



***Supplementary Table 2: The optimal RBS sequence for each integrase***

**Table 2:** The optimal RBS sequence for each integrase

Name	Optimal RBS sequence	Translation Initiation rate <sup>a</sup>
Int2	aacatagcgaatcgtaaggagttaaaag <b>atg</b>	7965
Int3	tcacacaggaagaaggctcg <b>atg</b>	15
Int4	agtaatttcaacacaataactaggattcga <b>atg</b>	2363
Int5	cagaggaaggaggctcg <b>atg</b>	529
Int7	agtaatttcaacaaaataactaggattcga <b>atg</b>	472
Int8	aaaacaataactaggattcga <b>atg</b>	4
Int9	tcacacaggatgaagcctag <b>atg</b>	13
Int10	tcaaacaggaagaaggctag <b>atg</b>	308
Int11	tcaaacaggacggaggctag <b>atg</b>	221
Int12	acacaggaagaaggctcg <b>atg</b>	40
Int13	agtaatttcaacaaaataacgagcattcga <b>atg</b>	1473

a. Calculated using the RBS calculator v1.1 (au)<sup>4</sup>.

**Supplementary Table 3: Recombinases and recognition sites used to build memory switches**

**Table 3.** Recombinases and recognition sites used to build memory switches

Name	NCBI No.	attB sequence <sup>a,b</sup>	attP sequence <sup>a,b</sup>
Int2	CBG73463	ggacggcgcagaaggggagtagctctt <b>cgcc</b> ggaccgtcgacatactgctcagctcgtc	gctcatgtatgtgtctacgcgagattc <b>cgcc</b> cgagaactctgcaaggcaactgctcttggt
Int3	NP_268897 <sup>1</sup>	gtttgtaaaggagactgataatggcatgtca <b>actataact</b> cgctcggtaaaaagcattcttat	atggataaaaaatacagcgtttttcatgtca <b>actataact</b> agttgtagtgctaaataatgctt
Int4	YP_002747001	ttccaaagagcgcaccaacgcgacctgaa <b>attg</b> aataagactgctgcttgtgtaaaggcgatgatt	aaaaattacaagttttcaacccttg <b>attg</b> aattagcggtoaaaataatttgaattcgttt
Int5	BAF03598	gagcgcggatcagggagtggaacg <b>ctggg</b> agcgtacacgctgtggctgcggtcggtgc	ccctaatacgcgaagtcgataactct <b>ctggg</b> agcgttgacaacttgcgcaccctgatctg
Int7	YP_003251752	agacgagaaaacgttccgtccgtctgggtcag <b>tg</b> ggcaagtgtatgacgggtcgtccgtt	gtgtataaacctgtgtgagagtttaagtttaca <b>tg</b> ccctaaccttaacttttacgcaggttcagctt
Int8	BAE05705	caatcatcagataactatggcggcagctgca <b>ta</b> ccacggttgtatcccgtctaaagtactcgt	ttaataaactatggaagtatgtacagctt <b>gca</b> atgttgagtgacaaaactccataaaaaat
Int9	BAF67264	tttataatgcaaaaaataatggcgaac <b>agg</b> taactggatacctcatccccaattaaaatttg	gtggtgtttttgtggaagtgtgtat <b>agg</b> taactgcatagttattccgaactccaatta
Int10	YP_003880342	agcacgctgataatcagcaagaccacca <b>ca</b> tttccaccaatgtaaaagctttaaccttagc	ggaaaaataaataatttttagtaacct <b>ca</b> ctcaatcaaggatagtaaaactctcactctt
Int11	YP_001886479	atggatttgcagattcccagatgccc <b>ct</b> acgaaagaggtacaaaactttattggaattaaat	gtttatagtttactaataagacgctctca <b>cc</b> ataaagctctattagtaaacatattccaact
Int12	YP_005759947	gttcgtggtaactatgggtgtacaggtg <b>cc</b> acattagttgtaccatttatgtttatgtggttaac	ttttgtatgttagttgtgctactgggtag <b>ac</b> ctaaatagtgacacaactgctattaaaatttaa
Int13	YP_001376196	gcatacattgtgtgtttttccagat <b>cc</b> agttggtcctgtaaatataagcaatccatgtgagt	caataacggtgtgtattttagaact <b>gca</b> cgttgttttagtaacataaatacaactccgaata
		IRL sequence	IRR sequence
HbiF	ABB83955 <sup>5</sup>	aatacaagacaattggggccaaactgtccatcat	ctctatgagtcaaaatggcccaaatgtttcatctttg
FimE	CAA27561 <sup>6</sup>	aatacaagacaattggggccattttgactcatagag	atgataggacagtttgcccaaatgtttcatctttg

a. The core sequence is shown in green.

b. The recognition sites of Int3, 5, HbiF and FimE are from literatures.

