SUPPLEMENTARY INFORMATION

Title

A descriptive guide for absolute quantification of produced shRNA pseudotyped lentiviral particles by real-time PCR

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Short running title

shRNA lentivirus quantification by RT-qPCR

Abbreviations

Amplification efficiencies (E); Cycle threshold (Ct); Human embryonic kidney (HEK); Human embryonic stem cells (hESCs); Melting temperature (Tm); Neuropilin 1 (NRP1); No reverse transcription (NRT); No template controls (NTC); Octamer-binding transcription factor 4 (OCT4); Plexin B1 (PLXNB1); Quantitative PCR (qPCR); Reverse transcription (RT); RNA interference (RNAi); Short hairpin RNA (shRNA); Standard error of the mean (SEM); The RNAi Consortium (TRC)

Table of content

Table S1. shRNA plasmid references and sequences

Description of key elements to identify the shRNA plasmids used in this work, such as the official gene symbol, TRC plasmid number, clone ID and detailed shRNA nucleotide sequence. Please note that these shRNAs were designed to target human genes. Nucleotides in the middle in bold represent the loop sequence within the shRNA.

Gene target	TRC Number	Clone ID	shRNA sequence	Abbreviation
Control **(SHC002)**			**CCGG**CAACAAGATGAAGAGCACCAA**CTCGAG** TTGGTGCTCTTCATCTTGTTG**TTTTT**	shControl
CDH8	TRCN0000054223	NM_001796.2-1784s1c1	**CCGG**GCCAAGTCATTCAAACTGTTA**CTCGAG** TAACAGTTTGAATGACTTGGC**TTTTTG**	ShCDH8
NRP1	TRCN0000063523	NM_003873.2-255s1c1	**CCGG**GCAACGATAAATGTGGCGATA**CTCGAG** TATCGCCACATTTATCGTTGC**TTTTTG**	shNRP1-23
	TRCN0000063524	NM_003873.2-369s1c1	**CCGG**CGGACCCATACCAGAGAATTA**CTCGAG** TAATTCTCTGGTATGGGTCCG**TTTTTG**	shNRP1-24
	TRCN0000063527	NM_003873.2-694s1c1	**CCGG**CAGCCTTGAATGCACTTATAT**CTCGAG** ATATAAGTGCATTCAAGGCTG**TTTTTG**	shNRP1-27
POU5F1 (OCT4)	TRCN0000004879	NM_002701.1-998s1c1	**CCGG**TCATTCACTAAGGAAGGAATT**CTCGAG** AATTCCTTCCTTAGTGAATGA**TTTTT**	shOCT4-79
	TRCN0000004881	NM_002701.1-797s1c1	**CCGG**CCCTCACTTCACTGCACTGTA**CTCGAG** TACAGTGCAGTGAAGTGAGGG**TTTTT**	shOCT4-81
PLXNB1	TRCN0000061534	NM_002673.3-5003s1c1	**CCGG**GCTTGAGTATTTCACTGACAT**CTCGAG** ATGTCAGTGAAATACTCAAGC**TTTTTG**	shPLXNB1-34
	TRCN0000061535	NM_002673.3-6152s1c1	**CCGG**CCCGATCAACAAACTTCTGTA**CTCGAG** TACAGAAGTTTGTTGATCGGG**TTTTTG**	shPLXNB1-35
	TRCN0000061536	NM_002673.3-132s1c1	**CCGG**CCAACTGCATTCACTCCCAAT**CTCGAG** ATTGGGAGTGAATGCAGTTGG**TTTTTG**	shPLXNB1-36
SERPINE1	TRCN0000052272	NM_000602.1-546s1c1	**CCGG**CATCATCAATGACTGGGTGAA**CTCGAG** TTCACCCAGTCATTGATGATG**TTTTTG**	shSERPINE1

Table S2. PCR primer references and sequences

Description of key elements to identify the primers used in this work: the acronym and official gene symbol of the target mRNAs, the number of targeted transcripts, the primer sequences (F: forward; R: reverse) and their melting temperatures (Tm), and the PCR product length. It is specified whether primers span an exon junction or if they are separated by at least one intron.

