Supplementary Information

Mutations in the Pre-Replication Complex cause Meier-Gorlin syndrome

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Supplementary Note

The phenotype spectrum of Pre-Replication complex mutations

In this and the accompanying paper mutations are found in several patients from a cohort of microcephalic dwarfism patients and subsequently in many cases of Meier-Gorlin syndrome. Considering both papers together, almost all cases have primordial dwarfism with substantial prenatal and postnatal growth retardation evident. However, not all cases have microcephaly, and conversely microtia and absent/hypoplastic patella is absent in some. Therefore patients with pre-replication complex mutations do not all fit into either the general diagnostic category of microcephalic dwarfism or the current definition of Meier-Gorlin syndrome. However, all the patients could be encompassed by redefining MGS as a wider phenotype spectrum in light of the molecular genetics findings reported here.

Are there genotype-phenotype correlations?

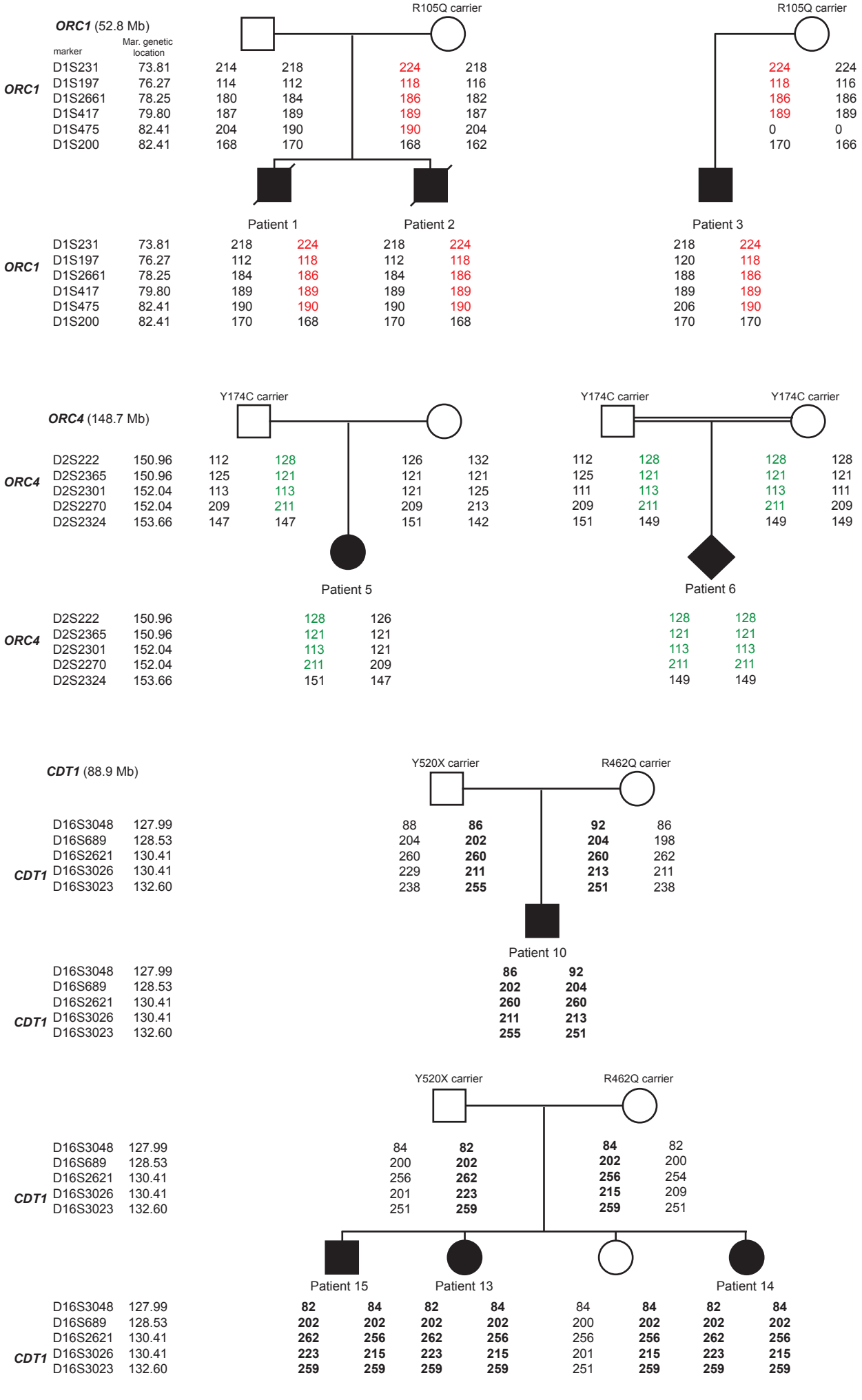
The large variation in phenotype associated with pre-replication complex mutations, also raises the question of phenotype-genotype correlations. Some preliminary observations may be made from the reported cases; however, further ascertainment of patients with preRC mutations will be necessary to substantiate these.