**Supplementary Table 4: Sequences of genetic parts used in this work**

**Table 4.** Sequences of genetic parts used in this work

Part name	Type	DNA sequence
BBa_J23119	promoter	ttgacagctagctcagtcctaggtataatgctagc
BBa_J23101	promoter	tttacagctagctcagtcctaggtattatgctagc
P <sub>Bad</sub>	promoter <sup>7</sup>	gaaaccaaattgtccatattgcatcagacattgcccactgctgcttttactggctcttctcgctaaccacacgggtaacc cgcttattaaaaagcattctgttaacaaagcgggaccaaagccatgacaaaacgcgtaacaaaagtgtctataatcacggcag aaaagtccacattgattatgtgacggcgtcacactttgctatgcatagcattttatccataagattagcggatcctacc tg
P <sub>Tac</sub>	promoter <sup>8</sup>	tgttgacaattaatcatcggctcgtataatgtgtggaattgtgagcgtcacaatt
P <sub>Tet</sub>	promoter <sup>8</sup>	tactccaccgttggctttttccctatcagtgatagagattgacatccctatcagtgatagagataatgagcac
P <sub>SrpR</sub>	promoter <sup>8</sup>	tctatgattggtccagattcgttaccaattgacagctagctcagtcctaggtatatacatacatgcttgttgtttaa
P <sub>BM3R1</sub>	promoter <sup>8</sup>	aatccgctgtagaggtctgattcgttaccaattgacgggaatgacggttcattccgataatgctagc
P <sub>PhIF</sub>	promoter <sup>8</sup>	cgacgtacgggtggaatctgattcgttaccaattgacatgatacgaacgtaccgtatcgttaagggt
RiboJ	insulator <sup>9</sup>	agctgtcaccggatgtgcttccggctctgatgagtcggtgaggacgaaacagcctctacaaaataatttgtttaa
SarJ	insulator <sup>9</sup>	agactgtcgcggatgtgtatccgacctgacgatgcccacaaaggccgaaacagtcctctacaaaataatttgtttaa
ScCJ	insulator <sup>9</sup>	agatgctgtagtgggatgtgtctcacctgaagagtacaaaagtcgaaacggtatcctctacaaaataatttgtttaa
LtsvJ	insulator <sup>9</sup>	agtacgtctgagcgtgatacccgctcactgaagatggcccgtagggccgaaacgtacctctacaaaataatttgtttaa
D/E20	spacer <sup>10</sup>	agttcgtgagagcgataac
Sequence of the reporter for characterization of the integrases (with the example of Int2) <sup>a</sup>	sequence	ttgacagctagctcagtcctaggtataatgctagcagttcgtgagagcgataacggacggcgcagaaggggagtagctctt cgccggaccgtcgacatcactgctcagctcgtcttaagcgtagtttctgctgcttggctgagccttttggatagttcatcca tgccatgtgtaaacccagcagctgttacaacctcaagaaggaccatggtgctctctttctggtgggactttcgaaaagggc agattgtgtggacaggtaatgggtgtctggttaaaaggacagggccatcgccaattggagatatttgggtgataatggctgct agttgaacgcttccactctcaatggtgtgcttaatttgaagtttaactttgattccattcttggttgtctgcatcgtgag atcacattgtgtgattatagttgtattccaactgtgtccaagaatgtttccatctctttaaatacaactccttttaactc gattctatatacaagttgatcaccttcaaacctgactcagcagcgtgtcttatagttcccgctcatcttggaaaaatagtt cttctcgtacataaacttcgggcatggcaactctgaaaaagtcagcggcttccatagatctgggtatcttggaaaagcatt gaaacccaataagtcaaagtagtgacaaggtgtggccatggaaacggtagtttccagtagtcaaaaataatgaaaggttaag tttccgtagctgcatcaccttccacctctccactgacagaaaaatttggccattacattaccatcctaatccaacaaga attgggacaactccagtcgaaaagttctctccttactcatctagttattctcctcttctctgagattaaaacaaaatttt gtagaggtgttctgctctcagcgactcatcagaccggaaagcacatccgggtgacagctagccaagagcagtgctctgcaga agttctcgggagagaatctcgcgtagacacatacatgac
<i>gfpmut3</i>	gene <sup>11</sup>	atgagtaaggagaagaacttttcaactgagttgtocaaattctgttgaattagatggtaatgttaatgggcacaaaat ctgtcagtgagaggggtgaaggtgatgacgacatacggaaaacttacccttaaatttatttgcactactggaacactaccgt tccatggccaacactgtcactacttgaactatggtgttcaatgctttcaagataccagatcataatgaaacggcatgac ttttcaagagtgccatgcccgaaggttatgacagaaagaaactatattttcaaaagtacgaggaactataagacacgtg ctgaagtcaggttgaaggtgatacacttgttaatagaaatcgagttaaaaggtatgtattttaaagaagatgaaacattct tggacacaagttggaatacaactataactcacacaagtatacatatgagcagacaaacaaaagaatggaatcaaaagttaac ttcaaaaattagacacaaactgaaagtggaagcgttcaactagcagaccattatcaacaaaataactccaattggcgtatggcc ctgtccttttacagacacacattacctgtccacacaaatctgccccttccgaaagatcccaacgaaaagagagaccacatggt cctcttgagtttgaacagctgctgggatatacatggcatggatgaactatacaaaaaggcctgcagcaaacgacgaaaaac tacgcttaa
<i>yfp</i>	gene <sup>11</sup>	atgggtgagcaagggcgagagctgttccacggggtggtgccatcctggtcgagctggacggcagcgtaaacggccacaagt tcagcgtgtccggcgagggcgagggcgatgcccactacggcaagctgacccctgaagttcatctgacacacggggtgctgccc cgtgcccctggcccaccctcgtgaccaccttccggctacggcctgcaatgcttgcggcctacccccgaccacatgaaagctgac gacttctcaagtccgcatgcccgaaggtcactgcccagggcgcacacctcttctcaaggacgacggcaactacaagacc cgccggaggtgaagttcgaagggcgacacctggtgaaccgcatcgagctgaaaggtgaaaggtgaaaggtgaaaggtgaa cctggggcacaagctggagtaacaactacaacgcaacacgtctatcatgcccgacaagcagaagaacggcatcaaggtg aacttcaagatccgccaacaacatcgaggacggcagcgtgacgctcggcagcaactaccagagaaccccccatcgccgacg gcccgtgctgctgcccgaacaacactaactgagctaccagtcgcccctgagcaaaacccccgagagacggcagatcacat ggtcctgctggagttcgtgaccgcccgggatcactctcggcatggacgagctgtacaagtaa
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*int4*

gene

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*int5*

gene

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*int7*

gene

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gene

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<i>int9</i>	gene	
<i>int10</i>	gene	
<i>int11</i>	gene	
<i>int12</i>	gene	
<i>int13</i>	gene	

