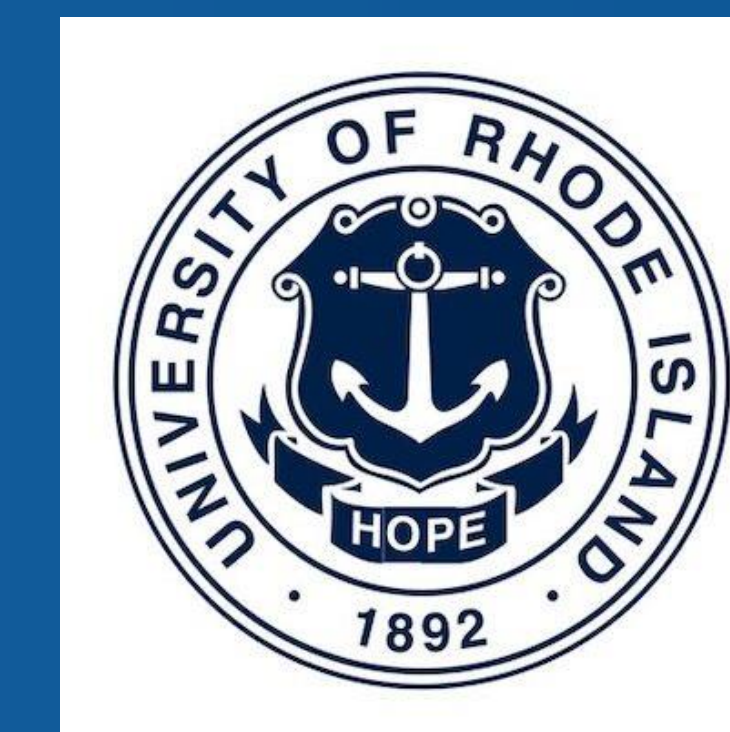
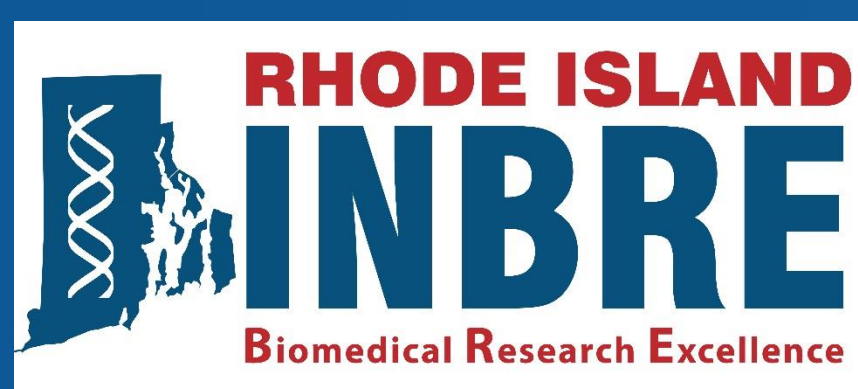


Studying the Function of Heterogeneous Ribosomes Using Antibiotics



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INTRODUCTION

Francisella tularensis

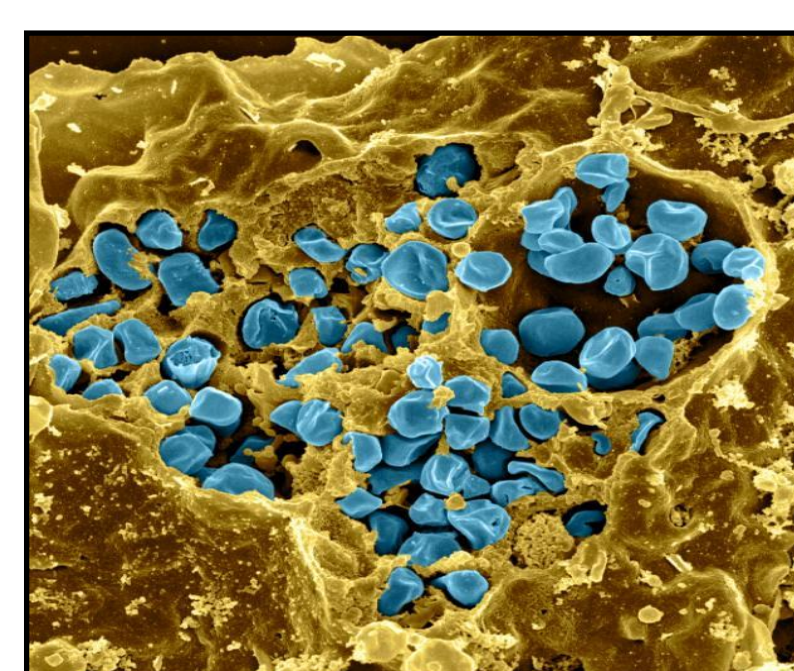
- > Gram negative bacterium
- > Causes tularemia, a potentially lethal illness
- > Potential bioweapon due to its highly infectious nature

