The Novel Chloroplast Outer Membrane Kinase KOC1 Is a Required Component of the Plastid Protein Import Machinery

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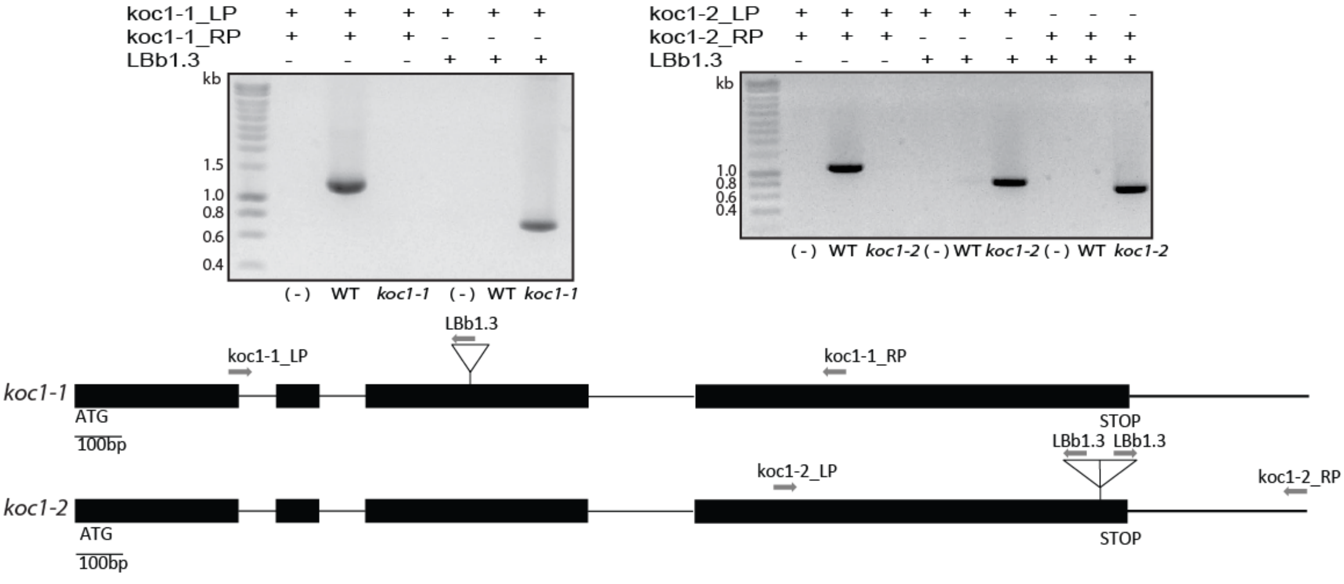

Figure S1.

FIGURE S1. Genotyping of *koc1* lines. Identification of the *koc1* homozygous T-DNA insertion mutants (*koc1-1* and *koc1-2*) by PCR. WT (Col-0) was used as a control, (-) negative control without DNA. Schematic representation of KOC1 gene indicating the T-DNA insertions (triangles), exons (black boxes) introns (thin lines) and primers koc1-1 or koc1-2 _LP or _RP and LBb1.3 (arrows).

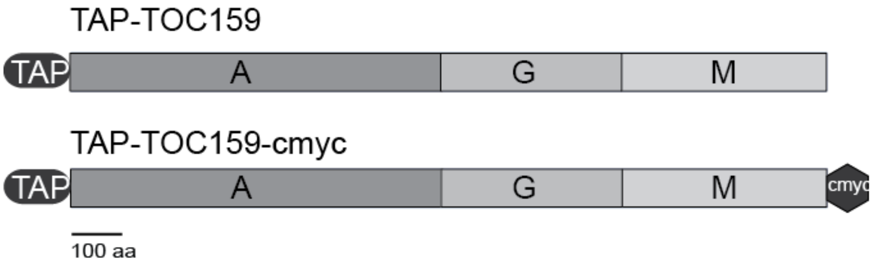

Figure S2.

