

Validation of Candidate Host Cell Entry Factors for Bovine Herpes Virus Type-1 Based on a Genome-Wide CRISPR Knockout Screen

Wenfang Spring Tan ^{1,*,+}, Enguang Rong ^{1,+}, Inga Dry ¹, Simon Lillico ^{2,3}, Andy Law ⁴, Paul Digard ¹, Bruce Whitelaw ^{2,4} and Robert G. Dalziel ¹

¹ Division of Infection and Immunity, the Roslin Institute, Easter Bush Campus, University of Edinburgh, Edinburgh EH259RG, UK; inga.dry@roslin.ed.ac.uk (I.D.); paul.digard@roslin.ed.ac.uk (P.D.); robert.dalziel@ed.ac.uk (R.G.D.)

² Division of Functional Genetics and Development, the Roslin Institute, Easter Bush Campus, University of Edinburgh, Edinburgh EH259RG, UK; simon.lillico@roslin.ed.ac.uk (S.L.); bruce.whitelaw@roslin.ed.ac.uk (B.W.)

³ Centre for Tropical Livestock Genetics and Health, the Roslin Institute, Easter Bush Campus, University of Edinburgh, Edinburgh EH259RG, UK

⁴ Division of Genetics and Genomics, the Roslin Institute, Easter Bush Campus, University of Edinburgh, Edinburgh EH259RG, UK; andy.law@roslin.ed.ac.uk

* Correspondence: wtan2@exseed.ed.ac.uk

+ These authors contributed equally to this work.

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Supplementary Materials, Methods and Data

Detailed CRISPR3i cloning protocol

Step1. Digest 10ug of pKLV2-U6gRNA5(BbsI)-PGKpuro2ABFP-W (referred to as PB_3i from now on, Addgene #67974) >=4hrs, gel purify vector and elute with 50ul H2O to obtain linear vector. Alternatively, use uncut PB_3i in Step3 directly but background may be higher if digestion is not complete.

Step2. Assemble the re-annealing reactions below;

Components	Volume (uL)
sgRNA sense + anti-sense oligo (50 µM)	1
10X NEB Buffer 2	0.5
ddH2O	3.5
Total	5

Anneal the oligos in a thermocycler by using the following parameters: 95 °C for 5 min; ramp down to 25 °C at 0.1°C/s, 4°C hold;

Mix 1ul of re-annealed oligos from each g1, g2 and g3 with 97ul nuclease free H2O for a 1:100 dilution;

Proceed with BbsI digest and ligation;

Step 3. Digestions and ligation:

Assemble the reactions in strip tubes below, replace oligo mix with H2O as negative control:

Components	Volume (uL)
PB_3i (~50 ng/ul, linear or circular)	0.25
TOPO_g1tracr_H1(100ng/ul)	0.5
TOPO_g2tracr_mU6(100ng/ul)	0.5
Diluted oligo duplex mixes from above	1
10X CutSmart buffer	1
NEB BbsI(10u/ul)	0.5
Quick T4 ligase(2000u/ul)	0.25
10mM ATP	0.5
20mM DTT	0.25
ddH2O	5.25
Total	10

Incubate reactions in a thermo cycler running the following program: 10 X (37°C, 5 min; 16°C, 10 min); 37°C, 30 min + 65°C, 10 min.

Step 4. Assemble the following plasmid-safe nuclease treatment and incubate reaction at 37°C for 30 min:

Components	Volume (uL)
Reaction from above	10
PlasmidSafe buffer	1.5
H2O	1.75
10mM ATP	1.25
Plasmid-Safe nuclease	0.5
Total	15

Step 5. Transform 2ul of the above reactions into 25 ul of Stbl3 or NEB Stable competent cells and plate the transformed E. Coli onto Carb⁵⁰ or Amp¹⁰⁰ plates.

Step 6. The second day, pick colonies for bacterial PCR and start 5ml of culture for minipreps using Qiagen miniprep columns and Plasmid plus buffers with endotoxin removal. Elute with 30ul of endotoxin-free, EB, TE or H₂O.

