Supplementary Materials for

Reconfigurable genetic inverters using contextual dependencies

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This supplementary information includes:

- Additional figures relating to the study.
- Additional tables relating to the study.
- Description of the codes and the data relating to the study.

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1 Decomposing fluorescence and scattering

In our experiments we aim to pick cells in the log-phase, this causes the cell-size distribution to be constant [1]. We do however see that there is some experiment to experiment variation of the cell size distribution. To negate the influence of the cell size variation into the fluorescence measures we decompose the fluorescence values in two components: a part that is experiment-dependent and a part that is scattering dependent. We evaluate the part that is scattering dependent on the context average. The raw flow cytometry files, along with the decomposed values are available at DOI:10.25405/data.ncl.12073479.

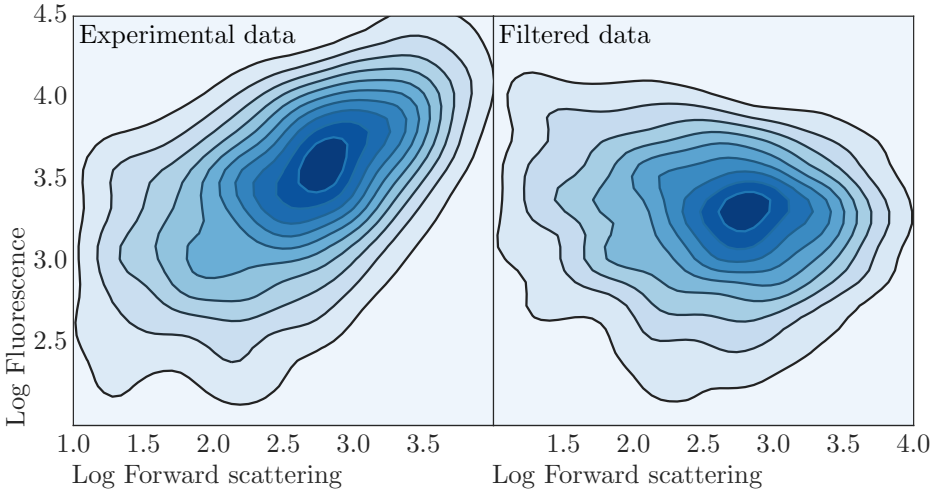


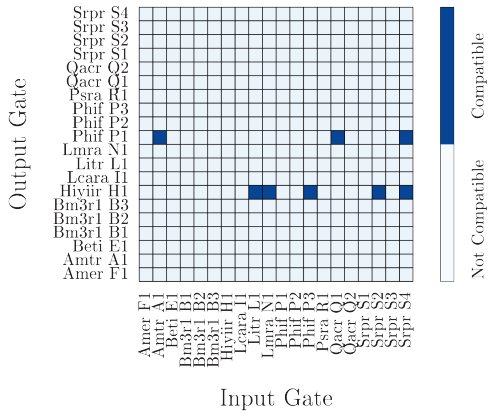
Figure S1: Removal of correlation between cell scattering and fluorescence

2 Compatibility tables for individual strains

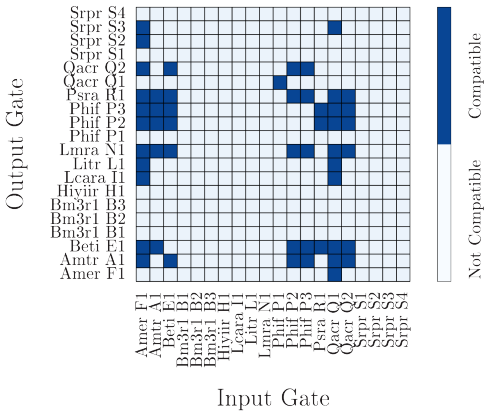
In order that the genetic circuits may be treated as logic NOT gates, the continuous variable (experimentally obtained standardised fluorescence) must be interpreted as a discrete variable (representing logic 1 or 0). Thresholds are therefore required to partition the input and output fluorescence values into groups that are to be interpreted as a logic value, or rejected as ambiguous. Compatibility between two gates is a qualitative measure of the agreement of these thresholds. In particular, the output thresholds of the 'input gate' must not lie in the group of inputs that would be rejected as ambiguous by the 'output gate'. Smaller ambiguous regions will increase the numbers of compatible pairs *in silico*, but circuits built from such pairs may behave unpredictably in the presence of noise or measurement error of a real system. Larger ambiguous regions will guard against the effect of noise, at the cost of flexibility in design. In this study we use a thresholding scheme that has used previously for the same library [2]. Further, we consider that two gates may only be connected if they are compatible, and a library with many compatible gates is desirable.

Here we show the compatibility between pairs of gates in individual strains, illustrating what can be achieved by incorporating backbone as a design parameter, without changing host. The superior performance found in the DH5α and CC118λpir *E. coli.* strains, in comparison to the *P.Putida* strain KT2440, may reflect the fact that the library components were initially selected for use in an *E. coli.* host, and that choice of host significantly impacts behaviour of genetic parts.

(a) Compatibility table for gates characterised in KT2440



(b) Compatibility table for gates characterised in CC118λpir



(c) Compatibility table for gates characterised in DH5α

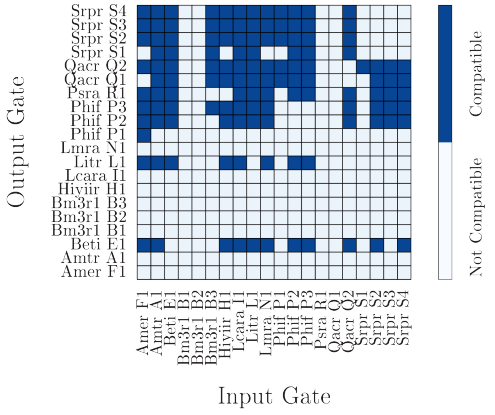


Figure S2: Compatibility tables for the library in different hosts. Input gate on the x-axis is the first gate, whose output provides the input for 'Output gate' on the y-axis. Two gates are compatible if their thresholds agree, and they do not use the same repressor molecule.

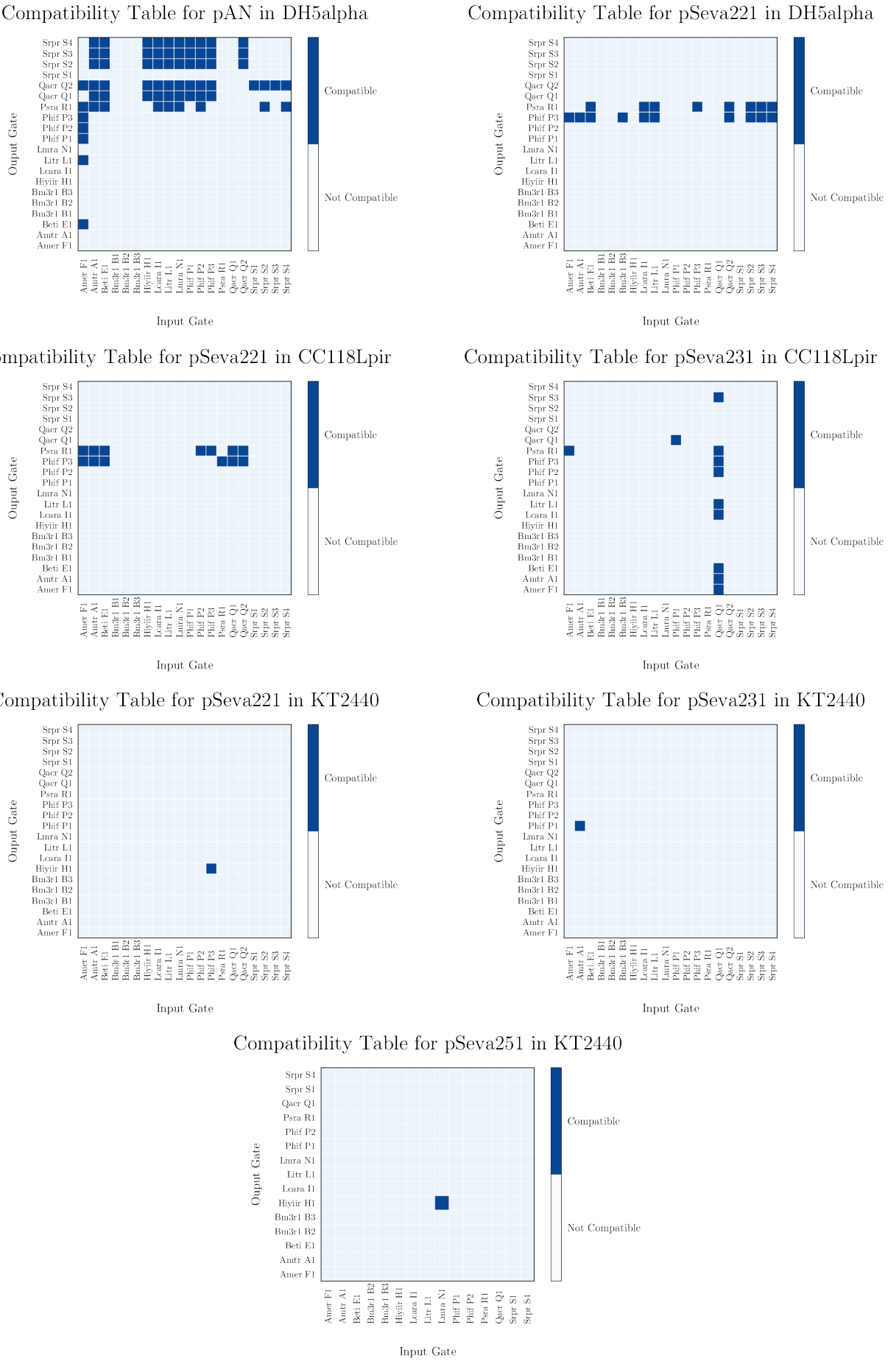


Figure S3: Compatibility tables for the library in each of the 7 contexts. Each table shows the compatible pairings for the library when placed in a given host and on a given backbone.

3 List of constructs

Table S1: 12 main gates and 8 variants that were obtained from the Cello work.

Inverters	Description	Study
pAN::AmeR-F1	NOT Gate	Nielsen et al[1]
pAN::AmtR-A1	NOT Gate	Nielsen et al[1]
pAN::BetI-E1	NOT Gate	Nielsen et al[1]
pAN::BM3R1-B1	NOT Gate	Nielsen et al[1]
pAN::BM3R1-B2	NOT Gate	Nielsen et al[1]
pAN::BM3R1-B3	NOT Gate	Nielsen et al[1]
pAN::HIyIIR-H1	NOT Gate	Nielsen et al[1]
pAN::lcaRA-I1	NOT Gate	Nielsen et al[1]
pAN::LitR-L1	NOT Gate	Nielsen et al[1]
pAN::LmrA-N1	NOT Gate	Nielsen et al[1]
pAN::PhIF-P1	NOT Gate	Nielsen et al[1]
pAN::PhIF-P2	NOT Gate	Nielsen et al[1]
pAN::PhIF-P3	NOT Gate	Nielsen et al[1]
pAN::PsrA-R1	NOT Gate	Nielsen et al[1]
pAN::QacR-Q1	NOT Gate	Nielsen et al[1]
pAN::QacR-Q2	NOT Gate	Nielsen et al[1]
pAN::SrpR-S1	NOT Gate	Nielsen et al[1]
pAN::SrpR-S2	NOT Gate	Nielsen et al[1]
pAN::SrpR-S3	NOT Gate	Nielsen et al[1]
pAN::SrpR-S4	NOT Gate	Nielsen et al[1]
pAN::1201	Autofluorescence	Nielsen et al[1]
pAN::1717	Standardisation	Nielsen et al[1]
pAN::1818	Promoter Activity	Nielsen et al[1]

Table S2: List of all libraries for context dependent inverters.

