Characterization of the Roco Protein Family in Dictyostelium discoideum

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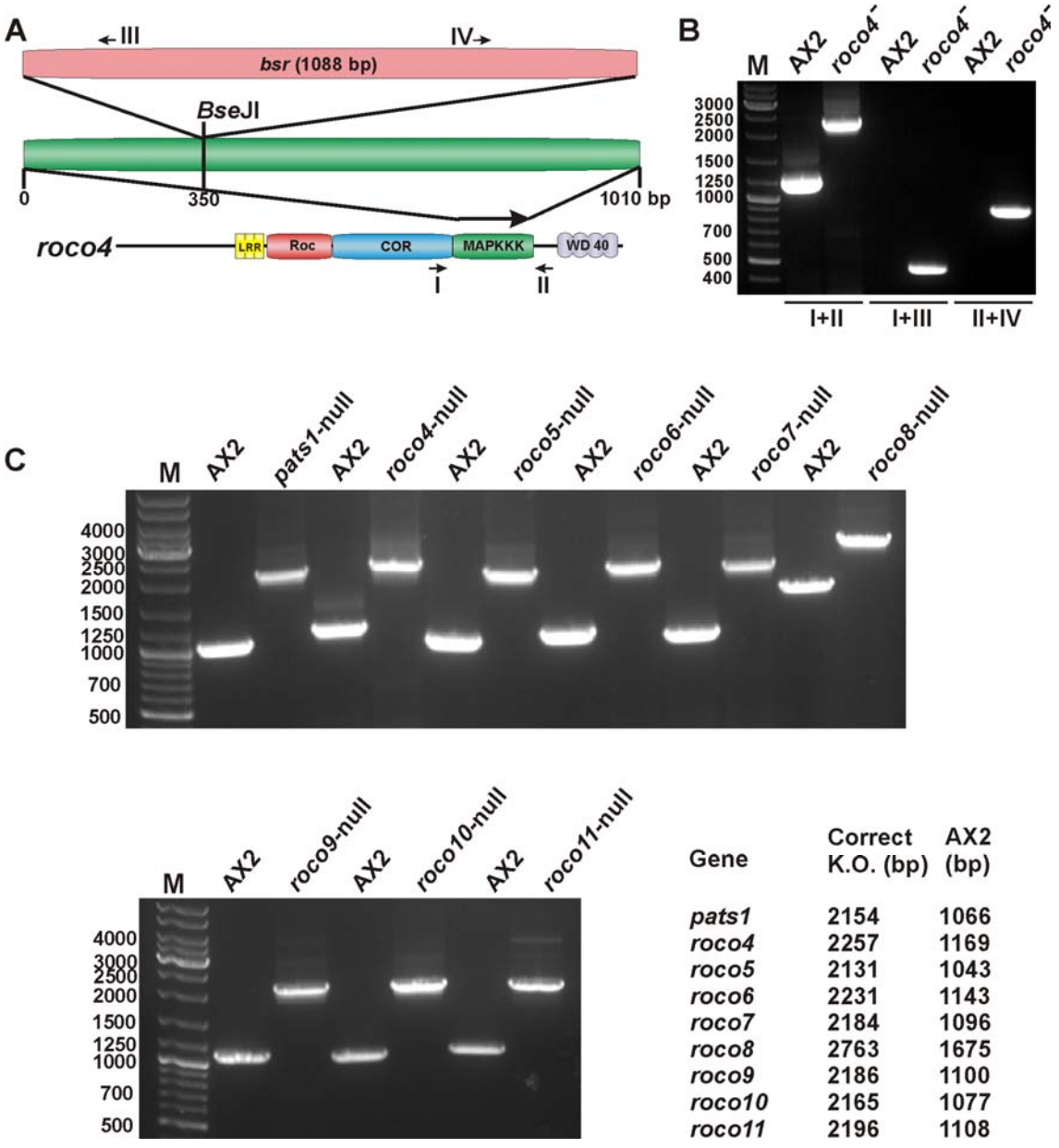
SUPPLEMENTAL INFORMATION



Gene	Correct K.O. (bp)	AX2 (bp)
pats1	2154	1066
roco4	2257	1169
roco5	2131	1043
roco6	2231	1143
roco7	2184	1096
roco8	2763	1675
roco9	2186	1100
roco10	2165	1077
roco11	2196	1108

