,842	44.8 kb	
	1	83,817	83,816	83.8 kb	
Total		242,470	14.4%		
MSC16	149,606	161,706	12,100	MC	<i>rps7</i> (ribosomal protein S7) JLV5-Del (Lilly <i>et al.</i> 2001)
	195,676	283,618	87,942	MC	
	347,550	362,669	15,119	MC	
	567,711	568,451	740	MC	
	572,554	622,942	50,388	MC	
	911,926	917,574	5,648	MC	
	22,564	25,270	2,706	44.8 kb	
	1	83,817	83,816	83.8 kb	
Total		258,459	15.3%		

MC = Master chromosome of 1.6 megabases (Mb).

44.8 kb = Extra-chromosome of 44.8 kb

83.8 kb = Extra-chromosome of 83.8 kb

*All locations are based on the mitochondrial sequence of Alverson *et al.* (2011).

Table S2 Fold change of copy number of mitochondrial and nuclear genes in wild-type inbred B and mitochondrial mutants MSC3, 12 and 16.

Gene	Line	Average Fold-change	S.E.	t-test
<i>gadph</i> ⁿ	Line B	0.89	0.06	CONTROL
<i>gadph</i> ⁿ	MSC3	0.86	0.10	0.4059
<i>gadph</i> ⁿ	MSC12	0.79	0.05	0.1267
<i>gadph</i> ⁿ	MSC16	0.83	0.05	0.2371
<i>actin3</i> ⁿ	Line B	1.00	0.06	CONTROL
<i>actin3</i> ⁿ	MSC3	1.21	0.08	0.0574
<i>actin3</i> ⁿ	MSC12	1.05	0.04	0.2899
<i>actin3</i> ⁿ	MSC16	1.08	0.08	0.2600
<i>nad9</i>	Line B	1.00	0.02	CONTROL
<i>nad9</i>	MSC3	1.38	0.22	0.0782
<i>nad9</i>	MSC12	2.60	0.25	0.0016*
<i>nad9</i>	MSC16	1.88	0.16	0.0031*
<i>cob</i>	Line B	1.00	0.02	CONTROL
<i>cob</i>	MSC3	1.29	0.05	0.0034*
<i>cob</i>	MSC12	1.47	0.20	0.0400*
<i>cob</i>	MSC16	1.32	0.08	0.0096*
<i>rps7</i>	Line B	1.02	0.15	CONTROL
<i>rps7</i>	MSC3	0.92	0.16	0.3371
<i>rps7</i>	MSC12	0.12	0.04	0.0021*
<i>rps7</i>	MSC16	0.12	0.05	0.0023*
<i>ccmFc</i>	Line B	1.00	0.04	CONTROL
<i>ccmFc</i>	MSC3	1.31	0.16	0.0715
<i>ccmFc</i>	MSC12	2.09	0.32	0.0143*

<i>ccmFc</i>	MSC16	2.38	0.75	0.0716
<i>nad7</i>	Line B	1.00	0.07	CONTROL
<i>nad7</i>	MSC3	1.04	0.12	0.4124
<i>nad7</i>	MSC12	1.23	0.18	0.1535
<i>nad7</i>	MSC16	1.08	0.12	0.2998
<i>ccmB</i>	Line B	1.05	0.23	CONTROL
<i>ccmB</i>	MSC3	1.30	0.21	0.2366
<i>ccmB</i>	MSC12	1.68	0.17	0.0482*
<i>ccmB</i>	MSC16	1.59	0.28	0.1039
<i>atp1</i>	Line B	1.03	0.16	CONTROL
<i>atp1</i>	MSC3	1.16	0.14	0.2794
<i>atp1</i>	MSC12	2.22	0.26	0.0089*
<i>atp1</i>	MSC16	2.42	0.24	0.0042*
<i>cox1</i>	Line B	1.01	0.10	CONTROL
<i>cox1</i>	MSC3	1.20	0.25	0.2568
<i>cox1</i>	MSC12	1.71	0.17	0.0116*
<i>cox1</i>	MSC16	2.04	0.28	0.0127*
<i>nad6</i>	Line B	1.01	0.10	CONTROL
<i>nad6</i>	MSC3	1.27	0.14	0.0985
<i>nad6</i>	MSC12	2.15	0.17	0.0023*
<i>nad6</i>	MSC16	2.38	0.25	0.0039*
<i>ubqcⁿ</i>	Line B	1.00	0.00	CONTROL
<i>ubqcⁿ</i>	MSC3	1.11	0.12	0.2041
<i>ubqcⁿ</i>	MSC12	1.05	0.09	0.2886
<i>ubqcⁿ</i>	MSC16	1.03	0.10	0.3820
<i>sdh3</i>	Line B	1.04	0.25	CONTROL

