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Characterization of the Roco Protein Family in Dictyostelium discoideum

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SUPPLEMENTAL INFORMATION

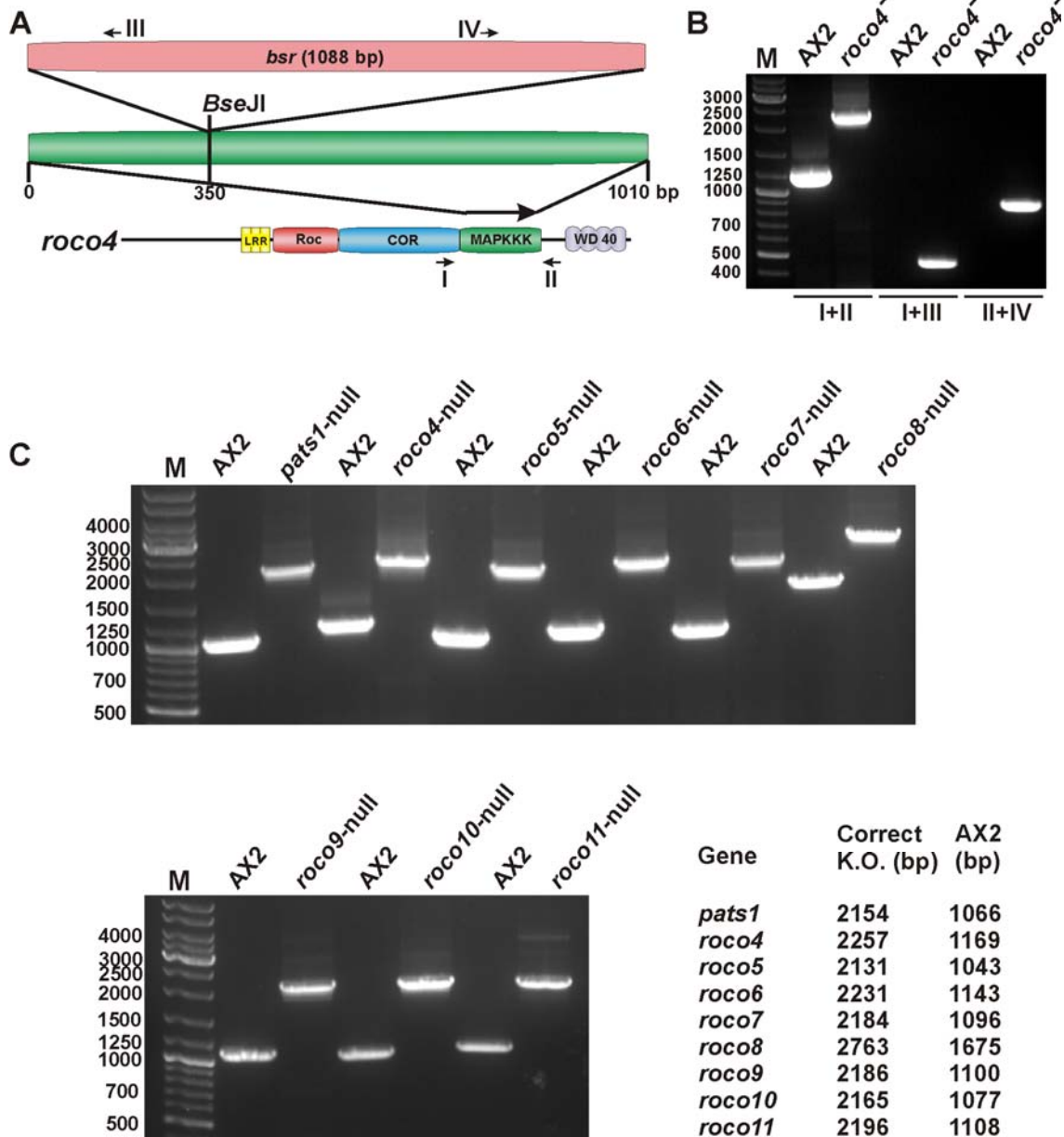


Figure S1. Gene disruption of all members of the *Dictyostelium* Roco family. (A) Schematic drawing of *roco4* gene disruption. A knockout construct was made by insertion of a *bsr* cassette in the *Bse*JI site of the kinase domain of Roco4. Roman symbols refer to primer annealing sites for identification 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.

