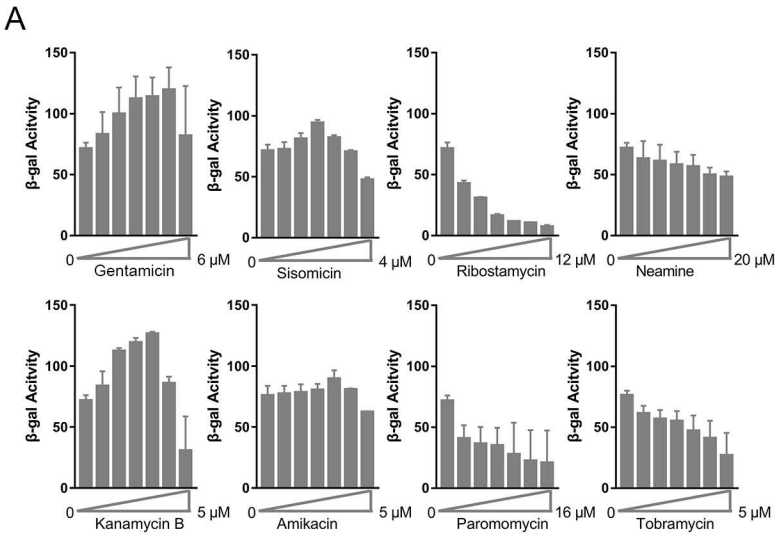
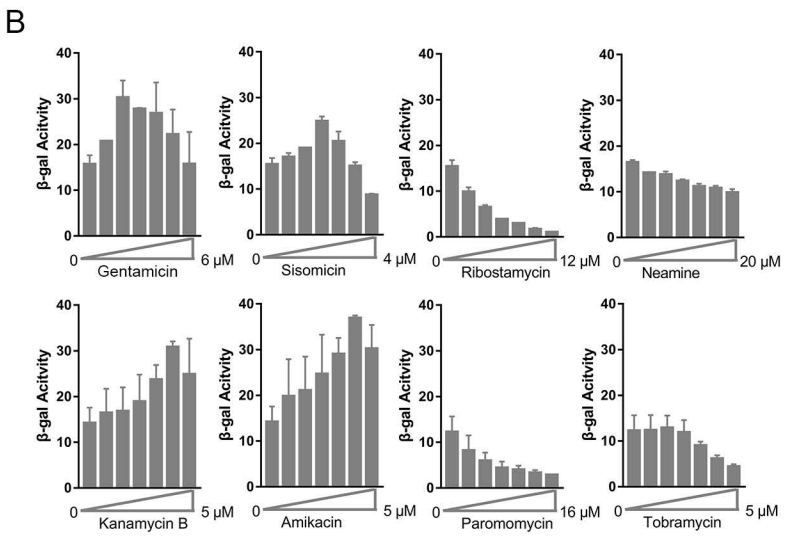
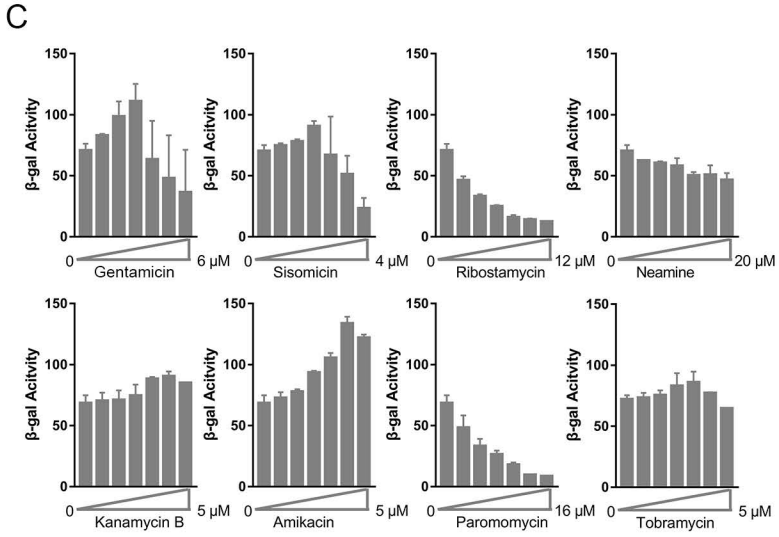