Ribosomal protein bS21

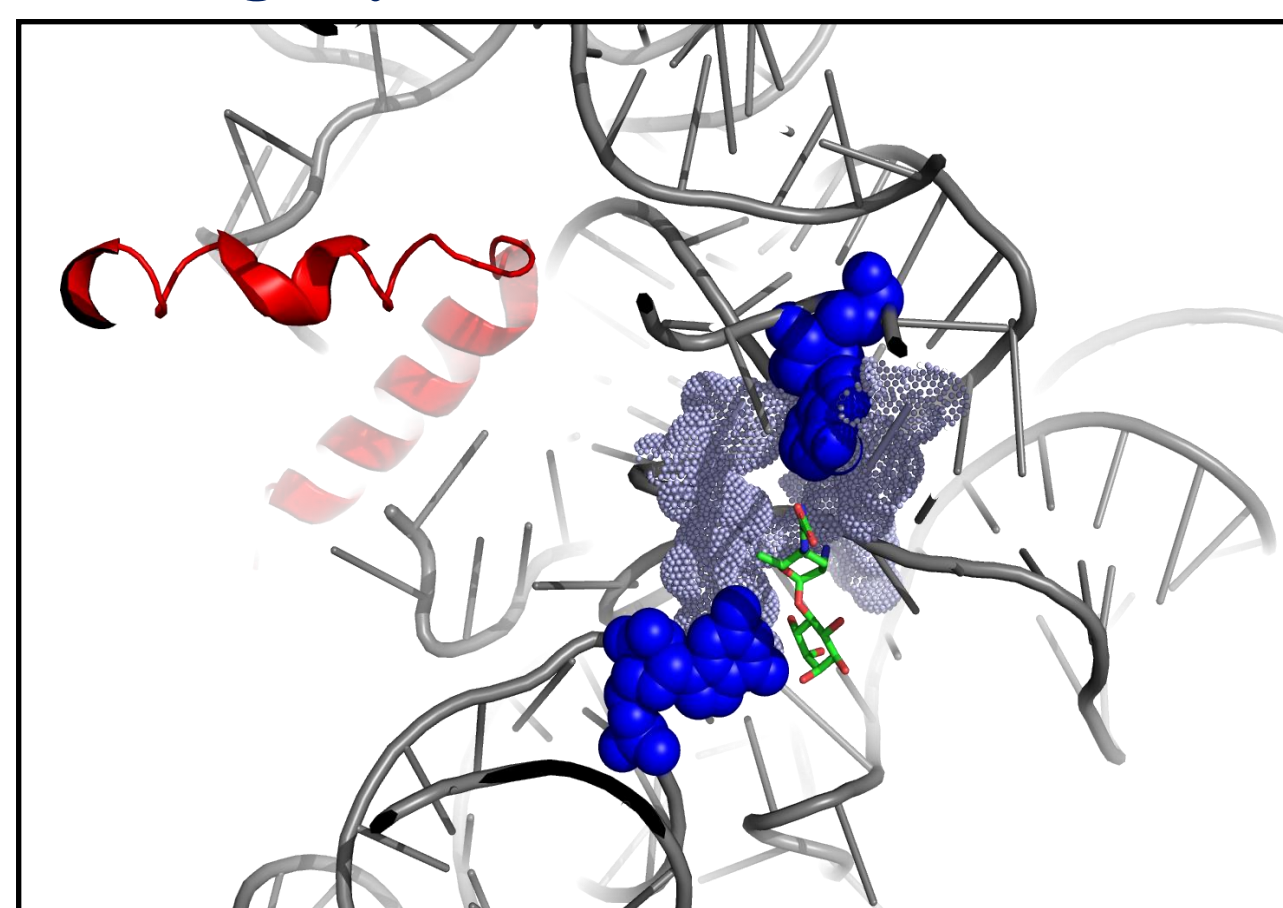
- > Involved in translation initiation
- > Three homologs encoded in the *F. tularensis* genome: *rpsU1*, *rpsU2*, and *rpsU3*
- > Strains were created to focus on each homolog individually

Antibiotics and Kasugamycin

- > Antibiotics effective against bacteria often inhibit ribosome function
- > Kasugamycin is an inhibitor of translation initiation
- > Antibiotics that bind to the 30S ribosome were studied with *F. tularensis*.
- > A previous student identified kasugamycin as the only drug that caused altered susceptibility within these strains among the tested 30S inhibitors.



Kasugamycin Binds on the Ribosome



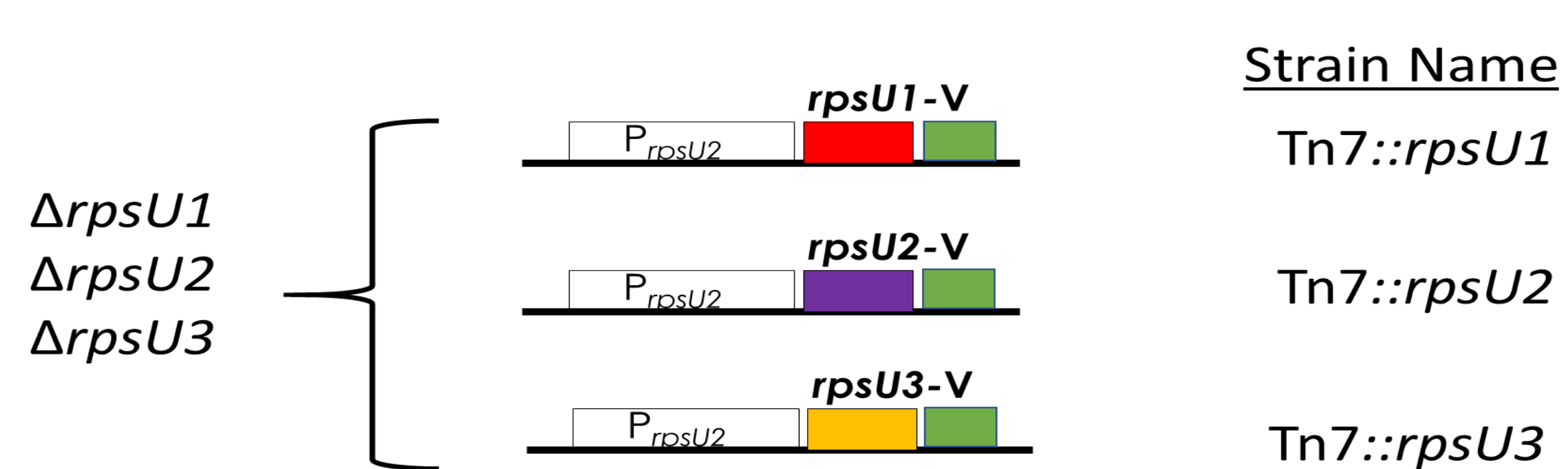
Green = kasugamycin Credit to Hannah Trautmann
Red = bS21
Grey = 16S rRNA
Blue = kasugamycin-binding pocket on 16S rRNA