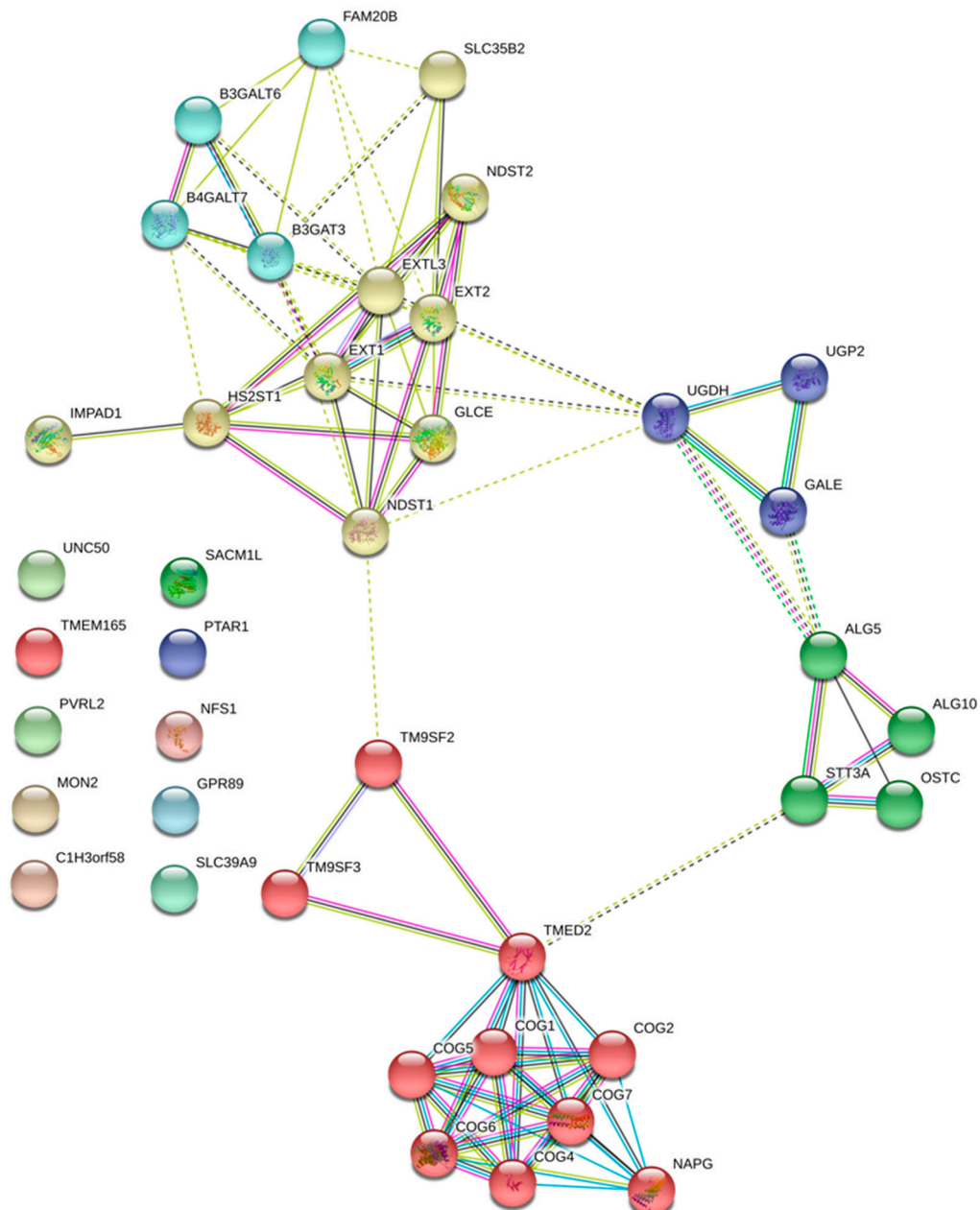


Figure S1. STRING analysis of all candidates with suspected roles in cell entry.