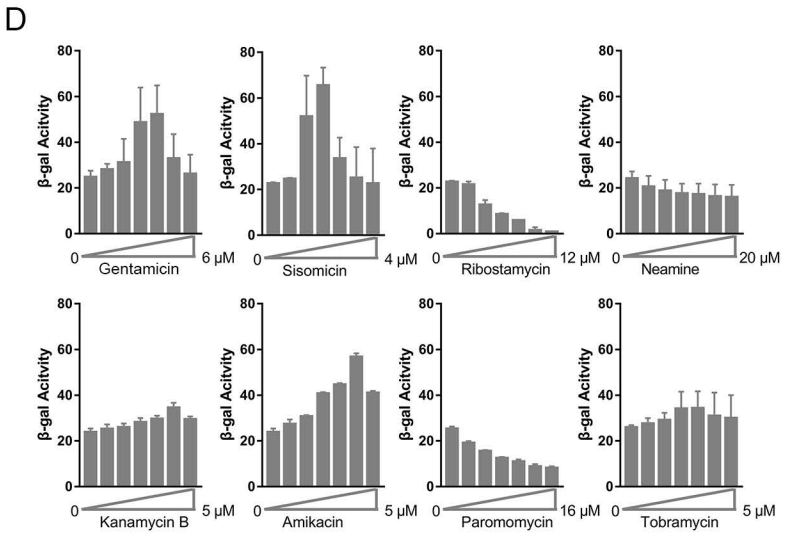
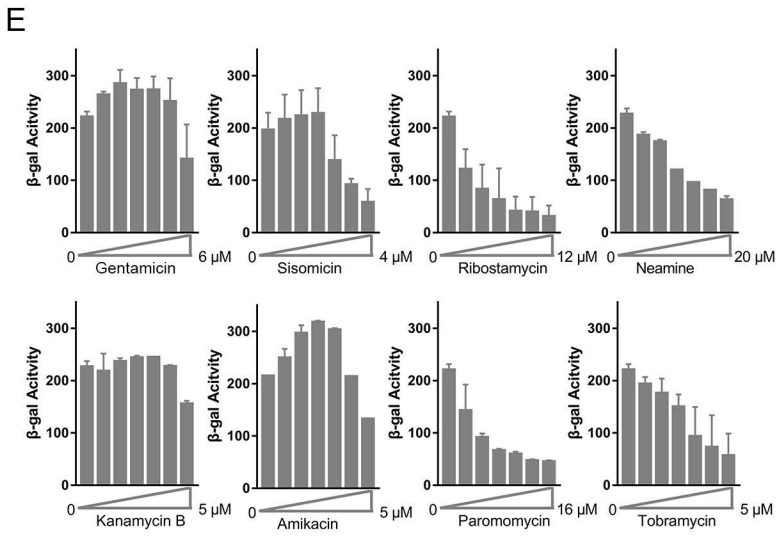

