Supplementary Online Material

Crucial contribution of the multiple copies of the initiator tRNA genes in the fidelity of $tRNA^{fMet}$ selection on the ribosomal P-site in *Escherichia coli*

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Running title: tRNA selection on ribosomal P-site

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Supplementary results

Mapping of the mutations in the suppressors strains D4 and D27:

P1 phage mediated transduction experiments showed that the mutation(s) responsible for the phenotypes of D4 and D27 were located at a single locus (*data not shown*). Genetic mapping of the locus was carried out by the method of generating miniTn*10* (confers resistance to kanamycin, Kan^R) insertion libraries in D4 and D27 using phage λNK1316 as delivery vehicle (Kleckner *et al.*, 1991). P1 phage lysates raised on these libraries were used to transduce *E. coli* KL16 harboring $pCAT_{am1}metY_{CUA/3GC}$ and selection of transductants on Kan. Transductants were then replica plated onto Kan plus Cm plates. P1 phage lysates were raised on many of the co-transductants (Cm^RKan^R), and used to transduce *E. coli* KL16 harboring $pCAT_{am1}metY_{CUA/3GC}$ and selection on Kan. This was followed by patching of transductants on Kan, and Kan plus Cm plates. The transductant (Kan^RCm^R) with the highest co-transduction frequency (~ 80% in D4 and ~75% in D27) were selected for mapping. High linkage of the chromosomal mutation conferring Cm^R with the Kan mini-transposon was further confirmed by back transductional crosses on D4 and D27 using P1 phage lysates raised on the selected transductants that showed Kan^RCm^S phenotype upon patching. To map the chromosomal location of the transposon, we followed the inverse PCR technique (Higashitani *et al.*, 1994). Sequence analysis of ~400 bp amplicons (Macrogen, S. Korea) revealed that the transposon was inserted within *csdA* in D4 (at 63.4 min) and *fucP* in D27 (at 63.2 min). To validate these locations, transductional crosses of D4 and D27 were performed with CAG12137 (Tet^R, 62.2 min) and CAG12135 (Tet^R, 63.6 min) strains which confirmed that the mutations in D4 and D27 lie closer to 63.6 min locus. Additional crosses between *csdA*::*kan*, CAG12135, D4 and D27 strains in various combinations limited the site of mutation between the 63.4 and 63.6 min region of the *E. coli* genome (Fig. S2). A high probability candidate within this region, the *metZWV* operon encoding for the majority of $tRNA^{fMet}$ in *E. coli*, was amplified by PCR (Experimental procedures) and subjected to DNA sequence analysis (Fig. S3) to reveal that D4 and D27 possessed novel mutations within the promoter region of *metZWV* (Fig. 2A).

Promoter mutations in *metZWV* in D4 and D27 independently confer the ability to initiate with the 3GC initiator tRNA:

To ensure that the promoter mutations (Fig. 2A) identified in D4 and D27, independently confer the ability to initiate with the 3GC tRNA, we targeted the promoter mutations to *E. coli* genome using λ recombineering (Datsenko and Wanner, 2000). The 0.45 kb amplicons harboring *metZWV* from D4 and D27 containing single mutations and the KL16 parent (as control) were electroporated into *E. coli* DY330 harboring $pCAT_{am1}metY_{CUA/3GC}$. Transformants were selected on Amp plus Cm plates. As shown in Fig. S4, *E. coli* DY330 transformants that appeared upon introduction of the amplicons from D4 and D27, grew on Cm plates like D4 and D27 (compare sectors 3 and 5; and 4 and 6). However, a few colonies that appeared on the plate wherein the amplicon from the KL16 parent was used as a control, failed to grow further on Cm plate (sectors 1 and 2). These observations suggest that the promoter mutations identified in D4 and D27 are sufficient to confer the ability to initiate with the 3GC mutant tRNA in *E. coli*.

Supplementary experimental procedures

Linear DNA transformations:

Amplicons (0.45 kp) of *metZWV* gene locus were prepared from *E. coli* D4, D27 or the KL16 genomic DNA by PCR using *Pfu* DNA polymerase and 20 pmols each of the forward (5' tgtttttgttcaaaatcatgcc 3') and reverse (5' ctggcgagaaggggatgataaaaaggcgctgaatgg 3') primers and electroporated into *E. coli* DY330 harboring $pCAT_{am1}metY_{CUA/3GC}$. Transformants were selected on Amp plus Cm (50 μg ml^{-1}) plates at 30 ^{o}C (Datsenko and Wanner, 2000).

