Supporting information for:

A Toolkit for Rapid Modular Construction of Biological Circuits in Mammalian Cells

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Supporting Information.

Supplementary Fig. 1. Comparison of constitutive promoters across cell lines and delivery methods.

Supplementary Fig. 2. Comparison of impact of 3' UTRs across cell lines and delivery methods.

Supplementary Fig. 3. **Generation of landing pads for human cell lines.**

Supplementary Fig. 4. Building a linear classifier to distinguish target sgRNA knockdown populations.

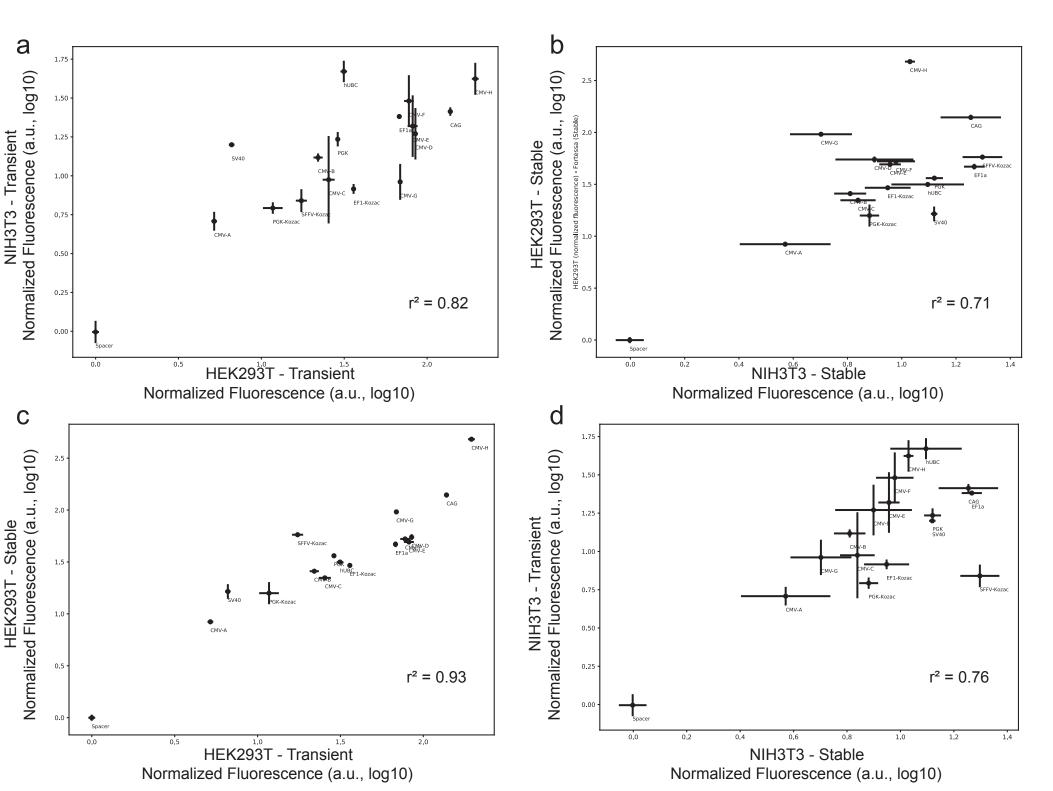
Supplementary Fig. 5. Generation and quality control of multicistronic constructs for Zaire ebolavirus ribonucleoproteins.

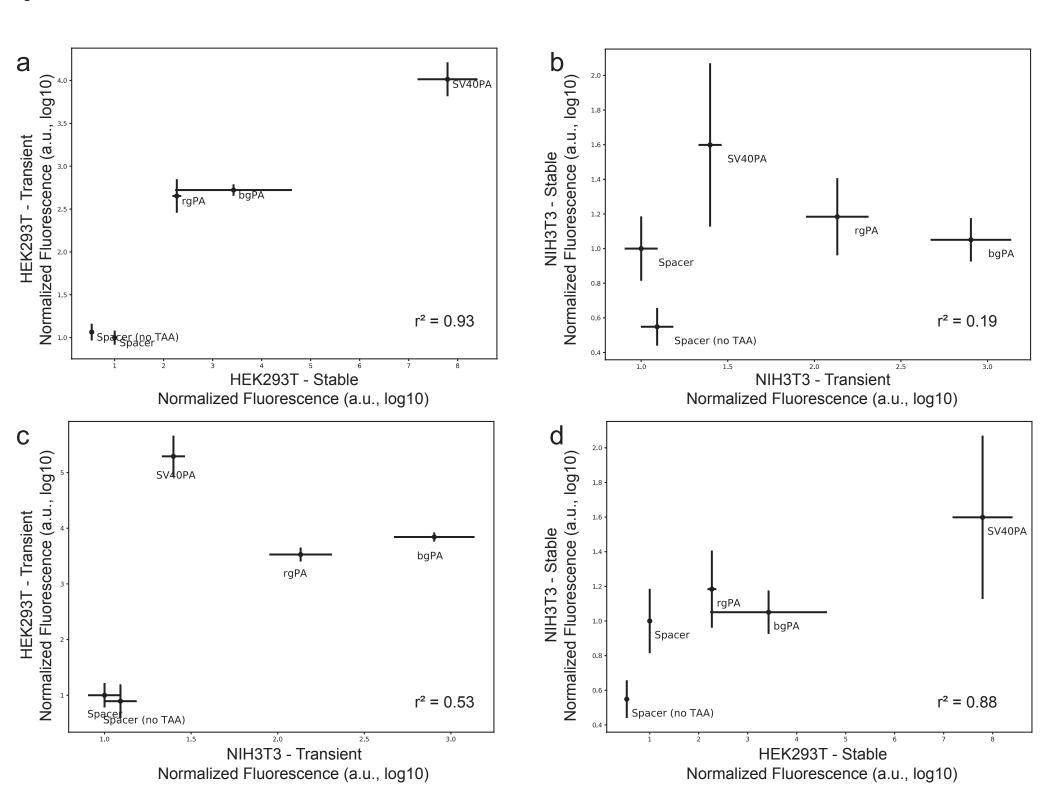
Supplementary Table 1. Name and description of MTK library plasmids.