Strain	Backbone	Stock Name	Library	Work	Definition
Ecoli DH5α	pAN	Tas74	Ecoli DH5α pAN::Amer-F1	This study	NOT Gate
		Tas75	Ecoli DH5α pAN::AmtR-A1	This study	NOT Gate
		Tas76	Ecoli DH5α pAN::BetI-E1	This study	NOT Gate
		Tas77	Ecoli DH5α pAN::BM3R1-B1	This study	NOT Gate
		Tas78	Ecoli DH5α pAN::BM3R1-B2	This study	NOT Gate
		Tas79	Ecoli DH5α pAN::BM3R1-B3	This study	NOT Gate
		Tas364	Ecoli DH5α pAN::HIyIIR-H1	This study	NOT Gate
		Tas81	Ecoli DH5α pAN::lcaRA-I1	This study	NOT Gate
		Tas82	Ecoli DH5α pAN::LitR-L1	This study	NOT Gate
		Tas83	Ecoli DH5α pAN::LmrA-N1	This study	NOT Gate
		Tas84	Ecoli DH5α pAN::PhIF-P1	This study	NOT Gate
		Tas85	Ecoli DH5α pAN::PhIF-P2	This study	NOT Gate
		Tas86	Ecoli DH5α pAN::PhIF-P3	This study	NOT Gate
		Tas87	Ecoli DH5α pAN::PsrA-R1	This study	NOT Gate
		Tas88	Ecoli DH5α pAN::QacR-Q1	This study	NOT Gate
		Tas89	Ecoli DH5α pAN::QacR-Q2	This study	NOT Gate
		Tas90	Ecoli DH5α pAN::SrpR-S1	This study	NOT Gate
		Tas91	Ecoli DH5α pAN::SrpR-S2	This study	NOT Gate
		Tas365	Ecoli DH5α pAN::SrpR-S3	This study	NOT Gate
		Tas93	Ecoli DH5α pAN::SrpR-S4	This study	NOT Gate
		Tas94	Ecoli DH5α pAN::1201	This study	Empty plasmid for autofluorescence
		Tas95	Ecoli DH5α pAN::1717	This study	RPU standard plasmid for standardization
		Tas366	Ecoli DH5α pAN::1818	This study	pTac activity plasmid for promoter activity
	pSEVA221	Tas385	Ecoli DH5α pSeva221::Amer-F1	This study	NOT Gate
		Tas386	Ecoli DH5α pSeva221::AmtR-A1	This study	NOT Gate
		Tas387	Ecoli DH5α pSeva221::BetI-E1	This study	NOT Gate
		Tas388	Ecoli DH5α pSeva221::BM3R1-B1	This study	NOT Gate
		Tas389	Ecoli DH5α pSeva221::BM3R1-B2	This study	NOT Gate
		Tas390	Ecoli DH5α pSeva221::BM3R1-B3	This study	NOT Gate
		Tas391	Ecoli DH5α pSeva221::HIyIIR-H1	This study	NOT Gate
		Tas392	Ecoli DH5α pSeva221::lcaRA-I1	This study	NOT Gate
		Tas393	Ecoli DH5α pSeva221::LitR-L1	This study	NOT Gate
		Tas394	Ecoli DH5α pSeva221::LmrA-N1	This study	NOT Gate
		Tas395	Ecoli DH5α pSeva221::PhIF-P1	This study	NOT Gate
		Tas396	Ecoli DH5α pSeva221::PhIF-P2	This study	NOT Gate
		Tas397	Ecoli DH5α pSeva221::PhIF-P3	This study	NOT Gate
		Tas398	Ecoli DH5α pSeva221::PsrA-R1	This study	NOT Gate
		Tas399	Ecoli DH5α pSeva221::QacR-Q1	This study	NOT Gate
		Tas400	Ecoli DH5α pSeva221::QacR-Q2	This study	NOT Gate
		Tas401	Ecoli DH5α pSeva221::SrpR-S1	This study	NOT Gate
		Tas402	Ecoli DH5α pSeva221::SrpR-S2	This study	NOT Gate
		Tas403	Ecoli DH5α pSeva221::SrpR-S3	This study	NOT Gate
		Tas404	Ecoli DH5α pSeva221::SrpR-S4	This study	NOT Gate

	Tas361	Ecoli DH5α pSeva221::1201	This study	Empty plasmid for autofluorescence
	Tas362	Ecoli DH5α pSeva221::1717	This study	RPU standard plasmid for standardization
	Tas363	Ecoli DH5α pSeva221::1818	This study	pTac activity plasmid for promoter activity
Ecoli cc118λpir / pSEVA221	Tas1	Ecoli cc118λpir pSeva221::Amer-F1	This study	NOT Gate
	Tas2	Ecoli cc118λpir pSeva221::AmtR-A1	This study	NOT Gate
	Tas3	Ecoli cc118λpir pSeva221::BetI-E1	This study	NOT Gate
	Tas4	Ecoli cc118λpir pSeva221::BM3R1-B1	This study	NOT Gate
	Tas5	Ecoli cc118λpir pSeva221::BM3R1-B2	This study	NOT Gate
	Tas6	Ecoli cc118λpir pSeva221::BM3R1-B3	This study	NOT Gate
	Tas7	Ecoli cc118λpir pSeva221::HlyIIR-H1	This study	NOT Gate
	Tas8	Ecoli cc118λpir pSeva221::lcaRA-I1	This study	NOT Gate
	Tas9	Ecoli cc118λpir pSeva221::LitR-L1	This study	NOT Gate
	Tas10	Ecoli cc118λpir pSeva221::LmrA-N1	This study	NOT Gate
	Tas11	Ecoli cc118λpir pSeva221::PhlF-P1	This study	NOT Gate
	Tas12	Ecoli cc118λpir pSeva221::PhlF-P2	This study	NOT Gate
	Tas13	Ecoli cc118λpir pSeva221::PhlF-P3	This study	NOT Gate
	Tas14	Ecoli cc118λpir pSeva221::PsrA-R1	This study	NOT Gate
	Tas15	Ecoli cc118λpir pSeva221::QacR-Q1	This study	NOT Gate
	Tas16	Ecoli cc118λpir pSeva221::QacR-Q2	This study	NOT Gate
	Tas17	Ecoli cc118λpir pSeva221::SrpR-S1	This study	NOT Gate
	Tas18	Ecoli cc118λpir pSeva221::SrpR-S2	This study	NOT Gate
	Tas19	Ecoli cc118λpir pSeva221::SrpR-S3	This study	NOT Gate
	Tas20	Ecoli cc118λpir pSeva221::SrpR-S4	This study	NOT Gate
	Tas65	Ecoli cc118λpir pSeva221::1201	This study	Empty plasmid for autofluorescence
	Tas68	Ecoli cc118λpir pSeva221::1717	This study	RPU standard plasmid for standardization
	Tas317	Ecoli cc118λpir pSeva221::1818	This study	pTac activity plasmid for promoter activity
	Tas367	Ecoli cc118λpir pSeva231::Amer-F1	This study	NOT Gate
	Tas368	Ecoli cc118λpir pSeva231::AmtR-A1	This study	NOT Gate
	Tas23	Ecoli cc118λpir pSeva231::BetI-E1	This study	NOT Gate
	Tas24	Ecoli cc118λpir pSeva231::BM3R1-B1	This study	NOT Gate
	Tas25	Ecoli cc118λpir pSeva231::BM3R1-B2	This study	NOT Gate
	Tas26	Ecoli cc118λpir pSeva231::BM3R1-B3	This study	NOT Gate
	Tas27	Ecoli cc118λpir pSeva231::HlyIIR-H1	This study	NOT Gate
	Tas28	Ecoli cc118λpir pSeva231::lcaRA-I1	This study	NOT Gate
	Tas29	Ecoli cc118λpir pSeva231::LitR-L1	This study	NOT Gate
	Tas30	Ecoli cc118λpir pSeva231::LmrA-N1	This study	NOT Gate
	Tas31	Ecoli cc118λpir pSeva231::PhlF-P1	This study	NOT Gate
	Tas32	Ecoli cc118λpir pSeva231::PhlF-P2	This study	NOT Gate
	Tas33	Ecoli cc118λpir pSeva231::PhlF-P3	This study	NOT Gate
	Tas34	Ecoli cc118λpir pSeva231::PsrA-R1	This study	NOT Gate
	Tas369	Ecoli cc118λpir pSeva231::QacR-Q1	This study	NOT Gate
	Tas36	Ecoli cc118λpir pSeva231::QacR-Q2	This study	NOT Gate
	Tas37	Ecoli cc118λpir pSeva231::SrpR-S1	This study	NOT Gate
	Tas38	Ecoli cc118λpir pSeva231::SrpR-S2	This study	NOT Gate
	Tas39	Ecoli cc118λpir pSeva231::SrpR-S3	This study	NOT Gate
	Tas40	Ecoli cc118λpir pSeva231::SrpR-S4	This study	NOT Gate

	Tas66	Ecoli cc118λpir pSeva231::1201	This study	Empty plasmid for autofluorescence
	Tas69	Ecoli cc118λpir pSeva231::1717	This study	RPU standard plasmid for standardization
	Tas370	Ecoli cc118λpir pSeva231::1818	This study	pTac activity plasmid for promoter activity
P_{Putida} KT2440	Tas193	Pputida KT2440 pSeva221::Amer-F1	This study	NOT Gate
	Tas194	Pputida KT2440 pSeva221::AmtR-A1	This study	NOT Gate
	Tas195	Pputida KT2440 pSeva221::BetI-E1	This study	NOT Gate
	Tas196	Pputida KT2440 pSeva221::BM3R1-B1	This study	NOT Gate
	Tas197	Pputida KT2440 pSeva221::BM3R1-B2	This study	NOT Gate
	Tas198	Pputida KT2440 pSeva221::BM3R1-B3	This study	NOT Gate
	Tas199	Pputida KT2440 pSeva221::HIyIIR-H1	This study	NOT Gate
	Tas200	Pputida KT2440 pSeva221::IcaRA-I1	This study	NOT Gate
	Tas201	Pputida KT2440 pSeva221::LitR-L1	This study	NOT Gate
	Tas202	Pputida KT2440 pSeva221::LmrA-N1	This study	NOT Gate
	Tas203	Pputida KT2440 pSeva221::PhlF-P1	This study	NOT Gate
	Tas204	Pputida KT2440 pSeva221::PhlF-P2	This study	NOT Gate
	Tas205	Pputida KT2440 pSeva221::PhlF-P3	This study	NOT Gate
	Tas206	Pputida KT2440 pSeva221::PsrA-R1	This study	NOT Gate
	Tas207	Pputida KT2440 pSeva221::QacR-Q1	This study	NOT Gate
	Tas208	Pputida KT2440 pSeva221::QacR-Q2	This study	NOT Gate
	Tas209	Pputida KT2440 pSeva221::SrpR-S1	This study	NOT Gate
	Tas210	Pputida KT2440 pSeva221::SrpR-S2	This study	NOT Gate
	Tas211	Pputida KT2440 pSeva221::SrpR-S3	This study	NOT Gate
	Tas212	Pputida KT2440 pSeva221::SrpR-S4	This study	NOT Gate
pSEVA221	Tas257	Pputida KT2440 pSeva221::1201	This study	Empty plasmid for autofluorescence
	Tas260	Pputida KT2440 pSeva221::1717	This study	RPU standard plasmid for standardization
	Tas266	Pputida KT2440 pSeva221::1818	This study	pTac activity plasmid for promoter activity
P_{Putida} KT2440	Tas213	Pputida KT2440 pSeva231::Amer-F1	This study	NOT Gate
	Tas214	Pputida KT2440 pSeva231::AmtR-A1	This study	NOT Gate
	Tas215	Pputida KT2440 pSeva231::BetI-E1	This study	NOT Gate
	Tas216	Pputida KT2440 pSeva231::BM3R1-B1	This study	NOT Gate
	Tas217	Pputida KT2440 pSeva231::BM3R1-B2	This study	NOT Gate
	Tas218	Pputida KT2440 pSeva231::BM3R1-B3	This study	NOT Gate
	Tas219	Pputida KT2440 pSeva231::HIyIIR-H1	This study	NOT Gate
	Tas220	Pputida KT2440 pSeva231::IcaRA-I1	This study	NOT Gate
	Tas221	Pputida KT2440 pSeva231::LitR-L1	This study	NOT Gate
	Tas222	Pputida KT2440 pSeva231::LmrA-N1	This study	NOT Gate
	Tas223	Pputida KT2440 pSeva231::PhlF-P1	This study	NOT Gate
	Tas224	Pputida KT2440 pSeva231::PhlF-P2	This study	NOT Gate
	Tas225	Pputida KT2440 pSeva231::PhlF-P3	This study	NOT Gate
	Tas226	Pputida KT2440 pSeva231::PsrA-R1	This study	NOT Gate
	Tas227	Pputida KT2440 pSeva231::QacR-Q1	This study	NOT Gate
	Tas228	Pputida KT2440 pSeva231::QacR-Q2	This study	NOT Gate
	Tas229	Pputida KT2440 pSeva231::SrpR-S1	This study	NOT Gate
	Tas230	Pputida KT2440 pSeva231::SrpR-S2	This study	NOT Gate
	Tas231	Pputida KT2440 pSeva231::SrpR-S3	This study	NOT Gate
	Tas232	Pputida KT2440 pSeva231::SrpR-S4	This study	NOT Gate
pSEVA231				