Compound heterozygous mutations appear to be associated with a more severe phenotype, particularly in the cases with *ORC1* mutations, when the presence of a frameshift mutation causes a severe developmental malformation syndrome. Similarly, a patient compound heterozygous for missense and null mutations in *ORC4* appears to be more severely affected: Patient P5, compound heterozygous for Y174C and A292fsX19 has absent patellae; while patients P6 and P7 with biallelic missense mutations (Y174C/Y174C) have patellae present, as well as milder growth impairment.
However other factors also appear to influence phenotype. For instance, case P18, with missense mutations in *CDC6* (T232R/T232R), has more severe growth impairment than some patients compound heterozygous for mutations in *CDT1*. This may imply that mutation of different genes contributes to variability. As well, it is possible that different genes result in different phenotypic manifestations. So far,

genu recurvatum and congenital emphysema have only been identified in *CDT1* and *ORC1* patients, while severe microcephaly (<-4 SD) is predominantly observed in *ORC1* patients.
Alternatively, the precise site and nature of a substituted amino acid may explain variable severity and phenotype expressivity. However, as intrafamilial variability is also seen, (for example P9 has hypoplastic patellae, while patellae are absent in his siblings), genetic background or stochastic effects may contribute as well to phenotype variability.

Finally, as mutations have only been identified in 18 of 33 patients screened, the genetic basis of a substantial proportion of Meier-Gorlin syndrome cases remains to be determined.

Supplementary Figure 1. Haplotype analysis of genes idnetified in MGS patients



Supplementary Figure 1. **Haplotype analysis of genes identified in MGS patients**.

Chromosomal haplotypes of microsatellites flanking the genomic location of *ORC1*, *ORC4* and *CDT1* in different family members. For *ORC1*, a common founder haplotype for the mutation R105Q was identified in two MGS families (highlighted in red). Analysis of *ORC4* identified a common haplotype in two MGS families for the Y174C mutation (highlighted in green). Analysis of *CDT1* did not identify any common haplotypes (mutation haplotypes highlighted in bold).

Supplementary Table 1. Major clinical findings in 18 Meier-Gorlin syndrome patients with mutations in the pre-replication complex

