Table S1: Luciferase reporter assays in the *rrnH* operon.

Strain[1]	Fusion[2]	Alleles	Luciferase[3]	16S/23S[4]
10921	*23S-aspU*	WT	13.4	
11281	*23S-aspU*	*nusAcs10*	9.7	
11280	*23S-aspU*	*nusB< >cat*	9.7	
11279	*23S-aspU*	*rnc< >spc*	11.9	
11282	*23S-aspU*	*nusAcs10 rnc< >spc*	15.3	
11283	*23S-aspU*	*nusB< >cat rnc < >spc*	16.9	
10920	*16S*	WT	28.3	1.0
11289	*16S*	*nusAcs10*	25.6	1.2
11288	*16S*	*nusB< >cat*	32.9	1.6
11287	*16S*	*rnc< >spc*	29.6	1.0
11290	*16S*	*nusAcs10 rnc < >spc*	34.5	0.9
11291	*16S*	*nusB< >cat rnc< >spc*	40.3	1.0

[1] Strains are derivatives of those described in Tables 1 – 3.

[2] *16S* - luciferase or *23S-aspU* – luciferase fusions in the *rrnH* operon (see Materials and Methods).

[3] Shown is a typical experiment. Assays were performed in LB at mid-log at 37ºC. Experiments were performed at least twice, with similar results.

[4] Luciferase values from the *16S* fusion are divided by the *23S-aspU* fusion for each strain. Data have been normalized to nus^+ (rnc^+ or rnc^-) levels taken as 1.0.

Table S2. Strain and Plasmid Constructs

Strain/Plasmid	Genotype	Source
W3110	*rph-1 (rrnD –rrnE)*inversion	NIH Collection
MC4100	*araD*139 Δ(*argF-lac*)U169 *rpsL*150 *relA*1	(Shiba *et al.*, 1986)
DY330	W3110 Δ*lacU*169 *gal*490* *pgl*Δ8 [λ cI857Δ(*cro-bioA*)]	(Yu *et al.*, 2000)
IQ85	MC4100 *secY24ts Tn10(tet)*	(Shiba et al., 1986)
IQ527	MC4100 *ssyB63/nusB63*	(Shiba et al., 1986)
IQ607	MC4100 *ssyG40*	(Shiba et al., 1986)
IQ626	MC4100 *ssyF29*	(Shiba et al., 1986)
IQ717	MC4100 *ssyE36*	(Shiba et al., 1986)
K1914	MC4100 *nusAcs10 argG*::Tn5(Kn^R)	This work
NB2	MC4100 *secY24ts rnc<>cat*	This work
NB3	MC4100 *ssyF29 rnc<>cat*	This work
NB4	MC4100 *ssyG40 rnc<>cat*	This work
NB6	MC4100 *nusAcs10 rnc<>cat*	This work
NB23	MC4100 *secY24ts nusAcs10*	This work
NB24	MC4100 *secY24ts nusA1*	This work
NB50	MC4100 *secY24ts ΔTn10*	This work
NB54	MC4100 *secY24ts ssyF29*	This work
NB56	MC4100 *secY24ts ssyE36*	This work
NB58	MC4100 *secY24ts ssyG40*	This work
NB60	MC4100 *secY24ts ssyB63*	This work
NB61	MC4100 *secY24ts nusB<>cat*	This work
NB70	MC4100 *secY24ts ssyB63 rnc<>cat*	This work
NB71	MC4100 *secY24ts ssyG40 rnc<>cat*	This work
NB72	MC4100 *secY24ts ssyF29 rnc<>cat*	This work
NB73	MC4100 *secY24ts ssyE36 rnc<>cat*	This work
NB74	MC4100 *secY24ts nusAcs10 rnc<>cat*	This work
NB75	MC4100 *secY24ts nusB<>cat rnc<>spc*	This work
NB83	MC4100 *ssyE36 rnc<>cat*	This work
NB97	MC4100 *rnc<>spc*	This work
NB363	W3110 mini-λ-*tet*	(Schweimer *et al.*, 2011)
NB371	DY330 *galK<>luc/amp*	(Schweimer et al., 2011)
NB375	W3110 *rrnH 16S-luc/amp*	(Schweimer et al., 2011)
NB377	W3110 *rrnH aspU-luc/amp*	This work
NB421	W3110 *nusB<>cat*	This work
NB452	W3110 *nusB<>cat nusAcs10*	This work
NB478	W3110 *rnc<>cat*	This work
NB479	W3110 *rnc<>spc*	This work
NB747	MC4100 *nusB<>cat*	(Bubunenko *et al.*, 2007)

