Construct information sheet

Gene name	**GLE1**
Uniprot ID	Q53GS7
Region	Q383 – S698

Description	RNA export mediator
Synonyms	GLE1L, LCCS, LCCS1, hGLE1
Construct ID	GLE1A-c200
Description of clone	This construct is based on the paper by *Lin et al (Nat Commun. 2018;9(1):2319),* reporting successful recovery of soluble GLE1 when co-expressed with a portion of NUP42. This construct is bi-cistronic, encoding the C-terminal domain (Q383-S698) of GLE1, followed by a second ORF encoding NUP42 GLE1-binding domain (I405-V448). GLE1 is fused to an N-terminal His_6-SUMO tag, which can be removed with SUMO protease, and a C-terminal avitag, which is effectively biotinylated when co-expressed with BirA in cultures supplemented with biotin. The DNA sequence is codon-optimized for expression in E. coli.
Protein sequence: SUMO-GLE1	*MCSSHHHHHHGSGSGSDQEAKPSTEDLGDKKEGEYIKLKVIGQDS* *SEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHT* *PKELGMEEEDVIEVYQEQTGG**QDITMQWYQQLQDASMQCVLTFE GLTNSKDSQAKKIKMDLQKAATIPVSQISTIAGSKLKEIFDKIHS LLSGKPVQSGGRSVSVTLNPQGLDFVQYKLAEKFVKQGEEEVASH HEAAFPIAVVASGIWELHPRVGDLILAHLHKKCPYSVPFYPTFKE GMALEDYQRMLGYQVKDSKVEQQDNFLKRMSGMIRLYAAIIQLRW PYGNRQEIHPHGLNHGWRWLAQILNMEPLSDVTATLLFDFLEVCG NALMKQYQVQFWKMLILIKEDYFPRIEAITSSGQMGSFIRLKQFL EKCLQHKDIPVPKGFLTSSFWRSASGLNDIFEAQKIEWHE * indicates the SUMO protease cleavage site. The biotinylation site is highlighted in green.
Second ORF: NUP42	MIATDNVLFTPRDKLTVEELEQFQSKKFTLGKIPLKPPPLELLNV
DNA sequence (coding sequence)	ATGTGCAGCAGCCATCATCATCATCATCACGGCAGCGGCAGCGGCTCTGA CCAGGAGGCAAAACCTTCAACTGAGGACTTGGGGGATAAGAAGGAAGGTG AATATATTAAACTCAAAGTCATTGGACAGGATAGCAGTGAGATTCACTTC AAAGTGAAAATGACAACACATCTCAAGAAACTCAAAGAATCATACTGTCA AAGACAGGGTGTTCCAATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGA GAATTGCTGATAATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGAT GTGATTGAAGTTTACCAGGAGCAAACGGGAGGTCAGGATATTACCATGCA GTGGTATCAGCAGCTGCAGGATGCAAGCATGCAGTGTGTTCTGACCTTTG AAGGTCTGACCAATAGCAAAGATAGCCAGGCCAAAAAAATCAAAATGGAT CTGCAGAAAGCAGCCACCATTCCGGTTAGCCAGATTAGCACCATTGCAGG TAGCAAACTGAAAGAGATCTTCGATAAAATTCATAGCCTGCTGAGCGGTA AACCGGTTCAGAGCGGTGGTCGTAGCGTTAGCGTTACCCTGAATCCGCAA GGTCTGGATTTTGTTCAGTATAAACTGGCCGAGAAATTTGTGAAACAGGG TGAAGAAGAGGTTGCAAGCCATCATGAAGCAGCATTTCCGATTGCAGTTG TTGCAAGCGGTATTTGGGAACTGCATCCGCGTGTTGGTGATCTGATTCTG GCACATCTGCACAAAAAATGTCCGTATAGCGTTCCGTTTTACCCGACCTT TAAAGAAGGTATGGCACTGGAAGATTATCAGCGTATGCTGGGTTATCAGG TGAAAGATAGCAAAGTTGAACAGCAGGATAACTTCCTGAAACGTATGAGC GGCATGATTCGTCTGTATGCAGCAATTATTCAGCTGCGTTGGCCGTATGG TAATCGTCAAGAAATTCATCCGCATGGCCTGAATCATGGTTGGCGTTGGC TGGCACAGATTCTGAATATGGAACCGCTGAGTGATGTTACCGCAACACTG