FIGURE S2. NTAP-TOC159 constructs. Schematic representation of TAP-TOC159 and TAP-TOC159-cmyc constructs. The domains of TOC159 are illustrated: the N-terminal A domain, G domain and M domain.

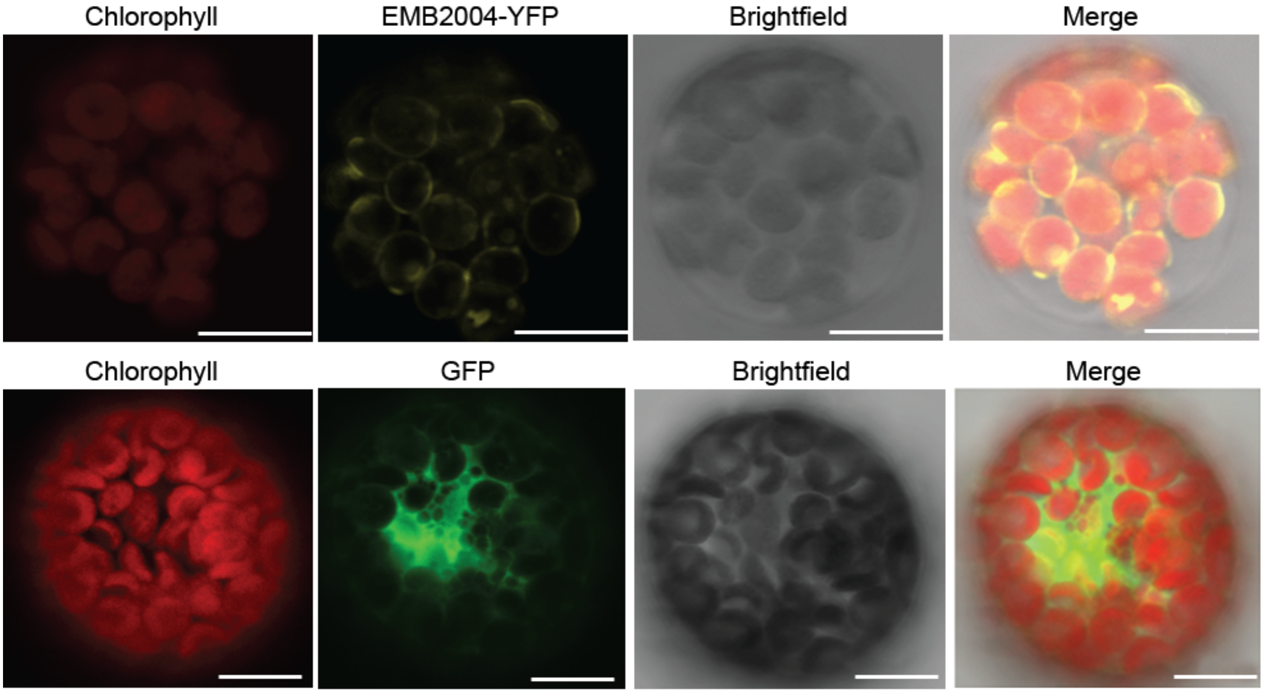

Figure S3.

FIGURE S3. Confocal microscopy images of an isolated representative protoplast expressing EMB2004-YFP and GFP. Chlorophyll fluorescence visualized in red identifying chloroplasts, EMB2004-YFP appears yellow, GFP in green. Intact protoplasts were visualized by bright field. Merge shows the overlay of chlorophyll, fluorescence of proteins and bright field images. Scale bars: 10µm.