Figure S1. Gene disruption of all members of the *Dictyostelium* Roco family. (A) Schematic drawing of *roco4* gene disruption. A knockout construct was made by insertion of a *bsr* cassette in the *Bse*JI site of the kinase domain of Roco4. Roman symbols refer to primer annealing sites for identification of correct integration events by PCR. **(B)** Identification of *roco4*-null cell line. gDNA was isolated from wild-type AX2 and potential knockout clones, and subjected to three PCR reactions. Primers I and II gave the expected products of 1169 and 2257 bp for AX2 and *roco4*-null respectively. Primers I and III and primers II and IV yielded no product for AX2 and bands of 451 and 862 bp for *roco4*-null respectively. **(C)** Identification of *roco* gene disruptions by PCR. Two primers that anneal just outside the knockout construct (Primers I and II for *roco4*) were used for PCR reactions with gDNA from wild-type AX2 and potential knockout clones as template. Clones with correct integration sites yield band shifts of around 1.1 kb, which is indicated in the figure.

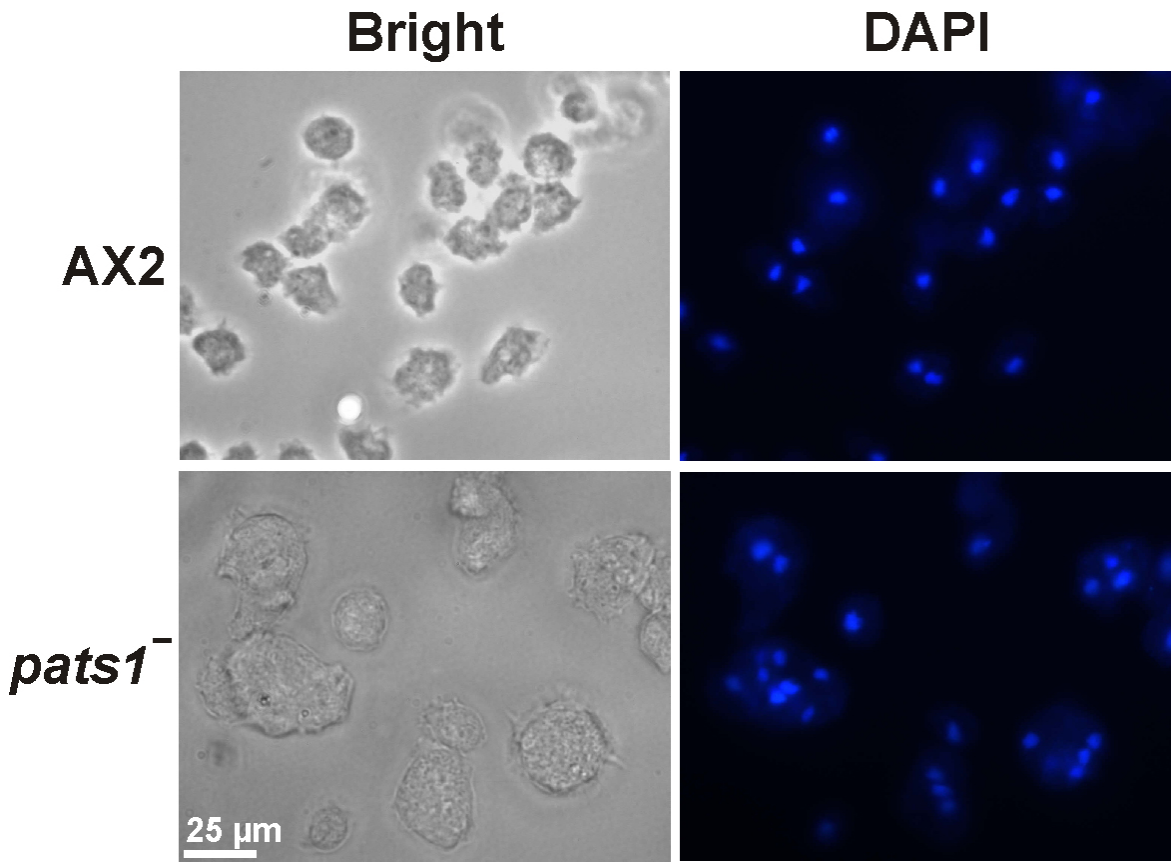


Figure S2. Visualization of nuclei in *pats1-null*. Wild-type and *pats1*-null cells were fixed with paraformaldehyde and stained with DAPI to visualize nuclei. A substantial fraction of *pats1*-null cells is multinucleated, while wild-type cells are mostly mononucleated.