A
β-gal Acitvity
Gentamicin 0 6 μM
Sisomicin 0 4 μM
Ribostamycin 0 12 μM
Neamine 0 20 μM
Kanamycin B 0 5 μM
Amikacin 0 5 μM
Paromomycin 0 16 μM
Tobramycin 0 5 μM


B
β-gal Acitvity
Gentamicin 0 6 μM
Sisomicin 0 4 μM
Ribostamycin 0 12 μM
Neamine 0 20 μM
Kanamycin B 0 5 μM
Amikacin 0 5 μM
Paromomycin 0 16 μM
Tobramycin 0 5 μM

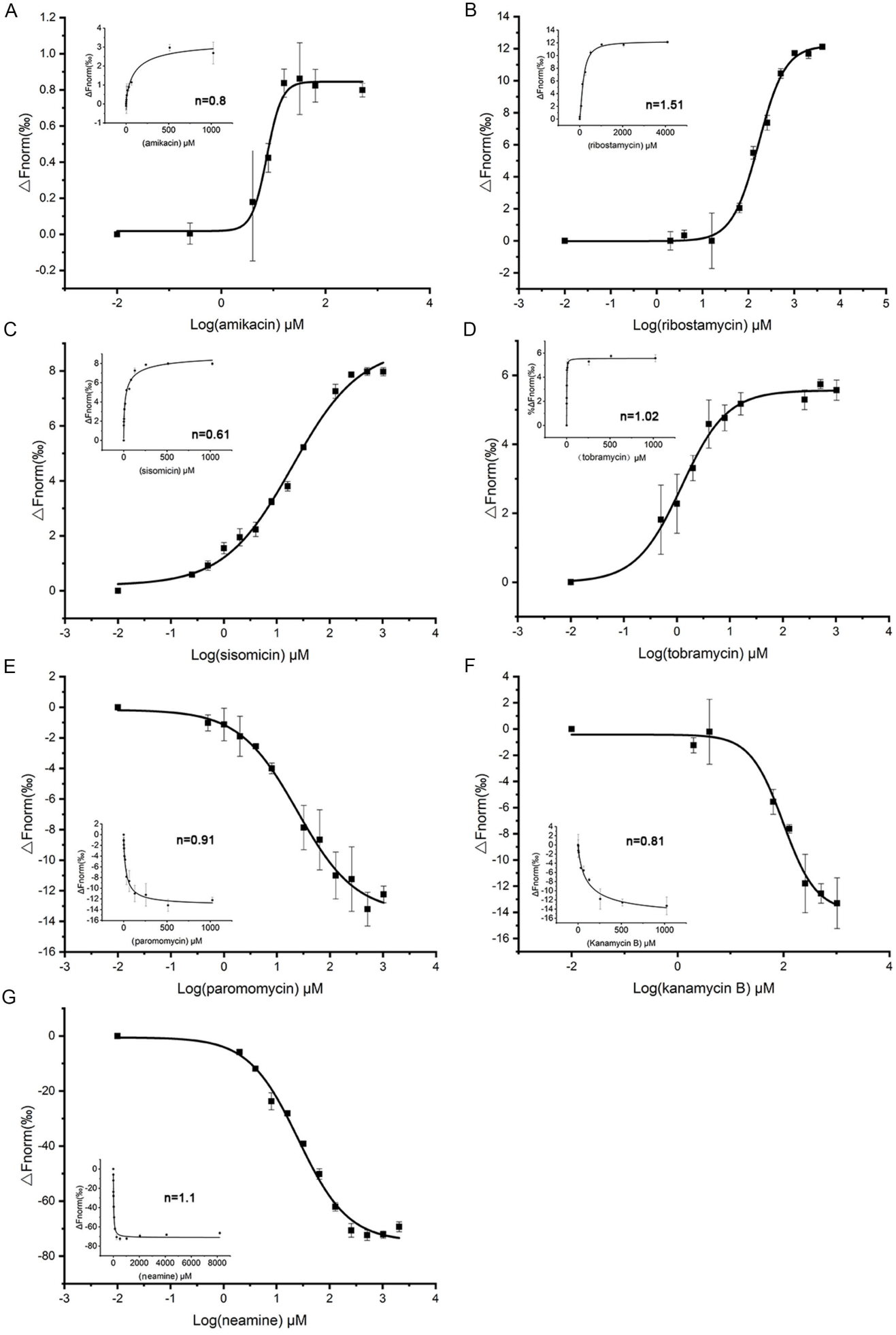

C
β-gal Acitvity
Gentamicin 0 6 μM
Sisomicin 0 4 μM
Ribostamycin 0 12 μM
Neamine 0 20 μM
Kanamycin B 0 5 μM
Amikacin 0 5 μM
Paromomycin 0 16 μM
Tobramycin 0 5 μM


D
β-gal Acitvity
Gentamicin 0 6 μM
Sisomicin 0 4 μM
Ribostamycin 0 12 μM
Neamine 0 20 μM
Kanamycin B 0 5 μM
Amikacin 0 5 μM
Paromomycin 0 16 μM
Tobramycin 0 5 μM


E
β-gal Acitvity
Gentamicin 0 6 μM
Sisomicin 0 4 μM
Ribostamycin 0 12 μM
Neamine 0 20 μM
Kanamycin B 0 5 μM
Amikacin 0 5 μM
Paromomycin 0 16 μM
Tobramycin 0 5 μM