NB755	W3110 *nusAcs10 argG::Tn5*(Kn) *galK*^{amb}	This work
NB777	MC4100 *rnc<>cat*	This work
NB778	MC4100 *ssyB63 rnc<>cat*	This work
NB836	W3110 *nusB<>cat* pACYC177	This work
NB837	W3110 *nusB<>cat* pAB36(*nusB*)	This work
NB840	W3110 *nusB<>cat rnc<>spc*	This work
NB853	MC4100 *nusB<>cat rnc<>spc*	This work
NB876	W3110 *nusAcs10 rnc<>cat*	This work
NBC971	MC4100 *secY24ts nusE71*	This work
RF27	W3110 *nusAcs10 argG::Tn5*(Kn) *galK*^{amb} pACYC184	This work
RF28	W3110 *nusAcs10 argG::Tn5*(Kn) *galK*^{amb} pA2-1(*nusAinfB*)	This work
10920	W3110 *rrnH-aspU<>luc/amp*	This work
10921	W3110 *aspU<>luc/amp*	This work
11279	W3110 *aspU<>luc/amp rnc<>spc*	This work
11280	W3110 *aspU<>luc/amp nusB<>cat*	This work
11281	W3110 *aspU<>luc/amp nusAcs*10	This work
11282	W3110 *aspU<>luc/amp nusAcs*10 *rnc<>spc*	This work
11283	W3110 *aspU<>luc/amp nusB<>cat rnc<>spc*	This work
11287	W3110 *rrnH-aspU<>luc/amp rnc<>spc*	This work
11288	W3110 *rrnH-aspU<>luc/amp nusB<>cat*	This work
11289	W3110 *rrnH-aspU<>luc/amp nusAcs*10	This work
11290	W3110 *rrnH-aspU<>luc/amp nusAcs*10 *rnc<>spc*	This work
11291	W3110 *rrnH-aspU<>luc/amp nusB<>cat rnc<>spc*	This work

