

Supplementary Materials

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A new family of transcriptional regulators activating biosynthetic gene clusters for secondary metabolites

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Aur1P	1	-----MNQRTMAVLEPHTVAAG-ANGGFSPAEGPRTPOHVPA
FarR1	1	-----MNQRTFAVLEPHTTAAGTANGAFLPAEGLRVPPHAPA
PgaR1	1	-----MNQQVIDPLGPHRTKRHRPSTPLPRHAATPTPRLPQASAA
Med-ORF30	1	-----
Chai	1	-----MRSDALLTVIARTEHQE
GcnR	1	-----MRNASVRPEAR
SchA25	1	MGEYRMSNQSTKFLPAIPQQLANRRAPTSPRPVSQHAQQHPPIQAQQLPPIQAQPQLRK

Aur1P	37	QSALWRLILIVESNSAEAEETLARGLRHGHVEVDIVGTGGEALRVYAEADLVLLDLELPDLD
FarR1	38	QSALWRLILIVESSTVEAEETLARGLRHGHVEVDIVGTGSEALRGYADADLVLLDLELPDLD
PgaR1	41	EHRALRVLVVENEAAAADALVQGLMRQGHHAHVDTGGAAMNAHNADLVLLDLELPDLD
Med-ORF30	1	----MRVLVVEDERDQAESLIDGLRRHGHDTIVAGSGAEATDAFENADLVLLDLELPDLD
Chai	18	TGGQMRVLVAEPDCESAKLLRQHMRRHGYETEIADTGAGALGSYQYEDLLMLDLELPDLD
GcnR	13	VRKPVRLAVESDSEVAKGLVRDLWRHGYQAESVPTGAQALQQYRNADLVLLDLELPDLD
SchA25	61	ARGVLRVLVVESGAPAAQSLVLSLARHGYQAVSVDTGSKALQKYREADVLVLLDLELPDLD

Aur1P	97	GLEVCRGIRAAHEIPVIAVTARGSELDRVLGLOAGAADDYLAKPYGFRELMARMEAVMRR-
FarR1	98	GLEVCRGIRAAHEIPVIAVTARGSELDRVLGLOAGAADDYLKPYGFRELMARMEAVMRR-
PgaR1	101	GLEVCRGIRAVSDTPIIAVTARGNELDRVLGLOAGSDDYLKPYGFRELMARIEAVMRR-
Med-ORF30	57	GLEVCRRMRAAGDTPIISFAGESAEELDRVLGLOAGSDDCLERPYSFRELMARIDAVMRR-
Chai	78	GLEVCRSIRTTSDPIIAFTSHGTELDRVLGLOAGCDDCLVKPFGFRELVARIEAMRR-
GcnR	73	GVELCRSIRALCDVPIIVVTARDELDRLVMSLRAGSDDYVVKPYSLAELIARIEAVMRR-
SchA25	121	GIEICRFVRAASGVGVIVVTARDELDRLVLSLKAGAADDYVVKPYSLELMARIEAVMRR-

Aur1P	156	ARPTR--PASATITCGPLSIDSGTREVTLDGVPVETTRKEFDMLYLLASHEDTVIPRKML
FarR1	157	ARPSR--PAAATITCGPLRIDAGTREVTLHGEPVETTRKEFDMLYLLASHEDTVIPRKML
PgaR1	160	SGPRT--ETARATSHGPLHIDSARREVTLDGDSVELTRKEFDLLHLLALRPGAVISRRL
Med-ORF30	116	VGARIGGQRRPTLSRGGLSLDSESRREVLDGRPIPLTRKEFDLLHYLASRPGVVSQRRL
Chai	137	IRPRA--VRSQRLSHGPLHIDRDRREVTLDHTQVKVTRKEFDLLQFLASQPTTVFSREQL
GcnR	133	VRQPA--SPDEVVVVGPLSIDPRARETRLDRTIEVTRKEFDLLYLLASRPGIVVTREQL
SchA25	180	VRPLP--LTTEVLTSSGPLRIDSRTRERLGDPEFVEVTRKEFDLLYLLASRPGSVVSRKQI