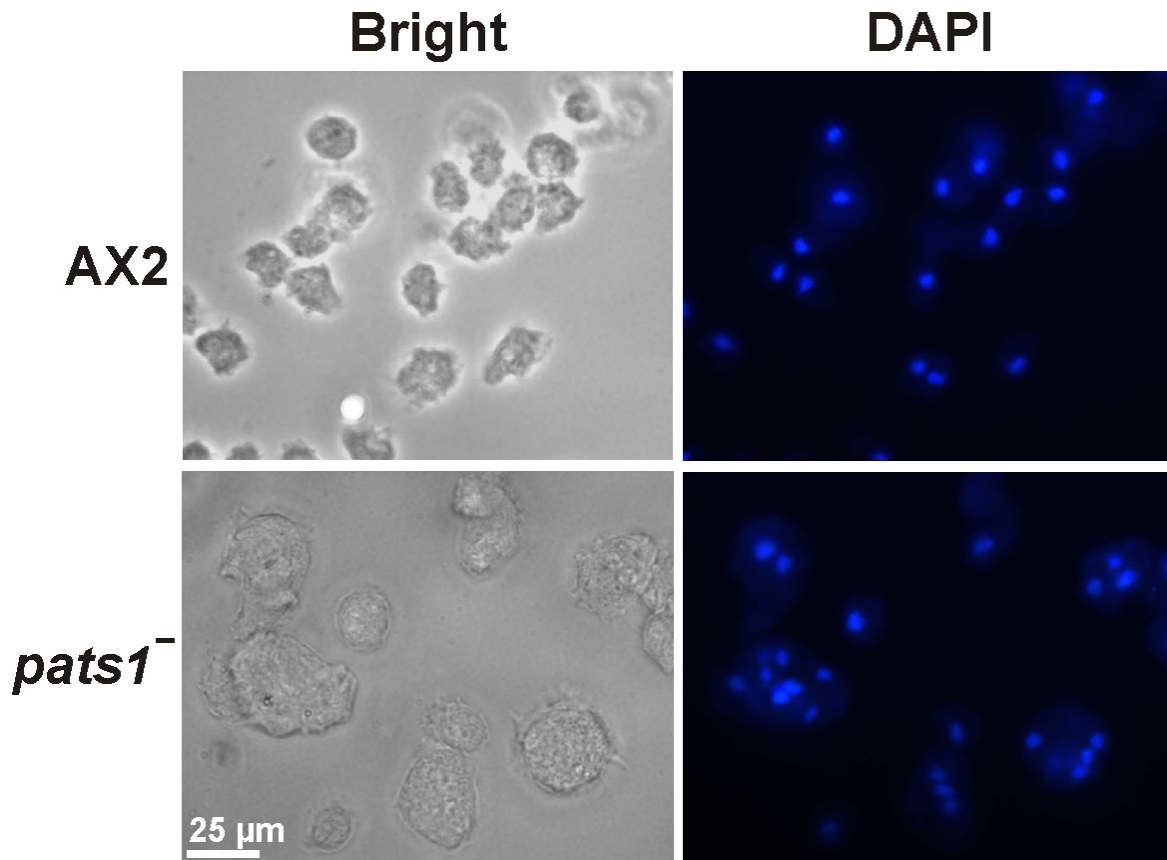


Figure S2. Visualization of nuclei in *pats1*-null. Wild-type and *pats1*-null cells were fixed with paraformaldehyde and stained with DAPI to visualize nuclei. A substantial fraction of *pats1*-null cells is multinucleated, while wild-type cells are mostly mononucleated.