		Tas258	Pputida KT2440 pSeva231::1201	This study	Empty plasmid for autofluorescence
		Tas261	Pputida KT2440 pSeva231::1717	This study	RPU standard plasmid for standardization
		Tas267	Pputida KT2440 pSeva231::1818	This study	pTac activity plasmid for promoter activity
	pSEVA251	Tas233	Pputida KT2440 pSeva251::Amer-F1	This study	NOT Gate
		Tas234	Pputida KT2440 pSeva251::AmtR-A1	This study	NOT Gate
		Tas235	Pputida KT2440 pSeva251::BetI-E1	This study	NOT Gate
		Tas237	Pputida KT2440 pSeva251::BM3R1-B2	This study	NOT Gate
		Tas238	Pputida KT2440 pSeva251::BM3R1-B3	This study	NOT Gate
		Tas239	Pputida KT2440 pSeva251::HIyIIR-H1	This study	NOT Gate
		Tas240	Pputida KT2440 pSeva251::lcaRA-I1	This study	NOT Gate
		Tas241	Pputida KT2440 pSeva251::LitR-L1	This study	NOT Gate
		Tas242	Pputida KT2440 pSeva251::LmrA-N1	This study	NOT Gate
		Tas243	Pputida KT2440 pSeva251::PhlF-P1	This study	NOT Gate
		Tas244	Pputida KT2440 pSeva251::PhlF-P2	This study	NOT Gate
		Tas246	Pputida KT2440 pSeva251::PsrA-R1	This study	NOT Gate
		Tas247	Pputida KT2440 pSeva251::QacR-Q1	This study	NOT Gate
		Tas249	Pputida KT2440 pSeva251::SrpR-S1	This study	NOT Gate
		Tas252	Pputida KT2440 pSeva251::SrpR-S4	This study	NOT Gate
		Tas259	Pputida KT2440 pSeva251::1201	This study	Empty plasmid for autofluorescence
		Tas262	Pputida KT2440 pSeva251::1717	This study	RPU standard plasmid for standardization
		Tas285	Pputida KT2440 pSeva251::1818	This study	pTac activity plasmid for promoter activity

Name	Sequence 5→3	Notes
Gate-Ctrl-Unvrsl-1	TCTAGGGCGGCGGATTTG	This is the position right before the MCS. Same for pSEVA221, pSEVA231, pSEVA251
Gate-Ctrl-Unvrsl-3	TGGTGAGCAAGGGCGAG	This is the position right after the multiplicity region of the gates This is same for pSEVA221, pSEVA231, pSEVA251
Gate-Ctrl-Unvrsl-4	ACCTTAGCTACCAGTCCGC	This is the position is same for pSEVA221, pSEVA231, pSEVA251
Gate-Ctrl-Unvrsl-5	ACAATCTTCTCGCGCAACG	This is the position is same for pSEVA221, pSEVA231, pSEVA251
Gate-Ctrl-Unvrsl-6	CGGTGAAGGGCAATCAGCT	This is the position is same for pSEVA221, pSEVA231, pSEVA251
Gate-Ctrl-Unvrsl-7	GAATGCATTATATGCACTCAGCGC	This is the position is the last one for covering the integrated region (when considered DNApol moves at least 600 bp) and same for pSEVA221, pSEVA231, pSEVA251
Gate-Ctrl-2-AmeR-F1	TTTTAGCAGCAAAAACGCACTGG	This is the 500th position for the AmeR-F1 gate
Gate-Ctrl-2-AmtR-A1	CCTGCTGAAAAGCACCGTTG	This is the 500th position for the AmtR-A1 gate
Gate-Ctrl-2-BetI-E1	GTCTGCATGCACTGCCG	This is the 500th position for the BetI-E1 gate
Gate-Ctrl-2-BM3R1-B123	CGGTCTGGCAAATGAACGTG	This is the 500th position for the BM3R1-B1 BM3R1-B2 and BM3R1-B3 gates and can be used in every cloning with these gates
Gate-Ctrl-2-HIyRII-H1	TGCCGAACTTTCTGGAAAAAAACC	This is the 500th position for the HIyIIR-H1 gate
Gate-Ctrl-2-lcaRA-I1	ACAAAAGCAACTATAGCATCGATGC	This is the 500th position for the lcaRA-I1 gate
Gate-Ctrl-2-litR-L1	CTGAACAAAGTGGAAAACGAGTTTCAC	This is the 500th position for the LitR-L1 gate
Gate-Ctrl-2-LmrA-N1	AAGAATATATCCGCCAGAAAATCGC	This is the 500th position for the LmrA-N1 gate
Gate-Ctrl-2-PhIF-P123	AAAATGAAAGCGAACAGGTGCG	This is the 500th position for the PhIF-P1 PhIF-P2 and PhIF-P3 gates
Gate-Ctrl-2-PsrA-R1	GGAACGTGAACTGGAACGTC	This is the 500th position for the PsrA-R1 gate
Gate-Ctrl-2-QacR-Q123	CAAATCAAATGCAAAACCAACCGC	This is the 500th position for the QacR-Q1 and QacR-Q2 gates
Gate-Ctrl-2-SrpR-S1234	TCCGCTGGAACTGGATTTTACAC	This is the 500th position for the SrpR-S1 SrpR-S2 SrpR-S3 and SrpR-S4 gates

Name	Sequence 5→3	Notes
PS1	AGGGCGGCGGATTTGTCC	
PS2	GCGGCAACCGAGCGTTC	
PS3	GAACGCTCGGTTGCCGC	
PS4	CCAGCCTCGCAGAGCAGG	
PS5	CCCTGCTTCGGGGTCATT	
PS6	GGACAAATCCGCCGCCCT	
Gate to pSEVA	CCTAGATTAATTAAAACACCCTTGTAT TACTGTTTATGTAAGC	Forward primer in order to pop out the gate
Gate to pSEVA	GTCTAAACTAGTCGTCCGGCGTAGAG GATC	Reverse primer in order to pop out the gate

Table S3: Primers List

In Table S1 there are 23 plasmids in total that are acquired from Cello study. 20 of these plasmids are NOT gates that have functional segments in common in all constructs. Those are LacI/P_{tac} expression system inducible with IPTG and *yfp* gene that is used for output readout. Gate specific units that are not shared for all are the RBS sites and gate proteins. Plasmids have kanamycin resistance gene and p15A origin of replication.

Autofluorescence plasmid is named as pAN::1201. This plasmid is used to measure background noise created by the backbone. The plasmid is composed of a constitutive P_{lac} promoter expressing lacZα gene. Also, two sensor proteins TetR and LacI are present at the same operon under P_{lacI} promoter. RPU standard plasmid is pAN::1717. This plasmid shares the same sensory proteins as in autofluorescence plasmid with the same P_{lacI}/LacI/TetR expression system, but the difference is that a *yfp* gene is expressed under constitutive promoter J23101. L3S2P21 terminator is used at the downstream of *yfp* for insulation of transcriptional read-throughs and native AraC terminator is used for insulation of *tetR* gene. pAN::1818 is the promoter activity plasmid. P_{lacI} constitutive promoter controls the expression of *lacI* and *tetR* genes. *yfp* gene is expressed at the downstream of P_{tac} promoter that is inducible with IPTG.

Table S2 shows the complete list of strains that are used in this study. We have constructed 7 different libraries changing in plasmid backbones, copy numbers and hosts. 7 libraries are consisting of 2 *Escherichia coli* strains (DH5α and CC118λpir) and 1 *Pseudomonas putida* strain (KT2440). DH5a is composed of two different backbones pAN and pSEVA. CC118λpir, whereas, it contains only pSEVA backbones however in two different copy numbers low and medium copy. Finally, KT2440 consists of low, medium and high copy numbers in pSEVA backbones. All 7 libraries have each of 20 NOT gates and 3 additional gates, autofluorescence plasmid, RPU standardization plasmid and Promoter activity plasmid. Only in KT2440 pSEVA251 (high copy) library we have encountered cloning problems in 5 of the gates which may have been resulted from a possible toxicity effect caused by them in higher copies. These failed gate clones are in pSEVA251 backbone BM3R1-B1, PhIF-P3, QacR-Q2, SrpR-S2 and SrpR-S3. Hence, in total 135 strains are generated in this study with NOT gates, and 21 strains that are to help to standardize outcomes and measure promoter activity, in total 156 strains listed in Table S2 are used in this work.

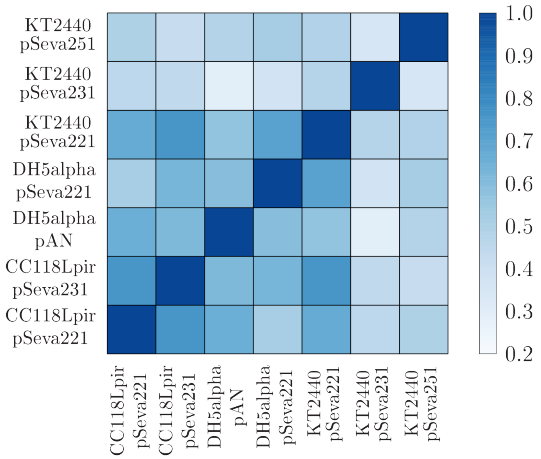
Table S3 gives detailed information about primers used in this study. There are four group of primers used here. First group is Gate-Unvrsl-Number primers, which are targeting various preserved regions in the expression system. These primers are to use for verification purposes of the clones and they are 6 in total. Second group of primers are called as Gate-Ctrl-2-Names. There are 12 of these plasmids which are named after the gate they are targeting. PCR verification of cloned gate can be done by using its corresponding group 2 primer. Third group is named with nomenclature PS-Number and they are 6 in total. These are general SEVA control primers targeting different regions of SEVA plasmids that help to confirm successful clones. Finally, in the fourth group there is a primer set used for pop-up of gates from pAN backbone to pSEVA backbone. These primers consist of gate homologous region and a flanking part that has PacI and SpeI restriction sides used for cloning into pSEVAs.

4 Context Similarity

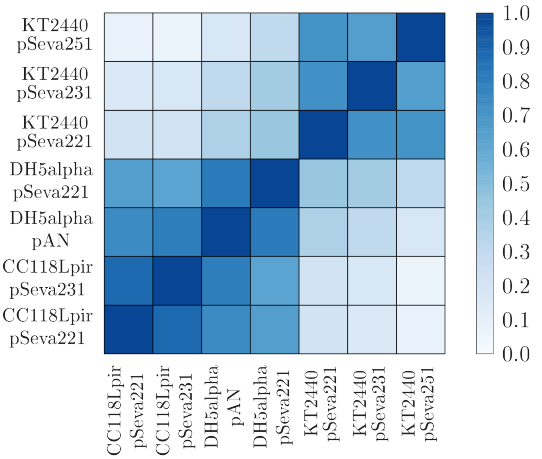
This study found that the same genetic logic gate can exhibit differing behaviour depending upon the context in which it was situated. The characterisation of the genetic logic gate may be both quantitatively and quantitatively different. Qualitative changes, such as to the shape of the response curve, are particularly interesting when considering the effects of context-circuit interplay, because they suggest these interactions are nonlinear phenomena.

We attempted to quantify changes in curve shape using a similarity measure as described in Methods of the manuscript. Log transformation of the curve ensures that deviations in the upper regions of input and output are not disproportionately penalised. The min-max normalisation in both input and output dimensions captures the shape information of the curve. Methods based on comparison of the gradient of the curves were also considered, and produce similar results.

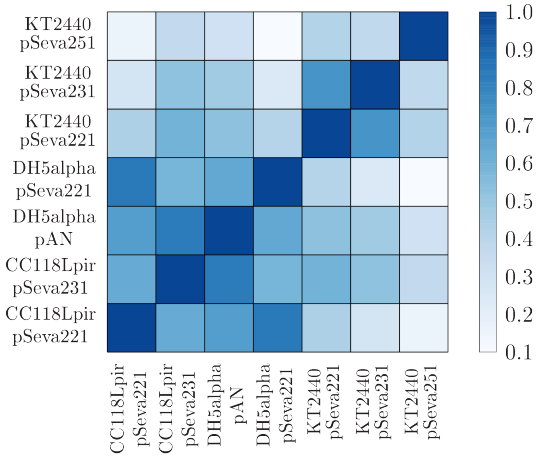
(a) Similarity scores heatmap for Amer F1 in 7 contexts.



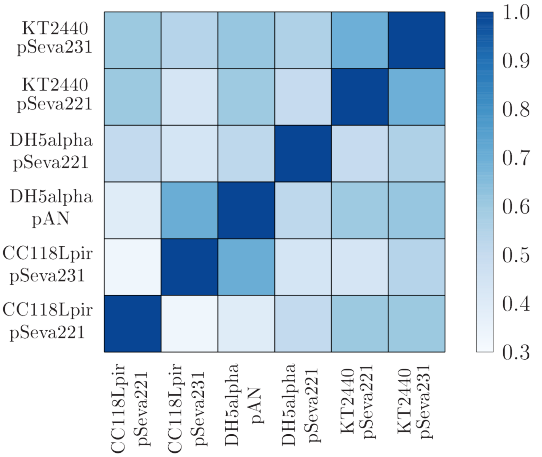
(b) Similarity scores heatmap for Amtr A1 in 7 contexts.