Aur1P	214	MQQVWGDS-----WSRR--TVDTHVSTLRNKIG-AGWIITIRGVGFRFGQG---
FarR1	215	MQQVWGDS-----WSRR--TVDTHVSTLRNKIG-ANWIITIRGVGFRFGQG---
PgaR1	218	MAQVWEDS-----WSRRGRTIDTHVSSLRGKLGSSSWIITVRGVGFRFRLGHP---
Med-ORF30	176	MIDVWGHPADV-GLSAQASRTIDTHVSSMRSKLRESGWITVRGVGFRFRQD---
Chai	195	MTEVWGASLPGGALGSNIARTIDTHVGTLRNKLGSSEWIITVRGVGFRFGGVQQS
GcnR	191	MVQVWRDS-----RSVPGRTIDTHVSSLRSKLGSSDWIITARGVGFRFLGRA---
SchA25	238	MTLVWGDS-----RSQPGRTIDTHVSSLRSKLGSSSWIITARGVGFRFGHA---

Figure S1. Comparison of Aur1P with similar atypical response regulators (ARRs) from the BGCs containing the *aur10* homologous gene (Figure 6). Protein sequences and accession numbers are: Aur1P of *S. lavendulae* subsp. *lavendulae* CCM 3239 (AAX57187), FarR1 of *S. lavendulae* FRI-5 (BAG74710), PgaR1 of *Streptomyces* sp. PGA64 (AHW57766), Med-ORF30 of *Streptomyces* sp. AM-7161 (BAC79018), Chai of *S. chattanoogensis* L10 (AIU99192), GcnR of *S. lusitanus* (AGO50631), SchA25 of *Streptomyces* sp. SCC-2136 (CAH10125). Identical residues are highlighted in black. Similar residues are shaded. The location of the secondary structure helical elements of homologous OmpR (Novakova et al. 2005) of the DNA-binding effector domain determined from its tertiary structure (Martinez-Hackert and Stock, 1997) are shown above the sequence. The numbers refer to the deposited amino acid sequences in databases.

References

- Martinez-Hackert, E; Stock, A.M. Structural relationships in the OmpR family of winged-helix transcription factors. *J. Mol. Biol.* **1997**, 269, 301-312.
- Novakova, R.; Homerova, D.; Feckova, L.; Kormanec, J. Characterization of a regulatory gene essential for the production of the angucycline-like polyketide antibiotic auricin in *Streptomyces aureofaciens* CCM 3239. *Microbiology-SGM* **2005**, 151, 2693-2706.

NCBI Multiple Sequence Alignment Viewer, Version 1.21.0

Sequence ID	Start	Alignment	End	Organism
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WP_031943728.1	(*)	1	293	Streptomyces lavendulae
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WP_007268981.1	(*)	1	293	Streptomyces sp. C
RPF25366.1	(*)	1	293	Streptomyces sp. Ag109...
RK986999.1	(*)	1	293	Streptomyces sp. 3211.6
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Sequence ID	Start	Alignment	End	Organism
Query_32537	(*)	1 101 110 120 130 140 150 160 170 180 190 200		
WP_031943728.1	(*)	AGCGVAAHLYTHRCGR	293	Streptomyces lavendulae
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Sequence ID	Start	Alignment	End	Organism
Query_32537	(*)	1	400	
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WP_125815156.1	(*)	1	400	Streptomyces sp. WAC0...
WP_007268981.1	(*)	1	400	Streptomyces sp. C
RPF25366.1	(*)	1	400	Streptomyces sp. Ag109...
RK986999.1	(*)	1	400	Streptomyces sp. 3211.6
WP_190187557.1	(*)	1	400	Streptomyces citratus
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MBO0735037.1	(*)	1	400	Streptomyces sp. CBM
WP_188280340.1	(*)	1	400	Streptomyces sp. CBM
WP_093715795.1	(*)	1	400	Streptomyces alni
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WP_189513472.1	(*)	1	400	Streptomyces narbonne...
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WP_073775780.1	(*)	1	400	Streptomyces sp. MJM1...
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WP_190853690.1	(*)	1	400	Actinomadura sp. RB99
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WP_193776487.1	(*)	1	400	Streptomyces sp. E2N166
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WP_169136273.1	(*)	1	400	Streptomyces sp. HNM...
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WP_206248469.1	(*)	1	400	Streptomyces bryophytor...
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WP_211305172.1	(*)	1	400	Streptomyces alibireticul...
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WP_106681612.1	(*)	1	400	Streptosporangium nond...
WP_069772989.1	(*)	1	400	Streptomyces sp. LUP30
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WP_211835093.1	(*)	1	400	Streptomyces violaceus...
WP_193463778.1	(*)	1	400	Streptomyces griseus
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WP_077057266.1	(*)	1	400	Streptomyces sp. MP131...
WP_075845427.1	(*)	1	400	Gaccharomonospora sp. NBRC...
WP_158920617.1	(*)	1	400	Streptomyces pini
SFL9842.1	(*)	1	400	Streptomyces talianensis
WP_149829908.1	(*)	1	400	Streptomyces sp. HNM...
WP_168442852.1	(*)	1	400	Streptomyces nashville...
WP_229885625.1	(*)	1	400	Streptomyces pini
WP_139238202.1	(*)	1	400	Streptomyces pini
WP_229843810.1	(*)	1	400	Streptomyces tanashi...
GHE66465.1	(*)	1	400	Streptomyces cellulosa...
WP_141760688.1	(*)	1	400	Streptomyces nanshensis
WP_207315326.1	(*)	1	400	Streptomyces lacuifiss...
WP_176148066.1	(*)	1	400	Streptomyces seoulensis

