

Supplementary Information

Recording gene expression order in DNA by CRISPR addition of retron barcodes

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This PDF file includes:

Supplementary Figure 1, Uncropped gels

Supplementary Tables 1 to 4

Supplemental Figure 1, Uncropped gels from main figures.

Figure 1c

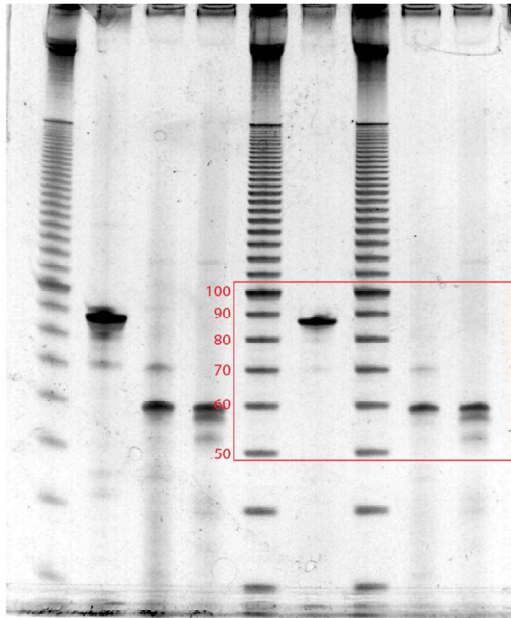


Figure 3c

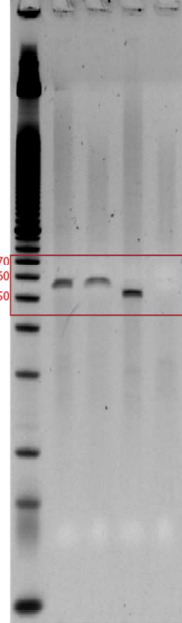
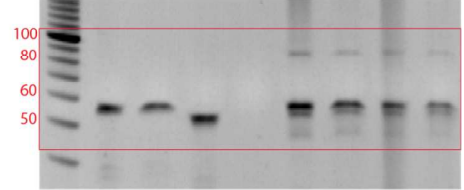