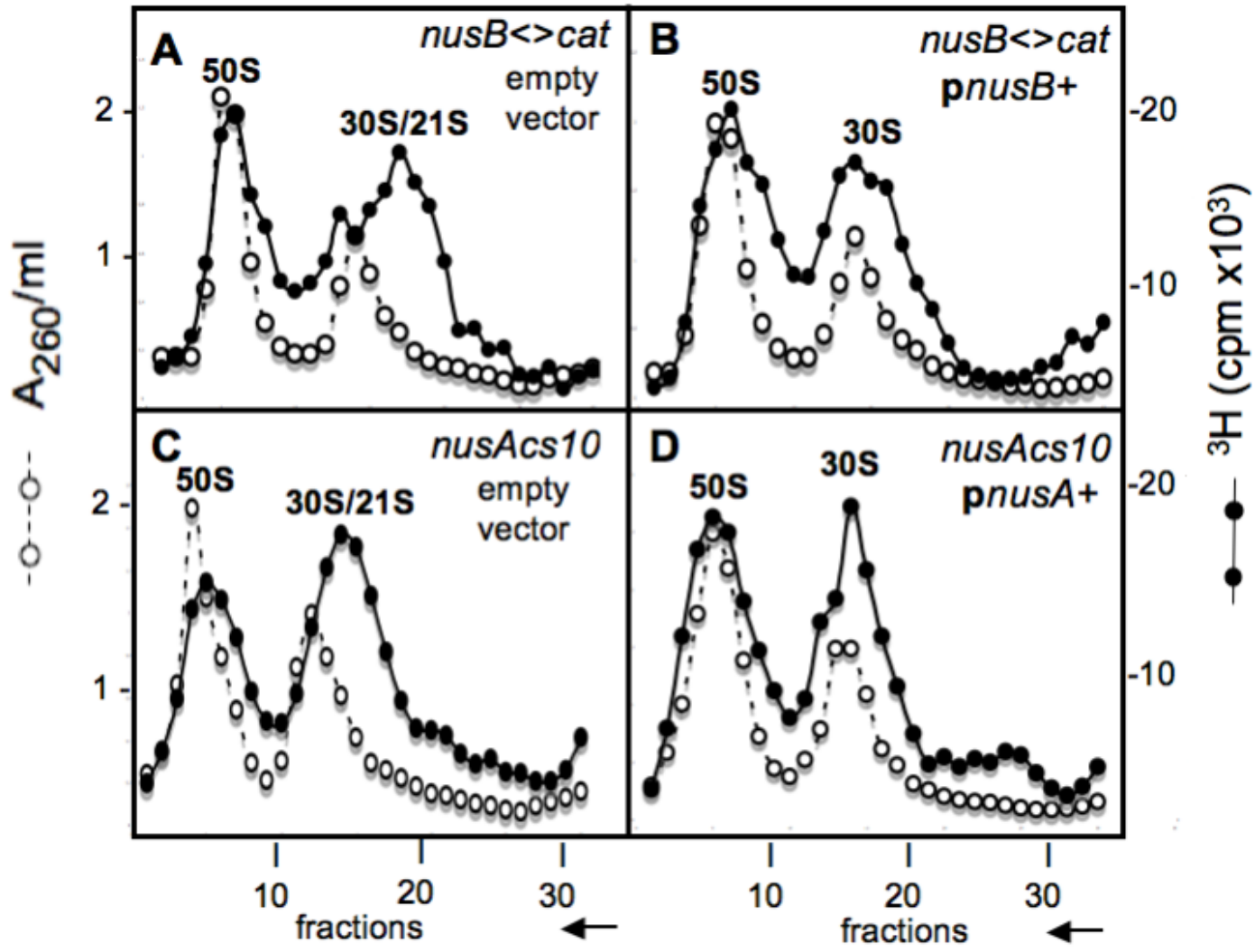
pCR-Script	colE1 *ori* Cm^{R}	Stratagene
pACYC177	p15A *ori* Ap^{R} Kn^{R}	Acc. # X06402
pACYC184	p15A *ori* Cm^{R} Tc^{R}	Acc. # X06403
pAB31	pCR-Script *nusB* Cm^{R}	This work
pAB36	pACYC177 *nusB* Kn^{R}	This work
pA2-1	pACYC184 *nusA-infB*	(Plumbridge & Springer, 1983)

Supplemental Fig. S1. Ribosome subunit profile of complemented *nus* cold-sensitive mutants. Sedimentation profiles of ribosome subunits prepared from strains NB836 (*nusB< >cat* /pACYC177) (panel **A**), NB837 (*nusB< >cat* /pAB36) (panel **B**), RF27 (*nusAcs10* /pACYC184) (panel **C**), and RF28 (*nusAcs10* /pA2-1) (panel **D**) labeled with [^{3}H]-uridine at 20° and displayed as described in Fig. 2.

Supplemental Fig. S2. The 21S precursor particles convert into 30S subunits. A culture of NB421 (Δ*nusB< >cat*) bacteria at 37° was transferred to a 20° shaking water bath. After two hours at 20°, [^{3}H]-uridine was added and the incubation was continued for 1h at which point 0.5mM non-radioactive uridine and rifampicin at a final concentration of 500 μg/ml were added. A bacterial sample was withdrawn at time 0 (panel **A**), and the rest of the culture was incubated further at 20° with bacterial samples withdrawn and harvested at 10min (panel **B**), 20min (panel **C**) and 30min (panel **D**) intervals. Bacterial crude extracts used for ribosome subunits isolation analyzed in panels A, B, C and D were prepared and sedimented on sucrose gradients as described in Fig. 2 and Methods.