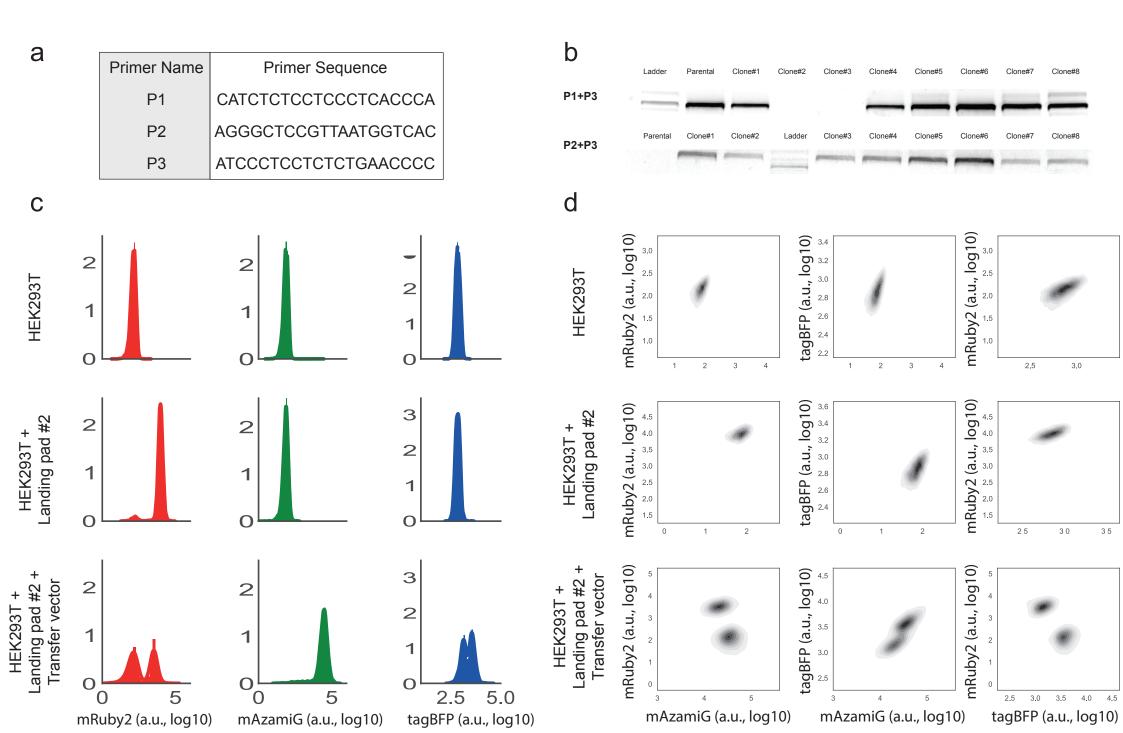
Supplementary Table 2. sgRNA sequences used in MTK library.

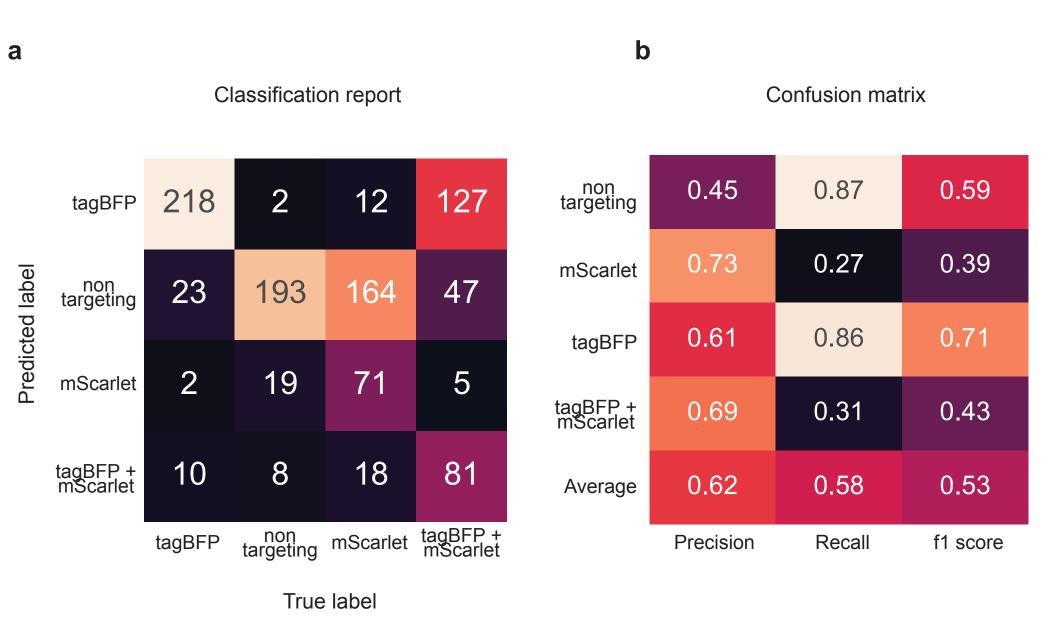
Supplementary Table 3. Primer sequences for part domestication.

Supplementary Table 4. Connector part overhang sequences.



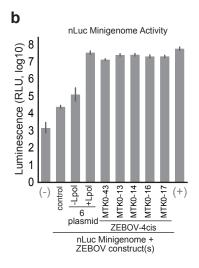


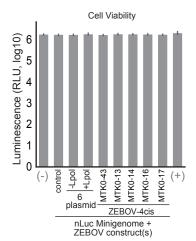


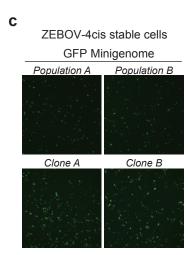


a

		ZEBC	V-4cis
Donor Vector	Donor Name	Colonies Screened	Colonies Correct
MTK0-43	PiggyBac Transposon	9	9
MTK0-13	PhiC31 Integrase	4	4
MTK0-14	BxBI Integrase	4	3
MTK0-16	PhiC31 tagBFP	4	4
MTK0-17	BxBI tagBFP	4	4







Supplementary Fig. 1. Comparison of constitutive promoters across cell lines and delivery methods. (a) The mAzamiGreen expression from transient transfection and PiggyBac integration of each promoter was assessed in HEK293T and (b) NIH3T3 cells. (c) mAzamiGreen expression of each promoter was compared between HEK293T and NIN3T3 in transient expression and (d) PiggyBac integration. Each point represents the mean of four biological replicates and error bars represent the standard deviation across replicates.

Supplementary Fig. 2. Comparison of impact of 3' UTRs across cell lines and delivery methods. (a) The mAzamiGreen expression from transient transfection and PiggyBac integration delivery of each 3' UTR was assessed in HEK293T and (b) NIH3T3 cells. (c) mAzamiGreen expression of each 3' UTR was compared between HEK293T and NIN3T3 in transient expression and (d) PiggyBac integration. Each point represents the mean of four biological replicates and error bars represent the standard deviation across replicates.

