Supplementary Information

Coupling retroviral production with a selectable event: selection of transcription factors that modulate γ-globin expression

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Supplementary Table 1:

TF_{ZF}	Zinc finger helices[a] F1	F2	F3	F4	F5	Target site	% Transduction[b]	Fold act. (luc)[c]	Fold act. (endogenous)[d]
Ala-VP64	ASAALVA	ASAALVA	ASAALVA	ASAALVA	ASAALVA	no DNA binding	10	1	1,5
gg1-VP64	DCRDLAR	RNDALTE	RSDHLTN	QSGDLRR	RKDNLKN DPGALVR	GTC AAG GCA AGG CTG GCC	75	5,8	16,7
#γA-VP64	DPGHLIR	QLAHLRA	TSGSLVR	DKKDLTR	TSGNLVR	GAT ACC GTT AGA GGC	73	3,9	3,7
#γB-VP64	DPGNLVR	DPGNLVR	DPGHLVR	HRTTLTN	SPADLTR	ACA AGT GGC GAC GAC	17	0,9	-
#γC-VP64	TSGHLVR	QKSSLIA	RSDHLTN	DSGNLRV	SPADLTR	ACA AAC AGG ATA GGT	17	1,4	-
#γD-VP64	HKNALQN	RTDTLRD	QSSNLVR	QRANLRA	DPGHLVR	GGC AAA GAA ACG ATT	11	1,2	-
#γE-VP64	QSSNLVR	QRANLRA	TSGSLVR	RTDTLRD	DKKDLTR	ACC ACG GTT AAA GAA	48	2,6	-
#γF-VP64	DCRDLAR	RNDALTE	QSGDLRR	RKDNLKN	DPGALVR	GTC AAG GCA CTG GCC	41	2,5	-
#γG-VP64	TTGNLTV	TSGHLVR	DSGNLRV	DKKDLTR	HKNALQN	ATT ACC AAC GGT AAT	20	2,3	-
#γH-VP64	DPGNLVR	ERSHLRE	RSDHLTN	HKNALQN	-	ATT AGG AGC GAC	15	1,3	-
#γJ-VP64	HRTTLTN	QSSNLVR	QLAHLRA	QRANLRA	RRDELNV	ATG AAA AGA GAA AGT	18	1,8	-
#γK-VP64	DPGNLVR	QSSSLVR	QRANLRA	SPADLTR	RTDTLRD	ACG ACA AAA GTA GAC	69	3,1	4,3
#γL-VP64	HKNALQN	QRANLRA	TSGSLVR	RTDTLRD	HRTTLTN	AGT ACG GTT AAA ATT	78	2,9	8,7
#γM-VP64	RRDELNV	DPGNLVR	SPADLTR	QSSNLVR	QSGDLRR	GCA GAA ACA GAC ATG	51	3,8	13,5
#γN-VP64	HKNALQN	TTGNLTV	QRAHLER	TSGHLVR	HRTTLTN	AGT GGT GGA AAT ATT	8	0,6	-
#γO-VP64	SPADLTR	RTDTLRD	DKKDLTR	QSSNLVR	TSGELVR	GCT GAA ACC ACG ACA	15	0,8	-
#γP-VP64	DKKDLTR	QRANLRA	HRTTLTN	ERSHLRE	QKSSLIA	ATA AGC AGT AAA ACC	17	1,4	-
#γA-opt-VP64	TSGHLVR	QAGHLAS	TSGSLVR	DCRDLAR	TSGNLVR	GAT GCC GTT TGA GGT	68	4,1	7,8

DNA interacting helices of 5-ZF clones from the fifth round of selection and their predicted target sites compared to control ZFs.

[a] Zinc finger helices are positioned in the anti-parallel orientation (C-terminal-F5 to F1-N-terminal) relative to the DNA target sequence. Amino acid positions -1 to +6 of each DNA recognition helix are shown. It should be noted that gg1-VP64 has six zinc finger domains rather than five. [b] Percentage of 293T cells that were GFP^+ in transduction efficiency test. [c] Fold increase in relative luciferase activity in HeLa cells standardized to the non-DNA binding Ala-ZF negative control. [d] Fold activation of human γ-globin in β-YAC BMCs containing selected zinc-fingers measured by RT-PCR.

Supplementary Methods

Construction of pPur-γ-gp

For the construction of pPur-γ-gp, expressing murine leukemia virus (MLV) gag and pol genes under the control of γ-globin promoter and enhancer region, oligonucleotides pPur-Linker-for (AATTAGATCTCGAAGTGCTAGCCGAAGTGGCCGGCCCGAAGTG) and pPur-linker-rev (AATTCACTTCGGGCCGGCCACTTCGGCTAGCACTTCGAGATCT) were annealed and the duplex was subcloned into the pPur plasmid (BD Biosciences) linearized with EcoR1; this introduced BglII, NheI and FseI sites. To introduce the γ-globin promoter and enhancer region (HS2/3 of LCR) into this plasmid, a fragment was amplified by PCR from the γβ-luc plasmid using oligonucleotides containing a NheI site (gb-luc-f, GCTCGACAGATCTCGAGCTGTGGATAAAGGAG, and gb-luc-Nhe-b, CCATTAGCTAGCTTGGACTAGGAGCTTATTGATA). This fragment was digested with NheI and BglII and ligated into the digested plasmid. Into the resulting plasmid a 220-bp PCR fragment containing SV40 poly-A (SV40pA-Fse-f, ATCAGCTAGCCGGGCCGGCCGCTTCGAGCAGACATGATAAGATAC, and SV40pA-EcoR-b, TCAAGAATTCTACGTTTACCACATTTGTAGAGG) was ligated after digestion with EcoRI and NheI, producing pPur-γ-pA and inserting a FseI site. A 5.2-kb PCR fragment containing murine leukemia virus gag-pol genes derived from the pHit60 plasmid was amplified using the oligonucleotides Hit-gp-Nhe-f (GAGGAGGAGGAGCTAGCATGGCCAGACTGTTACCACTCCCTTAAGTTTG) and Hit-gp-Fse-b (TCAGTGGCCGGCCTTAGGGGGCCTCGCGGGTTAACCT). This fragment was ligated into the pPur-γ-pA plasmid after digestion with FseI and NheI to create pPur-γ-gp.

Construction of #γA-opt

The modified protein, #γA-opt, was constructed using the SuperZif-plasmid. Briefly, three SuperZiF plasmids were synthesized containing all of the GNN, ANN, or CNN domains previously published; one SuperZiF plasmid each for the GNN, ANN, and CNN zinc-finger domains. For library assembly, /XhoI/ and /XmaI/ restriction sites are 5' to each finger and /AgeI/ and /SpeI/ sites are 3' to each finger. When creating zinc-finger libraries, the SuperZiF plasmids can be cut with /XhoI/ and /SpeI/ to release each finger’s coding sequence in equal molar quantities, thereby reducing library bias. For constructing designed proteins, unique restriction sites were placed between each finger, enabling the isolation of any individual finger from the appropriate SuperZiF vector.