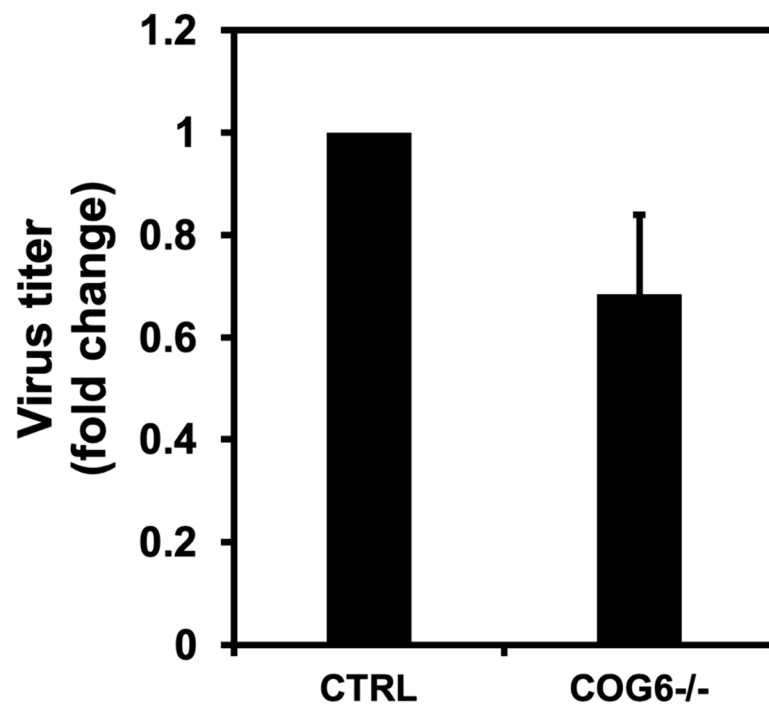


Figure S2. COG6KO reduced AIHV-1 replication. Plaque assay was conducted in Cas9^{+/+} control cells and COG6 KO cells infected with AIHV-1. Virus titers are presented as relative numbers with that in controls cells set as 1 (n=3, **p=0.016**).