Supplementary Fig. 3. **Generation of landing pads for human cell lines. (a)** PCR products from landing pad genotyping. P1+P3 indicate presence of WT hAVVS1 locus and P2+P3 indicate presence of BxBI landing pad in hAAVS1 locus. mRuby2, mAzamiGreen and tagBFP expression in populations of parental, Landing pad and Landing Pad with Transfer vector HEK293T cells. **(b)** mRuby2, mAzamiGreen and tagBFP expression in populations of parental, Landing pad #2 and Landing Pad #2 with Transfer vector HEK293T cells. In this clone, both wild type alleles of hAAVS1 locus were replaced by the landing pad construct, showing two populations as measured by fluorescence upon integration of the transfer vector and suggesting these two populations had one or two copies that integrated into the genome. mRuby2

expression indicates presence of hAAVS1 landing pad. mAzamiGreen and tagBFP expression indicates precise integration of transfer vector in hAAVS1 landing pad.

Supplementary Fig. 4. **Building a linear classifier to distinguish target sgRNA knockdown populations. (a)** Classification report of linear classifier. **(b)** Confusion matrix of linear classifier.

Supplementary Fig. 5. Generation and quality control of multicistronic constructs for Zaire ebolavirus ribonucleoproteins. (a) Multicistronic construct cloning efficiency. ZEBOV multicistronic construct containing 4 viral ORFs separated by P2A elements (ZEBOV-4cis) was BsmBl assembled directly into donor vectors for genome engineering. Specifically, ZEBOV-4cis was generated in donor vectors for PiggyBac transposon (MTK0-43), PhiC31 Integrase (MTK0-13, MTK0-16 tagBFP), and BxBI Integrase (MTK0-14, MTK0-17 tagBFP). The number of bacterial colonies screened and positive for correct construct by size (Notl digestions) is indicated. (b) Luminescence measurements of ZEBOV minigenome activity and cell viability. HEK293T cells were transfected with ZEBOV nLuc minigenome in combination with pCAGGs empty control plasmid (control), the ZEBOV 6 plasmids (with or without Lpol: +Lpol, -Lpol), or with ZEBOV-4cis in various part 0 donor vectors. Positive (+) and negative (-) controls include transfection of only pCAGGS-nLuc plasmid or pCAGGs empty plasmid, respectively. Nano luciferase activity was measured two days post transfection. Bar plots represent the mean of biological replicates (n=2). (c) GFP analysis of minigenome activity in stable cells. ZEBOV-4cis stable populations and clones were transfected with a T7-driven ZEBOV minigenome construct encoding the eGFP reporter along with T7 polymerase. After 2 days cells were imaged for GFP detection (Leica, 4X).