Supplementary references

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Supplementary figure legends

Figure S1: Growth of D4 and D27 harboring $pCAT_{am1}$ or $pCAT_{am1}$*metY*$_{CUA/3GC}$. Cultures were grown in LB containing Amp and streaked on LB-agar plates containing Amp, and Amp plus Cm, and incubated at 37 ^{o}C for 15 h.

Figure S2: Genetic fine mapping of the suppressor mutation in *E. coli* D4 and D27. The mutations responsible for the D4 and D27 were mapped to a location between *csdA* (63.4') and *recD* (63.6'). The *metZWV* locus (63.5 min) was PCR amplified as 0.45 kb (KL16 and D4) or ~1.27 kb (D27) fragment.

Figure S3: *Upper panel*, the 0.45 kb amplicons (Fig. S2) from the KL16 parent and D4, and the ~1.27 kb amplicon from D27 (Fig. S2) were subjected to DNA sequence analysis (Macrogen, S. Korea) with primers 5' tgtttttgttcaaaatcatgccaaatccgtgatcgg 3' for KL16 and D4, and 5' ctggctggttcaccagagagaa 3' for D27. Chromatograms corresponding to the sequence in the promoter region of the *metZWV* are shown. The arrows indicate the sites of mutations. *Lower panel:* Comparative analysis of the promoter regions of D4 and D27 *metZWV* locus with that of the parent (WT). There is a G to A mutation in D4 just upstream to the Pribnow box (-10) and a T to C mutation in D27 within the Pribnow box (-10). The mutations have been indicated by boxes between the sequences of the wild-type (WT) and the suppressor strain promoter. The -35 region, the spacing between the -10 and the -35 sequences and the transcription start site (+1) are also indicated.

Figure S4: The 0.45 kb amplicons (Fig. S2) from the KL16 parent or the D4 and D27 suppressor strains were introduced into *E. coli* DY330 harboring $pCAT_{am1}$*metY*$_{CUA/3GC}$ (Amp^R) and the transformants were selected on LB-agar plates containing Amp plus Cm at the permissive temperature of 30 ^{o}C. The colonies that appeared were grown in LB containing Amp and streaked on LB-agar plates containing Amp; Amp plus Cm (50 µg ml^{-1}); and Amp plus Cm (100 µg ml^{-1}), as indicated and incubated at 30 ^{o}C for approximately 36 h.

Figure S5: Initiation with elongator tRNA. D4 and D27 harboring $pCAT_{am1}$ (Amp^R, colE1 ori) and pACDH (vector alone) or pACDH*supE*, (Tet^R, pAC1 ori) compatible plasmids were grown in LB containing Amp plus Tet and streaked on LB-agar plates containing Amp plus Tet; and Amp plus Tet plus Cm (50 µg ml^{-1}) and incubated at 37 ^{o}C for 15 h.