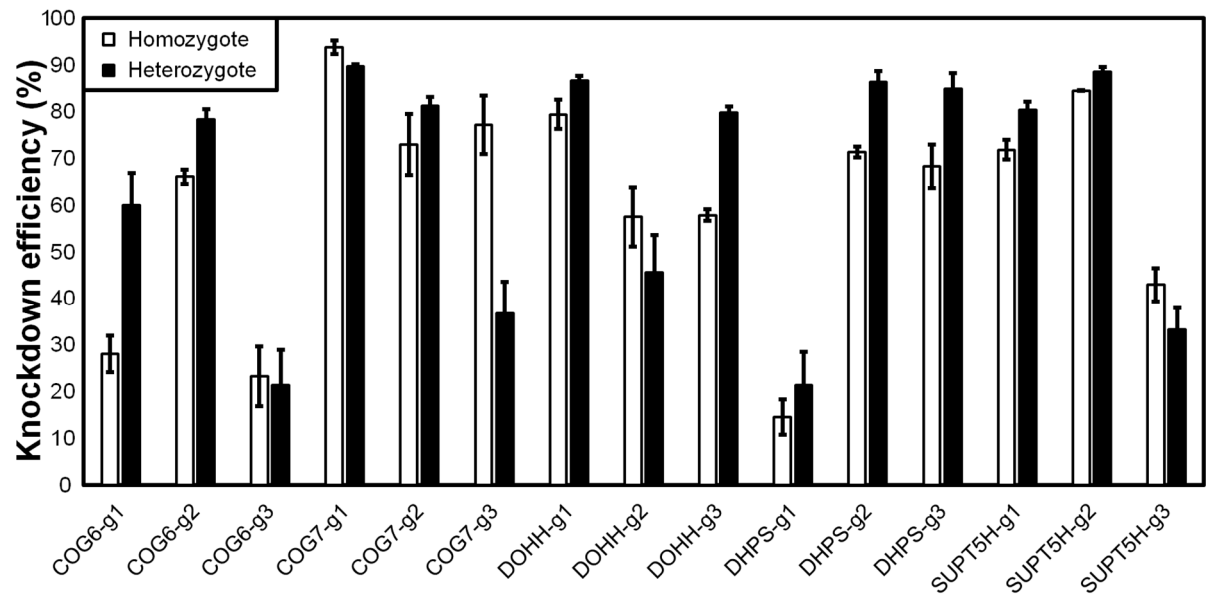


Figure S3. Comparison of CRISPRi KD efficiency in heterozygote and homozygote dCas9 cell lines. KD efficiency was assayed using RT-qPCR using mRNA samples collected from dCas9+/+ or dCas9/wt MDBKs transfected with PiggyBac vector expressing one of the three guides (g1,g2,g3) targeting one of the five genes tested. KD efficiency was calculated by $2^{-\Delta\Delta CT}$ using GAPDH as an internal control.

Table S1. Guide RNA sequences for CRISPRko and CRISPRi

Gene	I.D.	Guide sequence	purpose
PVR	NA	GCTCTTGTCAACCATTAGGGA	KO
PVRL2	NA	GTCGGCGGCGACAATCTCAA	KO
GLCE	NA	CAGAGTCAAGTGCATAAGTG	KO
HST2ST1	NA	GATGAATGTGTAGCCGAGGG	KO
COG6	NA	TTACGAAAGACTTTACCGGT	KO
COG7	NA	GTGTTGGTAGAAATCGACC	KO
COG6	g1	CGGGTTGCTGGTCTGCGCCG	CRISPRi
COG6	g2	GGACAACGACAAGGTGAGCG	CRISPRi
COG6	g3	AGTCGTAGTGCCCGCGACTG	CRISPRi
COG7	g1	CGTGAAGGAGTGGATCAACG	CRISPRi
COG7	g2	CCAAGAGGTGAACCACGCCG	CRISPRi
COG7	g3	ACATCAGCGCGCTGATCCAG	CRISPRi
DOHH	g1	AGTGAAGAATCAGCGCGCGG	CRISPRi
DOHH	g2	TCCGATCTGGTAGCTCACTG	CRISPRi
DOHH	g3	TGTGAAGGATCAGGTAAGTG	CRISPRi
DHPS	g1	CGAACTGTGCTTCAGCACGG	CRISPRi
DHPS	g2	GTGCGCGATAGTCCACGCCA	CRISPRi
DHPS	g3	GGTACAGCAAGTCAACGCCA	CRISPRi
SUPT5H	g1	CGAGGACAGCAACTTCTCCG	CRISPRi
SUPT5H	g2	AGGCTAAAGAGTCGCAAGAG	CRISPRi
SUPT5H	g3	CCGAAGTGGGAGTTGCGCTG	CRISPRi

Table S2. qPCR primers for viral genes and for CRISPRi validation

Gene	Forward	Reverse
UL23	CTCTGCTACCCCTTCGCCCCGCTACT	AGGGTGCACACGACGAGGTTGGC
COG6	GAGTTTGGAACAGCCAGAAGAAGTA	CGAGGTGTTCTTTCTCAGAAGCAGT
COG7	TGCTCCTCATTCCCAAGATGGATAG	TTCAGGGGGGAGGGACATGATATACT
DOHH	TGCTCAAGCATGAGCTGGCCTACT	CCACCTCAACAACAGGGTTCAGTAGA
DHPS	GTGGAGGAGGATTTTCATCAAGTGCT	CACTTCACACCCTCTGTGTTCTGCT
SUPT5H	ATTGCCTACCAGTTCACAGACACGC	ATCCGTCATCTCCTTGATAGGCACC