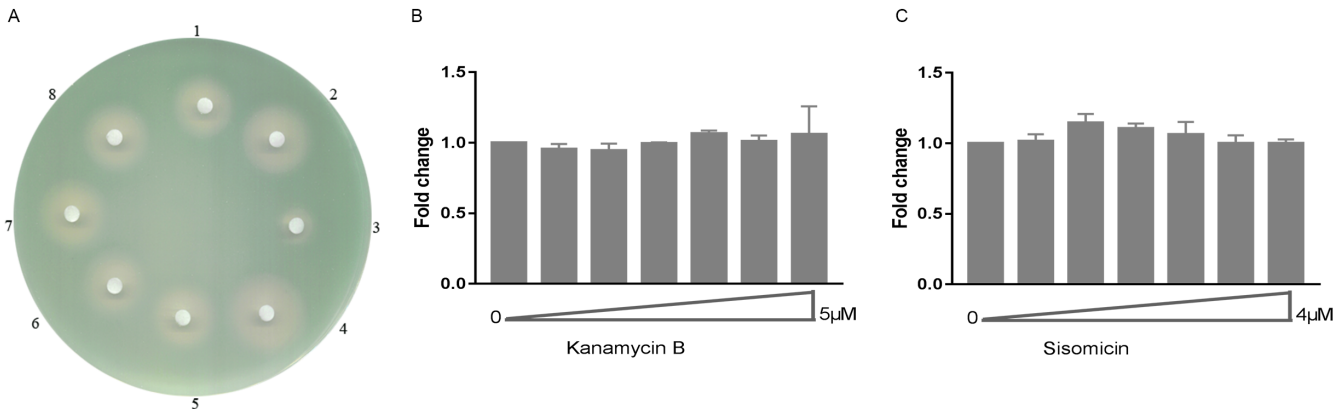
Supplementary Figure1.

The *β-gal* activity (Miller units) of the reporter gene with (A) aac-1, (B) aac-2, (C) aac-3, (D) aac-4, (E) aac-5 upon titration of aminoglycosides. Error bars are standard deviations of at least three independent experiments.


A
△Fnorm(‰)
Log(amikacin) μM
n=0.8
ΔFnorm(‰)
(amikacin) μM
B
△Fnorm(‰)
Log(ribostamycin) μM
n=1.51
ΔFnorm(‰)
(ribostamycin) μM
C
△Fnorm(‰)
Log(sisomicin) μM
n=0.61
ΔFnorm(‰)
(sisomicin) μM
D
△Fnorm(‰)
Log(tobramycin) μM
n=1.02
%ΔFnorm(‰)
（tobramycin）μM
E
△Fnorm(‰)
Log(paromomycin) μM
n=0.91
ΔFnorm(‰)
(paromomycin) μM
F
△Fnorm(‰)
Log(kanamycin B) μM
n=0.81
ΔFnorm(‰)
(Kanamycin B) μM
G
△Fnorm(‰)
Log(neamine) μM
n=1.1
ΔFnorm(‰)
(neamine) μM

Supplementary Figure 2.

Binding curves of aac-6 leader RNA riboswitch that bind with (A) amikacin, (B) ribostamycin, (C) sisomicin, (D) tobramycin, (E) paromomycin, (F) kanamycin B, (G) neamine. Inset is the Hill plot of aminoglycoside antibiotic binding and the Hill coefficient(n). Error bars are standard deviations of at least three independent experiments.


A
1
2
3
4
5
6
7
8
B
Fold change
1.5
1.0
0.5
0.0
0
5μM
Kanamycin B
C
Fold change
1.5
1.0
0.5
0.0
0
4μM
Sisomicin

Supplementary Figure3.

(A) Agar diffusion assays of cells transformed with the reporter plasmid containing the leader RNA of *ghoS* on plate with x-gal. Each filter disc was spotted with different aminoglycoside antibiotics: 1, sisomicin; 2, gentamycin; 3, neamine; 4, ribostamycin; 5, kanamycin B; 6, amikacin; 7, paromomycin; 8, tobramycin. (B and C) The *β-gal* activity (Miller units) of *ghoS* with (B) kanamycin B and (C) sisomicin

Supplementary Table 1. Leader RNA, control and covariance model sequences.

