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Supplementary information

Systematic molecular evolution enables robust biomolecule discovery

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Supplementary Information

Supplementary Table 1 | Method details for each experiment.

In all cases, the flow-through rate was 1 vol/hr. MP6[24] was used as the mutagenesis plasmid in all evolution experiments. See the Supplementary Table 4 for plasmid maps.

Experiment	Figure	Bacterial source	Replicate Layout	AP/Phage
Phage propagation with 96-head	SI Fig 7 (left)	Turbidostat	96 replicates in a dense 96-well plate. All wells inoculated with phage.	AP: pJC173b*. Phage: WT T7 RNAP selection phage
Phage propagation with 96-head	SI Fig 7 (right)	Turbidostat	96 replicates, every-other-column layout, spread over two plates. All wells inoculated with phage.	AP: pJC173b. Phage: WT T7 RNAP selection phage
Phage propagation with 96-head	Figure 1d (left), SI Fig 2a, SI Fig 7 (right)	Turbidostat	96 replicates, every-other-column, spread over two plates. Checker board inoculation pattern.	AP: pJC173b. Phage: WT T7 RNAP selection phage
Phage propagation with 8-channel head	SI Fig 8b	Turbidostat	96 replicates, every-other-column, spread over two plates. Checker board inoculation pattern.	AP: pJC173b. Phage: WT T7 RNAP selection phage
Phage propagation with chilled bacteria	Fig 1d (right)	Chilled bacteria	16 replicates, every-other-column layout.	AP: pJC173b. Phage: WT T7 RNAP selection phage
T7 → T3 RNAP evolution	Figure 2b, SI Fig 5b-d	Turbidostat	96 replicates, every-other-column layout, spread over two plates.	AP: pJC174b*. Phage: WT T7 RNAP selection phage
AARS evolution	Figure 3, SI Fig 2b	Turbidostat	24 replicates, every-other-column layout.	AP: pEC182a Phage: ECSP1x11, ECSP1x13 **
AGGG qtRNA evolution	Figure 4a-d, SI Fig 6e	Turbidostat	48 replicates, every-other-column layout.	AP: pED15x7, Phage: all 20 ESP1x phage libraries and !AGGG phage variants
TAGA qtRNA evolution	SI Fig 6	Turbidostat	48 replicates, every-other-column layout.	AP: pED15x1, Phage: all 20 ESP1x phage, and phage libraries
Feedback control qtRNA evolution	Figure 4e-i, Figure 5	Chemostats	48 replicates, every-other-column layout.	Lenient AP: pED7x163 + pED20x5. Moderate AP: pED15x1 + ES rope. Stringent AP: pED15x2 + ES rope. Phage: ESP1xR-1, ESP1xS-1, ESP1xH-1, ESP1xF-1.
10-day T7 evolution	Figure 6	Chilled Bacteria	32 replicates, every-other-column layout, 2 volumes per hour	pJC173b (T7), pJC174b (T3), pJC175e (psp-pIII), pEC201 (T3/TP6), pEC202 (T7/SP6), pEC203 (SP6), pEC204 (SP6/TP6), pEC205 (TP6), pEC206 (-3 variant), pEC207 (-5 variant)

** pJC173b, pJC174b, and WT T7 RNAP selection phage were gifts from Ahmed Badran.*
*** chPylRS AP and phage derived from pDB038a and pTECH-chPylRS. pTECH-chPylRS(IPYE) was a gift from Dieter Söll (Addgene plasmid # 99222 ; http://n2t.net/addgene:99222 ; RRID:Addgene_99222). pDB038a was a gift from David Liu (Addgene plasmid # 99211 ; http://n2t.net/addgene:99211 ; RRID:Addgene_99211).*

Supplementary Table 2 | Complete T7 RNAP clonal sequencing and conservation. Time required for evolution designated by early (t = 21h), mid (t = 23.5h), late (t = 26h). 4 replicates sampled for each time window, 3 clonal variants sequenced per replicate by sanger sequencing.