a. Anthropometric data

Patient	**P1**	**P2**	**P3**[1]	**P4**[2]	**P5**	**P6(twin)**[2]	**P7(twin)**[2]	**P8**[3]	**P9**[3]	**P10**[3]	**P11**	**P12**	**P13**[4]	**P14**[4]	**P15**[4]	**P16**	**P17**	**P18**[2]	***Total/range***
Family	F1	F1	F2	F3	F4	F5	F5	F6	F6	F6	F7	F8	F9	F9	F9	F10	F11	F12	
Gender	M	M	M	F	F	F	F	F	M	M	M	F	F	F	M	F	F	M	8M/10F
Mutations identified																			
Gene mutated	*ORC1*	*ORC1*	*ORC1*	*ORC1*	*ORC4*	*ORC4*	*ORC4*	*ORC6*	*ORC6*	*ORC6*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDC6*	
Amino acid alterations	R105Q	R105Q	R105Q	R105Q	Y174C	Y174C	Y174C	F86X	F86X	F86X	R462Q	A66T	R462Q	R462Q	R462Q	R462Q	Q361X	T323R	
	V667fsX24	V667fsX24	SS	SS	A292fsX19	Y174C	Y174C	Y232S	Y232S	Y232S	Y520X	SS/Q117H	Y520X	Y520X	Y520X	SS/Q117H	R453W	T323R	
Birth																			
Gestational age (weeks)	34	17	40	34	39	38	38	37	38	u	41	37	u	u	u	40	39	40	
Birth length (cm)	35	18	48	u	u	43	43	42	43	u	41	u	44	46	46	u	50	42.5	
Birth length SD[5]	-7.6 SD	NA	-3.3 SD			NA	NA	-4.1 SD	-4.5 SD		-6.9 SD		<-2SD	<-2 SD	<-3.5 SD		0 SD	-5.7 SD	-7.6 to 0 SD
Birth weight (kg)	1.18	0.13	<1.8	1.06	2.01	0.41	0.45	1.81	2.01	u	2.20	1.80	1.87	1.86	2.33	2.64	3.25	2.26	
Birth weight SD[5]	-5.5 SD	NA	<-5.9 SD	-5.9 SD	-4.1 SD	NA	NA	-3.8 SD	-3.9 SD		-3.9 SD	-3.8 SD	<-2 SD	<-2 SD	<-3.8 SD	-2.4 SD	-0.3 SD	-4.1 SD	<-5.9 to -0.3 SD
Birth head circumference (cm)	27	14	u	u	u	u	u	33	32	u	33	u	u	u	u	u	36.5	31	
Birth head circumference SD[5]	-3.6 SD	NA						-0.3 SD	-2.1		-2.2 SD		<-2 SD	>-2 SD			+1.5 SD	-3.7 SD	-3.7 to +1.5 SD
Current																			
Age	3m	NA	47y	15y	23y	5y 3m	5y 3m	14y 6m	15y 5m	4y 6m	8y 3m	7y 2m	16y 9 m	14y 4 m	17y 6m	4y 4m	5y	7y 0m	
Height (cm)	42	NA	132	123	127	94	95	143	154	94	104	100	137	139	166	93	108	103	
Height for age SD[6]	-9.6 SD		-6.6 SD	-6.9 SD	-6.4 SD	-4.2 SD	-4.1 SD	-3.3 SD	-2.4 SD	-3.2 SD	-4.7 SD	-5.1 SD	-4.7 SD	-3.9 SD	-1.6 SD	-3.3 SD	-0.4 SD	-4.1 SD	-9.6 to -0.4 SD
Weight (kg)	2.01	NA	72.1	18	23.6	11.3	11.4	48	34	14	12.6	11.6	31.1	28.8	49.9	10.3	15.5	11.9	
Weight for age SD[6]	-5.5 SD		+0.8 SD	-7.7 SD	-5.6 SD	-3.8 SD	-3.8 SD	-0.3 SD	-3.1 SD	-2.6 SD	-9.9 SD	-5.1 SD	-5.5 SD	-3.5 SD	-2.0 SD	-3.9 SD	-1.4 SD	-9.9 SD	-9.9 to +0.8 SD
Head circumference (cm)	28.5	NA	u	45.6	u	48.1	47	51.4	52	48.5	53	45.2	52	52.2	57.4	49.7	48	48	
Head circumference for age SD[6]	-9.8 SD			-4.0 SD		-2.1 SD	-3.0 SD	-1.6 SD	-2.1 SD	-2.3 SD	+0.1 SD	-5.0 SD	-1.3 SD	-1.0 SD	+1.7 SD	-0.5 SD	-2.1 SD	-3.3 SD	-9.8 to +1.7 SD

GA, gestational age; NA, not available; SD, standard deviation; SS, splice-site mutation; T, too young to ascertain; u, unknown.