Figure 3h



Supplementary Table 1, Statistics

Figure 1					
Panel	Biological Replicates	Comparison	Test	P value	P value summary
e	3	v32 with RT versus without RT	unpaired t test	<0.0001	****
	3	v35 with RT versus without RT	unpaired t test	0.0002	***
f	5	a1/a2 length: 12 versus 27	unpaired t test	0.0082	**
Figure 2					
Panel	Biological Replicates	Comparison	Test	P value	P value summary
b	3	Non-retron derived spacers: effect of condition	one-way ANOVA	0.5707	ns
		Retron derived spacers: effect of condition	one-way ANOVA	0.0004	***
		Follow-up: v35 "A" with RT versus without RT	Dunnett's multiple comparisons test (corrected)	<0.0001	****
		Follow-up: v35 "A" versus "B" 1	Dunnett's multiple comparisons test (corrected)	0.029	*
		Follow-up: v35 "A" versus "B" 2	Dunnett's multiple comparisons test (corrected)	0.0229	*
		Follow-up: v35 "A" versus "B" 3	Dunnett's multiple comparisons test (corrected)	0.0119	*
		Follow-up: v35 "A" versus "B" 4	Dunnett's multiple comparisons test (corrected)	0.0357	*
		Follow-up: v35 "A" versus "B" 5	Dunnett's multiple comparisons test (corrected)	0.0287	*
		Follow-up: v35 "A" versus "B" 6	Dunnett's multiple comparisons test (corrected)	0.2986	ns
		Follow-up: v35 "A" versus "B" 7	Dunnett's multiple comparisons test (corrected)	0.086	ns
		Follow-up: v35 "A" versus "B" 8	Dunnett's multiple comparisons test (corrected)	0.0007	***
Figure 3					
Panel	Biological Replicates	Comparison	Test	P value	P value summary
b	4-5	Effect of source (retron versus oligo)	two-way ANOVA	<0.0001	****
		Effect of location (LP/M/LD)	two-way ANOVA	<0.0001	****
		Follow-up: retron versus oligo, LP +/- ratio	Sidak's multiple comparisons test (corrected)	>0.9999	ns
		Follow-up: retron versus oligo, M +/- ratio	Sidak's multiple comparisons test (corrected)	0.9985	ns
		Follow-up: retron versus oligo, M +/- ratio	Sidak's multiple comparisons test (corrected)	<0.0001	****
d	2-5 comparison to oligo in panel b	Effect of source (retron versus oligo)	two-way ANOVA	0.0439	*
		Effect of location (LP/M/LD)	two-way ANOVA	<0.0001	****
		Follow-up: retron versus oligo, LP +/- ratio	Sidak's multiple comparisons test (corrected)	>0.9999	ns
		Follow-up: retron versus oligo, M +/- ratio	Sidak's multiple comparisons test (corrected)	>0.9999	ns
		Follow-up: retron versus oligo, M +/- ratio	Sidak's multiple comparisons test (corrected)	0.005	**
e	2-5	Effect of condition	one-way ANOVA	0.33	ns
		Follow-up: oligo versus retron -DBR1	Dunnett's multiple comparisons test (corrected)	0.2858	ns
		Follow-up: oligo versus retron +LDBR1	Dunnett's multiple comparisons test (corrected)	0.5715	ns
g	3	Effect of source (retron versus oligo)	two-way ANOVA	0.0394	*
		Effect of location (LP/M/LD)	two-way ANOVA	0.0083	**
		Follow-up: retron versus oligo, LP +/- ratio	Sidak's multiple comparisons test (corrected)	>0.9999	ns
		Follow-up: retron versus oligo, M +/- ratio	Sidak's multiple comparisons test (corrected)	>0.9999	ns
		Follow-up: retron versus oligo, M +/- ratio	Sidak's multiple comparisons test (corrected)	0.0053	**
i	3	Ecod with RT versus without RT	unpaired t test	0.0197	*
Figure 4					
Panel	Biological Replicates	Comparison	Test	P value	P value summary
b	4	"A" (aTc) versus "B" (Cho)	unpaired t test	0.2704	ns
c	4	Effect of Time on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.4172	ns
		Effect of Source on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.9312	ns
		Effect of Interaction Between Time and Source on Retron-Derived Acquisition Rate	two-way ANOVA	0.0007	***
		Follow-up: Rate of "A" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.005	**
		Follow-up: Rate of "B" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.0445	*