NCBI Multiple Sequence Alignment Viewer, Version 1.21.0

Sequence ID	Start	Alignment	End	Organism
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WP_125815156.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	282	Streptomyces sp. C
WP_007268981.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	286	Streptomyces sp. Ag109...
RPF25366.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	286	Streptomyces sp. A1136
RK096999.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	279	Streptomyces lavendulae
WP_190187557.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	277	Streptomyces sp. ISL-98
WP_136214074.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	298	Streptomyces sp. CBM
BAG74709.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	301	Streptomyces sp. CBM
WP_214921469.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	305	Streptomyces aini
MBO0735037.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	304	uncultured bacterium
WP_188280340.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	276	Streptomyces sp. HPF1...
WP_093715795.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	308	Streptomyces sp. AD198...
AEM44215.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	288	Streptomyces sp. 03/20...
WP_225844529.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	313	Streptomyces mashuensis
RPK83857.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	304	Streptomyces venezuelae
WP_206328142.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces venezuelae
WP_190132210.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	304	Streptomyces scabiei
WP_190623348.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	285	Streptomyces sp. 351M...
QES19388.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	248	Actinokineospora canju...
WP_143673252.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	284	Streptomyces sp. GMR22
WP_020141370.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces narbonensis
WP_121394609.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces sp. GCC ...
WP_199335066.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	304	Streptomyces brasiliac...
WP_189513472.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	315	Streptomyces camponot...
CAH10120.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	316	Streptomyces sp. FGA64
WP_216588930.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	304	Streptomyces sp. ISL-96
WP_189111507.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	281	Streptomyces boncuke...
AHW57770.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	292	Streptomyces sp. ISL-96
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WP_206441614.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	300	Actinomadura sp. RB99
WP_215129277.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	293	Actinomadura sp. RB99
WP_073775780.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	281	Streptomyces sp. 2238
MBO2892417.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	225	Streptomyces tubercidicus
WP_190835390.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	286	Streptomyces sp. E2N166
BAJ07840.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	299	Micromonospora sp. MW...
WP_218039315.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	295	Streptomyces roseovortic...
WP_193776487.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	266	Streptomyces sp. HNM...
WP_128763710.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	309	Streptomyces sp. TK08
WP_235485517.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	289	Streptomyces sp. tea 10
WP_169136273.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces
BAV16996.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	289	Streptomyces sp. 072-7A
MBX7550227.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	278	Streptomyces sp. IB201...
WP_120759153.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	296	Streptomyces sp. CB02...
WP_233878960.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	240	Actinomadura rayongensis
WP_143644090.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	284	Streptomyces sp. Ru72
OKID7325.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	292	Streptomyces abyssalis
WP_161101252.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	256	Streptomyces
WP_219822337.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	335	Streptomyces actuosus
WP_070010968.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	338	Streptomyces griseospo...
WP_124265853.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	304	Streptomyces globosporus
WP_162655397.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	299	Streptomyces globosporus
WP_190219409.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	286	Actinopolyspora biskrensis
WP_123947562.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	302	Streptomyces sp. MP131...
ROV69876.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	320	Streptomyces sp. st115
WP_179537186.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	331	Streptomyces showdoe...
ONK09315.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	290	Streptacidiphilus jiangxi...
WP_098025994.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	281	Thermomonospora echl...
WP_165591042.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	246	Streptomyces bryophytor...
WP_052438253.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	319	Streptomyces baamensis
WP_200827536.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	285	Streptomyces euroclidicus
WP_206248469.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces lydicus
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WP_221505294.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	293	Streptomyces albulus
WP_052687242.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces chatanoo...
WP_207630589.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	285	Streptomyces albireticul...
AIAD7021.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	305	Streptomyces exfoliatus
WP_053921784.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	283	Streptomyces sp. NRRL...
WP_211305172.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	320	Streptomyces albus
WP_051894184.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	302	Streptomyces asiaticus
WP_059130311.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	283	Streptomyces sp.
WP_050504914.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	292	Streptomyces albireticul...
WP_211831744.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	285	Streptomyces albireticul...
ARO44670.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	293	Streptomyces albulus ZPM
ARZ71616.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	291	Streptomyces sp. BHT-5-2
WP_199843978.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	289	Streptomyces roseovortic...
AKA09042.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	297	Streptosporangium nond...
WP_221906375.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	285	Streptomyces sp. LUP30
WP_052393332.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	259	Streptomyces lydicus
WP_106681612.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	265	Streptomyces violaceus...
WP_069772989.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	320	Streptomyces griseus
AJT68429.3	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	322	Streptomyces sp. GN-593
WP_211835093.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	283	Streptomyces sp. MBT84
WP_193463778.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	252	Streptomyces sp. CB02...
WP_202233662.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	259	Streptomyces sp. MP131...
WP_220291340.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	279	Gaccharomonospora sp...
WP_199930774.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	253	Streptomyces sp. NBRC...
WP_077057266.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	315	Streptomyces pini
WP_075845427.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	292	Streptomyces talianensis
WP_158920617.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	261	Streptomyces sp. HNM...
SFL99842.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	336	Streptomyces nashville...
WP_149829908.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	300	Streptomyces pini
WP_169442852.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	336	Streptomyces tanashi...
WP_229885625.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	280	Streptomyces cellulosa...
WP_139238202.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	297	Streptomyces nanshensis
WP_229843810.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	277	Streptomyces laculatisp...
GHE66465.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A	288	Streptomyces seoulensis
WP_141760688.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A		
WP_207315326.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A		
WP_17614806.1	1	NR AGR T F T A G G D V R A C F A R R A Q A R A		