Identifier	Annotation	fmol on column (negative control, replicate 1)	fmol on column (eluate, replicate 1)	fmol on column (negative control, replicate 2)	fmol on column (eluate, replicate 2)	Complete chloroplast reference	Complete chloroplast reference (fmol)	Enrichment index 1st replicate	Enrichment index 2nd replicate	avrg enrichment index	avrg fmol in eluate	% of bait
AT4G32250	Protein kinase superfamily protein; FUNCTIONS IN: protein kinase activity, kinase activity, ATP binding; INVOLVED IN: protein amino acid phosphorylation; LOCATED IN: chloroplast;	0.62613333333	34.9472	0.33516666667	10.9877	not detected	n.a.	n.a.	n.a.	infinite	22.96745	100
AT4G02510	An integral membrane GTPase that functions as a transit-sequence receptor required for the import of proteins necessary for chloroplast biogenesis. Located in the outer chloroplast membrane.	N.D.	6.4308	N.D.	1.45093333	not detected	n.a.	n.a.	n.a.	infinite	3.94086667	17.1584859
AT5G53170	encodes an FtsH protease that is localized to the chloroplast and the mitochondrion	N.D.	1.05953333	N.D.	2.45403333	not detected	n.a.	n.a.	n.a.	infinite	1.75678333	7.64901342
AT1G22530	PATELLIN 2 (PATL2); FUNCTIONS IN: transporter activity; INVOLVED IN: transport; LOCATED IN: plasma membrane, chloroplast	N.D.	1.61993333	N.D.	1.7089	not detected	n.a.	n.a.	n.a.	infinite	1.66441667	7.24685007
AT5G13490	Encodes mitochondrial ADP/ATP carrier	N.D.	2.2424	N.D.	0.9345	not detected	n.a.	n.a.	n.a.	infinite	1.58845	6.91609212
AT4G17530	AtRabD2c encodes a Rab GTPase, which plays important roles in pollen development, germination and tube elongation.	N.D.	2.14786667	N.D.	0.4689	not detected	n.a.	n.a.	n.a.	infinite	1.30838333	5.69668524
AT2G41840	Ribosomal protein S5 family protein; FUNCTIONS IN: structural constituent of ribosome; INVOLVED IN: translation; LOCATED IN: cytosolic small ribosomal subunit, cytosolic ribosome, nucleolus, membrane	N.D.	2.6055	N.D.		not detected	n.a.	n.a.	n.a.	infinite	1.30275	5.67215777
AT5G22640	EMB1211 is a MORN (multiple membrane occupation and recognition nexus) motif containing protein involved in embryo development and chloroplast biogenesis. The mRNA is cell-to-cell mobile.	N.D.	1.74823333	N.D.	0.74466667	not detected	n.a.	n.a.	n.a.	infinite	1.24645	5.42702825
AT2G36170	Ubiquitin supergroup;Ribosomal protein L40e; FUNCTIONS IN: structural constituent of ribosome; INVOLVED IN: translation; LOCATED IN: cytosolic large ribosomal subunit, nucleolus;	N.D.	1.76756667	N.D.	0.56953333	not detected	n.a.	n.a.	n.a.	infinite	1.16855	5.08785259
AT1G72150	novel cell-plate-associated protein that is related in sequence to proteins involved in membrane trafficking in other eukaryotes The mRNA is cell-to-cell mobile.	N.D.	1.2719	N.D.	1.01236667	not detected	n.a.	n.a.	n.a.	infinite	1.14213333	4.97283474
AT3G05590	Encodes cytoplasmic ribosomal protein L18.	N.D.	0.47013333	N.D.	1.681	not detected	n.a.	n.a.	n.a.	infinite	1.07556667	4.68300428
AT5G57350	member of Plasma membrane H+-ATPase family	N.D.	2.0477	N.D.		not detected	n.a.	n.a.	n.a.	infinite	1.02385	4.45783054
AT3G19820	Involved in the conversion of the early brassinosteroid precursor 24-methylenecholesterol to campesterol. Brassinosteroids affect cellular elongation. Mutants have dwarf phenotype. DWF1 is a Ca2+ dependent calmodulin-binding protein.	N.D.	1.804	N.D.	0.23593333	not detected	n.a.	n.a.	n.a.	infinite	1.01996667	4.44092255