[1-4]; Patients previously reported (See supplementary references)

[5,6]; Growth references (See supplementary references)

b. Clinical Features

Patient	P1	P2	P3[1]	P4[2]	P5	P6(twin)[2]	P7(twin)[2]	P8[3]	P9[3]	P10[3]	P11	P12	P13[4]	P14[4]	P15[4]	P16	P17	P18[2]	*Total*
Family	F1	F1	F2	F3	F4	F5	F5	F6	F6	F6	F7	F8	F9	F9	F9	F10	F11	F12	
Gender	M	M	M	F	F	F	F	F	M	M	M	F	F	F	M	F	F	M	8M/10F
Mutations identified																			
Gene mutated	*ORC1*	*ORC1*	*ORC1*	*ORC1*	*ORC4*	*ORC4*	*ORC4*	*ORC6*	*ORC6*	*ORC6*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDT1*	*CDC6*	
Amino acid alterations	R105Q	R105Q	R105Q	R105Q	Y174C	Y174C	Y174C	F86X	F86X	F86X	R462Q	A66T	R462Q	R462Q	R462Q	R462Q	Q361X	T323R	
	V667fsX24	V667fsX24	SS	SS	A292fsX19	Y174C	Y174C	Y232S	Y232S	Y232S	Y520X	SS/Q117H	Y520X	Y520X	Y520X	SS/Q117H	R453W	T323R	
Neonatal problems																			
Congenital lung emphysema	+	u	+	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	6/17
Respiratory problems	+	u	+	-	+	+	+	-	+	+	+	+	-	+	-	+	+	-	12/17
Early feeding problems	u	u	+	+	+	+	+	-	u	+	u	u	+	-	-	u	+	+	9/12
Craniofacial features																			
Microtia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	18/18
Low-set ears	+	+	+	+	u	-	-	-	-	-	+	+	+	+	+	+	-	+	11/17
Abnormally formed ears	+	+	+	+	u	+	+	+	+	-	+	-	+	+	+	+	+	+	15/17
Small mouth	+	+	+	-	u	+	+	-	-	+	+	+	-	-	-	-	-	-	8/17
Full lips	-	+	+	+	u	-	-	+	+	+	+	-	+	+	+	+	+	+	13/17
Maxillary hypoplasia	-	-	-	-	u	-	-	+	+	+	+	-	+	-	-	-	+	+	7/17
Mandibular hypoplasia	-	-	+	+	u	+	+	+	+	+	+	+	+	+	+	+	-	+	14/17
Skeletal anomalies																			
Absent patellae	+	u	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	14/17
Small patellae	-	u	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	1/17
Delayed bone age	u	u	+	+	u	+	+	+	+	-	u	u	+	-	u	u	+	+	9/11
Slender long bones	+	u	+	+	u	+	+	u	+	u	+	u	+	+	+	-	-	+	11/13
Genu recurvatum	+	+	+	-	-	-	-	-	-	-	-	+	u	u	u	+	-	-	5/15
Genitourinary anomalies																			
Cryptorchidism	+	-	+	NA	NA	NA	NA	NA	+	+	+	NA	NA	NA	-	NA	NA	+	6/8
Micropenis	+	-	-	NA	NA	NA	NA	NA	-	-	-	NA	NA	NA	-	NA	NA	+	2/8
Mammary glands																			
Hypoplasia	NA	NA	NA	+	+	T	T	+	NA	NA	NA	T	+	+	NA	T	T	NA	5/5
Development																			
Mental retardation	T	T	mild	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/11
Deceased	3.5 months	17 weeks GA																	2/18

GA, gestational age; NA, not available; SD, standard deviation; SS, splice site mutation; T, too young to ascertain; u, unknown.

[1-4]; Patients previously reported (See supplementary references)

[5,6]; Growth references (See supplementary references)

Supplementary Table 2. Primer sequences used for sequencing of genes.