Supplemental Fig. S3. Ribosome subunit profiles of *nus* cold-sensitive mutants prepared at 37°. Sedimentation profiles of ribosome subunits are prepared from the same strains and using the same method as described in Fig. 2 and Fig. 3 except that the cells were labeled at 37°. The 20° data from Figs. 2 and 3 are provided here for comparison with the 37° profiles for wild type, *rnc* and the *nus* mutants.

Supplemental Fig. S4. Primer extension analysis of the 5'-end of 16S rRNA isolated from wild type and *nus* cold-sensitive mutant cells. Cultures of wild type strain W3110 and *nus* cold sensitive mutant strain W3110 *nusAcs10 nusB*< >*cat* (NB452) were grown in LB at 37° to OD_{600}=0.6. Total RNA was isolated using Qiagen RNA isolation kit, and elution was done in RNase free DEPC water and quantified using nanodrop. Equal amounts of total RNA from wild type W3110 and the *nus* cold sensitive W3110 *nusAcs10 nusB*< >*cat* mutant (NB452) were annealed with the primer DNA 5'GTTCGACTTGCATGTGTTAGGC-3**'**, which anneals within and just downstream of the 5' end of the mature 16S rRNA sequence. Extension and labeling of the DNA was done by reverse transcription (M-MuLV RT.RNase H^-**)** using dNTPs plus [^{32}P]-α-dTTP. The ** indicates the position of the precursor 5' end, and the * indicates the position of the mature 5' end. The first four lanes are sequencing lanes using purified mature 16S rRNA. Lanes 177 and 178 are 