d	4	Effect of Time on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.0002	***
		Effect of Source on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	>0.9999	ns
		Effect of Interaction Between Time and Source on Retron-Derived Acquisition Rate	two-way ANOVA	<0.0001	****
		Follow-up: Rate of "A" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	<0.0001	****
		Follow-up: Rate of "B" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.888	ns
e	4	Effect of Time on Non-Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.0633	ns
		Effect of Inducer Order on Non-Retron-Derived Acquisition Rate (overall)	two-way ANOVA	>0.9999	ns
		Effect of Interaction Between Time and Order on Non-Retron-Derived Acquisition Rate	two-way ANOVA	0.5455	ns
		Follow-up: Rate of "N" Acquisitions A->B, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.1602	ns
		Follow-up: Rate of "N" Acquisitions B->A, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.5567	ns
g	4	"A" (Sal) versus "B" (aTc)	unpaired t test	<0.0001	****
h	3	Effect of Time on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.0003	***
		Effect of Source on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	>0.9999	ns
		Effect of Interaction Between Time and Source on Retron-Derived Acquisition Rate	two-way ANOVA	0.005	**
		Follow-up: Rate of "A" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.3036	ns
		Follow-up: Rate of "B" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.0002	***
i	4	Effect of Time on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.0473	*
		Effect of Source on Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.392	ns
		Effect of Interaction Between Time and Source on Retron-Derived Acquisition Rate	two-way ANOVA	0.0002	***
		Follow-up: Rate of "A" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.1003	ns
		Follow-up: Rate of "B" Acquisitions, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.0004	***
j	4	Effect of Time on Non-Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.1855	ns
		Effect of Inducer Order on Non-Retron-Derived Acquisition Rate (overall)	two-way ANOVA	0.5823	ns
		Effect of Interaction Between Time and Order on Non-Retron-Derived Acquisition Rate	two-way ANOVA	0.0007	***
		Follow-up: Rate of "N" Acquisitions A->B, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.09	ns
		Follow-up: Rate of "N" Acquisitions B->A, 24h versus 48h	Sidak's multiple comparisons test (corrected)	0.0014	**
l	6	Effect of Order (A->B vs B->A)	two-way ANOVA	<0.0001	****
		Effect of Rule	two-way ANOVA	0.7179	ns
		Effect of Interaction	two-way ANOVA	0.0044	**
		Follow-up: A/N (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	<0.0001	****
		Follow-up: N/B (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	0.236	ns
		Follow-up: A/B (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	<0.0001	****
		Follow-up: All (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	0.0003	***
		A->B Overall Values Different Than 0	one-sample t test	0.0065	**
		B->A Overall Values Different Than 0	one-sample t test	0.0363	*
m	5	Effect of Order (A->B vs B->A)	two-way ANOVA	<0.0001	****
		Effect of Rule	two-way ANOVA	0.9252	ns
		Effect of Interaction	two-way ANOVA	0.0017	**
		Follow-up: A/N (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	0.0011	**
		Follow-up: N/B (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	0.1054	ns
		Follow-up: A/B (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	<0.0001	****
		Follow-up: All (A->B vs B->A)	Sidak's multiple comparisons test (corrected)	<0.0001	****
		A->B Overall Values Different Than 0	one-sample t test	0.0327	*
		B->A Overall Values Different Than 0	one-sample t test	0.0116	*