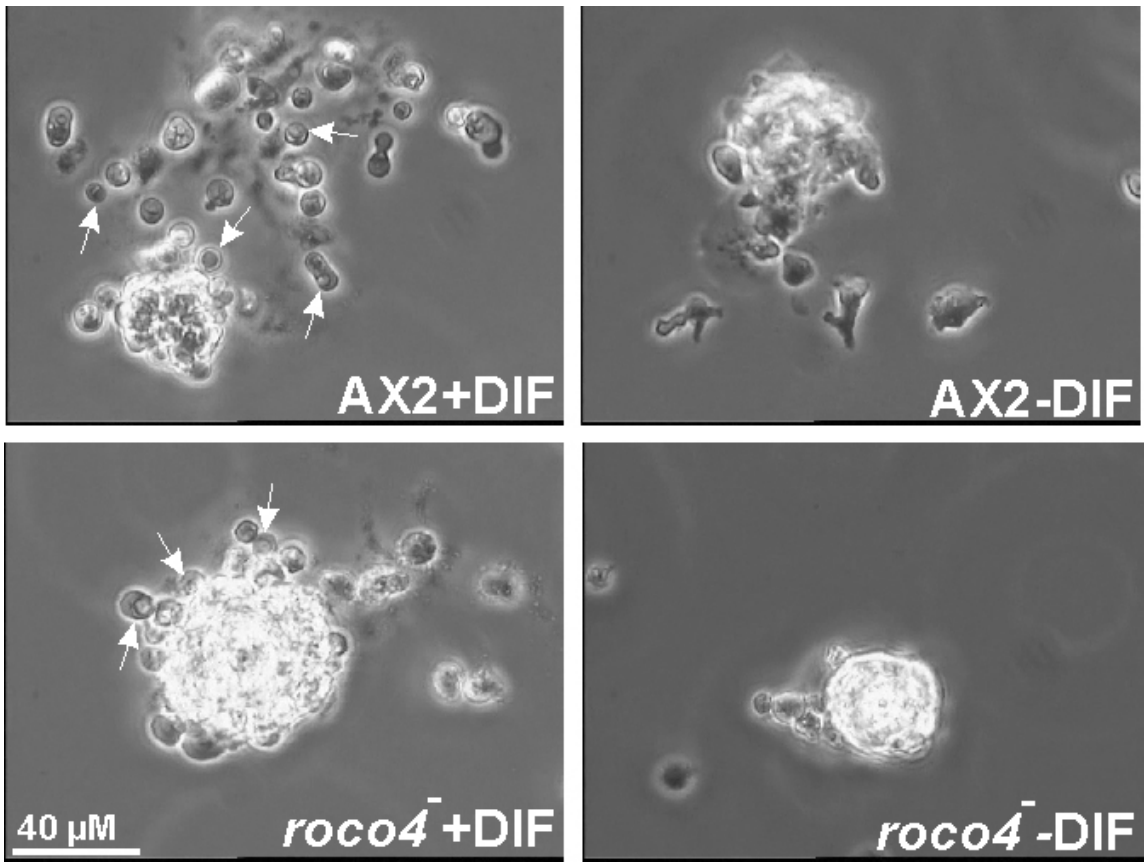


Figure S3. Differentiation of *roco4*-null in the presence of DIF. Exponentially growing cells in a 6-well plate were washed twice with phosphate buffer (PB) and incubated for 8 hours in PB+3mM cAMP at a density of 10^5 cells/cm^2. After two washes with PB, the cells were incubated in PB with or without 100 nM DIF. After 16 hours, cells were inspected for vacuolization and pictures were taken. Both wild-type and *roco4*-null cells were able to vacuolize in the presence of DIF, as appointed by white arrows.