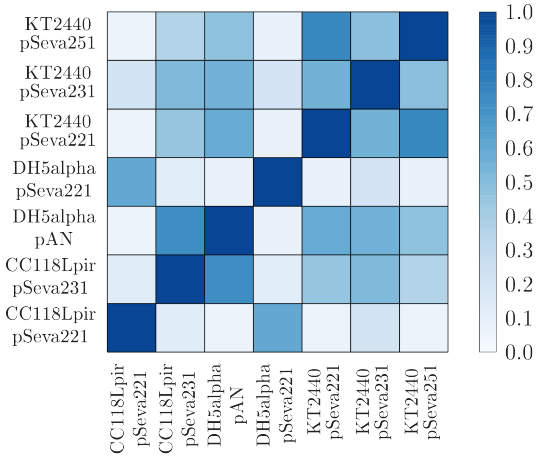
(c) Similarity scores heatmap for Beti E1 in 7 contexts.



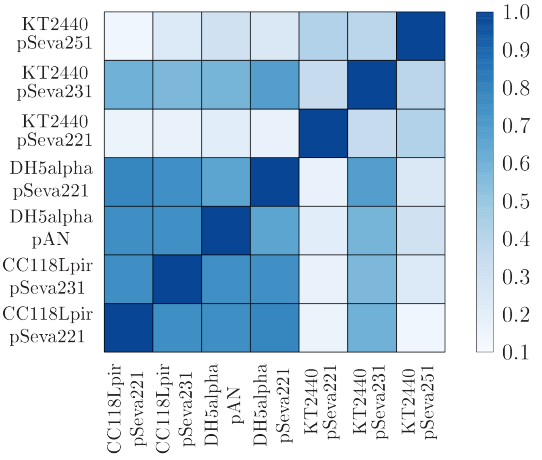
(d) Similarity scores heatmap for Bm3r1 B1 in 7 contexts.



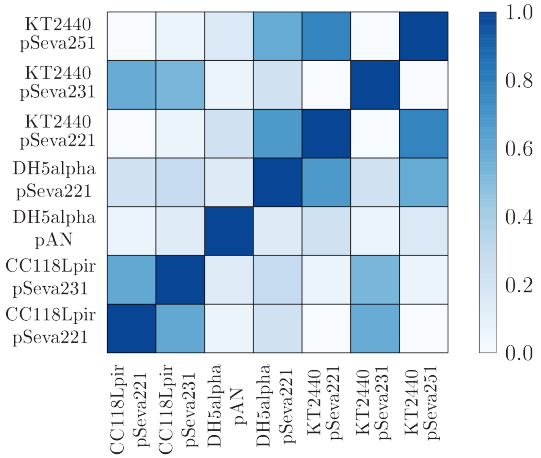
(e) Similarity scores heatmap for Bm3r1 B2 in 7 contexts.



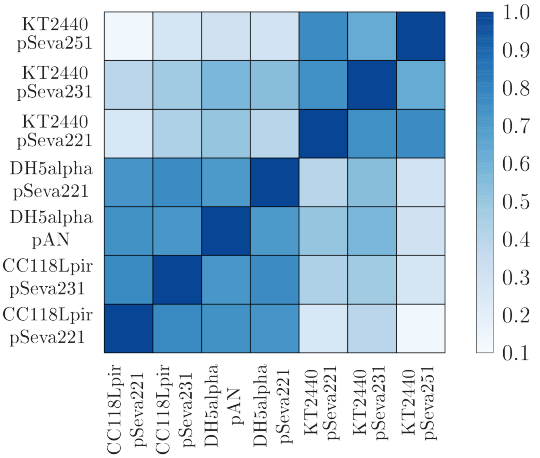
(f) Similarity scores heatmap for Bm3r1 B3 in 7 contexts.



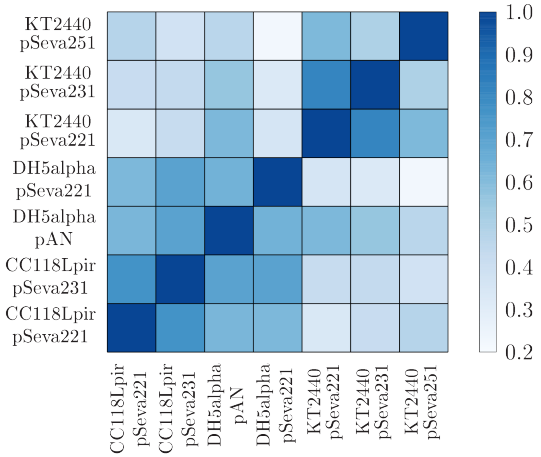
(g) Similarity scores heatmap for Hiyiir H1 in 7 contexts.



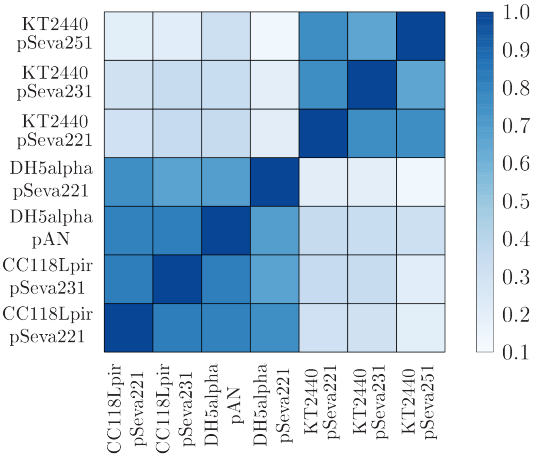
(h) Similarity scores heatmap for Lcara I1 in 7 contexts.



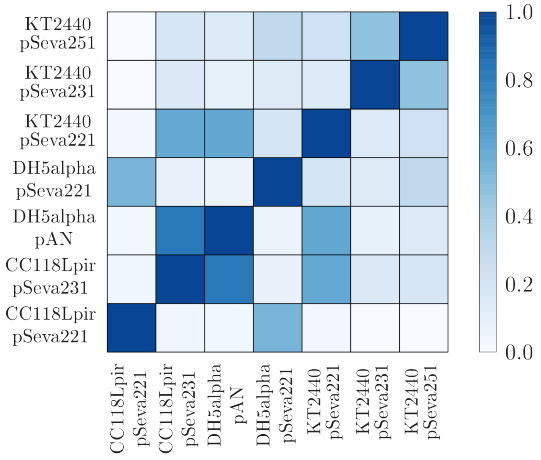
(i) Similarity scores heatmap for Litr L1 in 7 contexts.



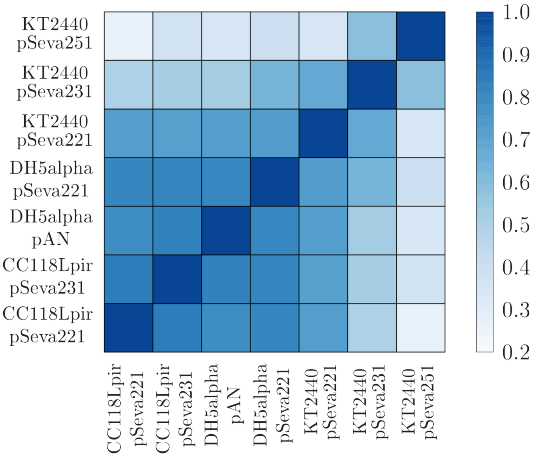
(j) Similarity scores heatmap for Lmra N1 in 7 contexts.



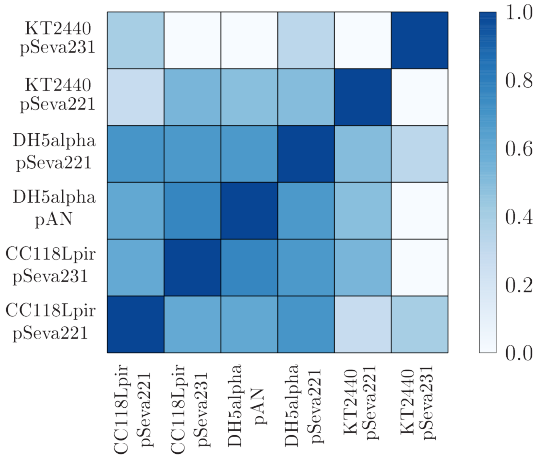
(k) Similarity scores heatmap for Phif P1 in 7 contexts.



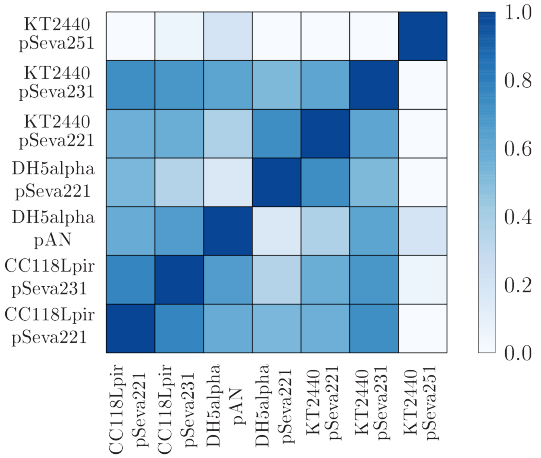
(l) Similarity scores heatmap for Phif P2 in 7 contexts.



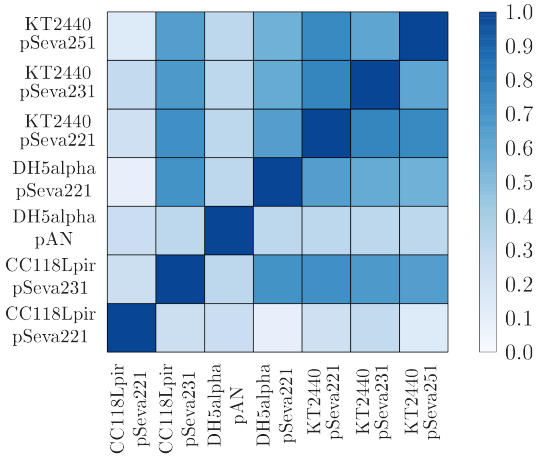
(m) Similarity scores heatmap for Phif P3 in 7 contexts.



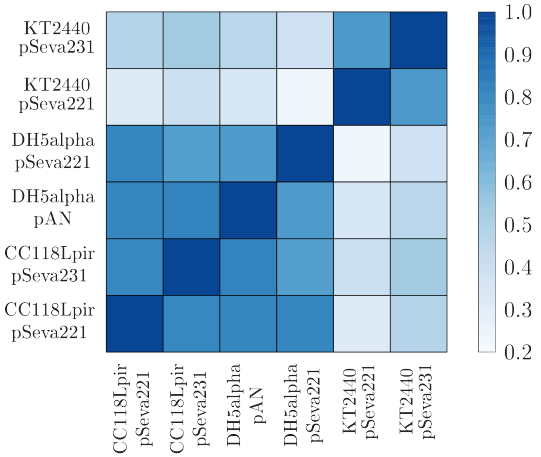
(n) Similarity scores heatmap for Psra R1 in 7 contexts.



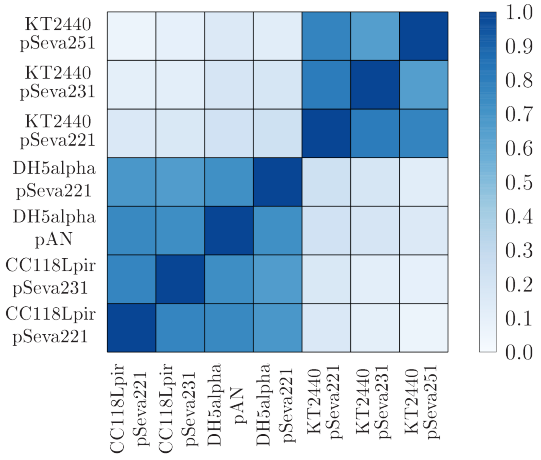
(o) Similarity scores heatmap for Qacr Q1 in 7 contexts.



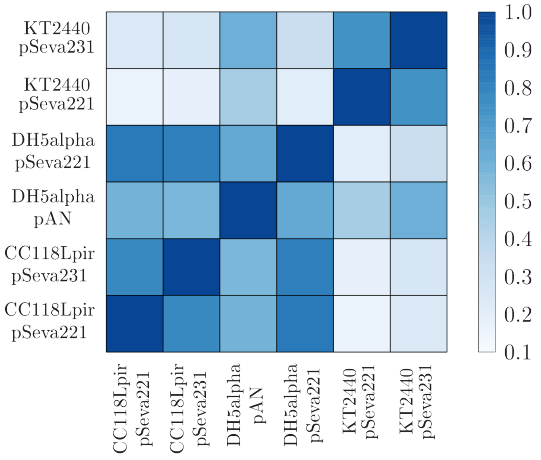
(p) Similarity scores heatmap for Qacr Q2 in 7 contexts.