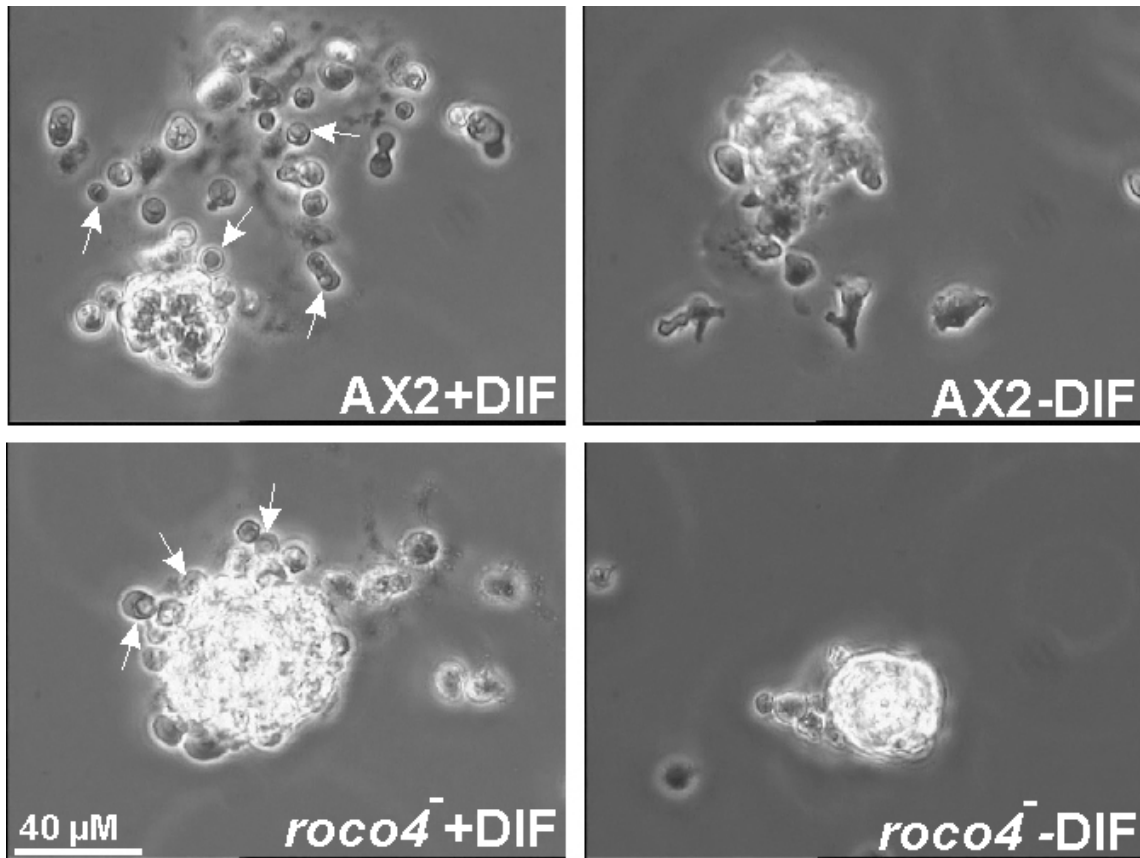


Figure S3. Differentiation of *roco4*-null in the presence of DIF. Exponentially growing cells in a 6-well plate were washed twice with phosphate buffer (PB) and incubated for 8 hours in PB+3mM cAMP at a density of 10^5 cells/cm². After two washes with PB, the cells were incubated in PB with or without 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.