ORC1-ex2F	TCATTTGAAGGGAGGGACTG	ORC4-ex2F	TGAAGCCATCGAAGATTTTG
ORC1-ex2R	GAACAGGCTAGATACAAGTTGACTG	ORC4-ex2R	TGTGCTGGCATCTCGTTAAG
ORC1-ex3F	GGATCTGAAGAGCTTGACCC	ORC4-ex3F	TGTGCTGGCATCTCGTTAAG
ORC1-ex3R	ATCCACAATCTCCCCACCTC	ORC4-ex3R	CAAAACCTGGAAGGCATCTG
ORC1-ex4F	GAATTGCTCAGTAAAGTGTCCTTG	ORC4-ex4F	GCTAGGCCAAGTACCCAGAG
ORC1-ex4R	CATGAAGTTCAGGAGGCAGAC	ORC4-ex4R	CACTAGGAGGACAAATAAGGGG
ORC1-ex5F	TACTCCTTGGCTCTCTTGGG	ORC4-ex5F	AATTTGAAAACTTACTTGTTAAAGAGG
ORC1-ex5R	TTTTATCACATAACCCTTTAGCCC	ORC4-ex5R	TGGTGTGCTAAGTAAAACATGG
ORC1-ex6F	TGTGATGAGATTGTGGGTTTG	ORC4-ex6F	AACTGGAAAACAACCTGCTG
ORC1-ex6R	TTTGACTATGACTCACATACTTCACG	ORC4-ex6R	GAATTTCTGAACCTCTCCCTACC
ORC1-ex7F	AGACTGGGAGCCAATCAGG	ORC4-ex7F	TTTGAGAATCAGACAGCCATC
ORC1-ex7R	CAATGGATTGGCAAATAAACAG	ORC4-ex7R	TTTGGCAAAAGCTTGTATTACC
ORC1-ex8F	CAGGAGGGACCCTGCAATAC	ORC4-ex8F	CCAAAGTAGGCTATCAGAATGTTTAC
ORC1-ex8R	TGTCCTCAGATTCCAGCTGTTAC	ORC4-ex8R	AACTAGCAGTGTCAGCAATTAAGC
ORC1-ex9F	ACATTTGCTGGTGTGTCCTG	ORC4-ex9F	AGCAAATGCCTGGAGGTG
ORC1-ex9R	GTGAATGAAAGCAGAGGCCC	ORC4-ex9R	TCCTTCAGCAATATTAGAAACTTTG
ORC1-ex10F	TGAGTAGATGGTTGGGGAATG	ORC4-ex10F	ACCAACCAGTAAGGCACAGG
ORC1-ex10R	TGAACTGTAACACCCAAGGC	ORC4-ex10R	AGTTCGCAACCAGTCTGAGC
ORC1-ex11-F	GTCTCGGTCCTTCCCTCAG	ORC4-ex11F	TCAGAAGTTTTGCACAGTATCTCC
ORC1-ex11R	CATGAACATGCAATACACGC	ORC4-ex11R	GCATTATGCCCACGTTAATTG
ORC1-ex12F	AAGCCCAAGGGAGTAAGACC	ORC4-ex12&13F	TGACCTTCTTCACCCCTCAG
ORC1-ex12R	CTTGCTCCTGAGGTACAGCC	ORC4-ex12&13R	GGGCAGTATACCTCCACAAAC
ORC1-ex13F	TGAAGCACCAGCTCAGTCTC	ORC4-ex14F	GCTTCCCAGTACTCTGTTCTGC
ORC1-ex13R	GCCACCACACCTGGACC	ORC4-ex14R	GGACAATAGTTTTCCGTTCTCTAC
ORC1-ex14F	CCACCTGGATAATCACTGGG	ORC6-ex1F	CGTCCTGTCCTAACCAATCC
ORC1-ex14R	CCAGCAGGTACTCAGCAAAG	ORC6-ex1R	TGAAGTAGGCCCTAAACCCC
ORC1-ex15F	ATCTCTTGGTTCTTCTGGGC	ORC6-ex2F	CCAACGTGTTGAGCAAACTTC
ORC1-ex15R	TGCAGGTTGAATGAGTAAAAGC	ORC6-ex2R	CCAAAAGTTAAAAGAGCTTCAATG
ORC1-ex16F	TCTTTGGCTTTCCTCTGGG	ORC6-ex3F	TGAAGCTAAACCTTTAGGTAGAGTGAC
ORC1-ex16R	CCATGTATTTTAAAAGCGTAAGTCC	ORC6-ex3R	CTGACCACATGATCCACCC
ORC1-ex17AF	CCAAATGAAGAAAGAAAGGAACA	ORC6-ex4F	TGGTTGATACTTTCAGTCTCTTTTC
ORC1-ex17AR	AGCCTGAGAAGTCAAGGCTG	ORC6-ex4R	TTAGAAGTATTCCTTGGGGATG
CDC6-ex2F	TTTGGATGTGAAGCAAAAGTG	ORC6-ex5F	TATCTGGTTGCCCAGAGAAG
CDC6-ex2R	AAAGCTCCTTCAAAGGACACAC	ORC6-ex5R	TTGACTTTTACAAGCAAAAGGACTAC
CDC6-ex3F	AAGAGGCAGAGGTCTTGCAC	ORC6-ex6F	CCAAAACATGCCCAAATACTG
CDC6-ex3R	TTTGGAGTCATCAGCCTTGG	ORC6-ex6R	AGCACTGGGCTGTTCACTG
CDC6-ex4F	CTCCAAATGAAACCACCCAC	ORC6-ex7F	GCTGTTTCTCCATGTCATTTCAG
CDC6-ex4R	CACCCAAGTATCATTTTACCAAG	ORC6-ex7R	TAAACAAAATCCCAAAGCCG