Evolution Timepoint	Well	Mutations
Early	H8	M219R,E477G,V521A
	F10	M219R,A373S,N748D
	F11	M219R,A373S,N748D
	F12	M219R,A323T
	C8	E222K,N748D
	C9	A382S,E694G,N748D
	C10	E222K,N748D
	F1	H205R,E683G,N748D,E775K
	F2	V177I,H205R,A323V,N748D,E775K
	F3	M219R,N748D
Mid	D9	M219R,K329R,V625I,N748D
	D10	M219R,A260V,Y385C,D388G,N748D
	C1	M219R,A304V,N748S
	C2	M219R,A304V,N748S
	C3	M219R
	E2	M219R
	E3	A144T,E222K
	B6	Y312H,N748D
	B7	K098R
	B8	K098R,V214L
Late	A11	E207K,N748D
	A12	E207K,N748D
	D5	E222G,N748D
	D6	M219R,D388N
	D7	M219R,T282A,D388N
	C12	M219R,I718T,N748S
	D1	M219R
	D2	M219R
	C5	M219R,N748S
	C6	M219R
	C7	M219R

Supplementary Table 3 | Long Term PRANCE Evolution subclone genotypes.
Genotypes of all subclones obtained from the 10-day T7 PRANCE on TP6, -3 Variant and -5 variant promoters. Bolded subclones for each replicate were chosen for luminescence assay validation.

Trajectory	Condition	AP	Rep	Subclone	Mutation
SP6	12h	TP6	1	1	R31S R57H D66E V177G M219R A253V D471G S633P M696L A724S R756C
				2	R31S R57H D66E V177G M219R A253V D471G E504W S633P M696L A724S R756C
				3	M219R N370S A519T L532F N748D
				4	**R31S R57H D66E E108K V177G M219R A253V S633P**
				5	R31S D66E V177G M219R A253V V574A S633P A724S
			2	1	F51S M219R E683G A691D N748D
				2	F51S S139G M219R F511V E683G A691D N748D
				3	E56G R96L K98R K172Q M219K T282A V340I E504W S527I S606A S641A L706M A822E T831A
				4	F51S R99G M219R E683G A691D N748D
				5	**F51S M219R E683G A691D N748D**
				6	T3P E40G F51S E63G A65G T75P M81K R84P R99G S139R A691D N748D
				7	F51S A144T K179Q M219R E683G A691D N748D M750I A808E S813Y L870K F880L A881P
			3	1	A7T M219R C540G A724V
				2	M219R R357C Q648K W682R N748S
				3	M219R R357C Q648K W682R
				4	C540G
				5	**M219R C540G**
				6	T76I C540G
				7	M1N N2E T76I K98R C540G S628R F825L
			4	1	M54L H161N M219R A354V A622T A658D T688M P862S
				2	V174A A247E I254L A354V L562I A622T T688M
				3	M219R R292C A354V S495F A622T T688M
				4	A622T T688M
				5	A622T T688M
				6	**K98R A622T T688M**
SP6	No Pulse	TP6	1	1	E222V E356D D388G A586V N748D I875L
				2	D13A E222A A615V T688M
				3	M219R Y623C T688M
				4	E222V
				5	E356D N748D I875L
				6	**D13A L170F E222A I875L**
			2	1	S43A S128R E222K
				2	D366N L526P E643K T688M N748D
				3	S43A S128R E222K E309G G449S V470I
				4	S128R
				5	**Q239R G803E**
			3	1	M219R
				2	T127A M219R
				3	E222K
				4	**E222K H799P A827P**
				5	K8N K98R A144T K172Q S495A E498G T566I V574A P818A T831A Y846C D851V P862A L870K N871C R873S L876S S878L D879V F880L F882L
			4	1	E222K A260S V574A
				2	M219K V574A E600G
				3	D13V M219R V574A
				4	V574A
				5	V574A
				6	**R34C V574A**
				7	M219R A260V W682R
SP6	Pre-Pulse	TP6	1	1	K98R D208A E403D K448R S684F N748D
				2	R96L K98R K172Q M219K I244V P371S A373T S641A A822E