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<i>hbiF</i>	gene <sup>5</sup>	<p>atgagcaccggttgaatttcagaccgttgaagatgaagtgatcccgaaaaaagcgaacgtgaaatgctggaagccagctgc  agcaaatgcacgtaaacgtgaaaaatcagaaaagcatgggcaagcagatctgatgagcgacgatgaatttgaaaaactgat  ggttgaaacccgtgaaacctacgatgaatgtaaacagaaactggaaagctgtgaggatccgatcaaaatcgatgaaacctat  ctgaaagaaattgtgtatatgtttcaccagacctttaaagatctggaaagcgaaaaacagaaagaaatttatcagcaagttca  ttcgcaccattcgtataaccgttaaagaacagcagccgattcgtccggataaaaagcaaaacccggtaaaaggcaaacagaaggt  gattattaccgaggtggaattctaccagtaa</p> <p>atgacgagaaaaatctcacacaggatgaagtctacaggctgatggatgcagcccagagcagatgtcctttcctgaaagaatc  gctgtctgattatgatggcttccattcacgggttttagggccagtgaaacttcttgatttacgtttatccgatattgatgcac  tggaacaacttaatatcggcgtattaaaaatgggttctcgcacaacacatcctcttctcctgatgaatataatttgatc  aagttgtggcttaagcagaggaagttaattgaaaacggagttgaaggagactggcttttctcctccggaaaacgcgcctta  tcagcagacaacatTTTTTTTctatcattcgtgaggctggaaaacgtgcaggattagctgtaaaagcacatcctcatatgtt  acgtcatgcctgtggtttgcctcggctgacaatgggtgcgatacccgactcttcaggattacctgggtcatcgaaatatt  cagcatactgtcagatacacagccagtaatgctgctcgttttaagggggtggaaaaaaaagcctcgttaa</p> <p>atgatgcaggcggttgttacggggcaacgggagccagagattatgtcttattctgttggcatatcggcatgggatgcgta  ttagtgaactgcttgatctgcattatcaggaccttgaccttaatgaaggtagaataaaatattcgcgactgaagaacggatt  ttctaccgttcaccggttacgttttgatgagcgtgaagccgtggaacgctggaccaggaacgtgctaactggaagcgct  gaccggactgacgctatatttattctcgcgcgggagtcggcttctcgcagcaggcctatcgcatattcgcgatgccg  gtattgaagctggaaccgtaacgcagactcatcctcatatgttaaggcatgcttgcggttatgaattggcggagcgtgggtgc  agatactcgtttaattcaggattatctcgggcacgaaatattcgccatactgtgcgttataccgccaagtaatgctgctcgt  tttgcggattatgggaagaataatctcataaacgaaaaataaaaagagaagaggtttga</p>
<i>fimE</i>	gene <sup>7</sup>	

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a. color code of the sequence: brown, promoter J23119; blue, spacer D/E20; green, gfp mut3; magenta, RBS; black, att B/P sites ;purple, RiboJ.



**Supplementary Table 5: Orthogonality matrix ( % ON )**

**Table 5: Orthogonality matrix (% ON)**

Sites <sup>a</sup>		2	3	4	5	7	8	9	10	11	12	13	FimE	HbiF
Int2		<b>97.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>2.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.7	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
Int3		<b>0.0</b>	<b>97.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.1</b>	<b>0.7</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.6	0.0	0.0	0.0	0.0	0.2	0.5	0.0	0.0	0.0	0.0	0.0
Int4		<b>0.0</b>	<b>0.1</b>	<b>94.7</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.9</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.0	1.7	0.0	0.0	0.0	0.0	1.7	0.0	0.1	0.0	0.0	0.0
Int5		<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>93.8</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.1	0.0	3.4	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
Int7		<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>94.1</b>	<b>1.3</b>	<b>0.0</b>	<b>32.5</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.0	0.0	0.0	0.5	1.4	0.0	5.3	0.0	0.0	0.0	0.0	0.0
Int8		<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>98.5</b>	<b>0.4</b>	<b>9.5</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.1	0.0	0.0	0.0	0.3	0.2	5.9	0.0	0.0	0.0	0.0	0.0
Int9		<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>69.6</b>	<b>24.6</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.0	0.0	0.0	0.0	0.7	5.0	9.7	0.0	0.1	0.0	0.0	0.0
Int10		<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>92.6</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.4	0.0	0.0	0.0	0.0	0.0
Int11		<b>0.0</b>	<b>0.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.6</b>	<b>0.0</b>	<b>8.7</b>	<b>81.1</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.2	0.0	0.0	0.0	0.5	0.0	3.2	1.8	0.0	0.0	0.0	0.0
Int12		<b>0.0</b>	<b>0.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.5</b>	<b>0.0</b>	<b>9.6</b>	<b>0.0</b>	<b>96.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.5	0.0	0.0	0.0	1.7	0.0	3.4	0.0	0.5	0.0	0.0	0.0
Int13		<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>0.1</b>	<b>90.6</b>	<b>0.0</b>	<b>0.0</b>
		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.5	0.0	0.0
FimE		<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.5</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>97.9</b>	<b>0.4</b>
		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.2	0.2
HbiF		<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>29.0</b>	<b>90.1</b>
		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	3.3	2.1

a. The average (upper row, bold) and standard deviation (lower row) of three independent triplicates are shown.