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-956 accgggtcaaa tagtgtggtg cctgtaaaac aaaataaaat
-916 aagatattat tatatTTTTa aaaaatataa acaaaaataa
-876 aataaaatta aaaatagtat tctcaaggta attagggttaa
-836 aatattacac acattgtatt atgatgaatt tgtTTTTgtt
-796 gtcacatcat atTtcattca cTcacttcct tTtaattTgt
-756 taagTTTTTT tTTTaaaata taagTTTTTT accaaaattt
-716 aaTTTTTTTg tgcataTTaa gtcacatcat tTTTTTaaaa
-676 acatatggat atTTTTgtgt gtgaatataa atgtgCGTgg
-636 ataaaaccca aaaatagagt gtgTTTggaa aattataaaa
-596 tattatcTTT tTTTTTTTTT tTTTTTTTaa aatttattTa
-556 tTtattattt tTTTTtattt tTtgaatggg tatcattata
-516 tTacataTat aTTTTTTTTT tTaaataata cacataacat
-476 aaaattTtaa tTtattagTt tTTTTttatt tTattttatt
-436 atTTTTtatt tTTTTtggtt cTTTTTTTca tTtattatat
-396 tTaaattatt atTTTTtatt tattTTTaat caataatata
-356 gaacCTTaat aatagatata tTattTTTTT aaaaaaaaaa
-316 aaaaaaaaaa atTTTatacc cacataCTaa tTTTaattTc
-276 tTTTTTTTTT tTTTcTTTTT tTTTcTTTTT tTTTTTaaaa
-236 aaaaaaataa tTataacaat aatataGtaa tacaacttat
-196 aaatataata tTaatagtgt ataaatagat aaatagtaat
-156 actatatagt tTatatagaa atatataaat aaatagataa
-116 tTaatTaata aataaataaa aaaaaaatat atatatataa
-76 tatcagTaac attaaaaaag aaaggTtaa aaaaaaaaaa
-36 aaaaaaataa taaaaaaaaa ataaataaat aaaaaaATGg
5 attcatcaca acaattacaa gaa M
D S S Q Q L Q E

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Figure S4. Sequence of the putative *roco4* promoter. The starts of the promoter 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 S1. Primer sequences for expression of kinase domains and fabrication of KO-constructs.

Primer	Sequence (5'-3')
Pats1kinasefw	AGATCT <i>AAAAAAAA</i> <u>ATG</u> ACCTATGATGCAAATGTTAG
Pats1kinaserv	CACTAGT AATATTTGATAAATGAATATCAGGAAAC
Roco4kinasefw	GGGATCC <i>AAAAAAAA</i> <u>ATG</u> TCAATTCCAGTGCATCG
Roco4kinaserv	CACTAGT ACTACCATCATCAGCATTGAAGAGGTGG
Roco5kinasefw	GAGATCT <i>AAAAAAAA</i> <u>ATG</u> CCTGAAATCATTGAAAAAGTTGGTG
Roco5kinaserv	ACTAGT TTGAATTTGAGTTAAAACCAGTAGAGATTGTAGAC
Roco6kinasefw	AGATCT <i>AAAAAAAA</i> <u>ATG</u> CAACCAACAAGTGATGAATC
Roco6kinaserv	CACTAGT ACTAACAACCTGAAACACCATTACC
Roco7kinasefw	GGATCC <i>AAAAAAAA</i> <u>ATG</u> ATCGATATCTATTTCATTGGC
Roco7kinaserv	CACTAGT ATTTATTTGATCTTCATTAATTGGTGG
Roco8kinasefw	GAGATCT <i>AAAAAAAA</i> <u>ATG</u> AAATGGTTTTCCCTTTG
Roco8kinaserv	CACTAGT TTTTCGAATTGATTGTTGAATCTTTAATTTCC
Roco9fw	CGGATCC <i>AAAA</i> <u>ATG</u> ACATCAATTGCTAATTTATTTG
Roco9rv	CTCTAATAAAAATTGGAATTGATAAACC
Roco10kinasefw	GAGATCT <i>AAAAAAAA</i> <u>ATG</u> CCAATTCGATCACTATTATTAG
Roco10kinaserv	TCTAGAG TTATTATCCGACGCTAAACTCTTATAAC
Roco11kinasefw	GGATCC <i>AAAAAAAA</i> <u>ATG</u> GATTCAACTACCCCAGTCCG
Roco11kinaserv	CACTAGT 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
<i>pats1</i>	<i>Eco32I</i>
<i>roco4</i>	<i>BseII</i>
<i>roco5</i>	<i>SfiI</i> *
<i>roco6</i>	<i>Eco105I</i>
<i>roco7</i>	<i>BglIII</i> *
<i>roco8</i>	<i>NdeI</i> *
<i>roco9</i>	<i>BglIII</i> *
<i>roco10</i>	<i>StyI</i> *
<i>roco11</i>	<i>MfeI</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	CACTAGGTCAAACCAATGTAATTTGTAAAGCACAAGTGGTGG
Roco7korv	GGTTGTTGTTGTTGGGCGGCTTGTGATTGTGGAGGTGG
Roco8kofw	CCTTGCTCATGTAGTTGCGAATGTCGTGATTACCACACC
Roco8korv	CGATTCTTACCTTAAATATTTATAACACCTAAAAGTCC
Roco9kofw	CCATCATTGTTGGTGGTATTTAAAATTTCGCACACACCTCAC
Roco9korv	GAATATGAATTACAACAACAACCACTACTAC
Roco10kofw	GAAAAGTTGAAATTTATAGAGATGATTCATTTTTGGTAAGATC
Roco10korv	GATAAATGATTGAACCACCAGATGGTAATGGATGGTCAG
Roco11kofw	TCACAATTATTGCTTGGTAAATCACAATTGGTATGTGG
Roco11korv	GAGTAAATGAATATCATTATTACCATTTCATTATAT