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	CTGTTTGATTTTCTGGAAGTTTGTGGTAATGCCCTGATGAAACAGTATCA GGTTCAGTTTTGGAAAATGCTGATCCTGATCAAAGAGGATTATTTTCCGC GTATTGAAGCAATTACCAGCAGCGGTCAGATGGGTAGCTTTATTCGTCTG AAACAGTTCCTGGAAAAATGCCTGCAGCATAAAGATATTCCGGTTCCGAA AGGTTTTCTGACCAGCAGCTTTTGGCGTAGCGCAAGCGGTCTGAATGATA TTTTTGAAGCCCAGAAAATTGAGTGGCACGAGTAAGAAATAATTTTATTT ATAAGGAGATATACCATGATTGCCACCGATAATGTTCTGTTTACACCGCG TGATAAACTGACCGTTGAAGAACTGGAACAGTTTCAGAGCAAAAAATTCA CCCTGGGTAAAATTCCGCTGAAACCGCCTCCGCTGGAACTGCTGAATGTT TAA
Construct accession No.	

Vector information:

pSUMO-LIC	pET expression vector with His6 and SUMO (96-aa sequence derived from SUMO1 protein) tags followed by a SUMO protease cleavage site. If no stop codon is included in reverse primer, protein of interest will be fused to biotinylation tag followed by additional His_6 tag.
Antibiotic resistance	Kanamycin, 50 μg/ml
Promoter	T7 - lacO
N-terminal fusion – seq.	MCSSHHHHHHGSGSGSDQEAKPSTEDLGDKKEGEYIKLKVIG QDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRI ADNHTPKELGMEEEDVIEVYQEQTGG* (* - SUMO cleavage site)
N-terminal fusion – MW	12577.9 Da
Additional features	Optional non-cleavable biotinylation and His_6 tags if reverse primer does not encode stop codon.
Protease cleavage	SUMO
Preferred expression host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase. For effective biotinylation, the host should overexpress BirA (e.g. from plasmid pCDF-BIRA, GenBank: JF914075)
Genebank:	Reference: PMID:29899397

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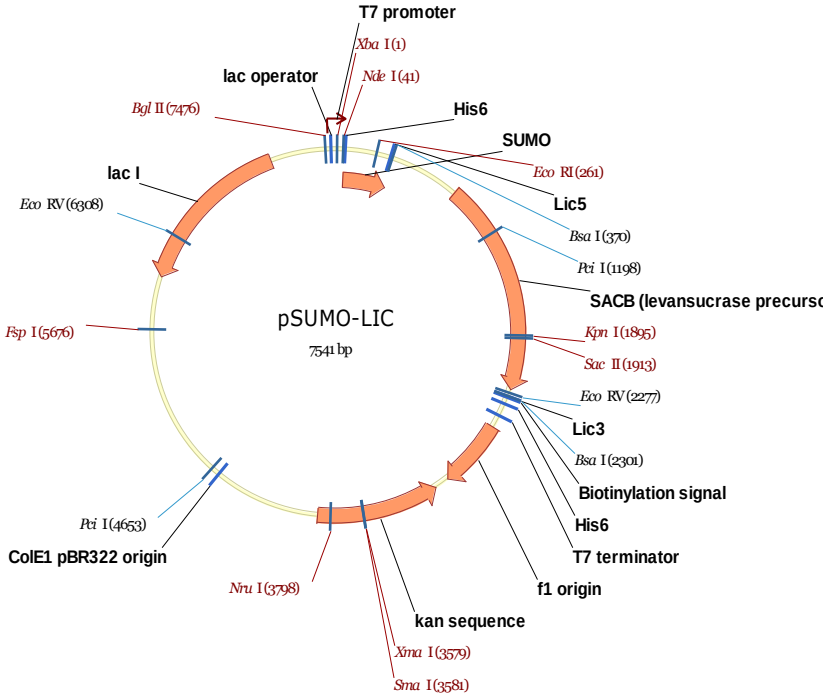

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