**Supplementary Table 6: Orthogonality matrix in average fluorescence**

**Table 6: Orthogonality matrix (average fluorescence)**

	Sites <sup>a</sup>												
	2	3	4	5	7	8	9	10	11	12	13	FimE	HbiF
<b>Int2</b>	<b>1181</b>	<b>9.4</b>	<b>7.5</b>	<b>7.2</b>	<b>8.6</b>	<b>13.3</b>	<b>7.7</b>	<b>25.7</b>	<b>7.5</b>	<b>7.8</b>	<b>7.3</b>	<b>7.2</b>	<b>6.9</b>
	73.5	0.9	0.7	0.9	0.3	3.7	1.0	5.5	0.5	0.4	0.3	0.2	0.2
<b>Int3</b>	<b>7.3</b>	<b>1190</b>	<b>7.1</b>	<b>7.5</b>	<b>8.3</b>	<b>10.4</b>	<b>9.4</b>	<b>22.6</b>	<b>7.6</b>	<b>7.4</b>	<b>7.6</b>	<b>7.2</b>	<b>7.2</b>
	0.1	141.0	0.5	0.5	0.4	1.9	3.2	2.7	0.4	0.4	0.0	0.2	0.1
<b>Int4</b>	<b>7.4</b>	<b>8.1</b>	<b>661.5</b>	<b>8.0</b>	<b>7.8</b>	<b>11.1</b>	<b>7.7</b>	<b>21.1</b>	<b>7.4</b>	<b>7.5</b>	<b>7.8</b>	<b>7.3</b>	<b>7.1</b>
	0.3	0.3	188.5	1.0	0.2	1.1	0.4	4.2	0.1	0.1	0.3	0.1	0.4
<b>Int5</b>	<b>8.0</b>	<b>14.8</b>	<b>8.3</b>	<b>241.9</b>	<b>8.7</b>	<b>13.6</b>	<b>8.5</b>	<b>22.2</b>	<b>7.9</b>	<b>8.7</b>	<b>8.1</b>	<b>7.6</b>	<b>7.6</b>
	0.5	3.2	0.7	38.3	0.4	2.9	0.8	0.6	0.4	0.7	0.1	0.2	0.2
<b>Int7</b>	<b>7.1</b>	<b>9.5</b>	<b>7.3</b>	<b>7.0</b>	<b>1292</b>	<b>17.8</b>	<b>7.3</b>	<b>57.9</b>	<b>7.1</b>	<b>7.2</b>	<b>7.2</b>	<b>7.3</b>	<b>6.8</b>
	0.2	0.9	0.2	0.4	117.9	5.7	0.3	6.1	0.3	0.2	0.1	0.1	0.3
<b>Int8</b>	<b>7.6</b>	<b>10.4</b>	<b>7.2</b>	<b>7.1</b>	<b>8.0</b>	<b>2669</b>	<b>7.6</b>	<b>41.0</b>	<b>7.2</b>	<b>7.5</b>	<b>7.3</b>	<b>7.0</b>	<b>6.6</b>
	0.6	1.7	0.6	0.7	0.3	286	0.7	4.7	0.3	0.5	0.2	0.3	0.2
<b>Int9</b>	<b>6.9</b>	<b>8.1</b>	<b>7.0</b>	<b>6.8</b>	<b>7.8</b>	<b>20.4</b>	<b>251.7</b>	<b>50.1</b>	<b>6.9</b>	<b>7.3</b>	<b>7.1</b>	<b>6.6</b>	<b>6.7</b>
	0.2	0.3	0.3	0.2	0.6	4.9	50.7	16.0	0.3	0.2	0.4	0.2	0.2
<b>Int10</b>	<b>6.9</b>	<b>8.4</b>	<b>7.2</b>	<b>7.1</b>	<b>7.8</b>	<b>11.5</b>	<b>7.3</b>	<b>943.7</b>	<b>7.3</b>	<b>7.4</b>	<b>7.5</b>	<b>7.0</b>	<b>7.2</b>
	0.3	0.3	0.3	0.3	0.3	1.0	0.2	405.4	0.5	0.3	0.3	0.1	0.2
<b>Int11</b>	<b>6.9</b>	<b>9.4</b>	<b>7.2</b>	<b>7.1</b>	<b>7.7</b>	<b>12.1</b>	<b>7.3</b>	<b>28.4</b>	<b>346.9</b>	<b>7.1</b>	<b>7.4</b>	<b>7.1</b>	<b>7.4</b>
	0.2	0.5	0.0	0.0	0.3	1.8	0.1	2.4	18.4	0.2	0.2	0.0	0.3
<b>Int12</b>	<b>6.9</b>	<b>14.4</b>	<b>7.4</b>	<b>7.2</b>	<b>8.8</b>	<b>22.5</b>	<b>7.8</b>	<b>38.9</b>	<b>7.6</b>	<b>794.4</b>	<b>7.6</b>	<b>7.1</b>	<b>7.1</b>
	0.5	4.0	0.5	0.4	0.8	6.5	0.7	6.7	0.4	129.2	0.2	0.2	0.2
<b>Int13</b>	<b>7.1</b>	<b>9.5</b>	<b>7.3</b>	<b>7.2</b>	<b>7.9</b>	<b>11.0</b>	<b>7.5</b>	<b>17.2</b>	<b>7.6</b>	<b>8.4</b>	<b>390</b>	<b>7.6</b>	<b>7.7</b>
	0.3	2.6	0.3	0.4	0.5	1.4	0.4	1.1	0.4	0.8	28.4	0.2	0.3
<b>FimE</b>	<b>7.6</b>	<b>5.7</b>	<b>7.6</b>	<b>7.4</b>	<b>8.0</b>	<b>11.6</b>	<b>7.6</b>	<b>21.7</b>	<b>7.3</b>	<b>7.5</b>	<b>7.3</b>	<b>885.7</b>	<b>10.0</b>
	0.6	4.9	0.1	0.0	0.1	0.0	0.2	2.5	0.0	0.1	0.1	10.8	0.1
<b>HbiF</b>	<b>7.3</b>	<b>8.8</b>	<b>7.5</b>	<b>7.6</b>	<b>7.6</b>	<b>8.7</b>	<b>7.6</b>	<b>13.8</b>	<b>7.3</b>	<b>7.4</b>	<b>7.5</b>	<b>43.5</b>	<b>225.7</b>
	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.5	0.0	0.1	0.2	4.2	5.0