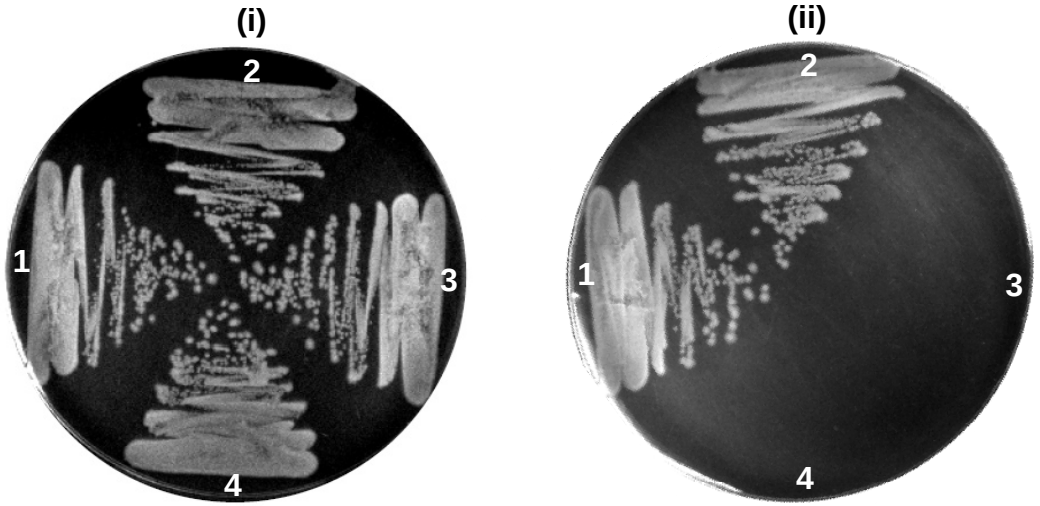
Table-S1

Strains /Plasmids	Genotype/Details	Reference/Source
***E. coli* strains**		
KL16	*E. coli* K-12, *thi1*, *relA*1, *spoT*1	Low, 1968
TG1	SupE hsdD5 thi D(lac-proAB) F_ [traD36 proAB+ lacIq lacZDM15]	Sambrook *et al.*, 1989
D4	A derivative of KL16, isolated as a suppressor of 3GC mutant tRNA.	This work
D27	A derivative of KL16, isolated as a suppressor of 3GC mutant tRNA.	This work
DY330	W3110 DlacU169 gal490 [lc1857D(cro-bioA)]	Lee *et al.*, 2001
TG1*ΔmetZVW::kan*	*E. coli* TG1 wherein *metZVW* operon has been replaced with a Kan^R encoding cassette.	This work
KL16*ΔmetZVW::kan*	*E. coli* KL16 wherein *metZVW* operon has been replaced with a Kan^R encoding cassette.	This work
KL16*ΔmetY::kan*	*E. coli* KL16 wherein *metY* has been replaced with a Kan^R encoding cassette.	This work
D4/D27*ΔmetZVW::kan*	*E. coli* D4 or D27 strains wherein *metZVW* operons have been replaced with Kan^R encoding cassettes.	This work
D4/D27*ΔmetY::kan*	*E. coli* D4 or D27 strains wherein *metY* have been replaced with Kan^R encoding cassettes.	This work
CAG12135	*recD*1901::Tn*10* at 63.6 min	CGSC, Singer *et al.*, 1989; Nichols *et al* ., 1998
CAG12173	*cysC*95::Tn*10* at 62.2 min	

Table-S2

Plasmid	Details	Reference/ Source
$pCAT_{am1}$ (Amp^R)	Renamed from $pRSVCAT_{am1.2.5}$. Harbors a CAT reporter gene with UAG initiation codon.	Varshney and RajBhandary (1990)
$pCAT_{am1}$*metY*$_{CUA}$ (Amp^R)	$pCAT_{am1}$ harboring *metY* gene which encodes $tRNA^{fMet}$ with CUA anticodon.	
$pCAT_{am1}$*metY*$_{CUA/3GC}$ (Amp^R)	Derivative of $pCAT_{am1}$*metY*$_{CUA}$ with additional mutations in *metY* at positions 29:41, 30:40 and 31:39 (from G:C, G:C and G:C to U:A, C:G and A:U).	Mandal *et al.*, (1996)
pACDH (Tet^R)	A cloning vector harboring ACYC Ori of replication, which is compatible with ColE1 Ori of replication plasmids.	Rao and Varshney, 2002
pKD4 (Amp^R, Kan^R)	Amp^R, Kan^R; *kan* marker is flanked by FRT sequences.	Datsenko and Wanner (2000)
pACDHN (Tet^R)	pACDH was digested with NcoI and EcoRI, end-filled and ligated.	Dastur and Varshney (*unpublished*)
pACDHN-CAT_{am1}*metY*$_{CUA/3GC}$ (Tet^R)	HindIII-XmnI fragment from $pCAT_{am1}$*metY*$_{CUA/3GC}$ harboring CAT_{am1} and *metY*$_{CUA/3GC}$ genes was cloned into HindIII and Bst1107I sites of pACDHN.	
pACDHN*metY*$_{CUA/3GC}$ (Tet^R)	pACDHN-CAT_{am1}*metY*$_{CUA/3GC}$ was digested with HindIII and BamHI (limited) and end-filled; the largest fragment lacking CAT_{am1} gene was eluted from agarose gel and ligated to generate pACDHN*metY*$_{CUA/3GC}$.	This work
pACDH*supE* (Tet^R)	*E. coli* TG1 *GlnVX* cloned into pACDH between NcoI and EcoRI sites.	This work
pTKCAT (Kan^R)	*E. coli*-mycobacteria shuttle vector generated by replacing *xylE* from pTKmx with promoterless CAT cassette.	Dastur *et al.,* (2002)
pTK-KL16-CAT (Kan^R)	pTKCAT containing promoter of *metZWV* from *E. coli* KL16, upstream of the CAT reporter cassette.	This work
pTK-D4-CAT (Kan^R)	pTKCAT containing promoter of *metZWV* from *E. coli* suppressor D4, upstream of the CAT reporter cassette.	This work
pTK-D27-CAT (Kan^R)	pTKCAT containing promoter of the *metZWV* from *E. coli* suppressor D27, upstream of the CAT reporter cassette.	This work