				3	R96L K98R D130N K172Q M219K T282A S606A S641A A822E C839Y
				4	R96L K98R H161Y V166I K172Q M219K T282A P371S S606A S641A N748S A822E
				5	R96L K98R H161Y V166I K172Q M219K T282A P371S S606A S641A N748S A822E
				6	R96L K98R H161Y V166I K172Q M219K T282A P371S S606A S641A N748S A822E
				7	**R96L K98R H161Y V166I K172Q M219K I244V T282A P371S S606A S641A N748S A822E**
			2	1	K98R D208A E403D K448R S684F N748D
				2	Y28C K98R D208A E403D S684F N748D
				3	K98R K120R D208A E403D S684F N748D
				4	K98R D208A E403D E504W Q505P S684F N748D
				5	K98R F162L K163R D208A E403D S684F N748D
				6	**K98R D208A E403D S684F N748D**
				7	E403D S684F N748D
			3	1	I154L M219R S641A N748S
				2	M219R R391S E504W Q505P S641A N748S A843S
				3	M219R S641A N748S
				4	M219R N748S
				5	M219R N748S A881G
				6	**M219R N748S**
				7	N748S
			4	1	E222K A354V A622T G675R T688M
				2	M219R A354V A622T T688M
				3	M81V M219R P275L A354V A622T T688M
				4	A354V A622T T688M
				5	A354V A622T T688M
				6	A234G A354V A622T T688M
				7	**E222A A354V A622T T688M**
T3	12h	Variant -3	1	1	L39I M219R P277Q S539A G618S N748D K765Q
				2	M219R A247T N748S
				3	M219R A247T N437T N748S R829S
				4	M219R A247T N748S
				5	M219R
				6	**A138T M219R A247T N748S**
			2	1	T23I M219R N370S A519T L532F A691T N748D
				2	M219R N370S A519T L532F N748D
				3	M46V R155H M219R V227A N370S A380V A519T L532F T668I N748D
				4	**M219R N370S A519T L532F N748D**
				5	M219R N370S E504G L532F N748D
				6	H161P N370S A519T L532F N748D
				7	N370S A519T L532F N748D
T3	12h	Variant -5	3	1	R96L K98R K172Q M219K M267I T282A A373S S606A S641A E768G A822E
				2	R96L K98R K172Q M219K T282A S606A S641A A822E
				3	R96L K98R K172Q M219K T282A S606A S641A A822E
				4	R96L K98R K172Q M219K T282A S606A S641A A822E
				5	R96L K98R K172Q M219K T282A S606A S641A A822E
				6	R96L K98R K172Q M219K T282A S606A S641A A822E
				7	**R96L K98R K172Q M219K T282A S606A S641A A822E**
			4	1	R96L K98R K172Q M219K Q239K T282A A383V S606A S641A D653Y A822E
				2	R96L K98R K172Q M219K T282A S606A S641A A822E
				3	R96L K98R K172Q M219K T282A S606A S641A A822E
				4	R96L K98R K172Q M219K T282A E504G S606A S641A A822E
				5	R96L K98R K172Q M219K T282A S606A S641A A822E
				6	R96L K98R K172Q M219K T282A S606A S641A A822E
				7	**R96L K98R K172Q M219K T282A S606A S641A A822E**

T3	No Pulse	Variant -3	1	1	H205R I244V M666I N748D D851G
				2	M219R R307L N748T
				3	N2S L191I C216S I244V D366N M666I N748D
				4	**A138T A188G M219R N748T**
			2	1	M219R M313T A622S V710I N748D S838Y L853S
				2	M219R M313T A622S V710I N748D S838Y
				3	M219R A622S V710I N748D S838Y
				4	M219R N588S
				5	M219R M313T A622S V710I N748D S838Y
				6	M219R M313T Q505A A622S N748D S838Y
				7	**M219R M313T A622S V710I N748D S838Y**
T3	No Pulse	Variant -5	3	1	R57C M219R S495F V574A E643K N748S
				2	A25T E35G R57C M219R V574A E643K N748S
				3	R57C A68S M219R P476S V521A V574A E643K N748S A863T
				4	R57C V574A E643K N748S
				5	R57C M219R V574A E643K N748S
				6	**R57C M219R V574A E643K N748S**
			4	1	E112G V134I T326A K378R M439I T440N A615T L621M L651M A708T E775K
				2	E108G E112G V134I K378R M439I A615T L621M L651M A708T E775K F849L
				3	V134I A247V W344R V594I L621M L651M E775K
				4	V134I L621M L651M E775K
				5	V134I L621M L651M E775K
				6	**V134I L621M L651M E775K**
			4	7	V134I L621M L651M E775K
T3	Pre-Pulse	Variant -3	1	2	I109V S241A E249G T598A N748D
				3	R57H M219R I367T A658T N748T
				4	N748D
				5	M219R
				6	**I109V A253V N748D H799R**
			2	1	E218A A513S S527N N748D
				2	E218A Q232R A255S A491V R720H N748D
				3	I4V F21S G62S E218A Y250C A255S I355L N748D
				4	N748D
				5	E218A A255S N748D
				6	**E218A N748D**
			2	7	T3M N86Y K98E G152C F182I
T3	Pre-Pulse	Variant -5	3	1	V134I A247V A295S E483K V594I N601T L621M L651M E775K
				2	V134I M219R V236I A247V T299N L621M L651M M666V N748D E775K
				3	V134I A247V A295S E483K V594I N601T L621M L651M E775K
				4	V134I A247V V594I N601T L621M L651M E775K
				5	V134I A247V V594I N601T L621M L651M E775K
				6	V134I A247V N601T L621M L651M E775K
				7	**V134I A247V V594I N601T L621M L651M E775K**
			4	1	A138T M219R N419S A558E N748T
				2	A18S M219R H346N H523Y I543V A558E N748T
				3	M219R H300L K450E N748S
				4	M219R V227F A558E N748T
				5	M219R A558E N748T
				6	**M219R A234G Q505E A558E N748T**
				7	E168A M219R I330L N748S