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		MTK Part Type	Description Encodes Cont.S as a Type 1 part to be used in the MTK system	Comments True 1 carts are 5 assembly connectors in the MTK. This part is identical to bYTK002 from Pumber VTK	Availability Core MTK
	MTK1_002 MTK1_003	Part 1 Part 1	Encodes Cont.1 as a Type 1 part to be used in the MTK system Encodes Cont.2 as a Type 1 part to be used in the MTK system		Core MTK
	MTK1_004		Encodes Cont.3 as a Type 1 part to be used in the MTK system	Type 1 parts are 5' assembly connectors in the MTK. This part is identical to pYTK005 from Dueber YTK	Core MTK
	MTK1_006	Part 1			Core MTK
	MTK1_007 MTK1_008	Part 1 Part 1	Encodes Cont.5° as a Type 1 part to be used in the MTK system Encodes Cont.5° and Christen hypersenselishiy side it in sizuation as a Type 1 part to be used in the MTK system	Type 1 parts are 5' assembly connectors in the MTK. This part is identical to pYTK008 from Dueber YTK Type 1 parts are 5' assembly connectors in the MTK.	Core MTK
	MTK1_009		Encodes Cont.1 and chicken hypersensitivity alte 4 insulator as a Type 1 part to be used in the MTK system Encodes Cont.2 and chicken hypersensitivity alte 4 insulator as a Type 1 part to be used in the MTK system Tencedes Cont.2 and chicken hypersensitivity alte 4 insulator as a Type 1 part to be used in the MTK system		Core MTK Core MTK
	MTK1_012	Part 1 Part 1			Core MTK
	MTK1_013 MTK1_014	Part 1 Part 1	Encodes ConLS and chicken hypersensitivity site 4 insulator as a Type 1 part to be used in the MTK system Encodes ConLS' and short flippase recognition target (FRT) site as a Type 1 part to be used in the MTK system	Type 1 parts are 5' assembly connectors in the MTK. Type 1 parts are 5' assembly connectors in the MTK.	Core MTK Core MTK
	MTK1_015	Part 1	Encodes ConLS, chicken hypersensitivity site 4 insulator and locus of X-over P1 (losP) as a Type 1 part to be used in the MTK system Encodes ConL6 as a Type 1 cert to be used in the MTK system	Type 1 parts are 5' assembly connectors in the MTK. Type 1 parts are 5' assembly connectors in the MTK.	
	MTK1_017 MTK1_018	Part 1 Part 1			Core MTK
	MTK1_019	Part 1	Encodes Cont.6 and chicken hypersensitivity site 4 insulator as a Type 1 part to be used in the MTK system Specific Cont.7 and distinct hypersensitivity site 4 insulator as a Type 1 part to be used in the MTK system.	Type 1 parts are 5' assembly connectors in the MTK.	Core MTK
Column	MTK1_022	Part 1			Core MTK
The color	MTK1_024	Part 1	Encodes Cont.1 and numan B1 instation as a Type 1 part to be used in the MIK system Encodes Cont.2 and human C3 instation as a Type 1 part to be used in the MIK system	Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/htt.3982 Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/htt.3982	Core MTK
Company	MTK1_026 MTK1_026	Part 1 Part 1	Encodes Cont.3 and human D1 insulator as a Type 1 part to be used in the MTK system Encodes Cont.4 and human E1 insulator as a Type 1 part to be used in the MTK system	Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/htt.3062 Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/htt.3062	Core MTK
The column The	MTK1_028	Part 1		Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/init.3062 Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/init.3062	Core MTK
The column The	MTK1_029 MTK1_030	Part 1 Part 1	Encodes ConL7 and human B2 insulator as a Type 1 part to be used in the MTK system Encodes ConL1 and picomavius peptide 2A (P2A) element as a Type 1 part to be used in the MTK system	Type 1 parts are 5' assembly connectors in the MTK. Insulator sequences from https://idoi.org/10.1038/nbt.3062 Type 1 parts are 5' assembly connectors in the MTK.	
Company	MTK1_031	Part 1 Part 1			Core MTK
Column	MTK1_033 MTK1_034	Part 1		Type 1 parts are 5' assembly connectors in the MTK. Type 1 parts are 5' assembly connectors in the MTK.	
The color	MTK2_001	Part 2	Encodes pTRE as a Type 2 part to be used in the MTK system Encodes at IAS (solidities) and a Type 2 part to be used in the MTK systems	Type 2 parts are promoters in the MTK Type 2 parts are promoters in the MTK	Core MTK
The color	MTK2_004	Part 2		Type 2 parts are promoters in the MTK	Core MTK
Company	MTK2_006	Part 2	Encodes pCAG as a Type 2 part to be used in the MIK system Encodes pSV40 as a Type 2 part to be used in the MTK system	Type 2 parts are promoters in the MTK	Core MTK
	MTK2_008	Part 2	Encodes human pLtbc as a Type 2 part to be used in the MTK system Encodes human pLtbc as a Type 2 part to be used in the MTK system	Type 2 parts are promoters in the MTK	Core MTK
Company	MTK2_013	Part 2		Type 2 parts are promoters in the MTK	Core MTK
Company	MTK2_014 MTK2_015	Part 2 Part 2	Encodes pLMS (minicIAM* core) as a Type 2 part to be used in the MTK system Encodes pSymTr (minicIAM* core) as a Type 2 part to be used in the MTK system	Type 2 parts are promoters in the MTK. Type 2 parts are promoters in the MTK. Promotor sequence from DOI: https://doi.org/10.1016/j.cell.2018.11.002	Core MTK
Column	MTK2_016	Part 2			Core MTK Core MTK
May	MTK2_018 MTK2_019	Part 2 Part 2			Core MTK Core MTK
Company	MTK2_020		Encodes truncated pCMV as a Type 2 part to be used in the MTK system Encodes truncated pCMV as a Type 2 part to be used in the MTK system	Type 2 parts are promoters in the MTK. Promotor sequence from https://doi.org/10.1016/j.ab.2012.06.001 Type 2 parts are promoters in the MTK. Promotor sequence from https://doi.org/10.1016/j.ab.2012.06.001	Core MTK
Company	MTK2_022	Part 2		Type 2 parts are promoters in the MTK. Promotor sequence from https://doi.org/10.1016/j.ab.2012.06.001	Core MTK
Column	MTK2_024 MTK2_026	Part 2 Part 2		Type 2 parts are promoters in the MTK	Core MTK
Column	MTK3_002	Part 3	Encodes bot (biastición-S deaminace) as a Type 3 part to be used in the MTK system Encodes both (biastición-S deaminace) as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK.	Core MTK
Company	MTK3_005				Core MTK
Company	MTK3_008	Part 3	Encodes numer resource ruce as w 1ype 3 pair to be used in the MTK system Encodes nuclear localized becarial EL 222 fused to activating domain of VP16 as Type 3 part to be used in the MTK system	Type 3 pants are generally reserved for coding sequences in the MTK. Type 3 pants are generally reserved for coding sequences in the MTK. Part sequence is from 10.1021/bH01040m.	Core MTK
Mary	MTK3_010	Part 3	Encodes Tech-On 30 transactivator profes as a Type 3 part to be used in the MTK system Encodes Tech-On 30 transactivator profesin as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from https://doi.org/10.1093/har/gkt002	Core MTK Core MTK
Mary	MTK3_011 MTK3_012	Part 3	Encodes HA-lagged PH-s fused to Tet Repressor protein as a Type 3 part to be used in the MTK system Encodes HA-lagged PYLcs fused to VP16 activator domain as a Type 3 part to be used in the MTK system		Core MTK Core MTK
Mary	MTK3_014	Part 3 Part 3	Encodes Flag-Bagged ABIcs fused to CML4 DNA binding domain as a Type 3 part to be used in the MTK system Encodes Go-copoled MMSD as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 10.1126/scisignal.2001449 Type 3 parts are generally reserved for coding sequences in the MTK. Coding sequence from https://doi.org/10.1073/jnns.0700293104	Core MTK
Company	MTK3_015	Part 3	Encodes Gi-coupled hM4D as a Type 3 part to be used in the MTK system		Core MTK
Column	MTK3_017	Part 3	Encodes Fluorescent probe for G1/S transition as a Type 3 part to be used in the MTK system		Core MTK
Column	MTK3_018 MTK3_019	Part 3 Part 3		Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 10.1038/ncomms12009 Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 10.1038/ncomms12009	Core MTK Core MTK
Mary	MTK3_020	Part 3	Encodes ERK Translocation Reporter (ERK KTR) as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 10.1038/ncomms12009 Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 11.1038/ncomms12009	Core MTK
March Marc	MTK3 022	Part 3	Encodes mTurquoise2 as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. This part is identical to pYTK032 from Dueber YTK	Core MTK