a. The average (upper row, bold) and standard deviation (lower row) of three independent triplicates are shown.

**Supplementary Table 7: Sequence of the memory array**

**Table 7. Sequence of the memory array<sup>a</sup>**

Memory array	<p>ggacgcgccagaaggggagtagctcttcgcccggaccgtcgacaactgctcagctcgtctgcccgaatggatccgagcggtgatccgg          taaaaccggatccggggaatagccaagagcagtgcocttgcagaagttctcgggagagaatctcgggttagacacatacatagagcggttgg          aaaggagactgataatggcaatgtacaactatactcgtcggtaaaaaggcaactctacggttagtcccggctgcctctctcgacgaaccgc          ttacatgcctagccgataagcattatttaggcaactacaactagtagttagtacaagaaacgctgtatTTTTTTTtccatttccaaa  <b>gagcgcccaacgcgacctgaaaattgaaataagactgctgcttggtaaaggcgatgatt</b>gttggaacatttagaggtcgtatccctatcg          cgaataattccataatgtaca<b>aaacgaattaca</b>aattatttgaccgctaattcaaa<b>tcaagggtg</b>aaaactttgtaatttttggagcgc  <b>cgatcagggagtgga</b>cgccctgggagcgtaca<b>cgctgtggctg</b>cggtg<b>cggtgc</b>aaaaagattgtagcccgctgcgcacatctagtt          aatgtccgcaagactc<b>cagatcaggg</b>tcgcag<b>agtg</b>tc<b>caacgctccc</b>aggag<b>gtta</b>to<b>gacttgc</b>gtattagg<b>gagacg</b>agaa<b>acgt</b>  <b>tccgtccgctgggtcag</b>ttggc<b>caag</b>ttg<b>atgac</b>cggt<b>ctccgtctc</b>ta<b>tcggtg</b>taggtcgtatttggagacagaatata<b>tg</b>gg          gccagtaagctta<b>aacgtg</b>aacctgcgt<b>aaaag</b>tt<b>aa</b>ggttag<b>gca</b>tg<b>taa</b>act<b>ta</b>act<b>ctca</b>acag<b>gtttata</b>ac<b>cca</b>at<b>ctc</b>  <b>agata</b>actat<b>ggcggc</b>acgt<b>gca</b>tt<b>aac</b>ac<b>ggtt</b>gt<b>atcc</b>gt<b>ctaa</b>ag<b>ta</b>ct<b>g</b>gt<b>gc</b>gttggtatactt<b>ca</b>gt<b>ctta</b>ac<b>ca</b>at<b>gc</b>ac          cggttt<b>gtg</b>ggat<b>gc</b>ct<b>at</b>ttt<b>tata</b>t<b>gga</b>gtt<b>gtt</b>ca<b>ctca</b>acatt<b>gca</b>ag<b>actgt</b>aca<b>cttcc</b>at<b>ag</b>tt<b>tata</b>tt<b>tata</b>t  <b>tgca</b>aaaa<b>taattgg</b>c<b>ga</b>ac<b>gag</b>gt<b>aa</b>ct<b>gg</b>at<b>ac</b>ct<b>ca</b>tc<b>cg</b>ca<b>at</b>ta<b>aa</b>att<b>g</b>ag<b>ca</b>cc<b>attg</b>cc<b>ctt</b>g<b>ca</b>cc<b>ctt</b>g<b>ca</b>g<b>ca</b>g<b>ca</b>g<b>g</b>          tgcctgagatccctagaa<b>taattg</b>ga<b>gtt</b>cg<b>ga</b>ata<b>acta</b>tg<b>caga</b>t<b>acctg</b>at<b>aca</b>act<b>tcca</b>ca<b>aaaa</b>ca<b>accaca</b>ag<b>ca</b>cg<b>gc</b>  <b>tgata</b>at<b>cag</b>ca<b>ag</b>ca<b>cc</b>ca<b>ac</b>att<b>cca</b>ca<b>atg</b>ta<b>aa</b>ag<b>ctt</b>ta<b>ac</b>ct<b>ta</b>g<b>ctt</b>ata<b>ca</b>ca<b>aa</b>at<b>ct</b>a<b>att</b>tt<b>cca</b>tg<b>ag</b>g<b>ct</b>g<b>ca</b>          cgacc<b>cg</b>tc<b>ca</b>at<b>ag</b>tc<b>aaa</b>ag<b>gtg</b>ag<b>gtt</b>ta<b>ct</b>at<b>cc</b>tt<b>g</b>at<b>g</b>ag<b>atg</b>tag<b>gtt</b>act<b>aaa</b>at<b>tata</b>ttt<b>cca</b>aa<b>tgga</b>  <b>ttttgc</b>ag<b>attcc</b>ag<b>atg</b>cc<b>ct</b>ac<b>aga</b>aa<b>gag</b>gt<b>aca</b>aa<b>ca</b>tt<b>t</b>at<b>t</b>g<b>ga</b>at<b>ta</b>at<b>ag</b>tt<b>og</b>tt<b>tt</b>g<b>g</b>at<b>tt</b>ac<b>og</b>tt<b>ct</b>          ctagcttatgatctgagcgta<b>agttg</b>aa<b>at</b>at<b>gtt</b>ta<b>ct</b>aa<b>ta</b>ag<b>actt</b>at<b>gg</b>gt<b>tg</b>ag<b>ag</b>cg<b>ctct</b>at<b>tag</b>ta<b>aa</b>ca<b>tata</b>aa<b>ac</b>gt<b>t</b>          cgt<b>g</b>ta<b>act</b>at<b>gg</b>gt<b>g</b>ta<b>cag</b>gt<b>g</b>ca<b>catt</b>ag<b>ttg</b>t<b>acc</b>at<b>tt</b>at<b>gtt</b>at<b>g</b>tt<b>g</b>tt<b>aa</b>cc<b>ag</b>g<b>aa</b>ca<b>ct</b>cc<b>gt</b>cc<b>gt</b>gc<b>gc</b>          actagga<b>aga</b>at<b>cc</b>ga<b>ag</b>gt<b>ccc</b>t<b>aa</b>at<b>tt</b>aa<b>tag</b>cag<b>ttg</b>tt<b>g</b>ta<b>ct</b>at<b>tt</b>ag<b>gt</b>ct<b>ccc</b>ag<b>tg</b>aca<b>ca</b>act<b>aa</b>ca<b>taca</b>aa<b>ac</b>  <b>aa</b>t<b>aac</b>g<b>gtt</b>g<b>tatt</b>g<b>tag</b>a<b>actg</b>ac<b>ag</b>tt<b>gtt</b>t<b>g</b>ta<b>aca</b>t<b>aa</b>t<b>aca</b>act<b>cc</b>g<b>aata</b>ct<b>ca</b>tag<b>cccc</b>cg<b>att</b>cg<b>cc</b>ag<b>gt</b>a          cttacgagga<b>a</b>tt<b>act</b>t<b>ata</b>tc<b>cg</b>act<b>ca</b>at<b>gga</b>tt<b>g</b>ct<b>tata</b>tt<b>ta</b>ca<b>gg</b>ac<b>ca</b>act<b>gga</b>ct<b>g</b>g<b>aaaa</b>ca<b>ca</b>ca<b>atg</b>t<b>atg</b>cg</p>