-956 **a**ccggtcaaa tagtgtggtg cctgtaaaac aaaataaaat
-916 aagatattat tatatttta aaaaatataa acaaaaataa
-876 aataaaatta aaaatagtat tctcaaggta attaggttaa
-836 aatatta**c**ac acattgtatt atgatgaatt tgttttt**g**tt
-796 gtcacatcat *atttcattca ctca*ctt**c**ct tttaatttgt
-756 taagtttttt ttttaaaata taagtttttt accaaaattt
-716 aaatttttg tgcatattaa gtcacatcat ttttttaaaa
-676 acatatggat atatttgtgt gtgaatataa atgtgcgtgg
-636 ataaaaccca aaaatagagt gtgtttggaa aattataaaa
-596 tattatcttt ttttttttt ttttttttaa aatttattta
-556 tttattattt ttttttattt tttgaatggg tatcattata
-516 ttacatatat aatttttttt ttaataataa cacataacat
-476 aaaatttaaa tttattagtt ttttttatt ttattttatt
-436 atttttattt ttttttggtt ctttttttca tttattatat
-396 ttaaattatt atttttatt tatttttaat caataa**t**ata
-356 gaaccttaat aatagatata ttatttttt aaaaaaaaaa
-316 aaaaaaaaaa attttatacc cacatactaa ttttaatttc
-276 ttttttttt ttttcttttt ttttcttttt ttttttaaaa
-236 aaaaaaataa ttataacaat aatatagtaa tacaacttat
-196 aaatataata ttaatagtgt ataaatagat aaatagtaat
-156 actatatagt ttatatagaa atatataaat aaatagataa
-116 ttaattaata aataaataaa aaaaaaatat atatatataa
-76 tatcagtaa**c** attaaaaaag aaaggttaaa aaaaaaaaaa
-36 aaaaaaataa taaaaaaaaa ataaataaat aaaaaa**ATG**g
5 attcatcaca acaattacaa gaa **M**
D S S Q Q L Q E

Figure S4. Sequence of the putative *roco4* promoter. The starts of the promotor fragments are indicated as **bold**/underlined; a putative regulatory element starting at -783 is underlined/*italic*. The ATG start codon at position 1 is shown in capitals/**bold**/underlined. Translated amino acids are presented as single letters under the sequence between position 1-27.

Table SI. Primer sequences for expression of kinase domains and fabrication of KO-constructs.

Primer	**Sequence (5'-3')**
Pats1kinasefw	**AGATCT***AAAAAA*ATGACCTATGATGCAAATGTTAG
Pats1kinaserv	C**ACTAGT**AATATTTGATAAATGAATATCAGGAAAC
Roco4kinasefw	G**GGATCC***AAAAAA*ATGTCAATTCCAGTGCGTATCG
Roco4kinaserv	C**ACTAGT**ACTACCATCATCAGCATTTGAAGAGGTGG
Roco5kinasefw	G**AGATCT***AAAAAA*ATGCTTGAAATCATTGAAAAAGTTGGTG
Roco5kinaserv	**ACTAGT**TGAATTTGAGTTAAAACCAGTAGAGATTGTAGAC
Roco6kinasefw	**AGATCT***AAAAAA*ATGCAACCAACAAGTGATGAATC
Roco6kinaserv	C**ACTAGT**ACTAACAACTGAAACACCATTTACC
Roco7kinasefw	**GGATCC***AAAAAA*ATGATCGATATCTATTCATTGGC
Roco7kinaserv	C**ACTAGT**ATTTATTTGATCTTCATTAATTGGTGG
Roco8kinasefw	G**AGATCT***AAAAAA*ATGAAATTGGTTTCCCTTTG
Roco8kinaserv	C**ACTAGT**TTTCGAATTGATTGTTGAATCTTTAATTTC
Roco9fw	C**GGATCC***AAA*ATGACATCAATTGCTAATTTATTTG
Roco9rv	CTCTAATAAAATTGGAATTGATAAACC
Roco10kinasefw	G**AGATCT***AAAAAA*ATGCCAATTCGATCACTATTATTAG
Roco10kinaserv	**TCTAGA**GTTATTATCCGACGCTAAACTCTTATAAC
Roco11kinasefw	**GGATCC***AAAAAA*ATGGATTCAACTACCCCAGTCCG
Roco11kinaserv	C**ACTAGT**TTTAGCAATTTGTAATTTTGGAACTCC

The sequences contain restriction sites in **bold**, Kozak sequences in *italic* and start codons are underlined.