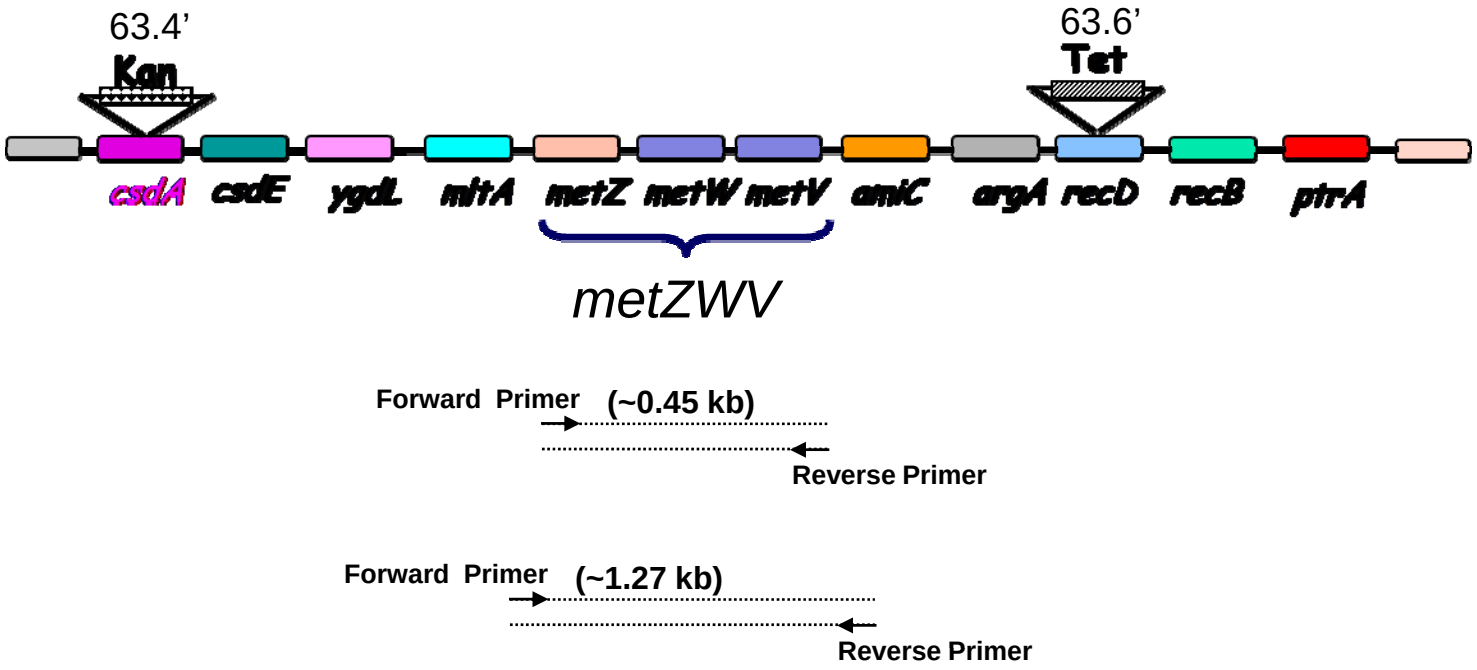
Kapoor *et. al.*, Fig. S1

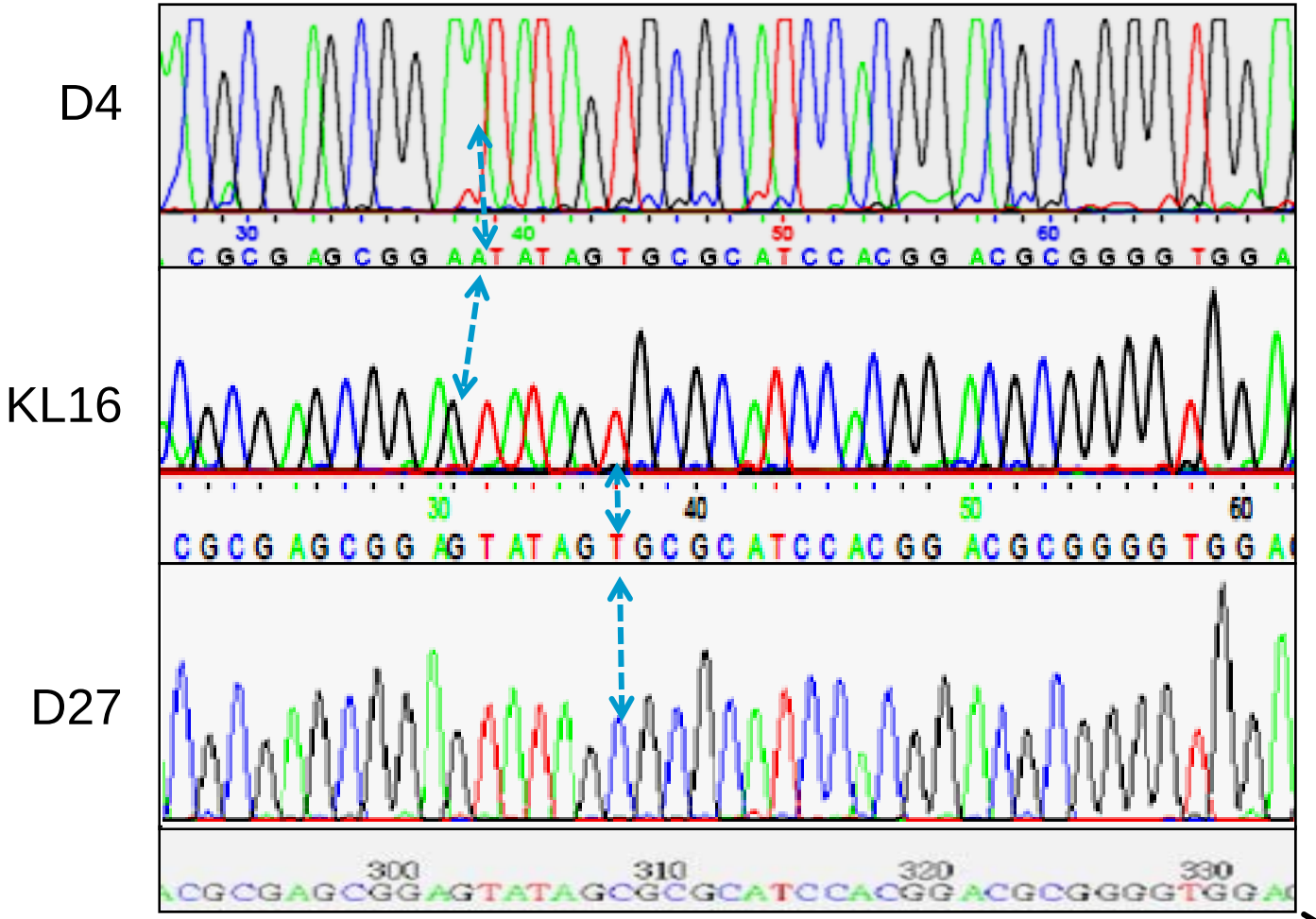


Amp^{100} **Amp^{100}+Cm^{30}**

Sectors:

1.D4+pCAT$_{am1}$*metY*$_{CUA/3GC}$
2.D27+pCAT$_{am1}$*metY*$_{CUA/3GC}$
3.D4+pCAT$_{am1}$
4.D27+pCAT$_{am1}$


63.4'
Kan
63.6'
Tet
csdA
csdE
ygdL
mltA
metZ
metW
metV
amiC
argA
recD
recB
ptrA
metZWV
Forward Primer (~0.45 kb)
Reverse Primer
Forward Primer (~1.27 kb)
Reverse Primer