<i>sdh3</i>	MSC3	1.23	0.07	0.2100
<i>sdh3</i>	MSC12	2.09	0.09	0.0048*
<i>sdh3</i>	MSC16	2.16	0.36	0.0265*
<i>nad3</i>	Line B	1.02	0.16	CONTROL
<i>nad3</i>	MSC3	1.43	0.16	0.0741
<i>nad3</i>	MSC12	2.00	0.08	0.0029*
<i>nad3</i>	MSC16	1.88	0.33	0.0402*
<i>rrn5</i>	Line B	1.01	0.12	CONTROL
<i>rrn5</i>	MSC3	1.05	0.10	0.4057
<i>rrn5</i>	MSC12	1.17	0.00	0.1311
<i>rrn5</i>	MSC16	1.26	0.05	0.0683
<i>rpl2</i>	Line B	1.00	0.04	CONTROL
<i>rpl2</i>	MSC3	1.00	0.08	0.4799
<i>rpl2</i>	MSC12	1.86	0.07	0.0003*
<i>rpl2</i>	MSC16	2.10	0.12	0.0006*
<i>rps3</i>	Line B	1.01	0.12	CONTROL
<i>rps3</i>	MSC3	1.40	0.16	0.0660
<i>rps3</i>	MSC12	2.31	0.20	0.0025*
<i>rps3</i>	MSC16	2.53	0.20	0.0015*
<i>rpl5</i>	Line B	1.00	0.07	CONTROL
<i>rpl5</i>	MSC3	1.38	0.19	0.0710
<i>rpl5</i>	MSC12	2.05	0.32	0.0171*
<i>rpl5</i>	MSC16	2.86	0.34	0.0030*
<i>nad5</i>	Line B	1.03	0.19	CONTROL
<i>nad5</i>	MSC3	0.36	0.11	0.0194*
<i>nad5</i>	MSC12	1.85	0.39	0.0661

<i>nad5</i>	MSC16	1.47	0.20	0.0931
<i>atp4</i>	Line B	1.03	0.19	CONTROL
<i>atp4</i>	MSC3	0.43	0.04	0.0182*
<i>atp4</i>	MSC12	1.69	0.31	0.0724
<i>atp4</i>	MSC16	1.88	0.19	0.0170*
<i>rrn5</i>	Line B	1.01	0.12	CONTROL
<i>rrn5</i>	MSC3	1.06	0.11	0.4046
<i>rrn5</i>	MSC12	1.29	0.06	0.0556
<i>rrn5</i>	MSC16	1.39	0.11	0.0432*
<i>aox^{nm}</i>	Line B	1.00	0.07	CONTROL
<i>aox^{nm}</i>	MSC3	1.21	0.22	0.2082
<i>aox^{nm}</i>	MSC12	1.00	0.07	0.4992
<i>aox^{nm}</i>	MSC16	1.24	0.10	0.0640
<i>porin^{nm}</i>	Line B	1.16	0.47	CONTROL
<i>porin^{nm}</i>	MSC3	34.74	33.31	0.1853
<i>porin^{nm}</i>	MSC12	0.80	0.20	0.2589
<i>porin^{nm}</i>	MSC16	2.34	1.03	0.1788
<i>cox2</i>	Line B	1.00	0.07	CONTROL
<i>cox2</i>	MSC3	1.12	0.30	0.3596
<i>cox2</i>	MSC12	1.79	0.14	0.0040*
<i>cox2</i>	MSC16	1.95	0.04	0.0002*
<i>rps13</i>	Line B	1.04	0.21	CONTROL
<i>rps13</i>	MSC3	1.33	0.23	0.1968
<i>rps13</i>	MSC12	2.19	0.00	0.0025*
<i>rps13</i>	MSC16	2.05	0.30	0.0253*
<i>mttb</i>	Line B	1.06	0.26	CONTROL

<i>mttb</i>	MSC3	1.42	0.11	0.1383
<i>mttb</i>	MSC12	2.46	0.06	0.0033*
<i>mttb</i>	MSC16	2.26	0.23	0.0130*
<i>rps10</i>	Line B	1.00	0.08	CONTROL
<i>rps10</i>	MSC3	1.12	0.05	0.1431
<i>rps10</i>	MSC12	2.02	0.24	0.0080*
<i>rps10</i>	MSC16	2.25	0.13	0.0007*
<i>atp8</i>	Line B	1.00	0.04	CONTROL
<i>atp8</i>	MSC3	1.15	0.09	0.0998
<i>atp8</i>	MSC12	1.55	0.04	0.0003*
<i>atp8</i>	MSC16	1.82	0.04	0.0001*
<i>rrnL</i>	Line B	1.00	0.04	CONTROL
<i>rrnL</i>	MSC3	1.25	0.15	0.0952
<i>rrnL</i>	MSC12	3.03	0.68	0.0207*
<i>rrnL</i>	MSC16	2.25	0.53	0.0400*

* Significant difference between an MSC mutant for a given mitochondrial or nuclear gene compared to wild-type B at $\alpha = 0.05$, Student's t-test.