Column	MTK3_024	Part 3	Encodes mRuty/2 as a Type 3 part to be used in the MTK system		Core MTK
Column	MTK3_026	Part 3	Encodes cAMP reporter Flaminds2 as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved or coding sequences in the MTK. Chaing sequence from doi: 10.1371/journal.pone.0100252	Core MTK
Company	MTK3 029	Part 3	Encodes PKA SPARK, a PKA activity reporter, as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 10.1128/scrisignat.2001449 Type 3 parts are generally reserved for coding sequences in the MTK. Coding sequence from doi: 10.1016/j.molcel.2017.12.008	Core MTK
Company	MTK3_031	Part 3			Core MTK
Company	MTK3_033	Part 3 Part 3	Encodes JNK Translocation Reporter (JNK KTR) fused to mRuby2 as a Type 3 part to be used in the MTK system Encodes Gibbereilin Receptor fused to VPR activation domain as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from 10.1016/j.cell.2014.04.039 Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from https://doi.org/10.1038/mneth.4042	Core MTK
Manual	MTK3_034 MTK3_035	Part 3 Part 3	Encodes Abasia acid Receptor fused to VPR activation domain as a Type 3 part to be used in the MTK system Encodes Imman E-Catherin as a Type 3 part to be used in the MTK system Encodes Imman E-Catherin as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Part sequence is from https://doi.org/10.1038/nmeth.4042 Type 3 parts are generally reserved for coding sequences in the MTK.	Core MTK
Company	MTK3_036 MTK3_037	Part 3	Encodes mScarlet as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Type 3 parts are generally reserved for coding sequences in the MTK. Coding sequence from DOI: 10.1128/urisingal.aat/3738	
Company	MTK3 038	Part 3	Encodes R-Finc-A, a cAMP reporter, as a Type 3 part to be used in the MTK system Encodes moves Relâ as a Type 3 part to be used in the MTK system	Type 3 parts are generally reserved for coding sequences in the MTK. Coding sequence from https://doi.org/10.1038/s41596-018-20251-1 Type 3 parts are generally reserved for coding sequences in the MTK.	Core MTK
Company	MTK3a_001	Part 3a		Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Column	MTK3a_003	Part 3a	Encodes Nuclear import sequence as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Column	MTK3a 005	Part 3a Part 3a	Encodes Colgi Targeting Sequence as a Type 3a part to be used in the MTK system Encodes Mitochondria Targeting Sequence as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Company	MTK3a_007	Part 3a	Encodes Peroxisome Targeting Sequence as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Property	MTK3a_008 MTK3a_009	Part 3a Part 3a	Encodes ERK Translocation Reporter (ERK KTR) as a Type 3a part to be used in the MTK system Encodes BxBl attP site as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system. Part sequence is from 10.1016/j.cell.2014.04.039 Type 3a parts are used for N-terminal coding sequences in the MTK system.	
Company	MTK3a_010 MTK3a_011	Part 3a Part 3a	Encodes Phi31 attP site as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Company	MTK3a_013 MTK3a_014	Part 3a Part 3a	Encodes msGFP as a Type 3a part to be used in the MTK system Finnelies Knamed Associated Ray representation from an as a Type 3a part to be used in the MTK system.	Type 3a parts are used for N-terminal coding sequences in the MTK system Type 3a parts are used for N-terminal coding sequences in the MTK system	
March Marc	MTK3a 015	Part 3a	Encodes Medicin and inconsistent and a Type Sa part to be used in the MTX system Encodes Medicin and inconsistent and a Type Sa part to be used in the MTX system	Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Company	MTK3a_017	Part 3a			Core MTK
Company	MTK3a_019	Part 3a	Encodes Absisic acid receptor as a Type 3a part to be used in the MTK system Encodes Absisic acid receptor as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system. Part sequence is from https://doi.org/10.1038/nmeth.4042	Core MTK
Company	MTK3a 021	Part 3a	Encodes Gitbernelin Receptor as a Type 3a part to be used in the MTK system Encodes HELB-COK2 Translocation Reporter as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system. Part sequence is from https://doi.org/10.1038/nmeth.4042 Type 3a parts are used for N-terminal coding sequences in the MTK system. Part sequence is from 10.1016/j.cell.2013.08.082	Core MTK
Property	MTK3a_023	Part 3a		Type 3a parts are used for N-terminal coding sequences in the MTK system. Part sequence is from https://doi.org/10.1038/nmeth.4042	Core MTK
Property	MTK3a_025	Part 3a Part 3a	Encodes spCAS9 tagged with NLS as a Type 3a part to be used in the MTK system Encodes spCAS9 tagged with NLS as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the MTK system Type 3a parts are used for N-terminal coding sequences in the MTK system	Core MTK
Company	MTK3a 027	Part 3a Part 3a	Encodes tadBFP as a Type 3a part to be used in the MTK system	Type 3a parts are used for N-terminal coding sequences in the NTK system. Part sequence is from https://doi.org/10.1038/nmeth.4042 Type 3a parts are used for N-terminal coding sequences in the NTK system	Core MTK
March Marc	MTK3a_029	Part 3a	Encodes mTurquoise2 as a Type 3a part to be used in the MTK system	Type 3a parts are for N-terminal coding sequences in the MTK. This part is identical to pYTK037 from Dueber YTK	Core MTK
March Marc	MTK3b_001 MTK3b_002	Part 3b Part 3b	Encodes human beta actin as a Type 3b part to be used in the MTK system Encodes human alpha tabulin 1 as a Type 3b part to be used in the MTK system	Type 3b parts are used for coding sequences in the MTK. Type 3b parts are used for coding sequences in the MTK.	Core MTK Core MTK
Miles Mile	MTK3b 004	Part 3b Part 3b			Core MTK Core MTK
Miles Mile	MTK3b_005 MTK3b_008		Encodes RFP670 as a Type 3b part to be used in the MTK system Encodes ladSPP as a Type 3b part to be used in the MTK system	Type 3b parts are used for coding sequences in the MTK. Type 3b parts are used for coding sequences in the MTK.	
Month Mont	MTK3b_007 MTK3b_008	Part 3b	Encodes bacterial light-advisated adelnylif cyclase (IEPAC) as a Type 3b part to be used in the MTK system Encodes EEPAC as a Type 5b part to be used in the MTK system	Type 3b parts are used for coding sequences in the MTK. Coding sequence from doi: 10.1074/jbc.M110.185496 Type 3b parts are used for coding sequences in the MTK.	Core MTK Core MTK
March Marc	MTK3b_009	Part 3b	Francisco miSnariat as a Tune 3h nort to be used in the MTK system	Type 3b parts are used for coding sequences in the MTK. This part is identical to pYTK046 from Dueber YTK Type 3b parts are used for coding sequences in the MTK.	
March Marc	MTK3b_011	Part 3b Part 3b	Francies IRFP713 as a Tune 3h nort to be used in the MTK system	Type 3b parts are used for coding sequences in the MTK. Type 3b parts are used for coding sequences in the MTK.	Core MTK
March Marc	MTK3h 013	Part 3b			Core MTK
Company Comp	MTK3b_015	Part 3b			Core MTK
Mich. 19	MTK3b_017	Part 3h	Encodes nat Connect 43 as a Type 30 part to be used in the MTK system	Type 30 parts are used for coding sequences in the MTK.	Core MTK
Minus Minu	MTK9h 019	Part 3b			Core MTK
Miles Mile	MTK3b_021	Part 3b	Encodes VPR activation domain as a Type 30 part to be used in the MTK system	Type 30 parts are used for coding sequences in the NTK. Type 30 parts are used for coding sequences in the NTK.	Core MTK
MFC 200 Per 200 Contact Amend Part of Part of Part of Section Per 200 Contact Amend Part of Part of Part of Section Per 200 Contact Amend Part of	MTK3b_024	Part 3b	Encodes human NFATC2 as a Type 3b part to be used in the MTK system	Type 30 parts are used for coding sequences in the MTK. Type 30 parts are used for coding sequences in the MTK.	Core MTK
MTCS, 201 Pet 1 Controls Pet 2 Controls Pet 2 Controls Pet 2 Control Pet 2 Controls Pet 2 Controls Pet 2 Control Pet 2 Controls Pet 2 Control	MTK3b_027 MTK3b_028	Part 3b	Encodes human TPS3 as a Type 3b part to be used in the MTK system Encodes (human TPS3 as a Type 3b part to be used in the MTK system)		Core MTK