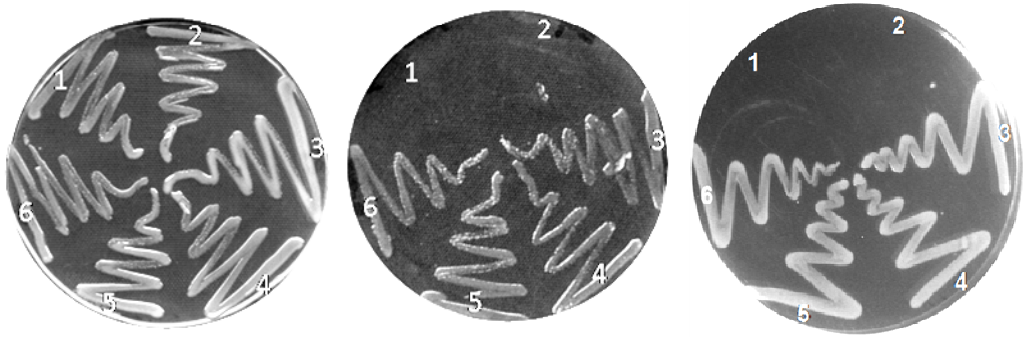
D4: GGTTGCATGAAAACGCGAGCGGAATATAGTGCGCATCCACGGACGC….

WT: GGTTGCATGAAAACGCGAGCGGAGTATAGTGCGCATCCACGGACGC….

D27: GGTTGCATGAAAACGCGAGCGGAGTATAGCGCGCATCCACGGACGC….

-35 16 bp -10 +1

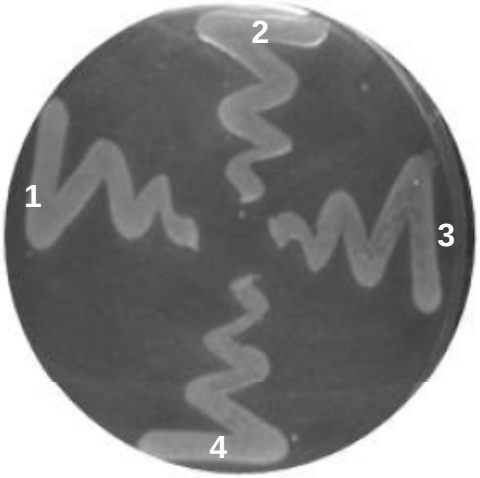
Kapoor *et. al.*, Fig. S4



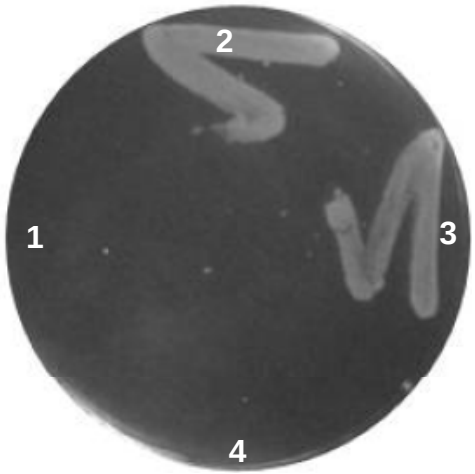
Amp^{100} Amp^{100}Cm^{50} Amp^{100}Cm^{100}

Sectors

1. DY330 (KL16) +pCAT$_{am1}$*metY*$_{CUA/3GC}$
2. DY330 (KL16) +pCAT$_{am1}$*metY*$_{CUA/3GC}$
3. D4+pCAT$_{am1}$*metY*$_{CUA/3GC}$
4. D27+pCAT$_{am1}$*metY*$_{CUA/3GC}$
5. DY330(D4)+pCAT$_{am1}$*metY*$_{CUA/3GC}$
6. DY330 (D27)+pCAT$_{am1}$*metY*$_{CUA/3GC}$



Amp^{100}Tet$^{7.5}$



Amp^{100}Tet$^{7.5}$Cm^{50}

Sectors:

1. D4+pCAT$_{am1}$+pACDH
2. D4+pCAT$_{am1}$+pACDH*supE*
3. D27+pCAT$_{am1}$+pACDH*supE*
4. D27+pCAT$_{am1}$+pACDH