Table S2. Unique restriction sites for KO-constructs.

Gene	**Site**
pats1	*Eco*32I
roco4	*Bse*JI
roco5	*Sfu*I*
roco6	*Eco*105I
roco7	*Bgl*II*
roco8	*Nde*I*
roco9	*Bgl*II*
roco10	*Sty*I*
roco11	*Mfe*I*

Unique restriction sites were used to insert the *bsr* cassette. Asterisks refer to sticky sites that were made blunt for the construction of the KO-constructs.

Table S3. Primer sequences for identification of correct integration events.

Primer	**Sequence (5'-3')**
Pats1kofw	GTGAGAATGCCGCTGTAAAAGGCACTGGTTATCTCAAGTG
Pats1korv	GGTGAAGTTGATGATGATTTACTTGGAGAGGATTGTTG
Roco4kofw	GTAGTTGTATTTCTGCACTTCAAGATGGTAAACCACATC
Roco4korv	CAAATGATCTAGATGGTGATAAAGCAATACTACTACCAC
Roco5kofw	CCTGAATTAATGATGTCTGATATTGGTCCAAACTTTACCC
Roco5korv	GTGGATGAGGTTGAGGTGGTGAGGTTGTACCACTGCCAC
Roco6kofw	GTGGTGGATCACAACCACCATCACCAAGAAGTGGTAAAG
Roco6korv	GGTTGTTGTTGTTGATGATAATGGAATGGTTGACTATCAGC
Roco7kofw	CACTAGGTCAAACCAATGTAATTTGTAAAGCACAACTAGGTGG
Roco7korv	GGTTGTTGTTGTTGGGCGGCTTGTGATTGTGGAGGTGG
Roco8kofw	CCTTGCTCATGTAGTTGCGAATGTCGTGATTTACCACACC
Roco8korv	CGATTCTTACCTTAAATATTTATAACACCTAAAAGTCC
Roco9kofw	CCATCATTTTGGTGGTATTTAAAATTCGCACACACCTCAC
Roco9korv	GAATATGAATTACAACAACAACAACCACTACTAC
Roco10kofw	GAAAAGTTGAAATTTATAGAGATGATTCATTTTTGGTAAGATC
Roco10korv	GATAAATGATTGAACCACCAGATGGTAATGGATGGTCAG
Roco11kofw	TCACAATTATTGCTTGGTAAATCACAATTGGTATGTGG
Roco11korv	GAGTAAATGAATATCATTATTACCCATTTTTCAATTATAT

Table S4. Primer sequences for RT-PCR.

Primer	**Sequence (5'-3')**
RTGbpCfw	CGTGAATTAGAAACTGGTGCTAGACC
RTGbpCrv	CCACTATATACACTGATCTCTCTG
RTPats1fw	GATGGTTAGAGTTGATAATAC
RTPats1rv	CCAATGCTTTAAATAATATACC
RTQkgAfw	GCAAGAGCATGTACATTAGGTG
RTQkgArv	GTTATTCTATTATTACTCATATCC
RTRoco4fw	CTCATGCTTGTACAGTTGGTGATG
RTRoco4rv	GGATATCCTTTGGTAATTCGGTG
RTRoco5fw	GGAATTCAACTACTCAAGCG
RTRoco5rv	CAGCTGGGAAAGAACTACCACTAC
RTRoco6fw	GATACCGTTATGGTTTCCAGAGG
RTRoco6rv	CGTAGGATCACCTTATGATCAATCG
RTRoco7fw	GATCAAGCTAAACAATGTTCAACTG
RTRoco7rv	CCCATTATAATTCTAGGTGATCC
RTRoco8fw	GAATGCGTTGATTGGATTTTTGG
RTRoco8rv	CAACAGCAGTTGATGATTTACTG
RTRoco9fw	CGTCAAGATAATGGTTTATCAATTCC
RTRoco9rv	CCCAATAACCACCATATTGTGAG
RTRoco10fw	CGTTTACCTGAACCAATTATAAGTG
RTRoco10rv	CATACGTTCTTCAGGTGATTGG
RTRoco11fw	CAATTATTAACAAAAGCGTGTACAACTGG
RTRoco11rv	GCTAATTCCAATGGTAAATCATCC
RTIG7fw	TTACATTTATTAGACCCGAAACCAAGCG
RTIG7rv	TTCCCTTTAGACCTATGGACCTTAGCG

Table S5. Primer sequences for expression cloning of Roco4, QkgA and Roco11.