Sequence after Int2/5/7/8 Induction	<p>ggacgcgccagaaggggagtagctcttcgcccggaccgtcgacaactgctcagctcgtctgcccgaatggatccgagcggtgatccgg          aacgctcggatccatttggcgacgacgagctgagcagtagtgcagcggctccggcgagaatctcgggttagacacatacatagagcggttgg          aaaggagactgataatggcaatgtacaactatactcgtcggtaaaaaggcaactctacggttagtcccggctgcctctctcgacgaaccgc          ttacatgcctagccgataagcattatttaggcaactacaactagtagttagtacaagaaacgctgtatTTTTTTTtccatttccaaa  <b>gagcgcccaacgcgacctgaaaattgaaataagactgctgcttggtaaaggcgatgatt</b>gttggaacatttagaggtcgtatccctatcg          cgaataattccataatgtaca<b>aaacgaattaca</b>aattatttgaccgctaattcaaa<b>tcaagggtg</b>aaaactttgtaatttttggagcgc  <b>cgatcagggagtgga</b>cgccctgggagcgtaca<b>cgctgtggctg</b>cggtg<b>cggtgc</b>aaaaagattgtagcccgctgcgcacatctagtt          aatgtccgcaagactc<b>cagatcaggg</b>tcgcag<b>agtg</b>tc<b>caacgctccc</b>aggag<b>gtta</b>to<b>gacttgc</b>gtattagg<b>gagacg</b>agaa<b>acgt</b>  <b>tccgtccgctgggtcag</b>ttggc<b>caag</b>ttg<b>atgac</b>cggt<b>ctccgtctc</b>ta<b>tcggtg</b>taggtcgtatttggagacagaatata<b>tg</b>gg          gccagtaagctta<b>aacgtg</b>aacctgcgt<b>aaaag</b>tt<b>aa</b>ggttag<b>gca</b>tg<b>taa</b>act<b>ta</b>act<b>ctca</b>acag<b>gtttata</b>ac<b>cca</b>at<b>ctc</b>  <b>agata</b>actat<b>ggcggc</b>acgt<b>gca</b>tt<b>aac</b>ac<b>ggtt</b>gt<b>atcc</b>gt<b>ctaa</b>ag<b>ta</b>ct<b>g</b>gt<b>gc</b>gttggtatactt<b>ca</b>gt<b>ctta</b>ac<b>ca</b>at<b>gc</b>ac          cggttt<b>gtg</b>ggat<b>gc</b>ct<b>at</b>ttt<b>tata</b>t<b>gga</b>gtt<b>gtt</b>ca<b>ctca</b>acatt<b>gca</b>ag<b>actgt</b>aca<b>cttcc</b>at<b>ag</b>tt<b>tata</b>tt<b>tata</b>t  <b>tgca</b>aaaa<b>taattgg</b>c<b>ga</b>ac<b>gag</b>gt<b>aa</b>ct<b>gg</b>at<b>ac</b>ct<b>ca</b>tc<b>cg</b>ca<b>at</b>ta<b>aa</b>att<b>g</b>ag<b>ca</b>cc<b>attg</b>cc<b>ctt</b>g<b>ca</b>cc<b>ctt</b>g<b>ca</b>g<b>ca</b>g<b>ca</b>g<b>g</b>          tgcctgagatccctagaa<b>taattg</b>ga<b>gtt</b>cg<b>ga</b>ata<b>acta</b>tg<b>caga</b>t<b>acctg</b>at<b>aca</b>act<b>tcca</b>ca<b>aaaa</b>ca<b>accaca</b>ag<b>ca</b>cg<b>gc</b>  <b>tgata</b>at<b>cag</b>ca<b>ag</b>ca<b>cc</b>ca<b>ac</b>att<b>cca</b>ca<b>atg</b>ta<b>aa</b>ag<b>ctt</b>ta<b>ac</b>ct<b>ta</b>g<b>ctt</b>ata<b>ca</b>ca<b>aa</b>at<b>ct</b>a<b>att</b>tt<b>cca</b>tg<b>ag</b>g<b>ct</b>g<b>ca</b>          cgacc<b>cg</b>tc<b>ca</b>at<b>ag</b>tc<b>aaa</b>ag<b>gtg</b>ag<b>gtt</b>ta<b>ct</b>at<b>cc</b>tt<b>g</b>at<b>g</b>ag<b>atg</b>tag<b>gtt</b>act<b>aaa</b>at<b>tata</b>ttt<b>cca</b>aa<b>tgga</b>  <b>ttttgc</b>ag<b>attcc</b>ag<b>atg</b>cc<b>ct</b>ac<b>aga</b>aa<b>gag</b>gt<b>aca</b>aa<b>ca</b>tt<b>t</b>at<b>t</b>g<b>ga</b>at<b>ta</b>at<b>ag</b>tt<b>og</b>tt<b>tt</b>g<b>g</b>at<b>tt</b>ac<b>og</b>tt<b>ct</b>          ctagcttatgatctgagcgta<b>agttg</b>aa<b>at</b>at<b>gtt</b>ta<b>ct</b>aa<b>ta</b>ag<b>actt</b>at<b>gg</b>gt<b>tg</b>ag<b>ag</b>cg<b>ctct</b>at<b>tag</b>ta<b>aa</b>ca<b>tata</b>aa<b>ac</b>gt<b>t</b>          cgt<b>g</b>ta<b>act</b>at<b>gg</b>gt<b>g</b>ta<b>cag</b>gt<b>g</b>ca<b>catt</b>ag<b>ttg</b>t<b>acc</b>at<b>tt</b>at<b>gtt</b>at<b>g</b>tt<b>g</b>tt<b>aa</b>cc<b>ag</b>g<b>aa</b>ca<b>ct</b>cc<b>gt</b>cc<b>gt</b>gc<b>gc</b>          actagga<b>aga</b>at<b>cc</b>ga<b>ag</b>gt<b>ccc</b>t<b>aa</b>at<b>tt</b>aa<b>tag</b>cag<b>ttg</b>tt<b>g</b>ta<b>ct</b>at<b>tt</b>ag<b>gt</b>ct<b>ccc</b>ag<b>tg</b>aca<b>ca</b>act<b>aa</b>ca<b>taca</b>aa<b>ac</b>  <b>aa</b>t<b>aac</b>g<b>gtt</b>g<b>tatt</b>g<b>tag</b>a<b>actg</b>ac<b>ag</b>tt<b>gtt</b>t<b>g</b>ta<b>aca</b>t<b>aa</b>t<b>aca</b>act<b>cc</b>g<b>aata</b>ct<b>ca</b>tag<b>cccc</b>cg<b>att</b>cg<b>cc</b>ag<b>gt</b>a          cttacgagga<b>a</b>tt<b>act</b>t<b>ata</b>tc<b>cg</b>act<b>ca</b>at<b>gga</b>tt<b>g</b>ct<b>tata</b>tt<b>ta</b>ca<b>gg</b>ac<b>ca</b>act<b>gga</b>ct<b>g</b>g<b>aaaa</b>ca<b>ca</b>ca<b>atg</b>t<b>atg</b>cg</p>