Table S3. Genotypes of CRISPR KO clones

Gene	Clone I.D.	Genotype
PVR	23	-50/-50
PVR	25	-2/-11
PVR	29	-8/-8
PVR	32	-5/-33
PVRL2	A4	-1/+1
PVRL2	A11	-4/+1
PVRL2	C11	-4/+1
PVRL2	D5	-19/-19
GLCE	A1	-13/+1
GLCE	A7	+1/+1
GLCE	B2	-8/-8
GLCE	B8	+1/+1
HST2ST1	A5	+1/+1
HST2ST1	C1	-10/-11
HST2ST1	C2	-1/+1
HST2ST1	C4	+1/+1
COG6	A4	-13/-13
COG6	A6	-1/+1
COG6	A9	-20/-20
COG6	C3	-152/-152
COG6	C6	+1/+1
COG7	A3	-32/-32
COG7	C5	-37/-37
COG7	F3	-13/-13
COG7	F7	-5/-5

Table S4. Oligos used for CRISPRi cloning.

CRISPRi sequences are underscored	
Sequence Name	Sequence
3iG1_PTAR1::45886631+:NC_037335.1	CAC CGA <u>TAC</u> CTA GCT TCA GGA CAC <u>GTT</u>
3iG1_PTAR1::45886631-:NC_037335.1	CTT AAA <u>CGT</u> <u>GTC</u> CTG AAG CTA GGT <u>ATC</u>
3iG2_PTAR1::45886607+:NC_037335.1	ATC TGA <u>ACC</u> <u>AGG</u> ACT ATG GGA <u>CTC</u> <u>GTT</u> TAA
3iG2_PTAR1::45886607-:NC_037335.1	GCT CTT AAA <u>CGA</u> <u>GTC</u> CCA TAG TCC <u>TGG</u> <u>TTC</u>
3iG3_PTAR1::45886540+:NC_037335.1	TGT <u>TGG</u> <u>GAG</u> CTT GTT GTG <u>GAC</u> <u>ATA</u>
3iG3_PTAR1::45886540-:NC_037335.1	AAA <u>CTA</u> <u>TGT</u> CCA CAA CAA <u>GCT</u> <u>CCC</u>
3iG1_SLC35B2::17762949+:NC_037350.1	CAC <u>CGG</u> <u>GAT</u> <u>CGG</u> <u>GAG</u> AGA AGT <u>CGC</u> <u>GTT</u>
3iG1_SLC35B2::17762949-:NC_037350.1	CTT AAA <u>CGC</u> <u>GAC</u> <u>TTC</u> TCT CCC <u>GAT</u> <u>CCC</u>
3iG2_SLC35B2::17763261+:NC_037350.1	ATC <u>TGT</u> <u>TGC</u> ACT TCC <u>GCT</u> <u>GGC</u> <u>CGG</u> <u>GTT</u> TAA
3iG2_SLC35B2::17763261-:NC_037350.1	GCT CTT AAA <u>CCC</u> <u>GGC</u> CAG <u>CGG</u> AAG <u>TGC</u> <u>AAC</u>
3iG3_SLC35B2::17762887+:NC_037350.1	TGT TGA <u>GGG</u> <u>CGC</u> <u>CGC</u> <u>GCC</u> TCC <u>AAC</u>
3iG3_SLC35B2::17762887-:NC_037350.1	AAA <u>CGT</u> <u>TGG</u> <u>AGG</u> <u>CGC</u> <u>GGC</u> <u>GCC</u> <u>CTC</u>
3iG1_SLC39A9::81185607+:NC_037337.1	CAC <u>CGC</u> <u>TCC</u> TCC AAG TCT AGA <u>GTG</u> <u>GTT</u>