MTCS, 201 Pet 1 Controls Pet 2 Controls Pet 2 Controls Pet 2 Control Pet 2 Controls Pet 2 Controls Pet 2 Control Pet 2 Controls Pet 2 Control	m1K3b_030 MTK3b_030	Part 3b	Encodes mouse RelA as a Type 3b part to be used in the MTK system Encodes mouse RelA as a Type 3b part to be used in the MTK system	Type 30 parts are used for coding sequences in the MTK. Type 30 parts are used for coding sequences in the MTK.	Core MTK
MTKG 05 Pet 4 Control south trade platin prolesported are a Type 4 agent to be used in the MTK spares First process of the process and trade platin prolesported are a Type 4 agent to be used in the MTK spares First process of the	MTK3b 031	Part 3b Part 4			Core MTK Core MTK
MYCAL GOT PACE A Control and Angel segenteen and the MYC Speller MYCAL Speller	MTK4 003	Part 4	Encodes SV40 polyadenylation signal as a Type 4 part to be used in the MTK system Encodes rabbit beta-globin polyadenylation signal as a Type 4 part to be used in the MTK system	Type 4 parts are polyadenylation signals in the MTK	Core MTK
MYCAL GOT PACE A Control and Angel segenteen and the MYC Speller MYCAL Speller	MTK4_004 MTK4_006	Part 4 Part 4	Encodes spacer sequence as a Type 4 part to be used in the MTK system Encodes spacer sequence (multicistronic) as a Type 4 part to be used in the MTK system	Type 4 parts are polyadenylation signals in the MTK Type 4 parts are polyadenylation signals in the MTK	Core MTK Core MTK
MFMs_506 PM 44 Documents of the processor of the processo	MTK4a_001 MTK4a_002	Part 4a		Type 4a parts are used for C-terminal coding sequences in the MTK system Type 4a parts are used for C-terminal coding sequences in the MTK system	Core MTK Core MTK
First Section	MTK4a_003 MTK4a_004				Core MTK Core MTK
MTMs_879 Port 46 Conclus Premissions Targeting Reguence as a Type 4 agent to be used in the MTK system Port 46 Conclus MTMs_871 Port 46 Conclus MT	MTK4a_005 MTK4a_006	Part 4a Part 4a	Encodes Mitochondria Targetring Sequence as a Type 4a part to be used in the MTK system Encodes ER Targetring Sequence IN Terminal) as a Type 4a part to be used in the MTK owners	Type 4a parts are used for C-terminal coding sequences in the MTK system Type 4a parts are used for C-terminal coding sequences in the MTK system	Core MTK Core MTK
MTMs_871 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system MTMs_873 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system MTMs_874 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_875 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess an ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess An Ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess An Ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess An Ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 Concess An Ordan treatment in a Type 4 gaint to see and the TMT system Concess MTMs_877 Pint 4 gaint to see and the TMT system Pint 4 Concess An Ordan treatment in a Type 4 gaint to see and the TMT system Pint 4 Concess An Ordan treatment in a Type 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and the TMT system Pint 4 gaint to see and	MTK4a 007	Part 4a			Core MTK
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MTMs_677 PM 44 Conclus an onable concluse EFF2 conceptance consequence and a Type 4 agent to be used in the MTK system MTMs_679 PM 44 Conclus the Conclus Teach of T	MTK4a_016	Part 4a Part 4a	Encodes mstGFP as a Type 4a part to be used in the MTK system Encodes in sucher localized tolePFP concression cassette as a Type 4a part to be used in the MTK system	Type 4a parts are used for C-terminal coding sequences in the MTK system Type 4a parts are used for C-terminal coding sequences in the MTK system	Core MTK
MTML (20) Port 4 Conclusion be of FEST to get a Tiple 4 get the beautiful to MTM (20) Port 4 Conclusion beautiful to Section 1 and 1	MTK4a_017 MTK4a_018	Post 4o	Encodes a nuclear localized EBFP2 coexpression cassetts as a Type 4a part to be used in the MTK system Encodes a nuclear localized mTurquoise2 coexpression cassetts as a Type 4a part to be used in the MTK system		Core MTK
Mink 1	MTK4a_020	Part 4a			Core MTK
MTAGE_SER PM 44 Chooked Sections And Transporter to the Control Management of the Control Manag	MTK4a_021 MTK4a_023	Part 4a Part 4a	Encodes a nuclear localized and destabilized miRuthy2 coexpression cassette as a Type 4a part to be used in the MTK system Encodes Gibberellin Insensitive as a Type 4a part to be used in the MTK system	Type 4a parts are used for C-terminal coding sequences in the MTK system Type 4a parts are used for C-terminal coding sequences in the MTK system. Part sequence is from https://doi.org/10.1038/nmeth.4042	Core MTK Core MTK
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MTMS_600 Part discovered and the segretary in particular properties propriet as propriets propri	MTK4b_001	Part 4b	Encodes Bovins Clowth Homone polyadanylation signal as a Type 4b part to be used in the MTK system Encodes Bovins Clowth Homone polyadanylation signal as a Type 4b part to be used in the MTK system	тури че рассы все с-веттина courting sequences in the MTK system. Hart sequence is from responded org/10.1038/mmeth.4042. Туре 4b parts are polyadary/lation signate used when a type 4a has been included as a coding sequence.	Core MTK
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MTR, 500 Part 5 Conclusion Confered as a Type S part to secure in the MTK. This part is secured in the MTK (in your members) of the MTK. And MTR, 500 Part 5 Conclusion Confered as a Type S part to secure in the MTK. This part is secured as the MTK (in your members) of the MTK. This part is secured as the MTK. This part is secure	MTK4b_006	Part 4b	Encodes spacer sequence in ut 1ype 4b pair to be used in the MTK system Encodes spacer sequence (multicistronic) as a Type 4b pair to be used in the MTK system	туры чо рыть или розумонтуватол signass used when a type 4a hall blenn included as a coding sequence. Туре 4b parts are polyadenylation signals used when a type 4a has been included as a coding sequence.	Core MTK
MTR_80 Part 5 Conciones Confeit as a Type S part to sead on the MTK types man and Taxon section as the MTK. This part is desired as by Time Types and the manual ma	MTK5 001	Part 5 Part 5			Core MTK
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MTR_800 Part 5 Concider Care Type Sparts but used in the MTK spares in State of the MTK spares in Stat	MTKS_006 MTKS_006	Part 5 Part 5	Encodes ConRS as a Type 5 part to be used in the MTK system Encodes ConRE as a Type 5 part to be used in the MTK system	Type 5 parts are 3' assembly connectors in the MTK. This part is identical to pYTK071 from Dueber YTK Type 5 parts are 3' assembly connectors in the MTK. This part is identical to pYTK072 from Dueber YTK	Core MTK Core MTK
MTG_810 Pad 5 Conciones Confir as a Type Spart bot used on the MTC system Consecution in the MTC	MTK5_007 MTK5_008	Dod 6			Core MTK Core MTK
MTG_81 Part 5 Econolas Comilió ao a Tipo S parto bos usado in the MTK signam (Tipo S parto ao Visual Parto Auto, France Parto Parto Auto (Tipo S parto ao Visual Parto Auto, France Part	MTK5_009 MTK5_010	Part 5			Core MTK
MTG_000 Part 6 Scooding-(DAV-Basicotion resistance cassets as a type 6 part to be used to the MTX system Pyre 6 part on the components of the destination basicotion resistance cassets as a type 6 part to be used to the MTX Cone MTX.	MTKS_011 MTKS_012	Part 5 Part 5	Encodes ConR8 as a Type 5 part to be used in the MTK system Encodes ConR6: and the human A1 insulator as a Type 5 part to be used in the MTK system	Type 5 parts are 3' assembly connectors in the MTK. Type 5 parts are 3' assembly connectors in the MTK. Insulator sequences from https://doi.org/10.1038/hbt.3062	Core MTK Core MTK
	MTK8_003		Encodes pCMV-Blassición resistance cassette as a type 6 part to be used in the MTK system	Type 6 parts can be components of the destination backbone such as resistance cassettle, nuclear marker, integrase recognition sites in the MTK	Core MTK