a. The colors of the att sequences correspond to Figure 3a. The 50bp spacer sequences flanked by att sites are shown in grey.

***Supplementary Table 8: Spacer sequences used to construct the memory array***

**Table 8.** Spacer sequences used to construct the memory array

Flanking att sites	sequences
Int2	gtcgccaaatggatccgagcgttgatccggtaaaaccggattcggggaat
Int3	cggtagtcccggtcgcctctctcgacgaaccgcttacatgcctagccgat
Int4	gttgatgaacattagaggtcgtatcctatcgcgataattccatatacgtaca
Int5	aaaaagatttgatgcccgtcgcacacatctagttaatgtccgcaagactc
Int7	atcggtaggtcgatTTTTGGACAGAAATATAGGGGCCAGTAAGCTTAC
Int8	gcgttggtatacttcagtccttaaccaatgcaccggtttgtgcggatgcct
Int9	agcaccattgccttggcgatcagcatgaggctgcctgagatcctctagaa
Int10	atacacaatctaattttccatgtaggctgcacgaccccgtccaatagtc
Int11	agttcgtgttttgattgttacacgttctctagcttatgatctgagcgta
Int12	ccagaggaacactccgtcggtcgcgcactaggaagaattccgaaggtccc
Int13	ctcatagcccccgattcggccaggtacttacgaggaattacttatatccg