Target acronym	Target official name	Variants	Primer sequences (5'->3')	Tm	Product length	Exon junction	Intron
HPRT1	hypoxanthine phosphoribosyltransferase 1	1	**F** GCAGCCCTGGCGTCGTGATTAG **R** TCGAGCAAGACGTTCAGTCCTGTCC	60.2 60.5	143	X	
MAPK1	mitogen-activated protein kinase 1 (ERK2)	2 (1)*	**F** TCGCCGAAGCACCATTCAAGTTCG **R** AGCACGTCCAGTCCTCTGAGCC	60.3 60.2	150		X
UBC	ubiquitin C	1	**F** TGCGGAGGGATCTCCGTGGG **R** GCATTGTCAAGTGACGATCACAGCG	60 59.2	140	X	
NRP1	neuropilin 1	5	**F** GGATGACAGCAAACGCAAGG **R** AGAGAGCTGGAAAAGTCCGC	60.1 60	83		X
PLXNB1	plexin B1	2	**F** GGCCCTGCATGAACTCTACA **R** AATCTGCTGGAGCCGATAGC	59.8 60	115		X
POU5F1 (OCT4)	POU class 5 homeobox 1	3**	**F** ATGTGGTCCGAGTGTGGTTC **R** TGTGCATAGTCGCTGCTTGA	60 60	67		

* Primers recognise only variant 1

** Primers recognise also variant 1B

5,810 – AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGT
CAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG
CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCC
AGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCT**GGGGATTTGGGGTTG**
CTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGG
AACAGATTTGGAATCACACGACC**TGGATGGAGTGGGACAGAGAA**ATTAACAATTACACAAGCTTA
ATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAATTA
GATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGGTATATAAAAATTATTCA
TAATGATAGTAGGAGGCTTGGTAGGTTTAAGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGT
TAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGG
CCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACG
GATCTCGACGGTATCGATCACGAGACTAGCCTCGAGCGGCCGCCCCCTTCACCGAGGGCCTATT
TCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGA
CTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGC
AGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTT
GGCTTTATATATCTTGTGGAAAGGACGAAACA**CCGG**NNNNNNNNNNNNNNNNNNNNN**CTCGAG**N
NNNNNNNNNNNNNNNNNNNN**TTTTTGAATTC**TCGACCTCGAGACAAATGGCAGTATTCATCCAC
AATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGC
AACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACA
GGGACAGCAGAGATCCACTTTGGCCGCGGCTCGAGGGGG **– 7,086**

Primers / PCR product
EcoR1 site
Loop/shRNA extremities / shRNA sequence
Rev response element
U6 promoter

Figure S1. Partial pLKO.1-puro plasmid sequence

The 5,810 to 7,086 pLKO.1-puro plasmid sequence is represented and contains the following elements: qPCR primers (bold underline green, PCR product (green), EcoR1 site (bold underline red), loop and shRNA extremities (bold underline purple), shRNA (purple), rev response element (orange) and U6 promoter. The qPCR product was predicted to have a size of 124 base pairs.