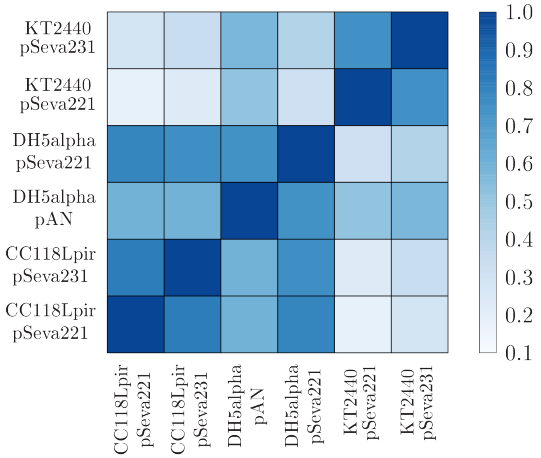
(q) Similarity scores heatmap for Srpr S1 in 7 contexts.



(r) Similarity scores heatmap for Srpr S2 in 7 contexts.



(s) Similarity scores heatmap for Srpr S3 in 7 contexts.



(t) Similarity scores heatmap for Srpr S4 in 7 contexts.

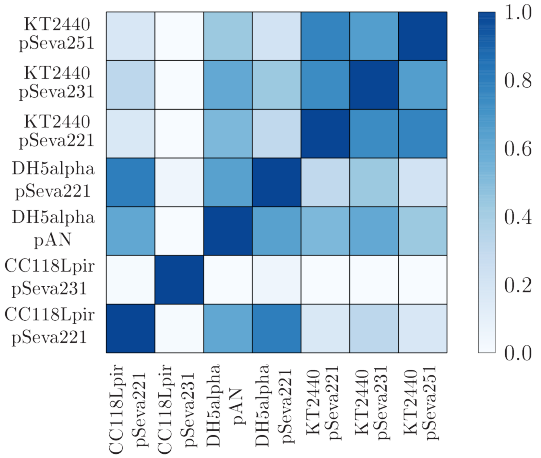


Figure S4: Similarity scores shown for all gates in all 7 contexts. A high similarity score (darker squares) is best. The minimum and maximum scores are 0 and 1, respectively.

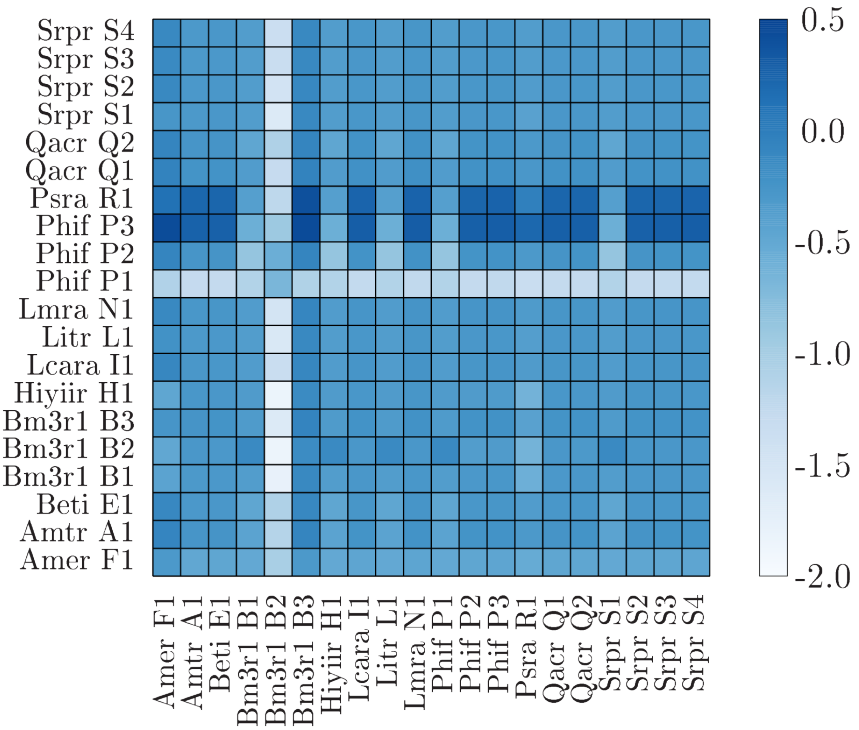
5 Compatibility Scoring

Whilst compatibility tables indicate which pairs of gates may be connected, they offer no indication as to which pairs are most or least compatible. It may be desirable, from a optimisation perspective, to select pairs of gates for which the first's output thresholds lie as far away from the second's ambiguous region as possible whose. Doing so will improve performance in spite of noise and provide a greater margin for error.

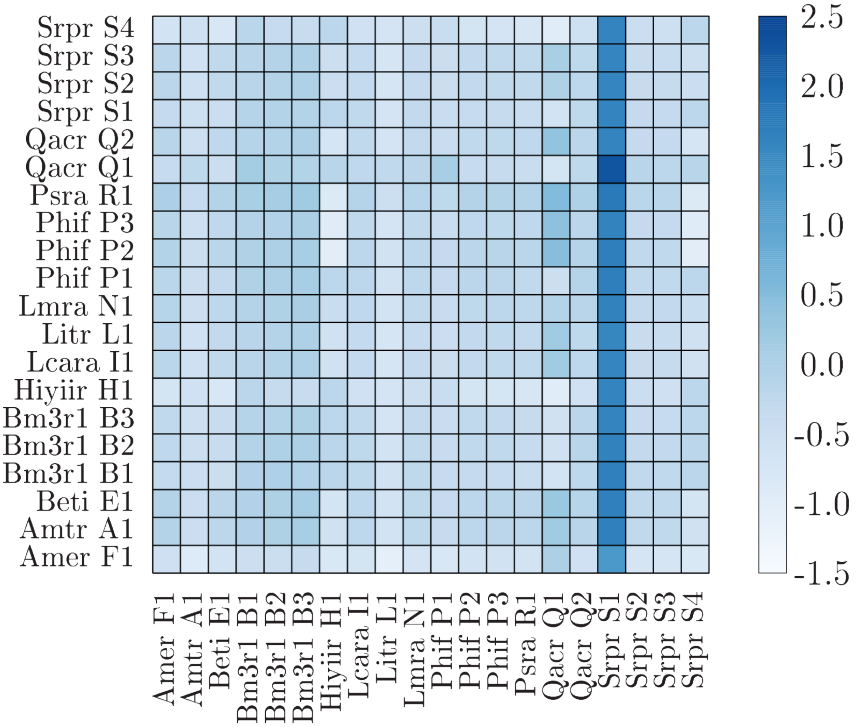
Conversely, we may wish to optimise the library for a specific context by redesign of the parts. In this case, we would like to know which pairs would be compatible with only small changes to their existing behaviour, such that the reward for our optimisation efforts are maximised.

We computed a compatibility score to measure these characteristics, as defined in Methods. A positive/negative score for a pair of gates indicated the pair is compatible/incompatible. Further, a positive score represents the minimum of the maximum perturbations to the thresholds that could be tolerated, whilst retaining the compatibility of the pair. A negative score represents the maximum of the minimum perturbations to the thresholds that would be required to make the pair compatible that could be allowed to any of thresholds. Thus, pairs of gates can be ranked in terms of compatibility.

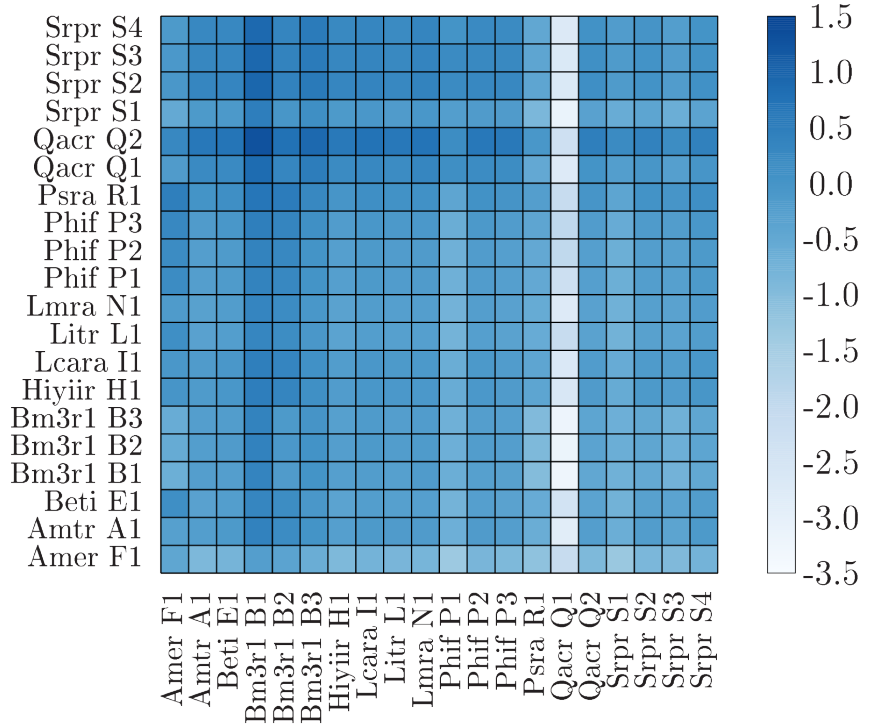
(a) Compatibility score heatmap for gates in the CC118λpir host with the pSeva221 backbone.



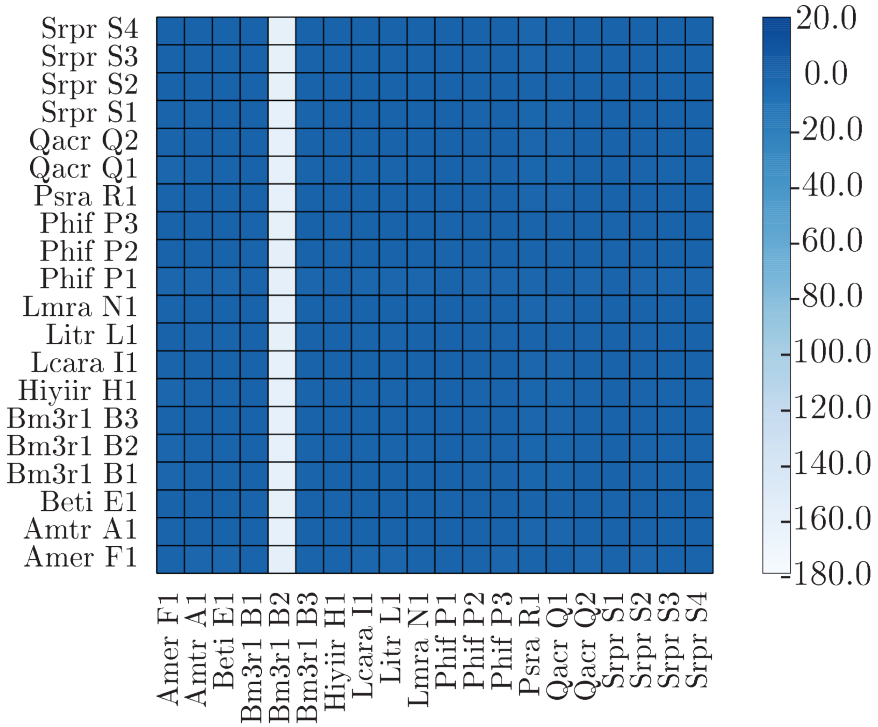
(b) Compatibility score heatmap for gates in the CC118λpir host with the pSeva231 backbone.



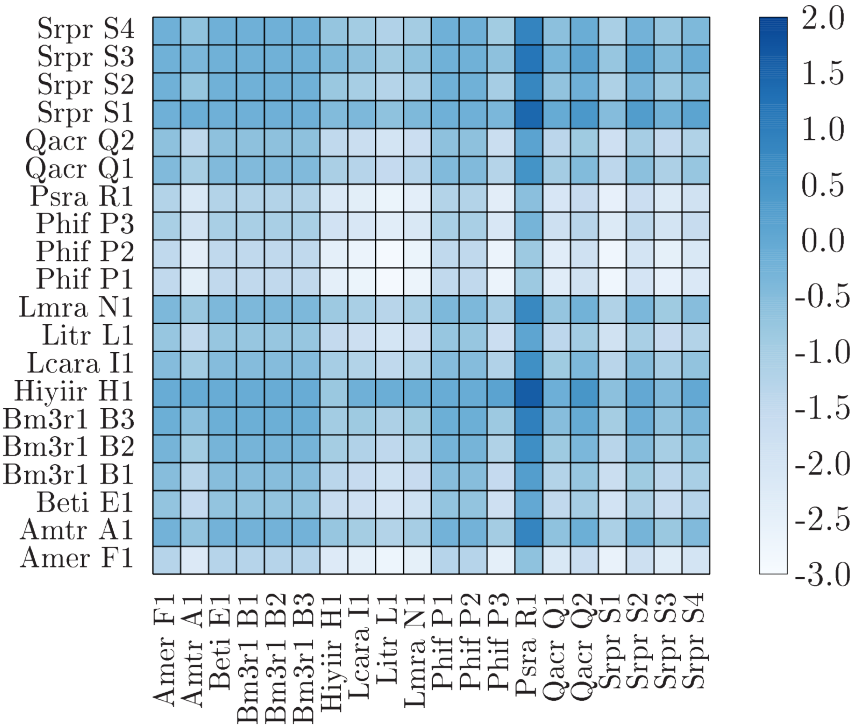
(c) Compatibility score heatmap for gates in the DH5α host with the pAN backbone.