ⁿ Nuclear-encoded gene with no function in the mitochondria.

^{nm} Nuclear-encoded mitochondrially targeted gene.

Table S3 Fold change in transcript levels of mitochondrial and nuclear genes from wild-type inbred B and mitochondrial mutants MSC3, 12 and 16.

Gene	Line	Average Fold-change	S.E.	t-test
<i>rps7</i>	Line B	1.12	0.18	CONTROL
<i>rps7</i>	MSC3	2.32	0.34	0.0044*
<i>rps7</i>	MSC12	0.21	0.07	0.0005*
<i>rps7</i>	MSC16	0.36	0.08	0.0016*
<i>rrn5</i>	Line B	1.05	0.12	CONTROL
<i>rrn5</i>	MSC3	1.67	0.49	0.1246
<i>rrn5</i>	MSC12	1.15	0.19	0.3181
<i>rrn5</i>	MSC16	1.16	0.16	0.2837
<i>rpl2</i>	Line B	1.09	0.37	CONTROL
<i>rpl2</i>	MSC3	1.76	0.47	0.0148*
<i>rpl2</i>	MSC12	2.00	0.29	0.0087*
<i>rpl2</i>	MSC16	2.00	0.31	0.0118*
<i>nad3</i>	Line B	1.31	0.32	CONTROL
<i>nad3</i>	MSC3	2.06	0.46	0.1028
<i>nad3</i>	MSC12	2.92	0.46	0.0041*
<i>nad3</i>	MSC16	4.75	1.14	0.0087*
<i>sdh3</i>	Line B	1.08	0.16	CONTROL
<i>sdh3</i>	MSC3	2.08	0.48	0.0395*
<i>sdh3</i>	MSC12	2.05	0.43	0.0256*
<i>sdh3</i>	MSC16	1.85	0.42	0.0583
<i>nad6</i>	Line B	1.23	0.28	CONTROL
<i>nad6</i>	MSC3	1.55	0.31	0.2295
<i>nad6</i>	MSC12	2.54	0.64	0.0379*

<i>nad6</i>	MSC16	2.57	0.66	0.0435*
<i>ubqcⁿ</i>	Line B	1.09	0.35	CONTROL
<i>ubqcⁿ</i>	MSC3	1.51	0.80	0.1613
<i>ubqcⁿ</i>	MSC12	1.11	0.21	0.4583
<i>ubqcⁿ</i>	MSC16	1.36	0.25	0.1873
<i>nad9</i>	Line B	1.43	0.48	CONTROL
<i>nad9</i>	MSC3	2.13	0.40	0.1391
<i>nad9</i>	MSC12	4.40	1.11	0.0122*
<i>nad9</i>	MSC16	5.21	1.71	0.0309*
<i>cob</i>	Line B	1.19	0.25	CONTROL
<i>cob</i>	MSC3	1.92	0.26	0.0301*
<i>cob</i>	MSC12	2.13	0.34	0.0156*
<i>cob</i>	MSC16	3.13	0.71	0.0142*
<i>actin3ⁿ</i>	Line B	3.46	2.54	CONTROL
<i>actin3ⁿ</i>	MSC3	3.15	0.86	0.4545
<i>actin3ⁿ</i>	MSC12	2.88	1.41	0.4222
<i>actin3ⁿ</i>	MSC16	3.99	0.87	0.4244
<i>rps10</i>	Line B	1.19	0.50	CONTROL
<i>rps10</i>	MSC3	1.90	1.32	0.1541
<i>rps10</i>	MSC12	1.89	0.41	0.0790
<i>rps10</i>	MSC16	2.64	0.53	0.0143*
<i>rps3</i>	Line B	1.09	0.17	CONTROL
<i>rps3</i>	MSC3	1.21	0.20	0.3276
<i>rps3</i>	MSC12	2.04	0.34	0.0097*
<i>rps3</i>	MSC16	1.93	0.37	0.0328*
<i>mttb</i>	Line B	1.07	0.16	CONTROL