Supplementary Table 2, Strains

Name	Species	Parental Line	Genotype	Method
NEB 5-alpha	<i>E. coli</i>	DH5α	<i>fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Δ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	
BL21-AI	<i>E. coli</i>	BL21	<i>F-ompT hsdSB (rB- mB-) gal dcm araB::T7RNAP-tetA</i>	
bSLS.114	<i>E. coli</i>	BL21-AI	<i>E. coli B F- ompT gal dcm lon hsdSB(rB⁻ mB⁻) [malB⁺]_{K-12} (λ^S) araB::T7RNAP-tetA ΔEco1</i>	lambda Red recombinase mediated insertion of chloramphenicol resistance, marker excision by FLP
bMS.346	<i>E. coli</i>	MG1655	<i>MG1655 galKL187TAAL188TGA Δexol ΔrecJ araB^A:pBAD>T7RNAP;Tet</i>	lambda Red recombination and phage P1 transduction

Supplementary Table 3, Plasmids

Name	Genes	Promoter	Inducer (working concentration)	Used in (panels)
pWUR.1+2	Cas1 + Cas2 (Yosef et al. 2012)	T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	3g-i
pSLS.400	Eco4 RT	pMphR	Erythromycin (400 μM)	3g-i
pSLS.402	Eco1 RT	pMphR	Erythromycin (400 μM)	1c, 1e-i, 2b-d, 3b-e, 3h
pSLS.405	1: Eco1 ncRNA + Eco1 RT; 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	1c, 3c, 3h
pSLS.407	1: Eco1 ncRNA v32; 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	1c, 1e
pSLS.408	1: Eco1 ncRNA v35; 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	1c, 1e-f
pSLS.416	1: Eco1 ncRNA v35, long a1/a2; 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	1f-i, 2b-d, 3b, 3d-e
pSLS.419	1: Eco4 ncRNA; 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	3g-i
pSBK.009	1: Eco1 ncRNA v35, long a1/a2, barcode 1 (CCT-AGG); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.010	1: Eco1 ncRNA v35, long a1/a2, barcode 2 (GCT-AGC); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.011	1: Eco1 ncRNA v35, long a1/a2, barcode 3 (CTG-CAG); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.012	1: Eco1 ncRNA v35, long a1/a2, barcode 4 (GTG-CAC); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.013	1: Eco1 ncRNA v35, long a1/a2, barcode 5 (ACG-CGT); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.014	1: Eco1 ncRNA v35, long a1/a2, barcode 6 (CAG-TAG); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.015	1: Eco1 ncRNA v35, long a1/a2, barcode 7 (GAG-CTC); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.016	1: Eco1 ncRNA v35, long a1/a2, , barcode 8 (GCT-TGC); 2: Cas1 + Cas2	1: T7/lac; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	2b-d
pSBK.079	1: Eco1 RT; 2: Cas1 + Cas2	1: J23115; 2: T7/lac	L-arabinose (2 mg/mL), IPTG (1 mM)	4b-e, 4g-j, 4l-m
pSBK.134	A: Eco1 ncRNA v35, long a1/a2; B: Eco1 ncRNA v35, long a1/a2, barcode 6	A: pTet*; B: pBetI	A: anhydrotetracycline (100 ng/mL); B: choline chloride (100 μM)	4b-e, 4l
pSBK.136	A: Eco1 ncRNA v35, long a1/a2; B: Eco1 ncRNA v35, long a1/a2, barcode 6	A: pSalTTC; B: pTet*	A: sodium salicylate (1 mM); B: anhydrotetracycline (100 ng/mL)	4g-j, 4m

Supplementary Table 4, Primers

Name	Sequence	Purpose
SPCR_MiSeq3_fow1	CTTCCCTACACGACGCTCTCCGATCTNCAATTAATAAATAGGTTATGTTAGAGGTGTTCC	Forward primer with Illumina edeptcr (#1 of set of 5) used to amplify BL21-AI CRISPR array for sequencing
SPCR_MiSeq3_fow2	CTTCCCTACACGACGCTCTCCGATCTNCAATTAATAAATAGGTTATGTTAGAGGTGTTCC	Forward primer with Illumina adapter (#2 of set of 5) used to amplify BL21-AI CRISPR array for sequencing
SPCR_MiSeq3_low3	CTTCCCTACACGACGCTCTCCGATCTNCAATTAATAAATAGGTTATGTTAGAGGTGTTCC	Forward primer with Illumina adapter (#3 of set of 5) used to amplify BL21-AI CRISPR array for sequencing
SPCR_MiSeq3_low4	CTTCCCTACACGACGCTCTCCGATCTNCAATTAATAAATAGGTTATGTTAGAGGTGTTCC	Forward primer with Illumina adapter (#4 of set of 5) used to amplify BL21-AI CRISPR array for sequencing
SPCR_MiSeq3_low5	CTTCCCTACACGACGCTCTCCGATCTNCAATTAATAAATAGGTTATGTTAGAGGTGTTCC	Forward primer with Illumina adapter (#5 of set of 5) used to amplify BL21-AI CRISPR array for sequencing
SPCR_MiSeq3_rev	GGAGTTCAGACGCTGTCTCTCCGATCTGTGCAACATCGTTCCTGATTGTC	Reverse primer with Illumina adapter used to amplify BL21-AI CRISPR array for sequencing
Eco1_v35_oligo	GTCAGAAAAACGGGTGGAGAGGTTGCTGCAACTCTCCATTTTCTGTAACTCAGA	Test acquisition behavior of Eco1 v35 by electroporation
Eco4_wt_oligo	AGCCGGGAACAACCTTTTGTATCCGCAACTGATTGCGGCTCAAAAGTTTGTCCGCAACTGTAATGTAATC	Test acquisition behavior of Eco4 by electroporation