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	MTK6_008 MTK6_009
	MTK8 010
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	### STORY 15 ### ST

Service of the control of the contro	JPEN536h				
The control of the co	JPF0536c	Encodes one insert Encodes one insert	Encodes pEF ta driving expression of TET30 P2A RFPP13, pEF ta expressing spdCast fused to KRAB domain P2A tag8FP, and an sgRNA targeting pUAS expressing mAzamiCinean in a PiggyBac destination vector Encodes pEF ta driving expression of TET30 P2A RFPP13, pEF ta expressing spdCast fused to VPR domain P2A tag8FP, and an sgRNA targeting pUAS expressing mAzamiCinean in a PiggyBac destination vector	Pleamid used to generate Fig. 5 Pleamid used to generate Fig. 5	MTK TUs and MTUs MTK TUs and MTUs
Services of the control of the contr	ARB365 ARB366	Encodes one insert Encodes one insert	Encodes pEF1a driving expression of TET3G P2A IRFP713, pEF1a expressing sadCas9 fused to mRuby/2 P2A mRuby/2, and an sgRNA targeting pUAS expressing mAzamiGreen in a PiggyBac destination vector Encodes pEF1a driving expression of TET3G P2A IRFP713, pEF1a expressing sadCas9 fused to KRAB domain P2A mRuby/2, and an sgRNA targeting pUAS expressing mAzamiGreen in a PiggyBac destination vector	Plasmid used to generate Fig. 5 Plasmid used to generate Fig. 5	MTK TUs and MTUs MTK TUs and MTUs
Services of the control of the contr	ARB367 ARB385	Encodes one insert Encodes one insert	Encodes pEF1a driving expression of TET3G P2A IRFP713, pEF1a expressing sadCas9 fused to VPR domain P2A mRuby2, and an sgRNA targeting pUAS expressing mAzamiGreen in a PiggyBac destination vector Encodes pEF1a driving expression of TET3G P2A IRFP713, pEF1a expressing sadCas9 fused to mRuby2 P2A mRuby2, and an sgRNA targeting pTRE expressing mAzamiGreen in a PiggyBac destination vector	Pleamid used to generate Fig. 5 Pleamid used to generate Fig. 5	MTK TUs and MTUs MTK TUs and MTUs
See Control of the Co	ARB386 ARB387	Encodes one insert Encodes one insert	Encodes (EFTs driving expression of TETSQ P2A RRPPTs, GETs expressing sadCas0 fused to KRAB domain P2A mRuby2, and an sigNAI surpsing pTRE expressing mXzam/Green in a PigyBac destination vector Encodes (EFT driving expression of TETSQ P2A RRPPTs, GETs are excression sadCas0 fused to VPR drowin P2A mRuby2, and an sigNAI surpsing pTRE expressing mXzam/Green in a PigyBac destination vector.	Plasmid used to generate Fig. 5 Plasmid used to generate Fig. 5	MTK TUs and MTUs MTK TUs and MTUs
See	MTK0_013-ZEBOV-4cis MTK0_014-ZEBOV-4cis	Encodes one insert Encodes one insert	Encodes ZEBOV NP-P2A-VP35-P2A-VP30-P2A-Lpoi in a PriC31 destination vector Encodes ZEBOV NP-P2A-VP35-P2A-VP30-P2A-Lpoi in a BxBl destination vector	Plasmid used to generate Fig. 6 Plasmid used to generate Fig. 6	MTK TUs and MTUs MTK TUs and MTUs
Margine Marg	MTK0_016-ZEBOV-4cis MTK0_017-ZEBOV-4cis	Encodes one insert Encodes one insert	Encodes ZEBOV NP-924-VP35-P2A-VP30-P2A-Lpol in a PNC31 destination vector with nuclear tag8FP Encodes ZEBOV NP-924-VP35-P2A-VP30-P2A-Lpol in a BxBl destination vector with nuclear tag8FP	Pleamid used to generate Fig. 6 Pleamid used to generate Fig. 6	MTK TUs and MTUs MTK TUs and MTUs
Service of the control of the contro	MTK0_043-ZEBOV-4cis ZEBOV-NP	Encodes one insert Encodes one insert	Encodes ZEBOV NP-P2A-VP35-P2A-VP30-P2A-Lpoi in a PiggyBac destination vector Encodes ZEBOV nucleocologist as a part 5 to be used in the MTK system	Plasmid used to generate Rig. 6 Plasmid used to generate Rig. 6	MTK TUs and MTUs MTK TUs and MTUs
	ZEBOV-VP35 ZEBOV-VP30	Encodes one insert	Encodes ZEBOV viral protein 35 as a part 3 to be used in the MTK system Formities ZEBOV viral novelen 30 as a next 3 to be used in the MTK system	Plasmid used to generate Fig. 6 Plasmid used to generate Fig. 6	MTK TUs and MTUs
	ZEBOV-Lpol MTK678-TIII SR1-CAG-ZEBOV-NP-on		Encodes ZEBOV large RNA dependent RNA polymerase as a part 3 to be used in the MTK system Financies connections I 9.81 and in 2.62 correspond 75-BONAID-snaper as a TII	Plasmid used to generate Fig. 6 Plasmid used to generate Fig. 6	MTK TUs and MTUs
	MTK678-TUL1R2P2A-sp-ZEBOV-VP35-sp	Encodes one insert	Encodes connectors L1 R2 and P2A-ZEBOV-VP35-spacer as a TU Encodes connectors L1 R2 and P2A-ZEBOV-VP35-spacer as a TU Encodes connectors L1 R2 and P2A-ZEBOV-VP35-spacer as a TU	Plasmid used to generate Eq. 6	MTK TUs and MTUs
	MTK678-TUL3REP2A-sp-ZEBOV-LPOL-sgPA	Encodes one insert	Encodes connectors L3 RE and P2A-ZEBOV-Lpot-spacer as a TU TEPOV at two missioners reporter disease by a T7 promoter.	Plasmid used to generate Fig. 6	MTK TUS and MTUS
	production	LILLUSAS GRETISES	APPORT HAVE HERISTONIAN TOWARD OF A 1.1 SECTIONAL	promission which and generation 1 right or	paint 102 and milos