Antibiotic Susceptibility of Strains with Single bS21 Homologs is Generally Unaffected

Drug	LVS	LVS Tn7::bS21-1	LVS Tn7::bS21-2	LVS Tn7::bS21-3
Kanamycin	53.5 +/- 0.6	55.3 +/- 0.2	53.6 +/- 0.4	53.2 +/- 0.5
Tetracycline	46.0 +/- 0.9	45.8 +/- 1.4	49.0 +/- 1.9	47.2 +/- 0.3
Streptomycin	55.1 +/- 3.4	54.9 +/- 0.7	56.1 +/- 0.1	58.0 +/- 1.8
Ciprofloxacin	56.5 +/- 1.3	56.0 +/- 1.2	55.1 +/- 0.1	56.0 +/- 0.1

METHODS AND MATERIALS

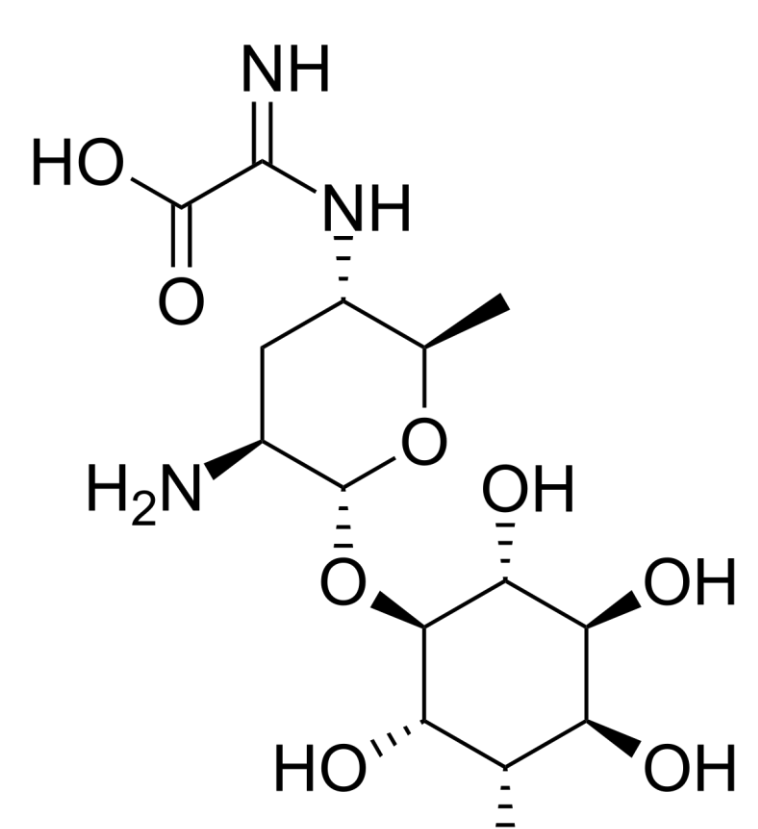
Strains Used



Strain Name
Tn7::rpsU1
Tn7::rpsU2
Tn7::rpsU3

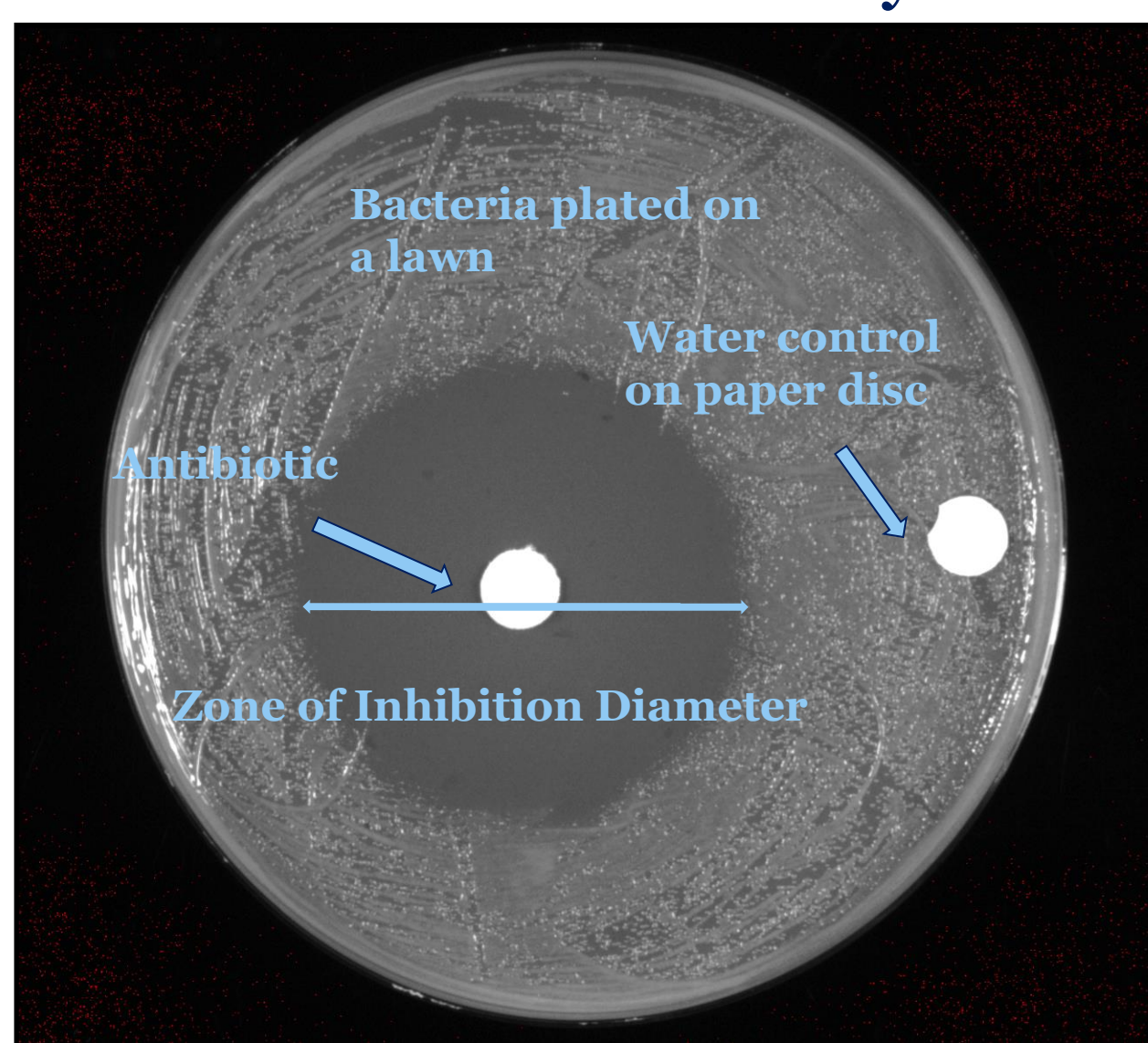
Credit to Hannah Trautmann

Kasugamycin



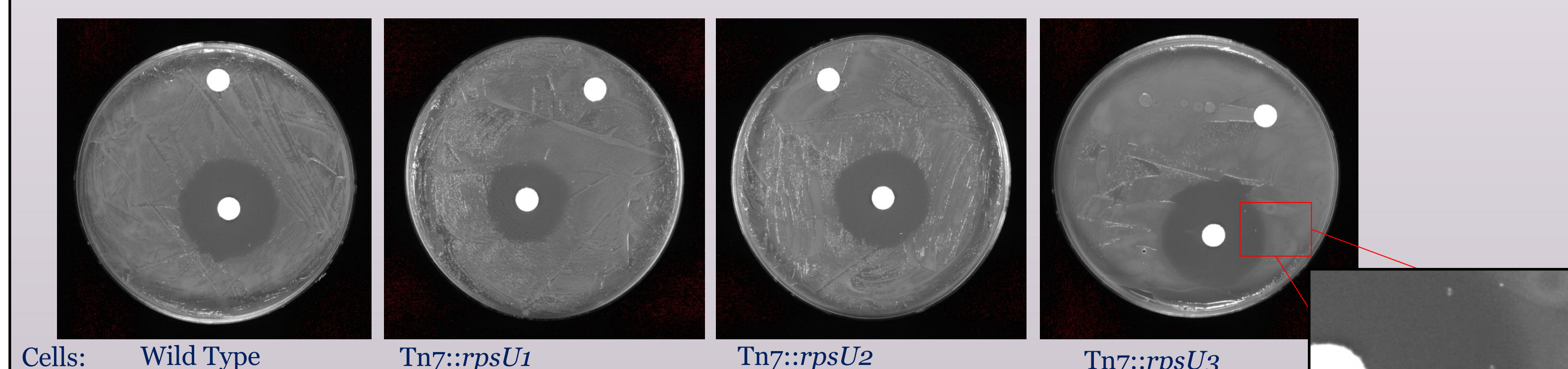
Dissolved in water and used at 50 mg/ml and 150mg/ml as indicated