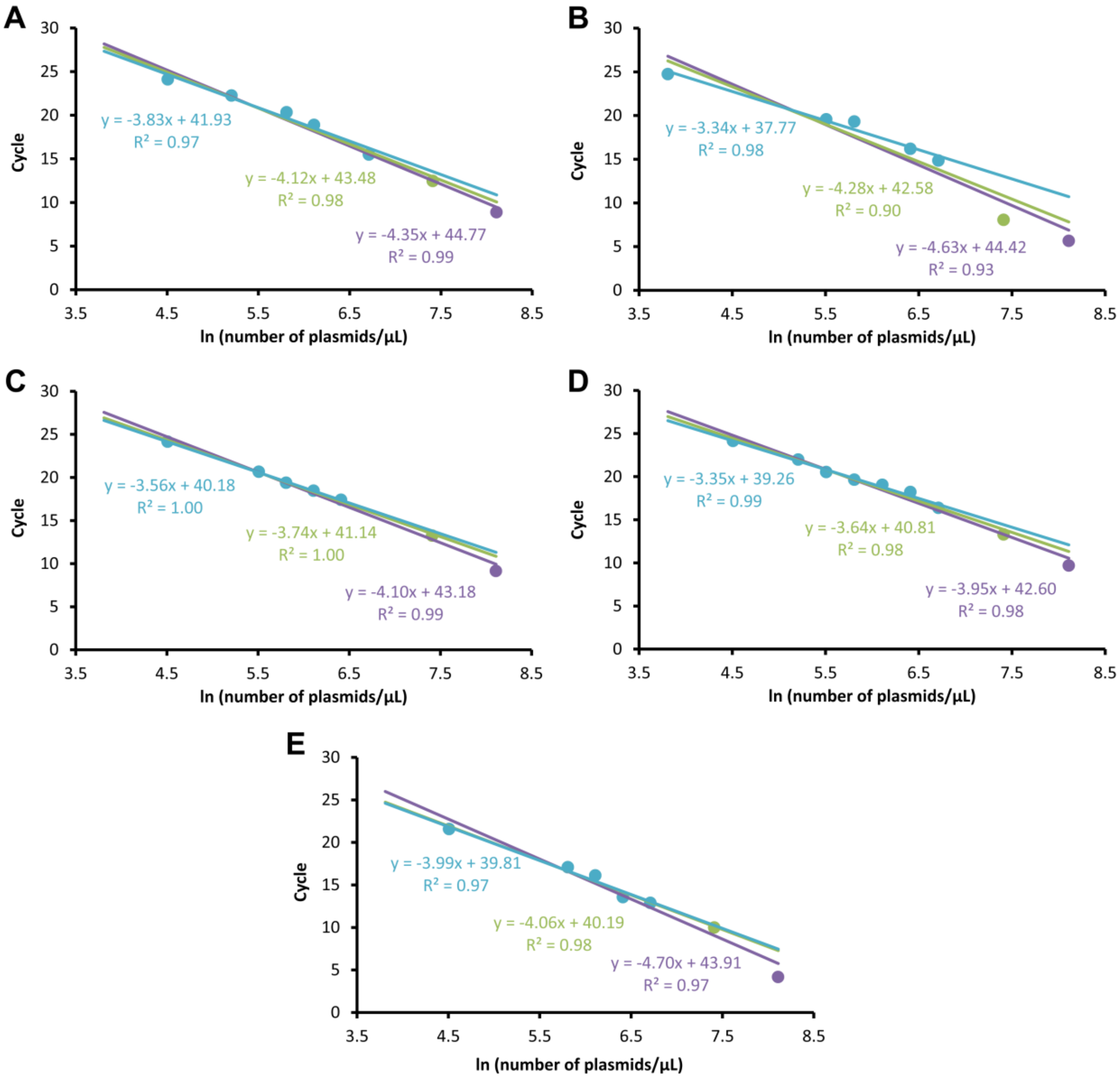

Figure S2 – Effect of the highest standard concentrations on qPCR efficiencies
Efficiency curves of purified linearised standards **A.** shPLXNB1-35, **B.** shOCT4-79, **C.** shNRP1-24, **D.** shCDH8 and **E.** shControl with the two highest plasmid concentrations (purple), without the highest concentration of 5 ng/reaction (green) without both highest tested concentrations of 5 and 1 ng/reaction (blue). Linear regression curves are given with their correlation coefficient R^2. The blue curves give a slope closer to -3.32, the ideal slope resulting in an amplification efficiency of 100%, compared to the purple and green ones.