Name	Accession number	annotation	sequence
AAC in *Pseudomonas fluorescens*	L06163.1	*Pseudomonas fluorescens*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CAGCACGGAGACACUUCAGCAUG
AAC-3	DQ393782.1	*Pseudomonas aeruginosa*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CAGCACAGAGCGACCAUUUCAUG
	EU886980.1	*Pseudomonas aeruginosa*	
	GU934609.1	*Pseudomonas aeruginosa*	
	GU966684.1	*Pseudomonas aeruginosa*	
	HM175875.1	*Pseudomonas aeruginosa*	
	HM366563.1	*Pseudomonas aeruginosa*	
	M29695.1	*Pseudomonas aeruginosa*	
	FJ917747	*Pseudomonas aeruginosa*	
	EF105290.1	*Riemerella anatipestifer*	
AAC-6	AJ878850.1	*Pseudomonas aeruginosa*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CAGCACAAAGAGCGUCCUCAUG
	FJ495083.1	*Pseudomonas aeruginosa*	
	GU784921.1	*Pseudomonas aeruginosa*	
AAC-2	KJ668707.1	*Enterobacter hormaechei*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CcCGCACGGAAUCAACA.UCUCAUG
AAC-4	AY623046.1	*artificial sequences*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CAUCACAAAGUACAGCAUCGUG
	AF439785.3	*Campylobacter jejuni*	
	AF439786.3	*Campylobacter jejuni*	
	Y11946.1	*Enterobacter cloacae*	

	Y11948.1	*Enterobacter cloacae*	
	EF370423.1	*Escherichia coli*	
	EU434618.1	*Psychrobacter pulmonis*	
	L25617.1	*Pseudomonas fluorescens*	
	L25666.1	*Pseudomonas fluorescens*	
	FJ715945.1	*Pseudomonas aeruginosa*	
	EU819087.1	*Pseudomonas aeruginosa*	
	JF412713.1	*Pseudomonas aeruginosa*	
	FN554982.1	*Pseudomonas aeruginosa*	
	FN554976.1	*Pseudomonas aeruginosa*	
	HQ832472.1	*Pseudomonas aeruginosa*	
	HQ832473.1	*Pseudomonas aeruginosa*	
	JN003857.1	*Pseudomonas aeruginosa*	
	FN554980.1	*Pseudomonas aeruginosa*	
	FN554979.1	*Pseudomonas aeruginosa*	
	X60321.1	*Pseudomonas aeruginosa*	
	FN554978.1	*Pseudomonas aeruginosa*	
AAC-5	AJ937775.1	*Yersinia sp.*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUGGG CGAACCCGGAGCCUCAUUAAUUG
>NZ_MCJD01000190.1	>NZ_MCJD01000190.1	*Citrobacter freundii*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CAGCACAAAGACCGUUCUCAUG
>NZ_NIOU01000114.1	>NZ_NIOU01000114.1	*Pseudomonas aeruginosa*	GGAGCAGCAACGAUGUUACGCAGCAG GGCAGUCGCCCUAAAACAAAGUUAGG CAACACAGAGCGACCGUUUCAUG

>NZ_NFFD01000041.1	>NZ_NFFD01000041.1	*Pseudomonas aeruginosa*	GGAGCAGUAACGAUGUUACGCAGCUGGGCAGUCGCCCGAAAACAAAGUUAGGCAUCACAAAGUACAGCAUCGUG
>NZ_CP024094.1	>NZ_CP024094.1	*Escherichia coli*	GGAGCAGCAACGAUGUUACGCAGCAGGGCGUCGCCCUAAAACAAAGUUAGGCAUCACAAAGUACAGCAUCGUG
>NZ_ADWQ01000050.1	>NZ_ADWQ01000050.1	*Escherichia coli*	GGAGCAGCAACGAUGUUACGCAGCAGGGCAGUCGCCCUAAAACAAAGUUAAACAUCAUGAGGGAUAGGCCGCAUG
>NC_012555.1	>NC_012555.1	*Enterobacter cloacae*	GGAGCAGCAACGAUGUUACGCAGCAGGGCAGUCGCCCUAAAACAAAGUUAGGcCCGCACGGAAUCAACAUCUCAUG
>NZ_LODH01000098.1	>NZ_LODH01000098.1	*Pseudomonas aeruginosa*	GGAGCAGCAACGAUGUUACGCAGCAGGGCAGUCGCCCUAAAACAAAGUUAGGCAGCAAAGAGCAGCCCCCUCCGUUAACGGAAGCUACGAAUG