Disc Diffusion Assay

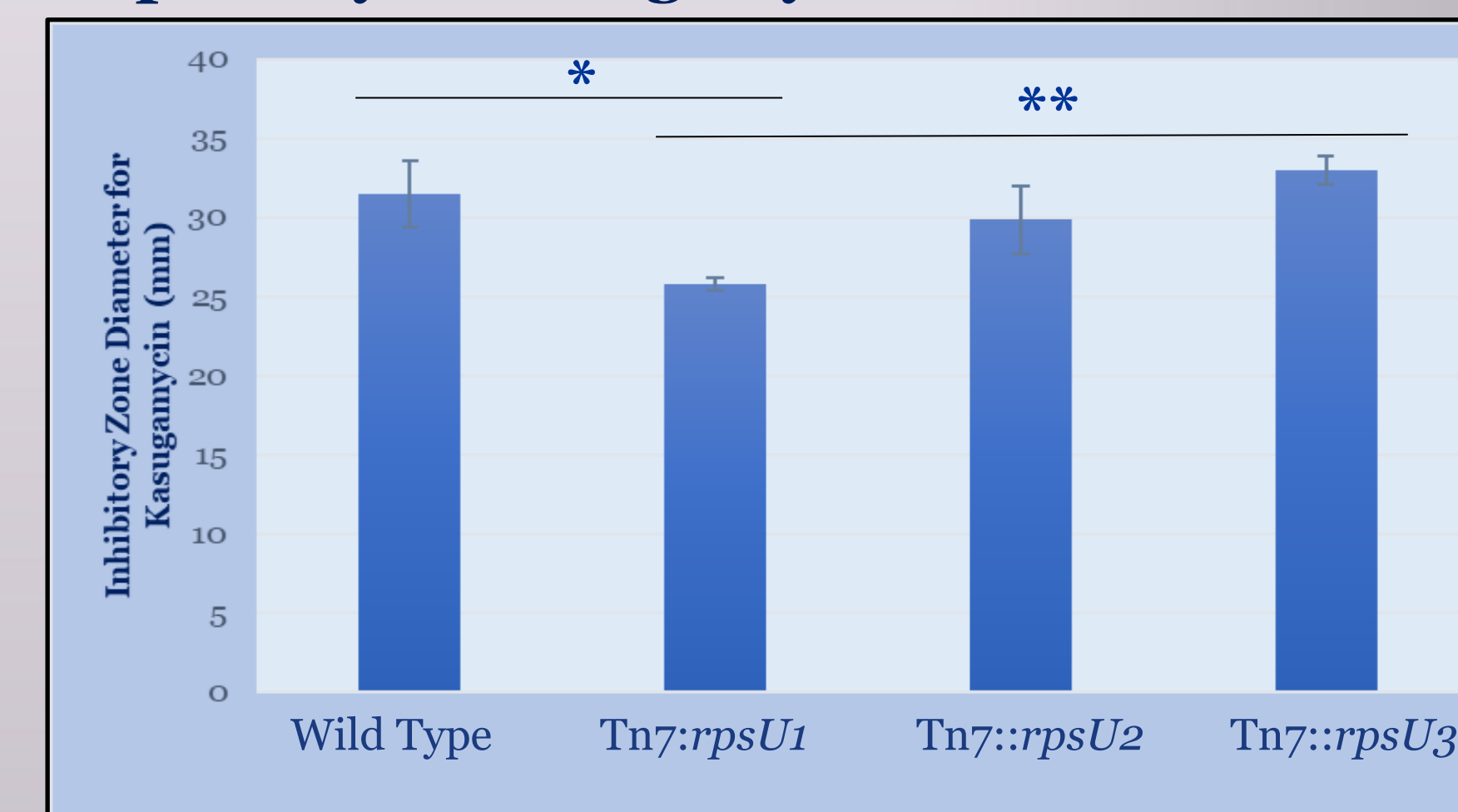


RESULTS

Cells with Different bS21 Content Have Altered Susceptibility to Kasugamycin



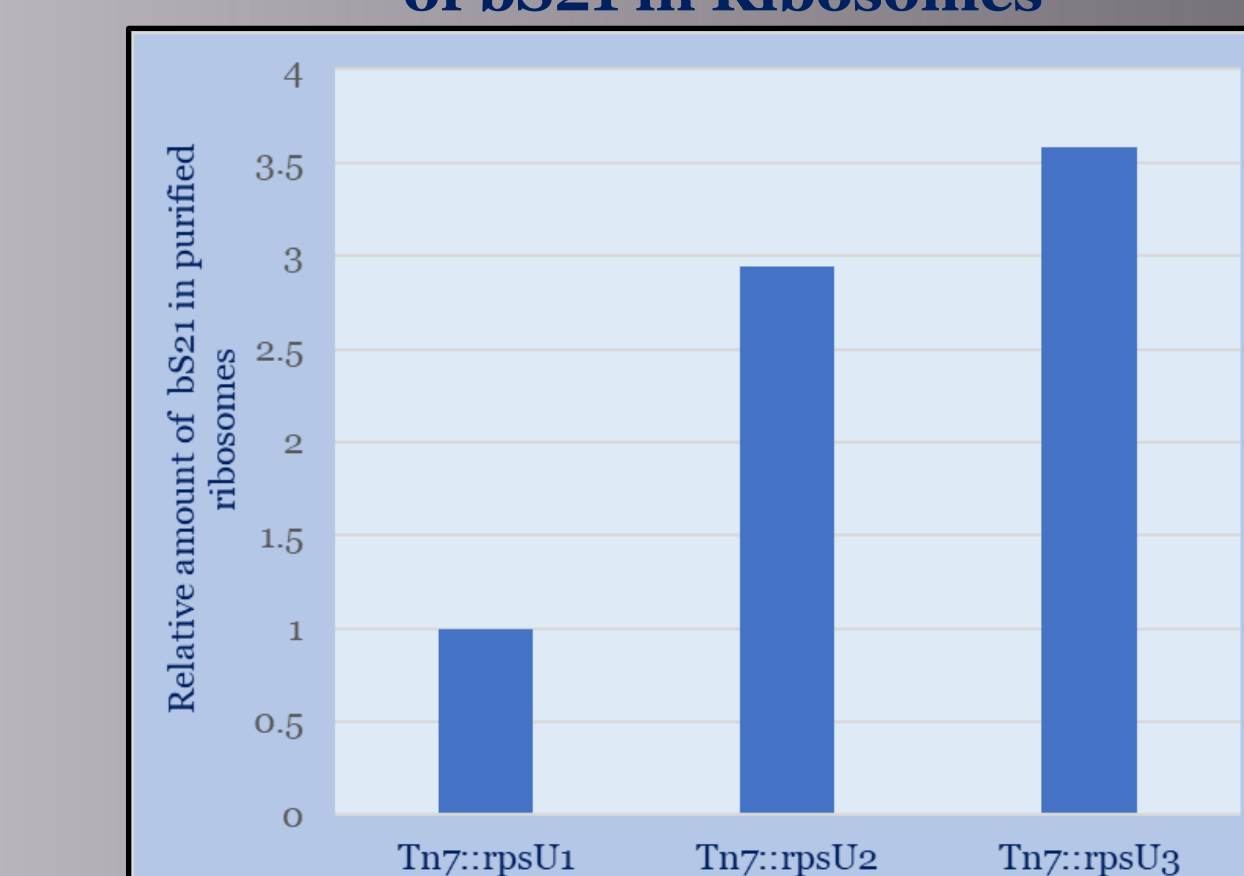
Cells: Wild Type Tn7::rpsU1 Tn7::rpsU2 Tn7::rpsU3