Supplementary Table 4 | Extended methods information.
Contains part numbers and all plasmids used in experiments throughout the manuscript.

Item	Company	Catalog no.	Notes
Cloning			
Mach1 Competent E coli	Thermo Fisher Scientific	C862003	
Turbo Competent E coli	New England BioLabs	C2984H	
NEB 5-alpha Competent E coli	New England BioLabs	C2988J	
NEB 10-beta Competent E coli	New England BioLabs	C3019H	
Illustra TempliPhi 100 Amplification Kit	GE Healthcare Life Sciences	89131-612	
Phusion U GreenHotStart DNA Poly (Box)	Thermofisher	F556L	Standard cloning polymerase
Phusion Hotstart Flex	New England BioLabs	M0535S	For Blunt end ligation
dNTP Mix, natural (10mM), 4mL	New England BioLabs	N0447L	
ET SSB	VWR	101417-992	PCR additive
Dpnl-5000 units/pack	New England BioLabs	New England BioLabs	For USER cloning
Enzyme, USER, 250Units (250ul/tu)	New England BioLabs	M5505L	For USER cloning
PCR purification	Qiagen	28106	For PCR purification
Blunt/TA Ligase Master Mix	New England BioLabs	M0367L	For Blunt end ligation
Filters for M13 bacteriophage			
mdi 0.22µm filter columns for phage purification	Thomas Scientific	1166U41	For filtering up to 500uL of phage supernatant
96-well 0.22µm filter plate for phage purification	Corning	3505	For filtering up to 150uL of phage supernatant in each well of a 96-well plate
Steriflip 50mL spin filter unit for for phage purification	Sigma Aldrich	Z380660	For filtering up to 50mL of phage supernatant
Plaque assays			
Bluo-Gal, 10g	Goldbio	CAS #97753-82-7. CAT #B-673-10.	Improves plaque contrast when plaquing in S2060-based strains (contains PSP-lacZ)
DMF (NN dimethyl formamide)	Sigma Aldrich	270547-100ML	Use to dissolve Bluo-Gal
1mL Hamilton tips. Clear, Sterile, Filter	Hamilton	Part # / Ref: 235821	Robotic plaque assays, PRANCE
300uL Hamilton tips. Clear, Sterile, Filter	Hamilton	Part # / Ref: 235832	Robotic plaque assays, PRANCE
24-well plates, in sleeves	VWR	82050-894	Robotic plaque assays
X-plates	VWR	25384308	Manual plaque assays
PACE supplies			
Plate reader plate, 96w Black clear Costar	Fisher Scientific	07-200-567	
1 mL syringe (green), pack of 100	VWR	53548-001	
50(60)mL syringe, pack of 30	VWR	80076-428	
See additional PACE materials: https://docs.google.com/spreadsheets/d/1hxYO9KX_xSYT4xRf5txSikVCFeMUECt3BztZKAgvbTE/edit#gid=0			
Chemicals & Media			
2XYT media powder	United States Biologicals	T9200	
Agar, Bacteriological; 10 KG	United States Biologicals	A0930	
L-Arabinose; 5 kg	GoldBio	A-300-5	MP inducer
IPTG	GoldBio	I2481C5	Inducer
DRM autoclaved component: Harvard Custom Media A	United States Biologicals	#CS050H-001	Autoclaved component of DRM