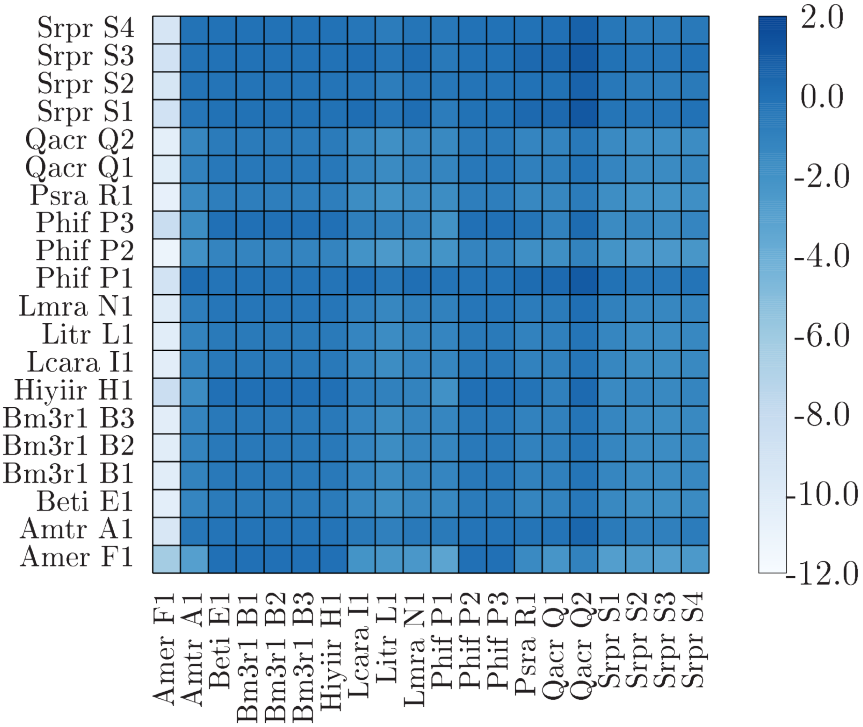
(d) Compatibility score heatmap for gates in the DH5α host with the pSeva221 backbone.



(e) Compatibility score heatmap for gates in the KT2440 host with the pSeva221 backbone.



(f) Compatibility score heatmap for gates in the KT2440 host with the pSeva231 backbone.



(g) Compatibility score heatmap for gates in the KT2440 host with the pSeva251 backbone.

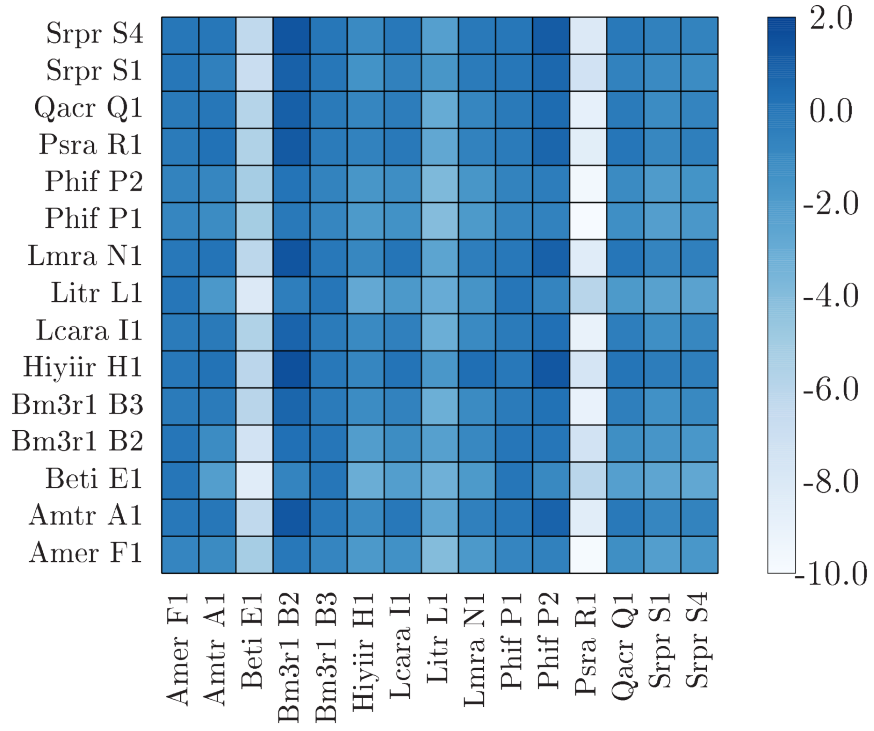


Figure S5: Compatibility scores between pairs of gates for all contexts. Higher scores are better and indicate more compatible pairs. Negative scores indicate incompatible pairs.

6 Numerical data

This section contains tables of numerical data that support the plots presented in the manuscript and in the rest of this supplementary material.

6.1 Gate Characterisation

The parameters of a hill equation are estimated from data for each gate. These parameters are then used for calculating the input and output thresholds as described in Methods. In the following tables, values of these parameters and thresholds are given, as well as whether the thresholds define a gate as a functional NOT gate. Values are omitted where the formula for a threshold produces a complex number, i.e. there are no solutions in the set of reals.

Table S4: AMER F1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.96	4.33	4.04	0.82	1.03	2.16	0.95	1.93	Yes
CC118Lpir	pSeva231	0.76	10.83	3.05	0.42	0.96	5.42	0.44	1.52	Yes
DH5alpha	pAN	1.19	8.03	2.83	0.76	1.32	4.01	0.86	2.37	Yes
DH5alpha	pSeva221	0.82	9.01	1.17	0.14	0.95	4.50	0.17	1.64	Yes
KT2440	pSeva221	9.41	14.42	0.48	0.01		7.21		18.82	No
KT2440	pSeva231	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	Yes
KT2440	pSeva251	2.71	3.81	0.04	0.00		1.91		5.41	No

Table S5: AMTR A1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.57	3.42	32.42	0.62	0.65	1.71	0.63	1.14	Yes
CC118Lpir	pSeva231	0.28	5.95	2.05	0.18	0.76	2.98	0.19	0.56	Yes
DH5alpha	pAN	0.26	1.38	1.30	0.21	0.53	0.69	0.31	0.52	Yes
DH5alpha	pSeva221	0.07	0.39	1.07	0.09	0.27	0.19	0.13	0.14	Yes
KT2440	pSeva221	0.30	1.67	0.85	0.07	0.30	0.84	0.12	0.61	Yes
KT2440	pSeva231	0.19	1.11	0.96	0.10	0.40	0.56	0.15	0.38	Yes
KT2440	pSeva251	0.12	0.28	2.97	0.24	0.16	0.14	0.47	0.25	No

Table S6: BETI E1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.62	3.90	43.73	0.64	0.66	1.95	0.65	1.24	Yes
CC118Lpir	pSeva231	0.28	7.22	2.41	0.34	1.27	3.61	0.35	0.56	Yes
DH5alpha	pAN	0.33	3.75	7.00	0.38	0.52	1.88	0.39	0.66	Yes
DH5alpha	pSeva221	0.12	2.96	28.13	0.13	0.14	1.48	0.13	0.24	Yes
KT2440	pSeva221	2.08	3.39	0.58	0.04		1.70		4.16	No
KT2440	pSeva231	1.44	2.02	1.45	0.11		1.01		2.88	No
KT2440	pSeva251	0.00	0.00	0.08	0.08	2.8×10^5	0.00	20.11	0.00	Yes

Table S7: BM3R1 B1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.64	0.76	2.00	0.00		0.38		1.27	No
CC118Lpir	pSeva231	0.28	0.91	1.65	0.28	0.32	0.45	0.49	0.55	No
DH5alpha	pAN	0.24	0.59	1.05	0.27	0.13	0.30	1.35	0.48	No
DH5alpha	pSeva221	0.11	0.14	2.00	0.00		0.07		0.23	No
KT2440	pSeva221	1.19	1.35	0.41	0.12		0.68		2.38	No
KT2440	pSeva231	0.99	1.20	1.03	0.04		0.60		1.98	No

Table S8: BM3R1 B2: Hill equation parameters and thresholds across all contexts. Values marked * are too large to be represented here.

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.14	0.67	0.57	3.96	23.21	0.34	10.33	0.28	Yes
CC118Lpir	pSeva231	0.29	1.05	2.75	0.41	0.48	0.52	0.55	0.59	No
DH5alpha	pAN	0.26	0.72	1.84	0.50	0.43	0.36	1.01	0.52	No
DH5alpha	pSeva221	0.02	0.12	0.00	0.00	*	0.06	*	0.04	Yes
KT2440	pSeva221	0.50	0.76	0.74	0.10		0.38		1.00	No
KT2440	pSeva231	1.03	1.27	0.77	0.04		0.63		2.06	No
KT2440	pSeva251	0.01	0.02	0.42	0.06	0.01	0.01	4.46	0.02	No

Table S9: BM3R1 B3: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.56	1.14	10.05	0.65	0.48	0.57	0.96	1.11	No
CC118Lpir	pSeva231	0.32	1.02	3.28	0.49	0.51	0.51	0.66	0.64	No
DH5alpha	pAN	0.27	0.66	3.90	0.36	0.28	0.33	0.56	0.55	No
DH5alpha	pSeva221	0.11	0.64	3.14	0.17	0.25	0.32	0.19	0.22	Yes
KT2440	pSeva221	0.24	0.36	0.00	0.00		0.18		0.47	No
KT2440	pSeva231	1.05	1.67	0.78	0.04		0.83		2.09	No
KT2440	pSeva251	0.45	0.60	0.00	0.00		0.30		0.90	No

Table S10: HIYIIR H1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.58	0.69	2.00	0.00		0.35		1.15	No
CC118Lpir	pSeva231	0.32	0.42	2.00	0.00		0.21		0.63	No
DH5alpha	pAN	0.22	2.57	3.34	0.27	0.54	1.28	0.29	0.43	Yes
DH5alpha	pSeva221	0.08	0.13	2.00	0.00		0.06		0.16	No
KT2440	pSeva221	0.02	0.52	1.05	0.10	1.61	0.26	0.11	0.05	Yes
KT2440	pSeva231	0.01	0.02	0.02	0.00		0.01		0.03	No
KT2440	pSeva251	0.02	0.46	1.04	0.07	1.50	0.23	0.07	0.03	Yes

Table S11: LCARA I1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.60	2.19	26.01	0.64	0.66	1.09	0.66	1.21	No
CC118Lpir	pSeva231	0.34	5.93	3.02	0.32	0.79	2.97	0.33	0.68	Yes
DH5alpha	pAN	0.23	2.26	9.79	0.38	0.47	1.13	0.39	0.46	Yes
DH5alpha	pSeva221	0.08	0.49	22.67	0.13	0.14	0.25	0.13	0.16	Yes
KT2440	pSeva221	0.52	4.59	0.90	0.05	0.41	2.30	0.06	1.04	Yes
KT2440	pSeva231	1.10	3.72	0.89	0.04	0.06	1.86	0.11	2.20	No
KT2440	pSeva251	0.37	1.38	1.10	0.11	0.17	0.69	0.22	0.75	No

Table S12: LITR L1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.63	1.25	2.00	0.00		0.63		1.26	No
CC118Lpir	pSeva231	0.35	6.05	1.44	0.12	0.80	3.02	0.13	0.70	Yes
DH5alpha	pAN	0.33	7.86	5.81	0.34	0.58	3.93	0.35	0.66	Yes
DH5alpha	pSeva221	0.14	1.60	32.33	0.13	0.14	0.80	0.13	0.27	Yes
KT2440	pSeva221	1.78	9.57	0.74	0.02	0.10	4.79	0.04	3.55	Yes
KT2440	pSeva231	0.99	5.14	0.93	0.03	0.09	2.57	0.04	1.97	Yes
KT2440	pSeva251	0.00	0.00	0.27	0.00	2.30	0.00	0.00	0.00	Yes