Colony identified within the zone of inhibition

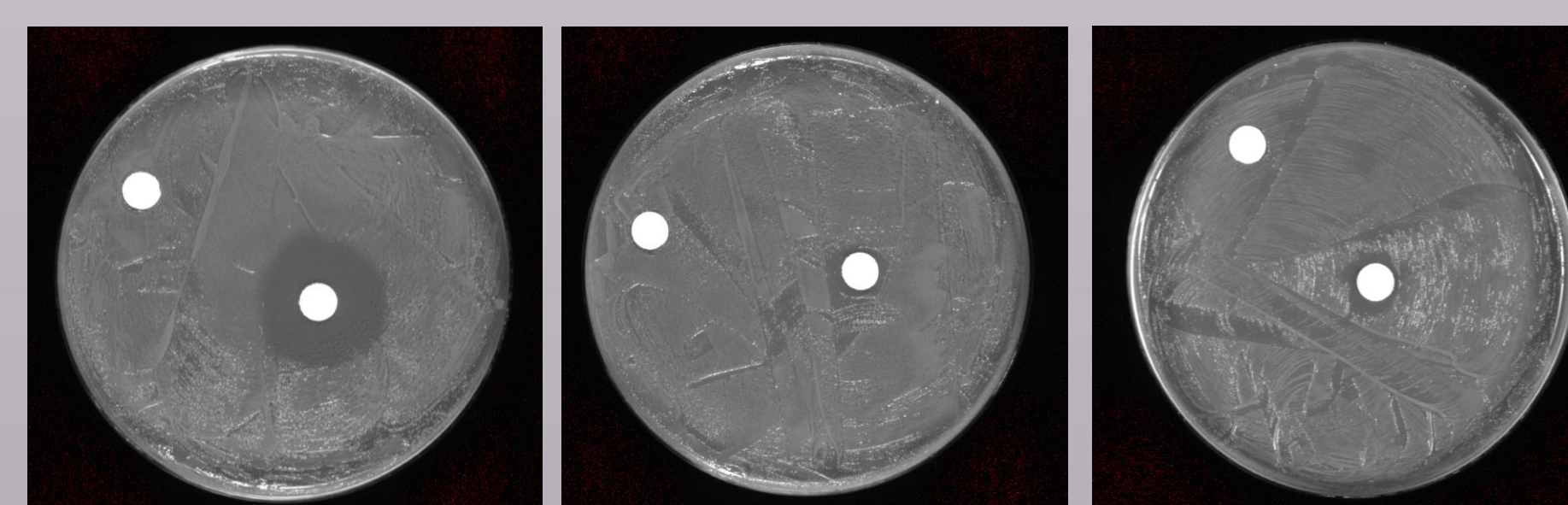
Disc diffusion assays were performed the indicated strains in biological triplicate using discs impregnated with 50 mg/ml kasugamycin. Plates were incubated for 48 hours, and zones of inhibition were measured in mm. Error bars represent standard deviation. *p<.002, **p<.0002

Isogenic Strains Have Different Amounts of bS21 in Ribosomes

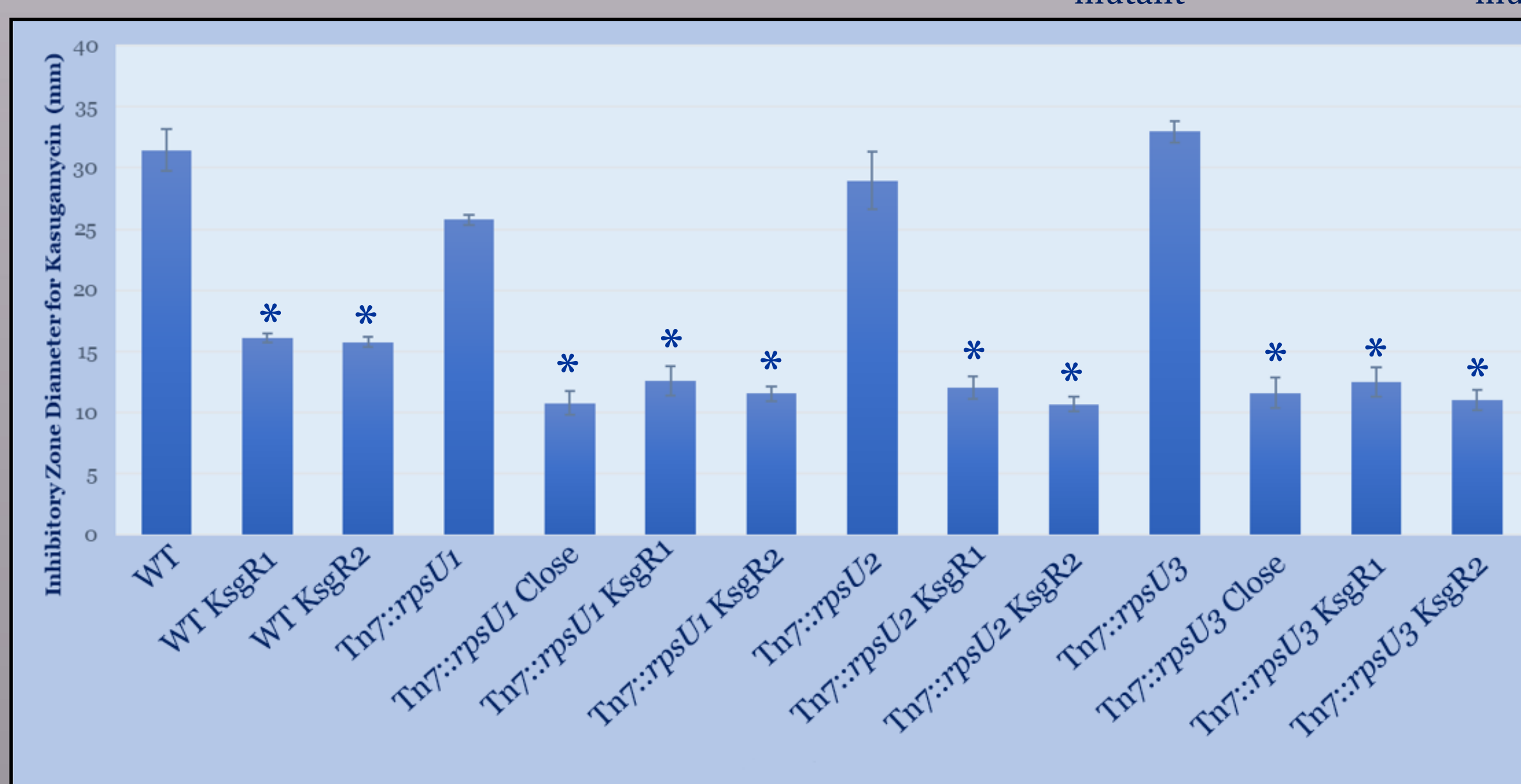


Amount of bS21 present in ribosomes isolated from indicated strains. VSV-G tagged bS21 homologs were detected by immunoblotting. Results were normalized to total protein in ribosome samples. Results from Hannah Trautmann.