1ul and 2ul of total wild type RNA used as template in reverse transcription, and 179 and 180 are 1ul and 2ul of total mutant RNA used as template in reverse transcription.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30S/21S
B
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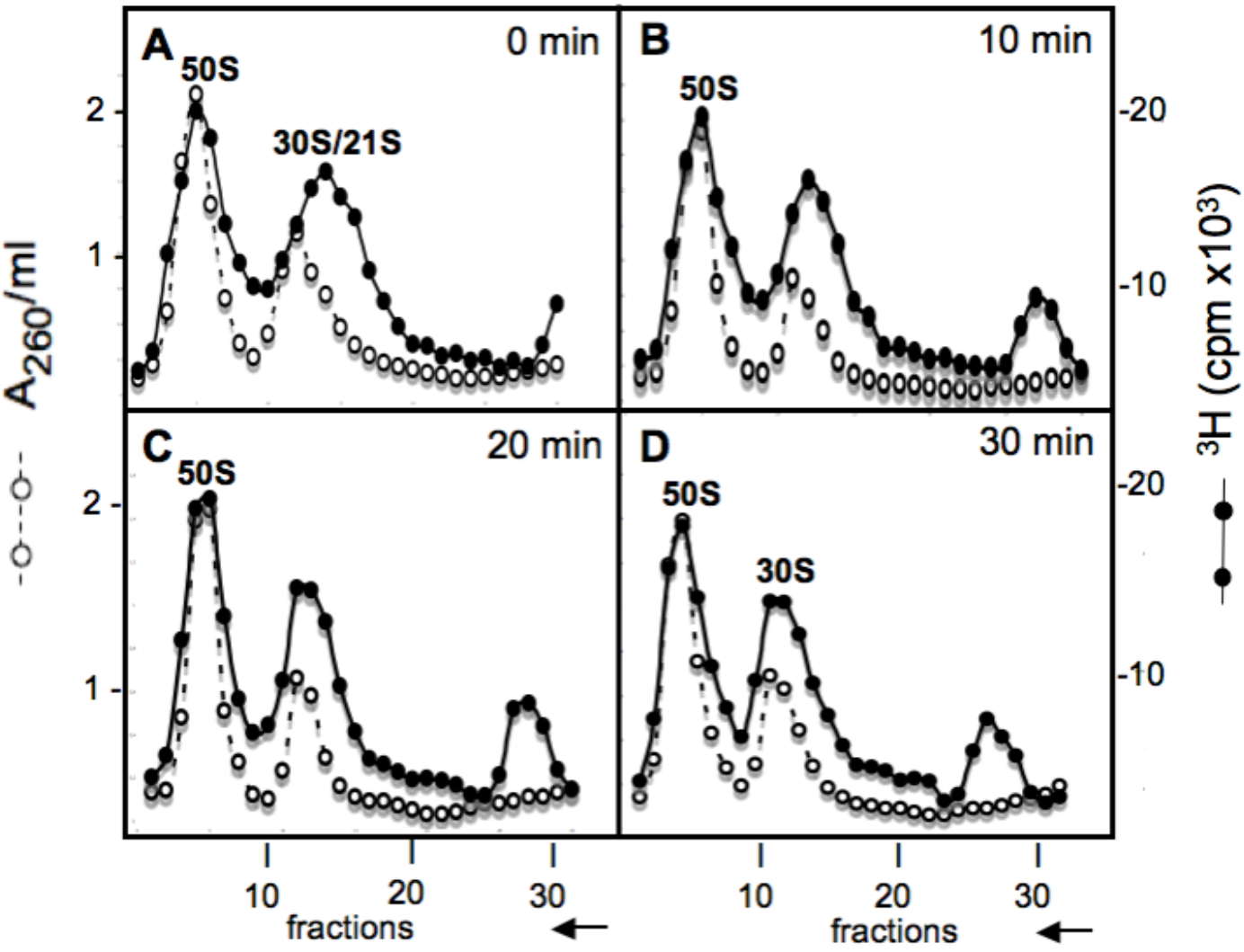
50S
30S
C
nusAcs10
empty
vector
50S
30S/21S
D
nusAcs10
pnusA+
50S
30S
A260/ml
3H (cpm x10³)
fractions
fractions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B
10 min
50S
C
20 min
50S
D
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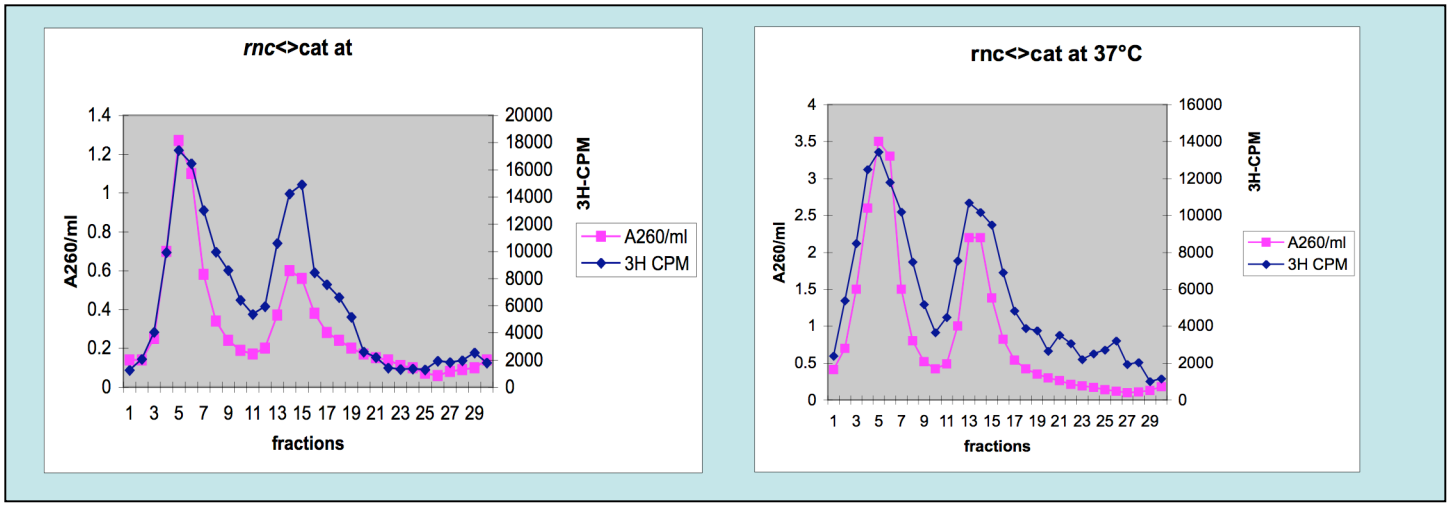
2
1
-20
-10
10
20
30
fractions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3H CPM