AT1G22780	S18 ribosomal protein involved in the binding of f-Met tRNA during initiation of mRNA translation. Expression restricted to meristems. Mutant phenotype-pointed first leaves,reduced fresh weight, growth retardation.	N.D.	1.60403333	N.D.	0.34843333	not detected	n.a.	n.a.	n.a.	infinite	0.97623333	4.25050815
AT2G45820	Remorin family protein; FUNCTIONS IN: DNA binding; INVOLVED IN: biological_process unknown; LOCATED IN: plasma membrane;	N.D.	1.3562	N.D.	0.58653333	not detected	n.a.	n.a.	n.a.	infinite	0.97136667	4.22931874
ATMG01190	ATPase subunit 1	N.D.	1.4399	N.D.	0.37563333	not detected	n.a.	n.a.	n.a.	infinite	0.90776667	3.95240511
AT1G33120	Ribosomal protein L6 family; FUNCTIONS IN: structural constituent of ribosome, rRNA binding; INVOLVED IN: translation; LOCATED IN: in 7 components	N.D.	1.51756667	N.D.	0.19406667	not detected	n.a.	n.a.	n.a.	infinite	0.85581667	3.72621543
AT3G04340	embryo defective 2458 (emb2458); FUNCTIONS IN: nucleoside-triphosphatase activity, ATPase activity, metalloendopeptidase activity, nucleotide binding, ATP binding; INVOLVED IN: embryo development ending in seed dormancy; LOCATED IN: chloroplast	N.D.	1.14196667	N.D.	0.2685	not detected	n.a.	n.a.	n.a.	infinite	0.70523333	3.07057742
AT1G79560	Encodes an FtsH protease that is localized to the chloroplast. Mutations in this locus result in embryo lethality.	N.D.	1.09513333	N.D.	0.30153333	not detected	n.a.	n.a.	n.a.	infinite	0.69833333	3.0405349
AT5G43745	Protein of unknown function (DUF1012); LOCATED IN: chloroplast, chloroplast envelope;	N.D.	0.8634	N.D.	0.4881	not detected	n.a.	n.a.	n.a.	infinite	0.67575	2.94220734
AT5G22830	Transmembrane magnesium transporter. One of nine family members.	N.D.	0.975	N.D.	0.3706	not detected	n.a.	n.a.	n.a.	infinite	0.6728	2.92936308
AT5G64580	AAA-type ATPase family protein; FUNCTIONS IN: nucleoside-triphosphatase activity, metalloendopeptidase activity, nucleotide binding, ATP binding; INVOLVED IN: embryo development; LOCATED IN: chloroplast, chloroplast envelope	N.D.	0.99913333	N.D.	0.26546667	not detected	n.a.	n.a.	n.a.	infinite	0.6323	2.75302657
AT5G42480	Shows homology to the cyanobacterial cell division protein Ftn2, mutant only has two mesophyll cell chloroplasts. Protein was localized to a ring at the center of the chloroplasts.	N.D.	0.9325	N.D.	0.18863333	not detected	n.a.	n.a.	n.a.	infinite	0.56056667	2.4407005
AT2G36160	Ribosomal protein S11 family protein; FUNCTIONS IN: structural constituent of ribosome; INVOLVED IN: translation; LOCATED IN: cytosolic small ribosomal subunit, cytosolic ribosome, plasma membrane, chloroplast, membrane;	N.D.	0.846	N.D.	0.2729	not detected	n.a.	n.a.	n.a.	infinite	0.55945	2.43583855
AT3G01290	SPFH/Band 7/PHB domain-containing membrane-associated protein family; INVOLVED IN: N-terminal protein myristoylation; LOCATED IN: mitochondrion, plasma membrane, vacuole, membrane	N.D.	0.51386667	N.D.	0.59723333	not detected	n.a.	n.a.	n.a.	infinite	0.55555	2.41885799
AT5G03910	member of ATH subfamily	N.D.	0.8718	N.D.	0.23696667	not detected	n.a.	n.a.	n.a.	infinite	0.55438333	2.41377834
AT5G56030	A member of heat shock protein 90 (HSP90) gene family. Expressed in all tissues and abundant in root apical meristem, pollen and tapetum. Expression is NOT heat-induced but induced by IAA and NaCl. Interacts with HsfA1d in the cytosol and the nucleus and	N.D.	1.0272	N.D.	0.04696667	not detected	n.a.	n.a.	n.a.	infinite	0.53708333	2.33845435
AT5G14030	translocon-associated protein beta (TRAPB) family protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown;	N.D.	0.8145	N.D.	0.18113333	not detected	n.a.	n.a.	n.a.	infinite	0.49781667	2.16748776