Table S13: LMRA N1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.60	1.56	37.90	0.65	0.65	0.78	0.68	1.19	No
CC118Lpir	pSeva231	0.30	3.33	2.66	0.29	0.68	1.67	0.32	0.59	Yes
DH5alpha	pAN	0.31	1.84	4.50	0.33	0.44	0.92	0.36	0.63	Yes
DH5alpha	pSeva221	0.12	0.34	44.51	0.13	0.13	0.17	0.13	0.24	No
KT2440	pSeva221	0.36	2.94	0.86	0.04	0.37	1.47	0.06	0.73	Yes
KT2440	pSeva231	0.49	1.93	1.23	0.07	0.12	0.97	0.13	0.99	No
KT2440	pSeva251	0.09	0.41	1.06	0.04	0.09	0.20	0.07	0.19	Yes

Table S14: PHIF P1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	5.93	9.76	0.00	0.00		4.88		11.85	No
CC118Lpir	pSeva231	0.27	1.27	1.24	0.16	0.36	0.64	0.25	0.54	Yes
DH5alpha	pAN	0.27	5.95	1.63	0.10	0.65	2.97	0.11	0.54	Yes
DH5alpha	pSeva221	4.44	6.21	0.05	0.01		3.10		8.87	No
KT2440	pSeva221	14.74	19.54	0.68	0.01		9.77		29.48	No
KT2440	pSeva231	0.06	1.11	1.30	0.10	0.86	0.56	0.11	0.12	Yes
KT2440	pSeva251	3.00	3.93	0.19	0.01		1.97		6.00	No

Table S15: PHIF P2: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.57	12.56	19.81	0.65	0.76	6.28	0.65	1.13	Yes
CC118Lpir	pSeva231	0.27	18.18	3.96	0.34	0.98	9.09	0.34	0.54	Yes
DH5alpha	pAN	0.27	10.85	7.08	0.35	0.58	5.43	0.35	0.54	Yes
DH5alpha	pSeva221	0.11	5.70	14.23	0.14	0.18	2.85	0.14	0.22	Yes
KT2440	pSeva221	14.33	21.55	0.49	0.01		10.78		28.66	No
KT2440	pSeva231	6.90	10.92	0.65	0.03		5.46		13.80	No
KT2440	pSeva251	1.70	3.83	0.73	0.08	0.01	1.91	1.50	3.39	No

Table S16: PHIF P3: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.17	5.60	41.99	0.67	0.73	2.80	0.67	0.34	Yes
CC118Lpir	pSeva231	0.31	15.76	3.73	0.36	1.02	7.88	0.36	0.63	Yes
DH5alpha	pAN	0.22	11.76	5.55	0.29	0.59	5.88	0.29	0.45	Yes
DH5alpha	pSeva221	0.03	3.08	41.24	0.13	0.14	1.54	0.13	0.05	Yes
KT2440	pSeva221	4.19	20.41	0.78	0.03	0.13	10.21	0.07	8.39	Yes
KT2440	pSeva231	0.01	0.02	2.00	0.00		0.01		0.02	No

Table S17: PSRA R1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.19	2.81	2.50	0.53	1.47	1.41	0.56	0.38	Yes
CC118Lpir	pSeva231	0.22	13.16	3.34	0.35	1.18	6.58	0.35	0.43	Yes
DH5alpha	pAN	0.15	8.12	1.39	0.16	2.90	4.06	0.17	0.29	Yes
DH5alpha	pSeva221	0.02	0.34	0.89	0.36	8.77	0.17	0.40	0.03	Yes
KT2440	pSeva221	8.15	16.92	0.91	0.11	0.01	8.46	4.19	16.29	No
KT2440	pSeva231	2.65	6.48	0.98	0.03	0.01	3.24	0.19	5.31	No
KT2440	pSeva251	0.14	1.33	0.06	0.00	438.85	0.67	0.00	0.29	Yes

Table S18: QACR Q1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.54	2.43	23.52	0.63	0.66	1.21	0.65	1.08	Yes
CC118Lpir	pSeva231	0.07	0.86	9.70	1.54	1.98	0.43	1.57	0.13	Yes
DH5alpha	pAN	0.00	1.81	0.94	0.11	509.71	0.90	0.11	0.00	Yes
DH5alpha	pSeva221	0.00	0.13	1.78	0.94	6.20	0.07	0.98	0.01	Yes
KT2440	pSeva221	0.62	3.61	0.85	0.08	0.40	1.81	0.14	1.25	Yes
KT2440	pSeva231	0.87	2.61	1.09	0.08	0.08	1.30	0.23	1.74	No
KT2440	pSeva251	0.25	0.78	2.12	0.19	0.20	0.39	0.30	0.50	No

Table S19: QACR Q2: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.57	3.89	37.08	0.65	0.68	1.95	0.65	1.13	Yes
CC118Lpir	pSeva231	0.32	8.44	4.87	0.40	0.77	4.22	0.41	0.64	Yes
DH5alpha	pAN	0.04	5.05	4.62	0.29	0.84	2.53	0.29	0.07	Yes
DH5alpha	pSeva221	0.10	0.53	44.57	0.13	0.13	0.27	0.13	0.20	Yes
KT2440	pSeva221	1.57	4.75	0.69	0.09	0.09	2.38	0.42	3.14	No
KT2440	pSeva231	1.67	3.50	0.99	0.05	0.00	1.75	1.09	3.34	No

Table S20: SRPR S1: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.63	1.12	2.00	0.00		0.56		1.26	No
CC118Lpir	pSeva231	0.32	0.88	0.06	0.00	0.00	0.44	25.54	0.64	No
DH5alpha	pAN	0.04	0.79	1.10	0.11	1.54	0.39	0.12	0.08	Yes
DH5alpha	pSeva221	0.11	0.17	2.00	0.00		0.09		0.23	No
KT2440	pSeva221	0.08	1.11	0.75	0.04	1.07	0.55	0.05	0.16	Yes
KT2440	pSeva231	0.05	0.36	1.22	0.08	0.32	0.18	0.10	0.09	Yes
KT2440	pSeva251	0.01	0.10	0.76	0.03	0.51	0.05	0.04	0.02	Yes

Table S21: SRPR S2: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.62	1.61	247.58	0.64	0.64	0.81	0.65	1.24	No
CC118Lpir	pSeva231	0.34	3.95	2.67	0.27	0.63	1.98	0.29	0.69	Yes
DH5alpha	pAN	0.02	2.21	4.04	0.32	0.98	1.10	0.32	0.05	Yes
DH5alpha	pSeva221	0.10	1.58	26.31	0.13	0.14	0.79	0.13	0.21	Yes
KT2440	pSeva221	0.33	1.27	0.76	0.12	0.26	0.64	0.31	0.67	No
KT2440	pSeva231	0.13	1.12	1.15	0.04	0.19	0.56	0.05	0.26	Yes

Table S22: SRPR S3: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.64	2.22	30.93	0.64	0.65	1.11	0.66	1.28	No
CC118Lpir	pSeva231	0.35	4.49	2.48	0.27	0.71	2.25	0.29	0.70	Yes
DH5alpha	pAN	0.01	2.14	3.27	0.28	1.69	1.07	0.28	0.01	Yes
DH5alpha	pSeva221	0.11	1.17	16.97	0.14	0.15	0.59	0.14	0.22	Yes
KT2440	pSeva221	0.14	1.24	0.82	0.07	0.73	0.62	0.09	0.28	Yes
KT2440	pSeva231	0.05	0.55	1.10	0.04	0.30	0.28	0.05	0.09	Yes

Table S23: SRPR S4: Hill equation parameters and thresholds across all contexts

Strain	Plasmid	Y min	Y max	n	k	IH	OH	IL	OL	Functional NOT
CC118Lpir	pSeva221	0.63	2.01	39.45	0.65	0.65	1.00	0.66	1.26	No
CC118Lpir	pSeva231	0.32	0.43	2.00	0.00		0.22		0.64	No
DH5alpha	pAN	0.05	1.97	4.24	0.39	0.91	0.98	0.40	0.10	Yes
DH5alpha	pSeva221	0.10	1.22	17.77	0.13	0.15	0.61	0.13	0.21	Yes
KT2440	pSeva221	0.28	1.19	0.79	0.10	0.26	0.59	0.21	0.56	Yes
KT2440	pSeva231	0.12	0.62	1.07	0.05	0.14	0.31	0.07	0.23	Yes
KT2440	pSeva251	0.05	0.28	0.50	0.04	0.63	0.14	0.10	0.10	Yes

6.2 Compatibility pairings

We calculated the number of compatible pairings for each strain and plasmid backbone combination, as well as aggregates of these combinations (e.g. for the strain DH5alpha, and all backbones which were characterised in this host, an aggregation of contexts DH5alpha pAN and DH5alpha pSeva221). Of interest for each context is:

- the number of gates which are valid, i.e. functional as NOT gates
- the number of compatible pairings as a percentage of all possible pairings
- the number of pairings which are 'inter-context', i.e. connections between gates which are on different backbones, or in different hosts

The maximum number of pairings possible depends on the number of gates available. We calculate this using the binomial coefficient as $2 \times \binom{n}{2}$, where n is the number of gates available for pairing. The binomial coefficient is doubled because each pairing can be inverted to produce another. The gates which could not possibly be connected, because they share a repressor molecule, or are the same gate, are then subtracted from this number.

Table S24: A table showing a breakdown of the number of compatible pairs in each of the 11 context dependent libraries we characterised. The context is specified by a strain and plasmid combination. 'All' indicates that all available plasmids for that particular strain are included. Analysis is done when all gates in a context are included, or when only 'Valid', i.e. functional NOT, gates are included.

Strain	Plasmid	Gates	Valid	Pairings(All)	Pairings(Valid)	Compatible Pairings	% (All)	% (Valid)
CC118Lpir	pSeva221	20	9	354	68	13	3.67	19.12
CC118Lpir	pSeva231	20	14	354	172	11	3.11	6.40
DH5alpha	pAN	20	17	354	252	67	18.93	26.59
DH5alpha	pSeva221	20	15	354	198	18	5.08	9.09
KT2440	pSeva221	20	10	354	84	1	0.28	1.19
KT2440	pSeva231	20	8	354	44	1	0.28	2.27
KT2440	pSeva251	15	7	204	40	1	0.49	2.50
DH5alpha	All	40	32	1416	904	203	14.34	22.46
KT2440	All	55	25	2700	514	13	0.48	2.53
CC118Lpir	All	40	23	1416	464	78	5.51	16.81
All	All	135	80	16188	5654	697	4.31	12.33

Table S25: A table showing how compatible pairings increases when additional backbones are utilised in the library. For each strain, the numbers of compatible pairings in which both gates use the same backbone ('single-backbone'), different backbones ('inter-backbone') and the sum of both ('total') are shown. Fold-change is between the single backbone and 'total' is also shown.

Strain	Compatible Pairs (single-backbone)	Compatible Pairs (inter-backbone)	Compatible Pairs (total)	Fold Change
CC118Lpir	24	54	78	3.25
DH5alpha	85	118	203	2.39
KT2440	3	10	13	4.33

Table S26: A table showing how compatible pairings increases when additional hosts are utilised in the library. For each host, the numbers of compatible pairings are shown. Also indicated is the number of inter-host pairings and the fold change between the total of single-host pairings and pairings across all contexts.

DH5alpha	CC118Lpir	KT2440	Compatible Pairs (single-host)	Compatible Pairs (inter-host)	Compatible Pairs (total)	Fold Change
203	78	13	294	403	697	2.37

Table S27: Numerical similarity scores for AMER F1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.75	0.65	0.52	0.67	0.45	0.50
CC118Lpir pSeva231	0.75	1.00	0.61	0.63	0.75	0.45	0.42
DH5alpha pAN	0.65	0.61	1.00	0.59	0.57	0.30	0.48
DH5alpha pSeva221	0.52	0.63	0.59	1.00	0.71	0.37	0.52
KT2440 pSeva221	0.67	0.75	0.57	0.71	1.00	0.48	0.49
KT2440 pSeva231	0.45	0.45	0.30	0.37	0.48	1.00	0.35
KT2440 pSeva251	0.50	0.42	0.48	0.52	0.49	0.35	1.00

Table S28: Numerical similarity scores for AMTR A1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.88	0.74	0.65	0.21	0.16	0.08
CC118Lpir pSeva231	0.88	1.00	0.80	0.63	0.21	0.18	0.07
DH5alpha pAN	0.74	0.80	1.00	0.82	0.37	0.30	0.18
DH5alpha pSeva221	0.65	0.63	0.82	1.00	0.44	0.41	0.31
KT2440 pSeva221	0.21	0.21	0.37	0.44	1.00	0.72	0.71
KT2440 pSeva231	0.16	0.18	0.30	0.41	0.72	1.00	0.65
KT2440 pSeva251	0.08	0.07	0.18	0.31	0.71	0.65	1.00

Table S29: Numerical similarity scores for BETI E1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.62	0.69	0.84	0.44	0.29	0.16
CC118Lpir pSeva231	0.62	1.00	0.82	0.58	0.59	0.52	0.37
DH5alpha pAN	0.69	0.82	1.00	0.64	0.53	0.48	0.31
DH5alpha pSeva221	0.84	0.58	0.64	1.00	0.41	0.25	0.10
KT2440 pSeva221	0.44	0.59	0.53	0.41	1.00	0.73	0.42
KT2440 pSeva231	0.29	0.52	0.48	0.25	0.73	1.00	0.37
KT2440 pSeva251	0.16	0.37	0.31	0.10	0.42	0.37	1.00