<i>mttb</i>	MSC3	1.57	0.34	0.1084
<i>mttb</i>	MSC12	2.01	0.32	0.0067*
<i>mttb</i>	MSC16	1.82	0.24	0.0113*
<i>atp4</i>	Line B	1.03	0.09	CONTROL
<i>atp4</i>	MSC3	0.47	0.07	0.0001*
<i>atp4</i>	MSC12	1.12	0.19	0.3112
<i>atp4</i>	MSC16	1.01	0.14	0.4523
<i>cox2</i>	Line B	1.03	0.18	CONTROL
<i>cox2</i>	MSC3	1.12	0.24	0.2669
<i>cox2</i>	MSC12	1.25	0.15	0.1114
<i>cox2</i>	MSC16	1.32	0.18	0.0917
<i>aox^{nm}</i>	Line B	1.03	0.10	CONTROL
<i>aox^{nm}</i>	MSC3	1.94	0.22	0.0017*
<i>aox^{nm}</i>	MSC12	2.33	0.27	0.0003*
<i>aox^{nm}</i>	MSC16	2.66	0.35	0.0007*
<i>rrnS</i>	Line B	1.06	0.13	CONTROL
<i>rrnS</i>	MSC3	1.35	0.18	0.1090
<i>rrnS</i>	MSC12	0.73	0.12	0.0383*
<i>rrnS</i>	MSC16	0.82	0.11	0.0905
<i>rps13</i>	Line B	1.10	0.17	CONTROL
<i>rps13</i>	MSC3	1.35	0.11	0.1150
<i>rps13</i>	MSC12	2.85	0.39	0.0003*
<i>rps13</i>	MSC16	2.82	0.71	0.0213*
<i>rpl5</i>	Line B	1.09	0.39	CONTROL
<i>rpl5</i>	MSC3	1.81	0.45	0.0112*
<i>rpl5</i>	MSC12	2.27	0.57	0.0396*

<i>rpl5</i>	MSC16	2.05	0.23	0.0026*
<i>atp8</i>	Line B	1.02	0.08	CONTROL
<i>atp8</i>	MSC3	1.09	0.23	0.3999
<i>atp8</i>	MSC12	1.33	0.23	0.0916
<i>atp8</i>	MSC16	1.45	0.22	0.0504
<i>nad5</i>	Line B	1.13	0.22	CONTROL
<i>nad5</i>	MSC3	0.14	0.02	0.0011*
<i>nad5</i>	MSC12	1.28	0.34	0.3535
<i>nad5</i>	MSC16	1.16	0.27	0.4686
<i>rrnL</i>	Line B	1.06	0.12	CONTROL
<i>rrnL</i>	MSC3	1.15	0.21	0.3519
<i>rrnL</i>	MSC12	1.19	0.30	0.3461
<i>rrnL</i>	MSC16	1.51	0.28	0.0843
<i>cox1</i>	Line B	1.13	0.41	CONTROL
<i>cox1</i>	MSC3	1.58	0.68	0.1262
<i>cox1</i>	MSC12	1.85	0.58	0.1347
<i>cox1</i>	MSC16	3.07	1.03	0.0500*
<i>atp1</i>	Line B	1.11	0.19	CONTROL
<i>atp1</i>	MSC3	2.05	0.24	0.0039*
<i>atp1</i>	MSC12	1.77	0.57	0.1510
<i>atp1</i>	MSC16	1.91	0.50	0.0849
<i>nad1</i>	Line B	1.03	0.08	CONTROL
<i>nad1</i>	MSC3	0.95	0.14	0.3251
<i>nad1</i>	MSC12	1.08	0.32	0.4367
<i>nad1</i>	MSC16	1.18	0.31	0.3286
<i>nad7</i>	Line B	1.05	0.11	CONTROL

<i>nad7</i>	MSC3	1.16	0.16	0.2910
<i>nad7</i>	MSC12	1.06	0.25	0.4889
<i>nad7</i>	MSC16	1.19	0.25	0.3109
<i>ccmB</i>	Line B	1.10	0.31	CONTROL
<i>ccmB</i>	MSC3	2.15	1.41	0.0784
<i>ccmB</i>	MSC12	1.55	0.19	0.0400*
<i>ccmB</i>	MSC16	2.30	0.40	0.0090*
<i>porin</i> ⁿ	Line B	1.08	0.15	CONTROL
<i>porin</i> ⁿ	MSC3	1.01	0.19	0.3941
<i>porin</i> ⁿ	MSC12	0.80	0.23	0.1624
<i>porin</i> ⁿ	MSC16	0.87	0.16	0.1690
<i>ccmFc</i>	Line B	1.08	0.15	CONTROL
<i>ccmFc</i>	MSC3	1.34	0.22	0.1781
<i>ccmFc</i>	MSC12	2.81	0.71	0.0176*
<i>ccmFc</i>	MSC16	2.34	0.47	0.0145*
<i>gadph</i> ⁿ	Line B	1.03	0.08	CONTROL
<i>gadph</i> ⁿ	MSC3	1.32	0.17	0.0829
<i>gadph</i> ⁿ	MSC12	1.17	0.17	0.2137
<i>gadph</i> ⁿ	MSC16	1.14	0.26	0.3495