AT2G19730	Ribosomal L28e protein family; FUNCTIONS IN: structural constituent of ribosome; INVOLVED IN: translation, ribosome biogenesis; LOCATED IN: in 6 components	N.D.	0.72103333	N.D.	0.2547	not detected	n.a.	n.a.	n.a.	infinite	0.48786667	2.12416558
AT4G23940	Encodes FtsHi1. Localizes to the chloroplast envelope membrane. Functions in chloroplast biogenesis and division.	N.D.	0.72396667	N.D.	0.25003333	not detected	n.a.	n.a.	n.a.	infinite	0.487	2.12039212
AT5G45775	Ribosomal L5P family protein; FUNCTIONS IN: structural constituent of ribosome; INVOLVED IN: translation; LOCATED IN: cytosolic large ribosomal subunit, vacuole	N.D.	0.73806667	N.D.	0.22373333	not detected	n.a.	n.a.	n.a.	infinite	0.4809	2.09383279
AT3G61260	Remorin family protein; FUNCTIONS IN: binding; INVOLVED IN: biological_process unknown; LOCATED IN: plasma membrane, vacuole; EXPRESSED IN: 24 plant structures; EXPRESSED DURING: 16 growth stages; CONTAINS InterPro DOMAIN/s: Remorin, C-terminal (InterPro:	N.D.	2.45243333	N.D.	1.38516667	not detected	0.077866667	31.4952911	17.7889555	24.6421233	1.9188	8.35443203
AT3G17970	Integral chloroplast outer membrane protein. Belongs to one of the 36 carboxylate clamp (CC)-tetratricopeptide repeat (TPR) proteins (Prasad 2010, Pubmed ID: 20856808) with potential to interact with Hsp90/Hsp70 as co-chaperones.	N.D.	1.86883333	N.D.	0.5175	AT3G17970	0.109733333	17.0306804	4.71597813	10.8733293	1.19316667	5.19503326
AT5G05000	Outer membrane GTPase protein that may function in import of nuclear encoded proteins into the chloroplast. Phosphorylation of the G-domains regulate translocon assembly.	N.D.	3.00213333	N.D.	1.28286667	AT5G05000	0.216633333	13.858132	5.92183413	9.88998307	2.1425	9.32841913
AT5G42020	Luminal binding protein (BiP2) involved in polar nuclei fusion during proliferation of endosperm nuclei.	N.D.	1.8882	N.D.	0.65423333	AT5G42020	0.1303	14.4911742	5.02097723	9.75607572	1.27121667	5.53486202
ATCG01130	YCF1.2; FUNCTIONS IN: protein binding; LOCATED IN: chloroplast, membrane; EXPRESSED IN: cultured cell, leaf; CONTAINS InterPro DOMAIN/s: Ycf1 (InterPro:IPR008896); BEST Arabidopsis thaliana protein match is: Ycf1 protein (TAIR:ATCG01000.1).	N.D.	3.65786667	N.D.	0.7971	ATCG01130	0.265366667	13.784198	3.00376837	8.39398317	2.22748333	9.69843554
AT3G49860	A member of ARF-like GTPase family. A thaliana has 21 members, in two subfamilies, ARF and ARF-like (ARL) GTPases.	N.D.	0.51023333	N.D.	0.1792	AT3G49860	0.071166667	7.16955504	2.51803279	4.84379391	0.34471667	1.5008922
AT1G10510	embryo defective 2004 (emb2004); INVOLVED IN: embryo development ending in seed dormancy; LOCATED IN: mitochondrion, chloroplast, plastid, chloroplast envelope	N.D.	1.73596667	N.D.	0.48246667	AT1G10510	0.336333333	5.16144698	1.43448959	3.29796829	1.10921667	4.82951597
AT1G02280	Encodes a GTP-binding GTP-ase. Component of the chloroplast protein import machinery. Required for import of POR B into plastids. Toc33 phosphorylation may not play an important role in vivo.	N.D.	3.2534	N.D.	0.7088	AT1G02280	0.653133333	4.98121874	1.08523017	3.03322446	1.9811	8.62568548
AT3G18420	Protein prenylyltransferase superfamily protein; FUNCTIONS IN: binding; INVOLVED IN: biological_process unknown; LOCATED IN: chloroplast, chloroplast envelope	N.D.	0.45073333	N.D.	0.30356667	AT3G18420	0.145233333	3.10351159	2.09019968	2.59685563	0.37715	1.64210655