Identification of Kasugamycin Resistant Mutants



Cells: Tn7::rpsU1 Tn7::rpsU1 Close KsgR mutant Tn7::rpsU1 Far KsgR mutant



Disc diffusion assays were performed the indicated strains in biological triplicate using discs impregnated with 50 mg/ml kasugamycin. Plates were incubated for 48 hours, and zones of inhibition were measured in mm. Error bars represent standard deviation. *p<.00005 compared to parental strain.

Mutations Identified that Correlate with Kasugamycin Resistance

Strain	Type of Mutation	Original Amino Acid	Mutation
LVS	-	-	-
LVS KsgR1	Missense	Arg217 (AGA)	Ile217 (ATA)
LVS KsgR2	Frameshift Deletion	His82 (CAT)	Deletion (-C)
Tn7::rpsU1	-	-	-
Tn7::rpsU1 Close	Nonsense	Glu206 (GAA)	Stop Codon (TAA)
Tn7::rpsU1 KsgR1	Missense	Thr48 (ACA)	Ile48 (ATA)
Tn7::rpsU1 KsgR2	Frameshift Deletion	His82 (CAT)	Deletion (-C)
Tn7::rpsU2	-	-	-
Tn7::rpsU2 KsgR1	Nonsense	Glu207 (GAA)	Stop Codon (TAA)
Tn7::rpsU2 KsgR2	Nonsense	Ser99 (TCA)	Stop Codon (TAA)
Tn7::rpsU3	-	-	-
Tn7::rpsU3 Close	Nonsense	Tyr202 (TAC)	Stop Codon (TAA)
Tn7::rpsU3 KsgR1	Frameshift Deletion	His82 (CAT)	Deletion (-C)
Tn7::rpsU3 KsgR2	Nonsense	Ser10 (TCG)	Stop Codon (TAG)

In all kasugamycin-resistant strains, we identified mutations in the *ksgA* gene by Sanger sequencing; no mutations were identified in the parental strains. The wild-type *KsgA* protein is 262 amino acids long.

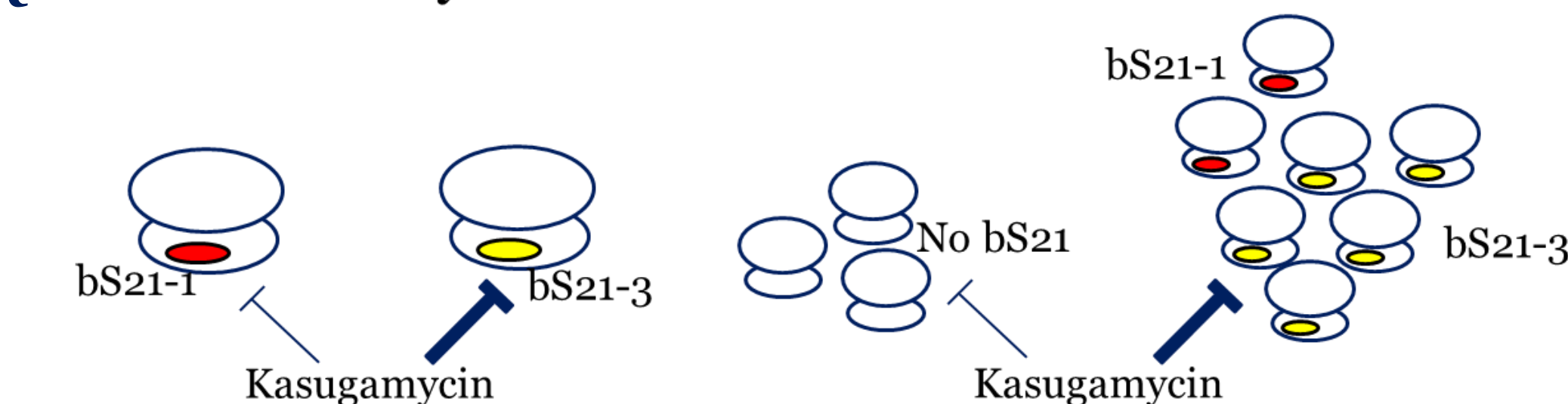
CONCLUSIONS AND FURTHER STEPS

Identified kasugamycin resistant mutants

Open Question: Identity

OR

Abundance



Exciting future direction: ribosome specific antibiotics?