* Significant difference between an MSC mutant for a given mitochondrial or nuclear gene compared to wild-type

B at $\alpha = 0.05$, Student's t-test.

ⁿ Nuclear-encoded gene with no function in the mitochondria.

^{nm} Nuclear-encoded mitochondrially targeted gene.

Table S4 Primer sequences that were used for quantitative-PCR.

Location	Gene	Forward Primer	Reverse Primer
Nuclear	<i>gadph</i>	AGGAAGAGTCCGAGGGAAAA	ACGAGGATGTGAAGCCAAAC
	<i>aox2</i>	GCACCATCAGCCCAAAAC	AGCATCCCTCCAACCATTTC
	<i>ubqc</i>	CACAGCTCGATATTGGACAGA	AATTTTCATCCCAACCCACA
	<i>actin3</i>	CCAAGGCGAATAGAGAGAAAA	GCAACATACATAGCGGGAGTG
Complex I	<i>nad9</i>	ACAAACCAGTGCAGACGAAG	CCGGATGATTGATGGAAGAA
	<i>nad7</i>	GCAGCACCTTACGATGTTCA	TGTCGCATCTCTTCGATACG
	<i>nad6</i>	TCCGTCTGGTTTTTGTCTTC	GTGGTTCTGTCGTCCTCCTC
	<i>nad3</i>	CACTCGGTCTTCCTTTTCCA	AACGACTTCTGGCATCACC
	<i>nad5 ex4</i>	AGGATATTAGGGGAAGCAGTGAG	CCAAGAAGATAGAGAGTCCCACA
Complex II	<i>sdh3</i>	TGGGTTTGATTTGCTTCACC	CGACGGATATTAGGATGAGCTTT
Complex III	<i>cob</i>	CTTGGTGAATAGGGCGAAAA	TGACAAAGCGGGAGGTGTAG
Complex IV	<i>cox1</i>	TTCCCATGCATTTCTTAGGG	AACGACGAATCCCAACTACG
	<i>cox2</i>	GATCTCAAGACGCAGCAACA	CATAAAGCGCGAACCAAGA
	<i>ccmFc</i>	GCCTCATTTCTTTCTTCC	TCACGTTTCGCTGAACTATG
	<i>ccmB</i>	CGGAATGGATCGGTAAACA	AAGAGCCGAACGAGAATGAA
Complex V	<i>atp1</i>	TGAAACACAAGCTGGAGACG	ACACGACTGACGGATAAGCC
	<i>atp4</i>	GCAGCAATTTCCAATCCT	TGCACTGTCTTTTCGCACTT
	<i>atp8</i>	TGCCTTTCTTCTTGACTTTCT	TCCTTGCTCCGTATGTTCTTC
Ribosome LU	<i>rpl2</i>	AGCCAAGAGGGGAGAGCA	CCGAGAAGAACGATTAGGG
	<i>rpl5</i>	GCCCGGAGAATTTAAGAAAGA	TGACCTAGCACGAGAAAGCA
	<i>rrnL</i>	GTCAGCGAGAAAATGGGAAC	CGGGTCAAATAGGAAGAACGA
Ribosome SU	<i>rrn5</i>	AGCGATCGACGTGAAAACAC	TCCTTCATTAAGCCGC
	<i>rrnS</i>	TGCGCTTTAGTTTGATTGCT	AGTCTGAGGACCCGTTTTGG
	<i>rps7</i>	TGACGATCCCTGGCTACAA	ATGGTTGAGGCCGTAGAGAA
	<i>rps13</i>	GTCTGCTCGTTCTCCCCTCT	AAAAAGCCATTACAGTTTCGTT
	<i>rps10</i>	GGAAGATTGGATTGCCTGAA	ATGCCTTTCTGTTTTATGACC
	<i>rps3</i>	GGAGCGAATACACAGGGAAG	CGAAGAAAGGAAAGAGCGAGA