AT2G43630	FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: chloroplast thylakoid membrane, chloroplast, nucleus, chloroplast envelope	N.D.	1.4308	N.D.	0.65796667	AT2G43630	0.484433333	2.95355398	1.35821923	2.1558866	1.04438333	4.54723242
AT5G14040	Encodes a mitochondrial phosphate transporter. Modulates plant responses to salt stress.	N.D.	0.89613333	N.D.	0.25456667	AT5G14040	0.3131	2.86213137	0.81305227	1.83759182	0.57535	2.50506695
AT3G46740	Component of the translocon outer membrane (TOC) complex. Forms the outer envelope translocation channel (beta-barrel). Plays a role in preprotein conductance. Imported into chloroplast. Expressed in young dividing photosynthetic tissues.	N.D.	8.04253333	N.D.	1.84283333	AT3G46740	2.8509	2.82105066	0.64640406	1.73372736	4.94268333	859.074187
AT5G23040	Encodes a protein that enables protochlorophillide's binding to pPORA's transit sequence, regulating pPORA's translocation into the plastid stroma, and blocking movement of the translocating polypeptide chain back into the cytosol.	N.D.	0.99363333	N.D.	0.31663333	AT5G23040	0.4071	2.44075985	0.77777778	1.60926881	0.65513333	2.85244262
AT3G12580	heat shock protein 70 (HSP70); FUNCTIONS IN: ATP binding; INVOLVED IN: in 9 processes; LOCATED IN: cytosol, mitochondrion, cell wall, plasma membrane.	-	0.2278	N.D.	0.16866667	AT3G12580	0.1409	1.61674947	1.19706648	1.40690797	0.19823333	0.86310554
AT4G00630	Encodes a K(+)/H(+) antiporter that modulates monovalent cation and pH homeostasis in plant chloroplasts or plastids.	N.D.	1.0542	N.D.	0.2698	AT4G00630	0.472666667	2.2303244	0.57080395	1.40056417	0.662	2.88234001
AT2G43950	Constitutes a peptide sensitive ion channel in chloroplast outer membranes. Accumulates in germinating seeds and developing embryos.	N.D.	2.5375	N.D.	0.7826	AT2G43950	1.199833333	2.11487707	0.65225726	1.38356716	1.66005	7.22783766
AT3G10840	alpha/beta-Hydrolases superfamily protein; FUNCTIONS IN: hydrolase activity, catalytic activity; LOCATED IN: chloroplast envelope	N.D.	0.81403333	N.D.	0.29863333	AT3G10840	0.4654	1.74910471	0.64167025	1.19538748	0.55633333	2.42226862
AT5G23890	LOCATED IN: mitochondrion, chloroplast thylakoid membrane, chloroplast, plastid, chloroplast envelope; EXPRESSED IN: 24 plant structures; EXPRESSED DURING: 14 growth stages; CONTAINS InterPro DOMAIN/s: S-layer homology domain (InterPro:IPR001119)	N.D.	1.30993333	N.D.	0.239	AT5G23890	0.653966667	2.00305826	0.36546205	1.18426016	0.77446667	3.37201852
AT1G77590	Encodes major plastidic long chain acyl-CoA synthetase with a slight substrate preference of oleic acid over any of the other fatty acids.	N.D.	1.2093	N.D.	0.30783333	AT1G77590	0.704	1.71775568	0.43726326	1.07750947	0.75856667	3.30279011
AT3G01500	Encodes a putative beta-carbonic anhydrase betaCA1. Together with betaCA4 (At1g70410) regulates CO2-controlled stomatal movements in guard cells.	N.D.	2.4029	0.59603333333	32.4081667	AT3G01500	16.37386667	0.14675214	1.97926167	1.06300691	17.4055333	75.783482