Figure S2. Comparison of Aur1O with similar proteins from unknown BGCs. Protein sequences and accession numbers are in the left column. Aur1O from *S. lavendulae* subsp. *lavendulae* CCM 3239 (AAK59995) is referred to as Query_32537. The Aur1O amino acid sequence comparison with the non-redundant protein database was performed using a BlastP browser and NCBI multiple sequence alignment viewer (<https://blast.ncbi.nlm.nih.gov/>). The numbers refer to the deposited amino acid sequences in databases.

Table S1. Oligonucleotides used in this study. Cloning sites are underlined.

Oligonucleotide	Sequence (5' – 3')
aur1AFw	CGCAGTCCGTCACCGAGACCC
aur1ARv	CGTCGTAGGTGATCTCCAGTCC
aur1OdDir	CCGACCAGCGACCACAGACACGAGCCGAGGGGGAACGACATTCCGGGG ATCCGTCGACC
aur1OdRev	CCTGCGCCCTGCGGGCGAAGCAGGCGCGGACGTCGCCCTCTGTAGGCTG GAGCTGCTTC
aur1OCla	CCCCATCGATCCGAGGGGGAACGACATGACG
aur1OXba	CCCCCTCTAGAGCTCAGGCGCGGGCCTGCGCCC
aur1AS1dir	CGTGGCCTTGACACTCCCTTGGC
aur1AS1rev	CGCCGCTCCAGACGTTCGAGGACG
aur1ApDir	CCCCGGATCCCAATTGCCGCTAGCCCCCTTAAGCCATTAATCCTTCGAAGGAC CCGCACAGCAACCGGTAGG
aur1ApRev	CCCCGGTACCCGCTCCGGAGACAGTGCACCG
aur1PpDir	CCCCGATATCCCCGATTTAACCCTTTTATCGGC
aur1PpRev	CCCCTTCGAAGGTCCGGCCGGGCTCAGCCCTGG
aur1OpRev	CCCCTTCGAAGGGAACGTCGTGCGGAAGACCGG
luxCrev	GCTCTCGGGGAAGATCTCGACCTGG
pMU1fd	CGGACGGTTCGGCCCCCTTTTTTGG
aur1PDHdir	GGGGTCTAGACCATATGAACCAGCGGACCATGGCTGTGC
aur1PDHrev	GGGGGTACCTCGAGTCAGCCCTGGCCGAAGCGGAATCC
aur1ODHdir	GGGGTCTAGACCATATGACGGTGACCGCGCTGTGCCACG
aur1ODHrev	GGGGGTACCTCGAGTCAGGCGCGGGCCTGCGCCCTGC
KT25dir	GCCATTATGCCGCATCTGTCC
KT25rev	GCGATTAAGTTGGGTAACGCC
UT18Cdir	GCGTGCCGAGCGGACGTTTCG
UT18Crev	CGGGGCTGGCTTAACCTATGC