DRM non-autoclaved component: Harvard Custom Media C	United States Biologicals	#CS050H-003	Filtered component of DRM
Assorted laboratory supplies			
96-well PCR plates (half skirt), Greiner Bio, pack of 10	VWR	652290	For PCR, 96-well phage filtration
2mL screw cap tubes (VWR), case 5000	VWR	10025-756	Recommended for phage storage
VWR® Aluminum Foils for 96-Well Plates	VWR	60941-126	
Porous sealign film: VWR® Rayon Films for Biological Cultures	VWR	60941-084	
2mL deep 96-well plate	Thomas scientific	1149J23	
"Fishtank pumps": INTLLAB 12V DC DIY Peristaltic Liquid Pump Dosing Pump for Aquarium Lab Analytical 3mm ID x 5mm OD	Amazon	link	
Liquid Handling Robot			
Hamilton STARlet	Hamilton Company	173020	
1000µl Pipetting Channels, 8 channels	Hamilton Company	173081	
CO-RE 96 channel Multi Probe Head	Hamilton Company	199090	
iSWAP Plate Handler	Hamilton Company	190220	
HEPA Flow Hood, UV	Hamilton Company	55502-01	
Peripheral Hardware			
CLARIOstar Multi-Mode Microplate Reader	BMG LABTECH	0430-101	
USB Microplate Orbital Shaker	Big Bear Automation	HT-91108-2	
3D printed components			
8-channel bacterial reservoir	See .stl files		Printed on a Formlabs Form 3 resin printer
96-channel bacterial reservoir			Printed on a Formlabs Form 3 resin printer
3D printed components			
8-channel bacterial reservoir	See .stl files		Printed on a Formlabs Form 3 resin printer
96-channel bacterial reservoir			Printed on a Formlabs Form 3 resin printer
Plasmid maps			
Download all .gb plasmid maps			
Name	Type	Resistance	Genotype
S2060	Bacterial strain	-	See addgene: https://www.addgene.org/105064/
pJC173b	Plasmid	Carb	T7 promoter driving pIII and luxAB
pJC174b	Plasmid	Carb	T3 promoter driving pIII and luxAB
pJC175e	Plasmid	Carb	PSP-pIII promoter driving pIII and luxAB
pEC201	Plasmid	Carb	T3/TP6 promoter driving pIII and luxAB
pEC202	Plasmid	Carb	T7/SP6 promoter driving pIII and luxAB
pEC203	Plasmid	Carb	SP6 promoter driving pIII and luxAB
pEC204	Plasmid	Carb	SP6/TP6 promoter driving pIII and luxAB
pEC205	Plasmid	Carb	TP6 promoter driving pIII and luxAB
pEC206	Plasmid	Carb	-3 variant promoter driving pIII and luxAB
pEC207	Plasmid	Carb	-5 variant promoter driving pIII and luxAB
pED20x5	Plasmid	Spec	Phage Shock Promoter driving T7 RNAP-12-203-TAGA
pED15x1	Plasmid	Kan	proD driving pIII-29-TAGA and luxAB-357-TAGA
pED15x2	Plasmid	Kan	proD driving pIII-29-34-TAGA and luxAB-357-TAGA
pED15x7	Plasmid	Kan	proD driving pIII-29-AGGG and luxAB-357-AGGG
ES rope	Plasmid	Spec	No payload; simply a extra plasmid with SpecR
pEC182a	Plasmid	Spec	Phage Shock Promoter driving pIII-29-TAG
ECSP1x11	Selection Phage	-	M13 ΔpIII phage expressing ChPylRS-VTHA
ECSP1x13	Selection Phage	-	M13 ΔpIII phage expressing ChPylRS-IP
WT T7 RNAP selection phage	Selection Phage	Kan	M13 ΔpIII phage expressing T7 RNAP