Table S1: Identification of KOC-interacting proteins and quantification by MSE. Proteins interacting with KOC were quantified by MSE and their quantities related to their abundance in a chloroplast reference proteome. The enrichment index is arbitrary and calculated from the abundance of the protein in the eluates divided by their abundance in complete chloroplasts without prior enrichment. Only those proteins identified in two biological replicates, no detection in the negative controls, an enrichment index above 1 and an absolute abundance of at least 2% of the bait were retained in the list and are displayed here.

Primer name	**Primer sequence**
KOC1_NheI_F	5’-GAGAGAGCTAGCATGGCTTCAAAGATTATT-3’
KOC1_NotI_R	5’-TCTCTCGCGGCCGCAAAGAACACAAGCCA-3’
KOC1_NcoI_F	5’-GAGACCATGGCTTCAAAGATTATTGC-3’
KOC1_NcoI_R	5’- TCTCCCATGGCTTGCAGATGATCCTTTGAA-3’
KOC1_NcoI_R2	5’- TCTCTCGCGGCCGCATCCTCCAGATGTTGA-3’
KOC1_FL_NcoI_F	5’-TACCATGGCTAGCATGGCTTCAAAG-3’
KOC1_FL_XbaI_R	5’-TCTCTAGACTAAAAGAACACAAGCCATTTAG-3’
LBb1.3 (Salk Institute)	5’-ATTTTGCCGATTTCGGAAC-3’
koc1-1_LP	5’- CAGGGGAGTCTCTAGTATCA-3’
koc1-1_RP	5’-TAG CAC AAA TCC ATC TGG GTC-3’
koc1-2_LP	5’-AGAACATGGATGTGCCAGAAG-3’
koc1-2_RP	5’-CGCTGCATATACCATGTGATG-3’
Toc159-StuI-F	5’-CAAATTCTCTATTGAGGCCTCAAGAACCATTG-3’
Toc159-cmyc-Gib-R	5’-CTTCTTCAGAGATCAGTTTCTGTTCTACGTACATGCTGTACTTGTC-3’
cmyc-uniGib-R	5’-CTGCAGGTCGACTCTAGATTACAGATCTTCTTCAGAGATC-3’
attB-KOC1-FW	5’- ACAAGTTTGTACAAAAAAGCAGGCTCCGCTTCAAAGATTATTGCTGG-3’
attB-KOC1-REV	5’- ACCACTTTGTACAAGAAAGCTGGGTCTAAAAGAACACAAGCCATT-3’
attB-EMB2004-FW	5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGCTTCTTCTTCCACCAG-3’
attB-EMB2004-REV	5’-GGGGACCACTTTGTACAAGAAAGCTGGGTGGAAGAAAATTTCTACTTCC-3’

TABLE S2: List of primers used for cloning and genotyping