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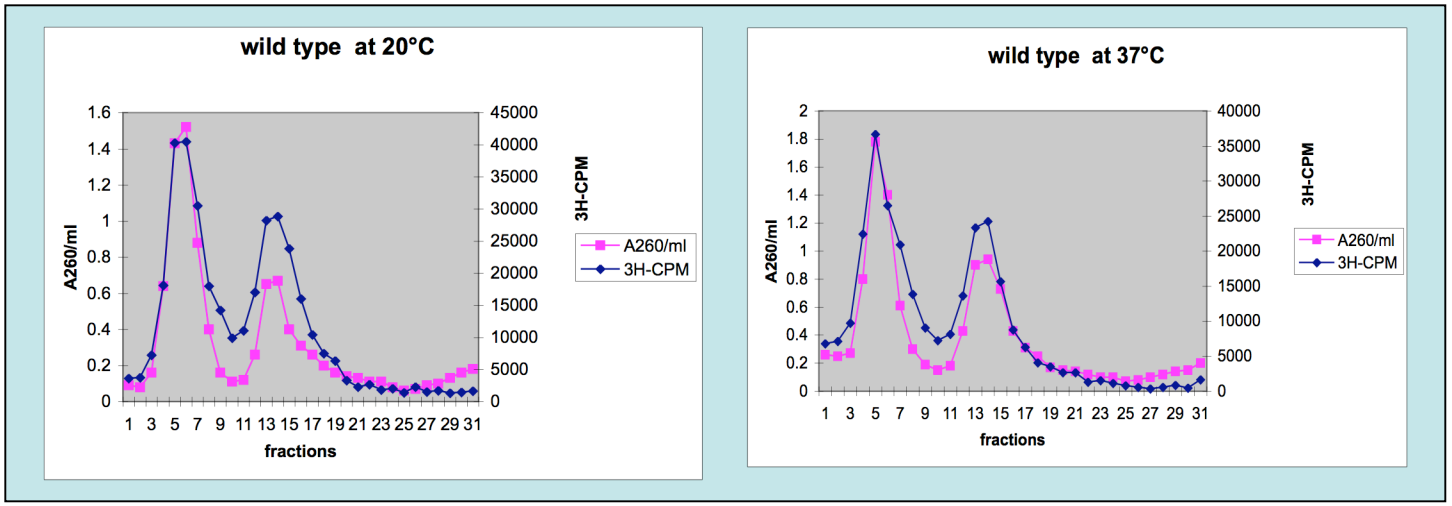
3H-CPM
fractions
A260/ml
3H CPM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fractions
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3H-CPM
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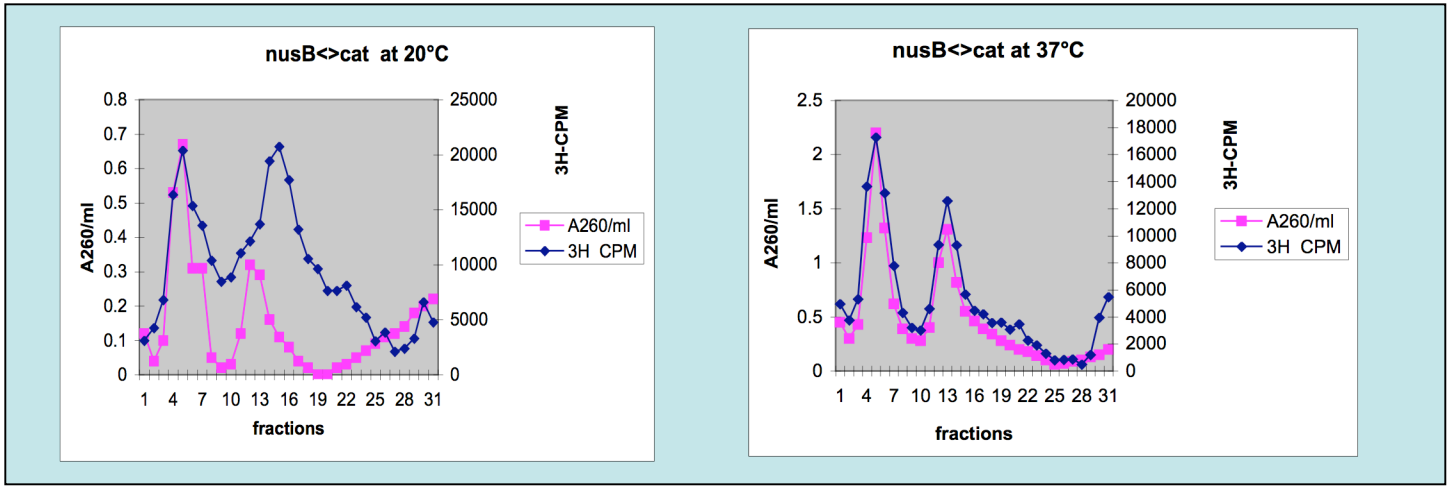
A260/ml
3H-CPM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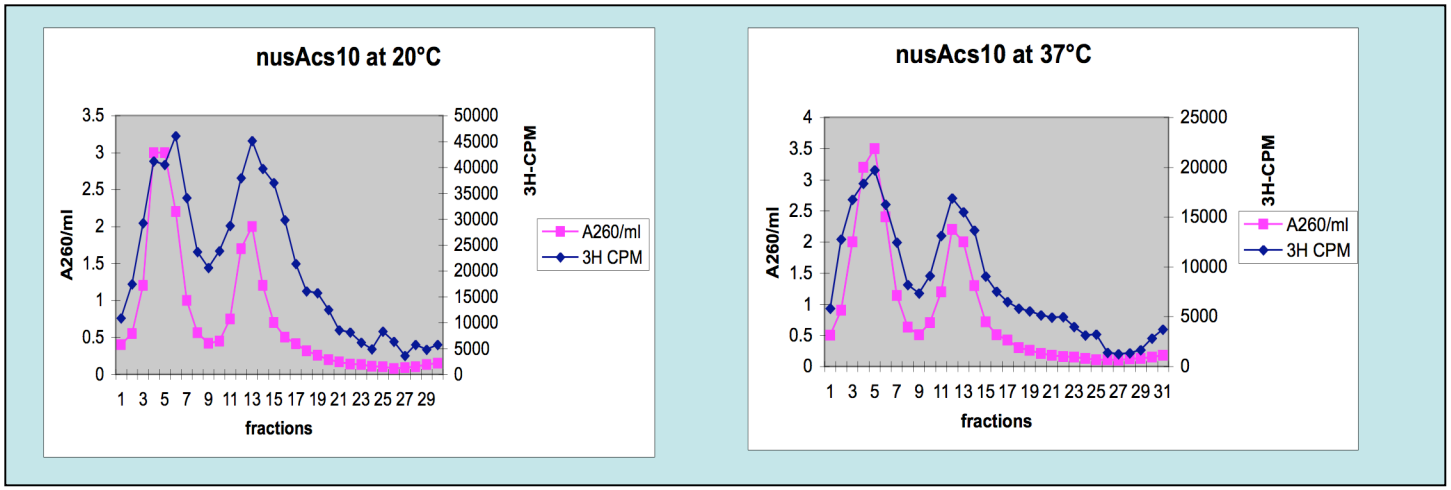
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3H-CPM
fractions
A260/ml
3H CPM
nusAcs10 at 37°C
A260/ml
3H-CPM
fractions
A260/ml
3H CPM


Mature 16S
wt
nusAB mutant
precursor 5’ 16S rRNA
mature 5’ 16S rRNA
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*
A C G T
177 178
179 180