Table S4. Primer sequences for RT-PCR.

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

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

Primer	Sequence (5'-3')
Roco4fwA	CGGATCCAAA <u>ATGGATTCATCACAACAATTAC</u>
Roco4rv1	CTCCAATGGTATATCTTCCAATAGATTACCACG
Roco4fw2	GAGTTAGATTTAAGTGATAATAAAATCACCG
Roco4rv2	CCCTTATGAACTAAACCAAAACCACCTTTACC
Roco4fw3	GTAGTTGTATTTCTGCACTTCAAGATGGTAAACCACATC
Roco4rvA	GGGATCCACGG AAAAATTTAATCTCGGTAAAATACC
QkgAfwA	CACTAGTAAA <u>ATGGATTTAGAACAAGATGAATGGATG</u>
QkgArv1	GTACCTGAACTACCAATGATGATCCACTACT
QkgAfw2	GCAAGAGCATGTACATTAGGTG
QkgArvA	ACTAGTA ATTGAAGCAGGATAATTTTTTAAAAATG
Roco11fwA	CTCTAGAAAA <u>ATGGAAACATCACAGATACGAAATGG</u>
Roco11rv1	CTTTTATACCAGTACCATTTGTACAAGATAC
Roco11fw2	TCTGGTCTATCTGTACCAATG
Roco11rv2	CTGGAGCAATATAGTCAATACG
Roco11fw3	CAACAATCGATACACTATTATCAGG
Roco11rvA	GTCTAGATTTAGCA ATTTGTAATTTTGGA ACTCC

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	CTCGAGACCGG TCAAATAGTGTGGTGCCTGTAAAAC
Prom4fwB	-829	CTCGAGCACAC ATTGTATTATGATG
Prom4fwC	-799	CTCGAGGTTG TACATCATATTTTC
Prom4fwD	-769	CTCGAGCCTTTT AATTTGTTAAG
Prom4fwE	-705	CTCGAGGCAT ATTAAGTTGTGTATG
Prom4fwF	-360	CTCGAGTATAG AACCTTAATAATAG
Prom4fwG	-67	CTCGAGACT TATATAGTTTATATAG

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

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

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