ESP1xA-1	Selection Phage	-	Phage expressing A-qtRNA homologue continging a !TAGA anticodon
ESP1xC-1	Selection Phage	-	Phage expressing C-qtRNA homologue continging a !TAGA anticodon
ESP1xD-1	Selection Phage	-	Phage expressing D-qtRNA homologue continging a !TAGA anticodon
ESP1xE-1	Selection Phage	-	Phage expressing E-qtRNA homologue continging a !TAGA anticodon
ESP1xF-1	Selection Phage	-	Phage expressing F-qtRNA homologue continging a !TAGA anticodon
ESP1xG-1	Selection Phage	-	Phage expressing G-qtRNA homologue continging a !TAGA anticodon
ESP1xH-1	Selection Phage	-	Phage expressing H-qtRNA homologue continging a !TAGA anticodon
ESP1xI-1	Selection Phage	-	Phage expressing I-qtRNA homologue continging a !TAGA anticodon
ESP1xK-1	Selection Phage	-	Phage expressing K-qtRNA homologue continging a !TAGA anticodon
ESP1xL-1	Selection Phage	-	Phage expressing L-qtRNA homologue continging a !TAGA anticodon
ESP1xM-1	Selection Phage	-	Phage expressing M-qtRNA homologue continging a !TAGA anticodon
ESP1xN-1	Selection Phage	-	Phage expressing N-qtRNA homologue continging a !TAGA anticodon
ESP1xP-1	Selection Phage	-	Phage expressing P-qtRNA homologue continging a !TAGA anticodon
ESP1xQ-1	Selection Phage	-	Phage expressing Q-qtRNA homologue continging a !TAGA anticodon
ESP1xR-1	Selection Phage	-	Phage expressing R-qtRNA homologue continging a !TAGA anticodon
ESP1xS-1	Selection Phage	-	Phage expressing S-qtRNA homologue continging a !TAGA anticodon
ESP1xT-1	Selection Phage	-	Phage expressing T-qtRNA homologue continging a !TAGA anticodon
ESP1xV-1	Selection Phage	-	Phage expressing V-qtRNA homologue continging a !TAGA anticodon
ESP1xW-1	Selection Phage	-	Phage expressing W-qtRNA homologue continging a !TAGA anticodon
ESP1xY-1	Selection Phage	-	Phage expressing Y-qtRNA homologue continging a !TAGA anticodon
ESP1xA-lib	Selection Phage	-	Phage expressing A-qtRNA homologue continging a randomized NNNN anticodon
ESP1xC-lib	Selection Phage	-	Phage expressing C-qtRNA homologue continging a randomized NNNN anticodon
ESP1xD-lib	Selection Phage	-	Phage expressing D-qtRNA homologue continging a randomized NNNN anticodon
ESP1xE-lib	Selection Phage	-	Phage expressing E-qtRNA homologue continging a randomized NNNN anticodon
ESP1xF-lib	Selection Phage	-	Phage expressing F-qtRNA homologue continging a randomized NNNN anticodon
ESP1xG-lib	Selection Phage	-	Phage expressing G-qtRNA homologue continging a randomized NNNN anticodon
ESP1xH-lib	Selection Phage	-	Phage expressing H-qtRNA homologue continging a randomized NNNN anticodon
ESP1xI-lib	Selection Phage	-	Phage expressing I-qtRNA homologue continging a randomized NNNN anticodon
ESP1xK-lib	Selection Phage	-	Phage expressing K-qtRNA homologue continging a randomized NNNN anticodon
ESP1xL-lib	Selection Phage	-	Phage expressing L-qtRNA homologue continging a randomized NNNN anticodon

ESP1xM-lib	Selection Phage	-	Phage expressing M-qtRNA homologue continging a randomized NNNN anticodon
ESP1xN-lib	Selection Phage	-	Phage expressing N-qtRNA homologue continging a randomized NNNN anticodon
ESP1xP-lib	Selection Phage	-	Phage expressing P-qtRNA homologue continging a randomized NNNN anticodon
ESP1xQ-lib	Selection Phage	-	Phage expressing Q-qtRNA homologue continging a randomized NNNN anticodon
ESP1xR-lib	Selection Phage	-	Phage expressing R-qtRNA homologue continging a randomized NNNN anticodon
ESP1xS-lib	Selection Phage	-	Phage expressing S-qtRNA homologue continging a randomized NNNN anticodon
ESP1xT-lib	Selection Phage	-	Phage expressing T-qtRNA homologue continging a randomized NNNN anticodon
ESP1xV-lib	Selection Phage	-	Phage expressing V-qtRNA homologue continging a randomized NNNN anticodon
ESP1xW-lib	Selection Phage	-	Phage expressing W-qtRNA homologue continging a randomized NNNN anticodon
ESP1xY-lib	Selection Phage	-	Phage expressing Y-qtRNA homologue continging a randomized NNNN anticodon
ESP1xA-1	Selection Phage	-	Phage expressing A-qtRNA homologue continging a !TAGA anticodon