***Supplementary Table 9: Primer sequences used to characterize the memory array***

**Table 9.** Primer sequences used to characterize the memory array

Name	Sequences
Int2_fwd	caaaatggtctttacgatgccattggg
Int2_rev	tccgagcgttgatccggtaaaac
Int3_fwd	gcccgatcaactcgcgc
Int3_rev	tctctcgacgaaccgcttacatgc
Int4_fwd	gcgtagacacatacatgagcgtttgtaaagg
Int4_rev	cattagaggtcgtatcctatcgcgataaattcc
Int5_fwd	ttttatccatttccaaagagcgcgc
Int5_rev	atgcccgctgcgcacat
Int7_fwd	gtaatttttgagcgcggatcag
Int7_rev	cgattttgacagaaaatatggggcc
Int8_fwd	gggagacgagaaacgttccgtcc
Int8_rev	gtatacttcagtcttaaccaatgcaccggttg
Int9_fwd	gtttataacacccaatcatcagataactatggcg
Int9_rev	gcatgaggctgctgagatcctcta
Int10_fwd	atacttccatagttttattaatttatattgcgaaaaataattggcg
Int10_rev	ctaattttccatgtaggctgcacgacc
Int11_fwd	caacaaaaacaaccacaagcagct
Int11_rev	gtgttttggattgttacacgttctctagcttatgatc
Int12_fwd	tatttatattttccaaatggattttgcagattccc
Int12_rev	cactccgtcggtcgcgc
Int13_fwd	gtaaacatataaacgttcgtggtaactatgggtgttac
Int13_rev	atagcccccgattcgcga

***Supplementary Note 1: Discovery strategy when prophages occur within a conserved gene***

Our method to identify the recognition sites of an LSTP integration is based on a genome comparisons of those regions that occur up- and downstream of the prophage. This requires a reference genome that lacks the prophage. We also developed a complimentary method that does not require a reference genome when the prophage occurs within a conserved gene. In these cases, the truncated gene fragments corresponding to the N- and C-terminal protein fragments can be identified up- and downstream of the prophage. The truncated protein fragments are compared against the NCBI protein database using a BLASTP search (default parameters). If a protein is found that is longer than the fragments, it can be used as the reference protein. The 5'- and 3'- gene fragments corresponding to the protein fragments are aligned with the gene for the reference protein (**Supplementary Fig.1**). This yields the 2-18bp core sequence that occurs where the protein coding sequence splits. The *attL* and *attR* sites were defined by taking about 60bp around the core sequence. The *attB* and *attP* sites were reconstructed using *attL* and *attR* sequences, as described in **Figure 1b**. Eight out of the 34 members of integrase-*attB/P* library was discovered using this strategy and they are demarcated in Table 1. For some integrases both strategies can be applied.

**Supplementary Note 2: Weak promoter activity of the Int13 attP site**

When building all the memory switches, the first construct that was attempted by placing the *attP* site upstream of the *gfp* coding sequence. For the majority this was not a problem, but we found that the Int13 site generated a weak constitutive promoter (**Supplementary Fig.2**). This activity was sufficient to move the OFF cell population past the threshold that we used across the memory switches to determine the % of cells that turn on. Unintentional promoters appearing within parts or at part junctions have been observed previously<sup>13</sup>. We eliminated this problem by swapping the *att* sites so that *attP* occurs downstream of *gfp*. Note that after flipping, the resulting *attR* site is insulated by the spacer and RiboJ sequence.

### **Supplementary Note 3: Optimization of RBS sequence for each integrase**

Optimizing the dynamic range of each memory switch required tuning the ribosome binding site (RBS) controlling the expression of the integrase. If the expression is too high, then the switch turns on prior to the addition of inducer. If the expression is too low, then the switch cannot turn on or only a subpopulation of cells turns on.

The optimal level of expression depends on a number of factors and is not known *a priori*. Therefore, to identify the optimal RBS, degenerate RBS sequences consisting of 16-32 sequences were designed using the RBS Calculator for each integrase that encompassed translation initiation rates from  $10^0$  to  $10^4$  au<sup>4</sup>. The degenerate RBS sequences were synthesized in primers and inserted upstream of the coding sequence of each integrase gene using inverse PCR. In detail, the plasmid sequence was amplified using Phusion HF DNA polymerase (New England Biolabs), digested with DpnI restriction enzyme (New England Biolabs) and gel-recovered. The product was then phosphorylated by T4 Polynucleotide Kinase (New England Biolabs) and ligated by T4 ligase (Invitrogen) in a single reaction for 1 hour at room temperature. *E. coli* DH10b cells containing only the reporter plasmid were made electro-competent and transformed with the cognate controller plasmids with degenerate RBS by electroporation. For each integrase 32-64 colonies were picked and cultured in LB media plus 0.5% glucose, 34 µg/ml chloramphenicol and 50 µg/ml kanamycin for screening. The overnight (16 hour) cultures of transformants were washed twice and diluted 200 times in LB containing 0.5% glucose (control) or 1 mM arabinose (induction), and then grown for 6h before flow cytometry analysis. A clone was chosen to have an “optimal” RBS if it exhibited minimal leakage (<0.5% population expressing GFP) and maximal GFP expression when induced (**Supplementary Table 3**).



**Supplementary Note 4: The impact on growth when single or multiple integrases were induced**

In Figure 2G the impact on growth is measured when single integrases were induced. To investigate if the growth defect is additive when multiple integrases are used, cells containing 2-integrase cascade (**Supplementary Fig. 8**, Cas\_2+5), 3-integrase cascade (**Figure 3f**, Cas\_2+5+7) and 4-integrase operon (**Figure 3c**, Cis\_2+7+8+5) were compared with strains containing single integrases (**Figure 2**, Int2, Int5, Int7 and Int8) after 12 hours induction with various concentrations of arabinose (**Supplementary Fig. 9**). The final OD (600nm) decreased slightly in cells containing single integrases at high concentration of arabinose for cells expressing single integrases. However the same effect was not observed in cells incorporating multiple integrases, suggesting that using multiple integrases in parallel is not constrained by growth defect.

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