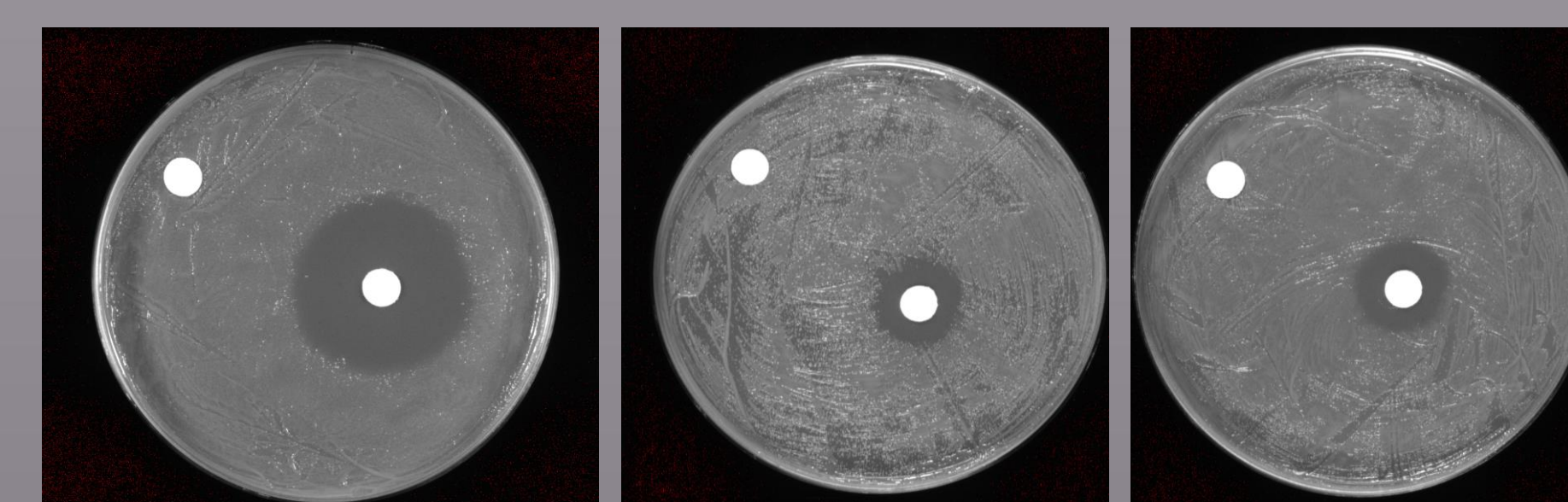
References

- Kasugamycin*:
Schuwirth, B. S. *et al.* Structural analysis of kasugamycin inhibition of translation. *Nat Struct Mol Biol* 13, 879–886 (2006).
- Translation initiation*:
Duval, M., Simonetti, A., Caldelari, I. & Marzi, S. Multiple ways to regulate translation initiation in bacteria: Mechanisms, regulatory circuits, dynamics. *Biochimie* 114, 18–29 (2015).

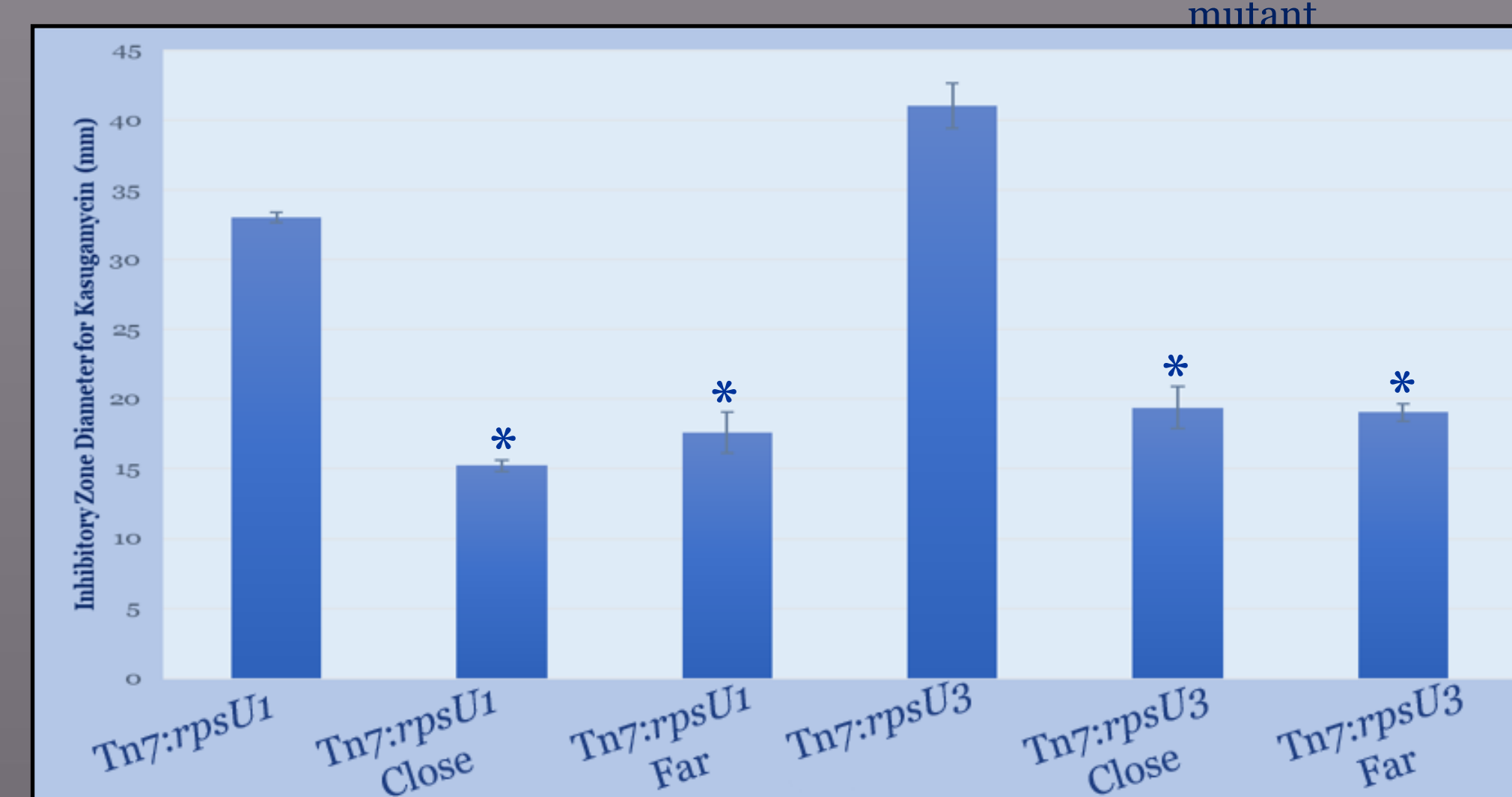
Acknowledgments

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Further Testing of Kasugamycin Resistant Mutants



Cells: Tn7::rpsU1 on 150 mg/ml Tn7::rpsU1 Close KsgR mutant Tn7::rpsU1 Far KsgR mutant



Disc diffusion assays were performed the indicated strains in biological triplicate using discs impregnated with 150 mg/ml kasugamycin. Plates were incubated for 48 hours, and zones of inhibition were measured in mm. Error bars represent standard deviation. *p<.0001 compared to parental strain.