Primer	**Sequence (5'-3')**
Roco4fwA	C**GGATCC***AAA*ATGGATTCATCACAACAATTAC
Roco4rv1	CTCCAATGGTATATCTTCCAATAGATTACCACG
Roco4fw2	GAGTTAGATTTAAGTGATAATAAAATCACCG
Roco4rv2	CCCTTATGAACTAAACCAAAACCACCTTTACC
Roco4fw3	GTAGTTGTATTTCTGCACTTCAAGATGGTAAACCACATC
Roco4rvA	G**GGATCC**ACGGAAAAATTTTAATCTCGGTAAAATACC
QkgAfwA	C**ACTAGT***AAA*ATGGATTTAGAACAAGATGAATGGATG
QkgArv1	GTACCTGAAACTACCAATGATGATCCACTACT
QkgAfw2	GCAAGAGCATGTACATTAGGTG
QkgArvA	**ACTAGT**AATTGAAGCAGGATAATTTTTTAAAAATG
Roco11fwA	C**TCTAGA***AAA*ATGGAAACATCACAGATACGAAATGG
Roco11rv1	CTTTTATACCAGTACCATTTGTACAAGATAC
Roco11fw2	TCTGGTCTATCTGTACCAATG
Roco11rv2	CTGGAGCAATATAGTCAATACG
Roco11fw3	CAACAATCGATACACTATTATCAGG
Roco11rvA	G**TCTAGA**TTTAGCAATTTGTAATTTTGGAACTCC

The sequences contain *Bam*HI (Roco4), *Bcu*I (QkgA) and *Xba*I (Roco11) sites in **bold**, Kozak sequences in *italic* and start codons are underlined.

Table S6. Primer sequences for *roco4* promoters.

Primer	**Start bp**	**Sequence (5'-3')**
Prom4fwA	-956	**CTCGAG**ACCGGTCAAATAGTGTGGTGCCTGTAAAAC
Prom4fwB	-829	**CTCGAG**CACACATTGTATTATGATG
Prom4fwC	-799	**CTCGAG**GTTGTCACATCATATTTC
Prom4fwD	-769	**CTCGAG**CCTTTTAATTTGTTAAG
Prom4fwE	-705	**CTCGAG**GCATATTAAGTTGTGTATG
Prom4fwF	-360	**CTCGAG**TATAGAACCTTAATAATAG
Prom4fwG	-67	**CTCGAG**ACTATATAGTTTATATAG

The sequences contain *Xho*I sites in **bold**.

Table S7. Locus tags for phylogenetic analysis of the deduced Roco proteins.

Gene	***Dictyostelium discoideum***	***Dictyostelium purpureum***	***Dictyostelium fasciculatum***	***Polysphondylium pallidum***
gbpC/roco1	DDB0191359	DPU_G0059624	DFA_03461	PPL_12173
qkgA/roco2	DDB0185215	*Not present*	*Not present*	*Not present*
pats1/roco3	DDB0191503	DPU_G0070698	DFA_06290	PPL_08658
roco4	DDB0191509	DPU_G0058498	DFA_11519	PPL_09273
roco5	DDB0232931	DPU_G0063182	DFA_03850	PPL_10521
roco6	DDB0214834	DPU_G0065240	DFA_08323	PPL_12503
roco7	DDB0191295	DPU_G0059300	DFA_09719	PPL_05273
roco8	DDB0191480	DPU_G0058976	DFA_00686 DFA_00687 DFA_00688	PPL_04837
roco9	DDB0191512	DPU_G0072160	DFA_09477	PPL_07407 PPL_07408
roco10	DDB0201665	DPU_G0063892	DFA_00911	PPL_02805 PPL_02806
roco11	DDB0191297	*Not present*	*Not present*	*Not present*