CDC6-ex5F	GCTCTTCAAAGACATTTTAGGC	CDT1-ex1F	CCGCCTCTTCCTCCCTTC
CDC6-ex5R	ATGGGCAACAGACCTTGC	CDT1-ex1R	CTCAGTTTCCCCGAGCC
CDC6-ex6F	TCAGCTTTAGGAAAGTGACCTG	CDT1-ex2-3F	TCCCAGTTAACTCAGAGCGG
CDC6-ex6R	CGGCAGAAAGCTGGTTTAAG	CDT1-ex2-3R	CTCCCTGGAAGGAGCAGAC
CDC6-ex7F	GCTTTCAGAAGATTTAGTTTTCCC	CDT1-ex4-5F	CTGCTGTGGCGTTGGAG
CDC6-ex7R	TTTCCTAGCACAGGTGATCC	CDT1-ex4-5R	ACCCACCCTGGAAACTGTG
CDC6-ex8F	TTGGGACCTAAATTACAGAGGG	CDT1-ex6F	GACTGGTCACGGGTGGG
CDC6-ex8R	CAGAGCCTCTTAAGCCACATTC	CDT1-ex6R	GGTTAGGTGCTGAGGCAGTG
CDC6-ex9F	CTGGTGGTTTGGTGTGTTTG	CDT1-ex7F	CGTAAGCACAGGCCTACCTC
CDC6-ex9R	TTCATCTTTCTACCTCTTTCCTCAG	CDT1-ex7R	AAGCTCATCACCAAGGCTTC
CDC6-ex10F	TTGAATTTCCTTTAGCTCAAACTTAG	CDT1-ex8-9F	AGAGATACCGGGGACTCCTG
CDC6-ex10R	TGAAGTATCATAGGCCTTTTGG	CDT1-ex8-9R	CTGCAGCACCAGGCAATTC
CDC6-ex11F	TTTTGCAAACCCAGACTCAG	CDT1-ex10F	GTTTCAAGTGCTGCCCG
CDC6-ex11R	AACGACTTGTTTAACTAACTGTGGTC	CDT1-ex10R	AAAGAAAATGCTGGTGGGC
CDC6-ex12F	CTGACAACTTTGCTTTTGTGAG		
CDC6-ex12R	GCACTAAAATGAAGACTGTAGCTCTC		

Supplementary References

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