3iG1_SLC39A9::81185607-:NC_037337.1	CTT AAA <u>CCA</u> <u>CTC</u> TAG ACT TGG <u>AGG</u> <u>AGC</u>
3iG2_SLC39A9::81185495+:NC_037337.1	ATC <u>TGC</u> <u>ATG</u> TTA CTC CAC TCC <u>GAG</u> <u>GTT</u> TAA
3iG2_SLC39A9::81185495-:NC_037337.1	GCT CTT AAA <u>CCT</u> <u>CGG</u> AGT GGA <u>GTA</u> <u>ACA</u> <u>TGC</u>
3iG3_SLC39A9::81185759+:NC_037337.1	TGT <u>TGC</u> <u>CAC</u> AAG CAA TGT TCC <u>CGA</u>
3iG3_SLC39A9::81185759-:NC_037337.1	AAA <u>CTC</u> <u>GGG</u> AAC ATT <u>GCT</u> TGT <u>GGC</u>
3iG1_TM9SF2::76343648+:NC_037339.1	CAC <u>CGG</u> <u>CGC</u> <u>CCG</u> TCA ACT TCT <u>GCG</u> <u>GTT</u>
3iG1_TM9SF2::76343648-:NC_037339.1	CTT AAA <u>CCG</u> CAG AAG TTG <u>ACG</u> <u>GGC</u> <u>GCC</u>
3iG2_TM9SF2::76343397+:NC_037339.1	ATC TGA <u>CCG</u> GAA CTC TCC <u>GTC</u> <u>GCG</u> <u>GTT</u> TAA
3iG2_TM9SF2::76343397-:NC_037339.1	GCT CTT AAA <u>CCG</u> CGA <u>CGG</u> AGA GTT <u>CCG</u> <u>GTC</u>
3iG3_TM9SF2::76343686+:NC_037339.1	TGT <u>TGG</u> <u>CGC</u> CCA CCT TGC ACT <u>CGT</u>
3iG3_TM9SF2::76343686-:NC_037339.1	AAA <u>CAC</u> <u>GAG</u> TGC AAG GTG <u>GGC</u> <u>GCC</u>
3iG1_TMED2::52090946+:NC_037344.1	CAC <u>CGC</u> <u>AGC</u> TAT <u>CCG</u> AGT CCT <u>GGG</u> <u>GTT</u>
3iG1_TMED2::52090946-:NC_037344.1	CTT AAA <u>CCC</u> CAG GAC TCG GAT AGC <u>TGC</u>
3iG2_TMED2::52091065+:NC_037344.1	ATC <u>TGT</u> <u>TCC</u> <u>TGG</u> ACA TCG <u>ACG</u> <u>TGG</u> <u>GTT</u> TAA
3iG2_TMED2::52091065-:NC_037344.1	GCT CTT AAA <u>CCC</u> <u>ACG</u> TCG ATG TCC <u>AGG</u> <u>AAC</u>
3iG3_TMED2::52091089+:NC_037344.1	TGT TGC <u>TCG</u> <u>GCC</u> ACC TCA AAG <u>ATG</u>
3iG3_TMED2::52091089-:NC_037344.1	AAA CCA TCT TTG <u>AGG</u> TGG <u>CCG</u> <u>AGC</u>
3iG1_TMED2::70838793+:NC_037333.1	CAC <u>CGC</u> <u>AAC</u> AGA GGA <u>CGT</u> <u>GAG</u> <u>ACA</u> <u>GTT</u>
3iG1_TMED2::70838793-:NC_037333.1	CTT AAA <u>CTG</u> TCT CAC <u>GTC</u> CTC TGT <u>TGC</u>