Supplementary Table 2

Figure	Target Locus	Protospacer Sequence	Cas9 Species (Part Plasmid)	PAM Sequence	Forward Oligo	Reverse Oligo	Backbone Vector	Source
	3 hAAVS1 (site 1)	GAGCCACATTAACCGGCCCT	S. pyogenes (MTK3_025)	GGG	5' TGTTTGGAGCCACATTAACCGGCCCTG	5' TAAACAGGGCCGGTTAATGTGGCTCCA	MTK234_002	This study
	3 hAAVS1 (site 2)	GAGCCACATTAACCGGCCCT	S. pyogenes (MTK3_025)	TGG	5' TGTTTGATTCCCAGGGCCGGTTAATGG	5' TAAACCATTAACCGGCCCTGGGAATCA	MTK234_002	This study
	3 hAAVS1 (site 3)	GGGGCCACTAGGGACAGGAT	S. pyogenes (MTK3_025)	TGG	5' TGTTTGGGGGCCACTAGGGACAGGATG	5' TAAACATCCTGTCCCTAGTGGCCCCCA	MTK234_002	This study
	4 tagBFP	CTACAACGTCAAGATCAGAG	S. pyogenes (MTK3_025)	GGG	5' TGTTTGCTACAACGTCAAGATCAGAGG	5' TAAACCTCTGATCTTGACGTTGTAGCA	MTK234_002	This study
	4 mScarlet	CCACAACGAAGATTATACCG	S. pyogenes (MTK3_025)	TGG	5' TGTTTGCCACAACGAAGATTATACCGG	5' TAAACCGGTATAATCTTCGTTGTGGCA	MTK234_002	This study
	4 human non-targeting	ACGGAGGCTAAGCGTCGCAA	S. pyogenes (MTK3_025)		5' TGTTTGACGGAGGCTAAGCGTCGCAAG	5' TAAACTTGCGACGCTTAGCCTCCGTCA	MTK234_002	Doench, J. G. et al. (2016)
	5 TRE	TACGTTCTCTATCACTGATA	S. pyogenes (MTK3_025)	GGG	5' TGTTTGTACGTTCTCTATCACTGATAG	5' TAAACTATCAGTGATAGAGAACGTACA	MTK234_002	Gao, Y. et al. (2016)
	5 TRE	GTTACTCCCTATCAGTGATA	S. aureus (MTK3b_019)	AGGAGT	5' TGTTTGGTTACTCCCTATCAGTGATAG	5' ATAACTATCACTGATAGGGAGTAACCA	MTK234_050	Gao, Y. et al. (2016)
	5 UAS	GAGCACTGTCCTCCGAACGT	S. pyogenes (MTK3_025)	CGG	5' TGTTTGGAGCACTGTCCTCCGAACGTG	5' TAAACACGTTCGGAGGACAGTGCTCCA	MTK234_002	This study
	5 UAS	GAACGTCGGAGCACTGTCCT	S. aureus (MTK3b_019)	CCGAAC	5' TGTTTGGAACGTCGGAGCACTGTCCTG	5' ATAACAGGACAGTGCTCCGACGTTCCA	MTK234_050	This study

Part	Forward Primer	Reverse Primer	Destination Vector	Notes	
1	5' gcatCGTCTCatcGGTCTCaCCCTCGTCTCaNNNN	5' gcatCGTCTCaGGTCTCaCGTT	MTK0_027	NNNN is barcode for connector, see supplemental table 4	
1 (reverse connectors)	5' gcatCGTCTCatcGGTCTCaCCCT	5' gcatCGTCTCaGGTCTCaCGTTCGTCTCtNNNN	MTK0_027	NNNN is barcode for connector, see supplemental table 4	
2	5' gcatCGTCTCatcGGTCTCaAACG	5' gcatCGTCTCaGGTCTCaCATA	MTK0_027		
3	5' gcatCGTCTCatcGGTCTCaTATG	5' gcatCGTCTCaGGTCTCaGGAT	MTK0_027		
За	5' gcatCGTCTCatcGGTCTCaTATG	5' gcatCGTCTCaGGTCTCaAGAA	MTK0_027		
3b	5' gcatCGTCTCatcGGTCTCaTTCT	5' gcatCGTCTCaGGTCTCaGGAT	MTK0_027		
4	5' gcatCGTCTCatcGGTCTCaATCCtaa	5' gcatCGTCTCaGGTCTCaCAGC	MTK0_027		
4a	5' gcatCGTCTCatcGGTCTCaATCC	5' gcatCGTCTCaGGTCTCaGCCA	MTK0_027	remove first 3 bases (taa) for multicistronic use	
4b	5' gcatCGTCTCatcGGTCTCaTCGG	5' gcatCGTCTCaGGTCTCaCAGC	MTK0_027		
5	5' gcatCGTCTCatcGGTCTCaGCTG	5' gcatCGTCTCaGGTCTCaTGTACGTCTCtNNNN	MTK0_027	NNNN is barcode for connector, see supplemental table 4	
5 (reverse connectors)	5' gcatCGTCTCatcGGTCTCaGCTGCGTCTCaNNNN	5' gcatCGTCTCaGGTCTCaTGTA	MTK0_027	NNNN is barcode for connector, see supplemental table 4	
6	5' gcatCGTCTCatcGGTCTCaTACA	5' gcatCGTCTCaGGTCTCaACTC	MTK0_027		
7	5' gcatCGTCTCatcGGTCTCaGAGT	5' gcatCGTCTCaGGTCTCaTCGG	MTK0_027		
8	5' gcatCGTCTCatcGGTCTCaCCGA	5' gcatCGTCTCaGGTCTCaAGGG	MTK0_027		
Ва	5' gcatCGTCTCatcGGTCTCaCCGA	5' gcatCGTCTCaGGTCTCaATTG	MTK0_027		
8b	5' gcatCGTCTCatcGGTCTCaCAAT	5' gcatCGTCTCaGGTCTCaAGGG	MTK0_027		
234	5' gcatCGTCTCatcGGTCTCaAACG	5' gcatCGTCTCaGGTCTCaCAGC	MTK0_027		
234 (SpCas9 sgRNA)	5' TGTTTGNNNNNNNNNNNNNNNNNNNN	5' TAAACN'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'	MTK234_002	N(20) are guide specifc sequence, N' is reverse complement	ıt
234 (SaCas9 sgRNA)	5 TGTTTGNNNNNNNNNNNNNNNNNNNN	5' ATAACN'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N	MTK234_050	N(20) are guide specifc sequence, N' is reverse complement	ıt

Connector	Overhang
LS, LS'	5' CTGA
L1, R1	5' CCAA
L2, R2	5' GATG
L3, R3	5' GTTC
L4, R4	5' GGTA
L5, R5	5' AAGT
L6, R6	5' CCCT
L7, R7	5' GCGG
L8, R8	5' TTTA
RE, RE'	5' AGCA