Table S30: Numerical similarity scores for BM3R1 B1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231
CC118Lpir pSeva221	1.00	0.33	0.40	0.51	0.61	0.60
CC118Lpir pSeva231	0.33	1.00	0.70	0.44	0.44	0.54
DH5alpha pAN	0.40	0.70	1.00	0.52	0.60	0.61
DH5alpha pSeva221	0.51	0.44	0.52	1.00	0.50	0.56
KT2440 pSeva221	0.61	0.44	0.60	0.50	1.00	0.70
KT2440 pSeva231	0.60	0.54	0.61	0.56	0.70	1.00

Table S31: Numerical similarity scores for BM3R1 B2 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.13	0.06	0.60	0.06	0.21	0.06
CC118Lpir pSeva231	0.13	1.00	0.73	0.13	0.45	0.51	0.35
DH5alpha pAN	0.06	0.73	1.00	0.08	0.59	0.55	0.47
DH5alpha pSeva221	0.60	0.13	0.08	1.00	0.08	0.21	0.08
KT2440 pSeva221	0.06	0.45	0.59	0.08	1.00	0.55	0.75
KT2440 pSeva231	0.21	0.51	0.55	0.21	0.55	1.00	0.48
KT2440 pSeva251	0.06	0.35	0.47	0.08	0.75	0.48	1.00

Table S32: Numerical similarity scores for BM3R1 B3 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.75	0.75	0.79	0.16	0.60	0.14
CC118Lpir pSeva231	0.75	1.00	0.75	0.75	0.17	0.57	0.24
DH5alpha pAN	0.75	0.75	1.00	0.66	0.21	0.59	0.31
DH5alpha pSeva221	0.79	0.75	0.66	1.00	0.17	0.69	0.25
KT2440 pSeva221	0.16	0.17	0.21	0.17	1.00	0.36	0.43
KT2440 pSeva231	0.60	0.57	0.59	0.69	0.36	1.00	0.39
KT2440 pSeva251	0.14	0.24	0.31	0.25	0.43	0.39	1.00

Table S33: Numerical similarity scores for HIYIIR H1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.60	0.07	0.22	0.00	0.58	0.00
CC118Lpir pSeva231	0.60	1.00	0.14	0.29	0.07	0.53	0.07
DH5alpha pAN	0.07	0.14	1.00	0.14	0.23	0.07	0.16
DH5alpha pSeva221	0.22	0.29	0.14	1.00	0.67	0.22	0.59
KT2440 pSeva221	0.00	0.07	0.23	0.67	1.00	0.00	0.78
KT2440 pSeva231	0.58	0.53	0.07	0.22	0.00	1.00	0.00
KT2440 pSeva251	0.00	0.07	0.16	0.59	0.78	0.00	1.00

Table S34: Numerical similarity scores for LCARA I1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.77	0.74	0.73	0.26	0.39	0.14
CC118Lpir pSeva231	0.77	1.00	0.72	0.76	0.43	0.48	0.28
DH5alpha pAN	0.74	0.72	1.00	0.71	0.51	0.57	0.31
DH5alpha pSeva221	0.73	0.76	0.71	1.00	0.40	0.54	0.30
KT2440 pSeva221	0.26	0.43	0.51	0.40	1.00	0.75	0.77
KT2440 pSeva231	0.39	0.48	0.57	0.54	0.75	1.00	0.63
KT2440 pSeva251	0.14	0.28	0.31	0.30	0.77	0.63	1.00

Table S35: Numerical similarity scores for LITR L1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.77	0.62	0.62	0.34	0.42	0.47
CC118Lpir pSeva231	0.77	1.00	0.71	0.70	0.42	0.44	0.38
DH5alpha pAN	0.62	0.71	1.00	0.64	0.62	0.56	0.46
DH5alpha pSeva221	0.62	0.70	0.64	1.00	0.36	0.33	0.23
KT2440 pSeva221	0.34	0.42	0.62	0.36	1.00	0.81	0.61
KT2440 pSeva231	0.42	0.44	0.56	0.33	0.81	1.00	0.50
KT2440 pSeva251	0.47	0.38	0.46	0.23	0.61	0.50	1.00

Table S36: Numerical similarity scores for LMRA N1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.82	0.81	0.75	0.31	0.30	0.21
CC118Lpir pSeva231	0.82	1.00	0.81	0.67	0.36	0.35	0.21
DH5alpha pAN	0.81	0.81	1.00	0.68	0.36	0.34	0.32
DH5alpha pSeva221	0.75	0.67	0.68	1.00	0.21	0.20	0.14
KT2440 pSeva221	0.31	0.36	0.36	0.21	1.00	0.76	0.76
KT2440 pSeva231	0.30	0.35	0.34	0.20	0.76	1.00	0.65
KT2440 pSeva251	0.21	0.21	0.32	0.14	0.76	0.65	1.00

Table S37: Numerical similarity scores for PHIF P1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.04	0.04	0.53	0.04	0.00	0.00
CC118Lpir pSeva231	0.04	1.00	0.82	0.09	0.59	0.16	0.18
DH5alpha pAN	0.04	0.82	1.00	0.07	0.60	0.09	0.16
DH5alpha pSeva221	0.53	0.09	0.07	1.00	0.20	0.14	0.31
KT2440 pSeva221	0.04	0.59	0.60	0.20	1.00	0.16	0.24
KT2440 pSeva231	0.00	0.16	0.09	0.14	0.16	1.00	0.47
KT2440 pSeva251	0.00	0.18	0.16	0.31	0.24	0.47	1.00

Table S38: Numerical similarity scores for PHIF P2 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.84	0.78	0.81	0.72	0.49	0.27
CC118Lpir pSeva231	0.84	1.00	0.83	0.82	0.72	0.52	0.38
DH5alpha pAN	0.78	0.83	1.00	0.81	0.72	0.53	0.34
DH5alpha pSeva221	0.81	0.82	0.81	1.00	0.73	0.63	0.40
KT2440 pSeva221	0.72	0.72	0.72	0.73	1.00	0.68	0.35
KT2440 pSeva231	0.49	0.52	0.53	0.63	0.68	1.00	0.59
KT2440 pSeva251	0.27	0.38	0.34	0.40	0.35	0.59	1.00

Table S39: Numerical similarity scores for PHIF P3 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231
CC118Lpir pSeva221	1.00	0.59	0.60	0.70	0.28	0.40
CC118Lpir pSeva231	0.59	1.00	0.76	0.68	0.53	0.00
DH5alpha pAN	0.60	0.76	1.00	0.68	0.49	0.00
DH5alpha pSeva221	0.70	0.68	0.68	1.00	0.50	0.32
KT2440 pSeva221	0.28	0.53	0.49	0.50	1.00	0.00
KT2440 pSeva231	0.40	0.00	0.00	0.32	0.00	1.00

Table S40: Numerical similarity scores for PSRA R1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.77	0.58	0.52	0.57	0.72	0.00
CC118Lpir pSeva231	0.77	1.00	0.66	0.35	0.57	0.69	0.06
DH5alpha pAN	0.58	0.66	1.00	0.17	0.37	0.62	0.20
DH5alpha pSeva221	0.52	0.35	0.17	1.00	0.73	0.52	0.00
KT2440 pSeva221	0.57	0.57	0.37	0.73	1.00	0.61	0.00
KT2440 pSeva231	0.72	0.69	0.62	0.52	0.61	1.00	0.00
KT2440 pSeva251	0.00	0.06	0.20	0.00	0.00	0.00	1.00

Table S41: Numerical similarity scores for QACR Q1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.25	0.26	0.09	0.24	0.30	0.15
CC118Lpir pSeva231	0.25	1.00	0.32	0.71	0.72	0.68	0.65
DH5alpha pAN	0.26	0.32	1.00	0.32	0.32	0.32	0.32
DH5alpha pSeva221	0.09	0.71	0.32	1.00	0.65	0.59	0.56
KT2440 pSeva221	0.24	0.72	0.32	0.65	1.00	0.77	0.75
KT2440 pSeva231	0.30	0.68	0.32	0.59	0.77	1.00	0.62
KT2440 pSeva251	0.15	0.65	0.32	0.56	0.75	0.62	1.00

Table S42: Numerical similarity scores for QACR Q2 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231
CC118Lpir pSeva221	1.00	0.81	0.81	0.81	0.32	0.48
CC118Lpir pSeva231	0.81	1.00	0.82	0.72	0.39	0.53
DH5alpha pAN	0.81	0.82	1.00	0.74	0.34	0.46
DH5alpha pSeva221	0.81	0.72	0.74	1.00	0.24	0.39
KT2440 pSeva221	0.32	0.39	0.34	0.24	1.00	0.74
KT2440 pSeva231	0.48	0.53	0.46	0.39	0.74	1.00

Table S43: Numerical similarity scores for SRPR S1 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.77	0.75	0.69	0.17	0.11	0.07
CC118Lpir pSeva231	0.77	1.00	0.73	0.66	0.18	0.12	0.10
DH5alpha pAN	0.75	0.73	1.00	0.72	0.22	0.20	0.16
DH5alpha pSeva221	0.69	0.66	0.72	1.00	0.24	0.19	0.13
KT2440 pSeva221	0.17	0.18	0.22	0.24	1.00	0.81	0.77
KT2440 pSeva231	0.11	0.12	0.20	0.19	0.81	1.00	0.65
KT2440 pSeva251	0.07	0.10	0.16	0.13	0.77	0.65	1.00

Table S44: Numerical similarity scores for SRPR S2 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231
CC118Lpir pSeva221	1.00	0.78	0.59	0.84	0.16	0.25
CC118Lpir pSeva231	0.78	1.00	0.57	0.81	0.19	0.27
DH5alpha pAN	0.59	0.57	1.00	0.64	0.46	0.61
DH5alpha pSeva221	0.84	0.81	0.64	1.00	0.22	0.34
KT2440 pSeva221	0.16	0.19	0.46	0.22	1.00	0.74
KT2440 pSeva231	0.25	0.27	0.61	0.34	0.74	1.00

Table S45: Numerical similarity scores for SRPR S3 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231
CC118Lpir pSeva221	1.00	0.82	0.60	0.79	0.18	0.29
CC118Lpir pSeva231	0.82	1.00	0.60	0.75	0.23	0.34
DH5alpha pAN	0.60	0.60	1.00	0.74	0.51	0.57
DH5alpha pSeva221	0.79	0.75	0.74	1.00	0.32	0.42
KT2440 pSeva221	0.18	0.23	0.51	0.32	1.00	0.75
KT2440 pSeva231	0.29	0.34	0.57	0.42	0.75	1.00

Table S46: Numerical similarity scores for SRPR S4 across all contexts. This data is plotted in Figure S4.

	CC118Lpir pSeva221	CC118Lpir pSeva231	DH5alpha pAN	DH5alpha pSeva221	KT2440 pSeva221	KT2440 pSeva231	KT2440 pSeva251
CC118Lpir pSeva221	1.00	0.01	0.61	0.80	0.17	0.32	0.18
CC118Lpir pSeva231	0.01	1.00	0.00	0.05	0.00	0.00	0.00
DH5alpha pAN	0.61	0.00	1.00	0.64	0.52	0.60	0.44
DH5alpha pSeva221	0.80	0.05	0.64	1.00	0.30	0.43	0.22
KT2440 pSeva221	0.17	0.00	0.52	0.30	1.00	0.74	0.78
KT2440 pSeva231	0.32	0.00	0.60	0.43	0.74	1.00	0.66
KT2440 pSeva251	0.18	0.00	0.44	0.22	0.78	0.66	1.00

7 Using the provided codes and data

The codes that implement the *in silico* methods from this study can be found and downloaded at `https://github.com/lgrozinger/pyolin`, along with instructions detailing its use.

In order to obtain the results from this particular study, only Python3.6 or greater is needed. However, to generate the figures used in the manuscript further requires gnuplot and LaTeX. Once these dependencies are installed, the command:

```
python3 produce_figures.py full-update
```

once run from the project root directory will perform the analysis and place figures in the appropriate subdirectories. Docker users may find it more convenient to use the Dockerfile associated with the project.

It should also possible to run the same analysis on a different dataset, if the data is provided as a csv file with the expected fields and format. The processed data from this study that is provided with the codes can act as a guideline.

The package is also capable of generating UCF files that can be used with the 'Cello' automated design framework [2].

More information can be found in the project README at `https://github.com/lgrozinger/pyolin/blob/master/README.md`.

The data used for this study can be obtained at `https://figshare.com/s/18e6a10d708d15839837`. A description of the files founds there can be found in the accompanying README file.

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