3iG2_TMEM165::70839078+:NC_037333.1	ATC TGT <u>GGC</u> GGT TCC TTG TTC CGG GTT TAA
3iG2_TMEM165::70839078-:NC_037333.1	GCT CTT AAA CCC GGA ACA AGG AAC CGC <u>CAC</u>
3iG3_TMEM165::70838985+:NC_037333.1	TGT TGC <u>AGC</u> CCG GGC GCA GAT GCG
3iG3_TMEM165::70838985-:NC_037333.1	AAA CCG CAT CTG CGC CCG GGC TGC
3iG1_TMEM41B::43311362+:NC_037342.1	CAC CGG GAT CCT TAC AAC ACC CAG GTT
3iG1_TMEM41B::43311362-:NC_037342.1	CTT AAA <u>CCT</u> GGG TGT TGT AAG GAT CCC
3iG2_TMEM41B::43311459+:NC_037342.1	ATC TGG TCG AAA GAT CGC AAA CGG GTT TAA
3iG2_TMEM41B::43311459-:NC_037342.1	GCT CTT AAA <u>CCC</u> GTT TGC GAT CTT TCG ACC
3iG3_TMEM41B::43311568+:NC_037342.1	TGT TGA <u>GAC</u> CTG GGA ACC AGC GCG
3iG3_TMEM41B::43311568-:NC_037342.1	AAA <u>CCG</u> CGC TGG TTC CCA GGT <u>CTC</u>
3iG1_UGDH::58620020+:NC_037333.1	CAC CGT AGA AGT GGT TGC AGC ACT GTT
3iG1_UGDH::58620020-:NC_037333.1	CTT AAA <u>CAG</u> TGC TGC AAC CAC TTC TAC
3iG2_UGDH::58620252+:NC_037333.1	ATC TGG GAG CAG GGA TGC AAC CGA GTT TAA
3iG2_UGDH::58620252-:NC_037333.1	GCT CTT AAA <u>CTC</u> GGT TGC ATC CCT GCT <u>CCC</u>
3iG3_UGDH::58620127+:NC_037333.1	TGT TGG CAT <u>CCC</u> GAG AAG GGT GTG
3iG3_UGDH::58620127-:NC_037333.1	AAA CCA CAC CCT TCT CGG GAT <u>GCC</u>
3iG1_UGP2::62288834+:NC_037338.1	CAC CGA AGA TAG TTA TGT ACT CGG GTT
3iG1_UGP2::62288834-:NC_037338.1	CTT AAA <u>CCC</u> GAG TAC ATA ACT ATC TTC
3iG2_UGP2::62288971+:NC_037338.1	ATC TGT <u>TCT</u> CAG CCG CAG AAC GCG GTT TAA
3iG2_UGP2::62288971-:NC_037338.1	GCT CTT AAA <u>CCG</u> CGT TCT GCG GCT GAG AAC
3iG3_UGP2::62288921+:NC_037338.1	TGT TGT <u>TGC</u> TAA AGT ATC TGT GGA
3iG3_UGP2::62288921-:NC_037338.1	AAA <u>CTC</u> CAC AGA TAC TTT AGC AAC
3iG1_UNC50::3824412+:NC_037338.1	CAC CGC TGC GTG CTC AAG GTA GCG GTT
3iG1_UNC50::3824412-:NC_037338.1	CTT AAA <u>CCG</u> CTA CCT TGA GCA CGC AGC
3iG2_UNC50::3824300+:NC_037338.1	ATC TGC CTA AGA CCG CGA ACT CGG GTT TAA
3iG2_UNC50::3824300-:NC_037338.1	GCT CTT AAA CCC GAG TTC GCG GTC TTA <u>GGC</u>
3iG3_UNC50::3824390+:NC_037338.1	TGT TGC <u>CTG</u> GCG CTC GGA TGA CCG
3iG3_UNC50::3824390-:NC_037338.1	AAA CCG GTC ATC CGA GCG CCA GGC
3iG1_nc_guide74+	CAC CGC GCG GCG TCC ATA TCA CGT GTT
3iG1_nc_guide74_	CTT AAA CAC GTG ATA TGG ACG CCG CGC
3iG2_nc_guide95+	ATC TGC GTA CTC GTA TAC TAC TCG GTT TAA
3iG2_nc_guide95-	GCT CTT AAA <u>CCG</u> AGT AGT ATA CGA GTA <u>CGC</u>
3iG3_nc_guide606+	TGT TGT <u>GAC</u> GCG TGG TCG AAC CGC
3iG3_nc_guide606-	AAA <u>CGC</u> GGT TCG ACC ACG CGT <u>CAC</u>