

# Transcriptional Analysis of the *tpr* Genes of *Treponema pallidum* subsp. *pallidum*

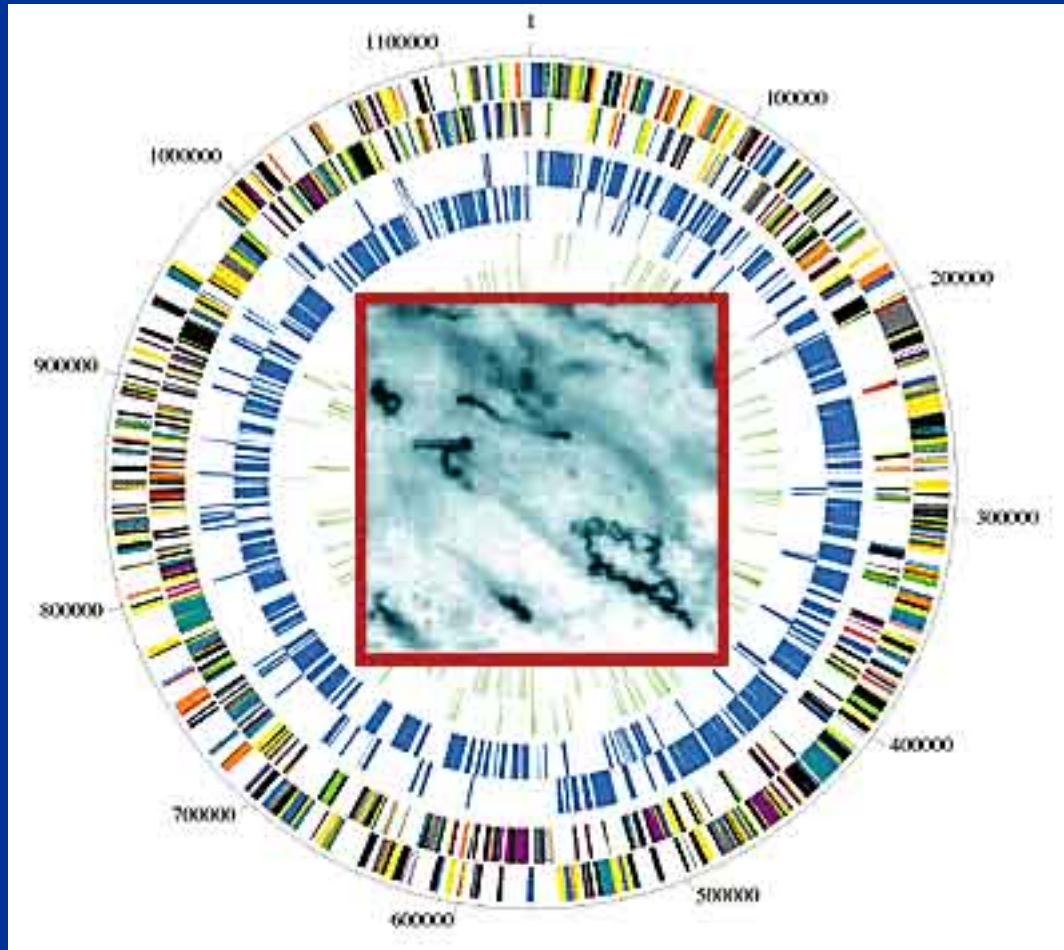
Lorenzo Giacani, Ph.D.

# *T. pallidum* subsp. *pallidum*



- Gram negative
- Not cultivable *in vitro*  
↓
- Lack of genetic systems

# *T. pallidum* Genome



Fraser, C. M., S. J. Norris, G. M. Weinstock, *et al.* 1998. **Complete genome sequence of *Treponema pallidum*, the syphilis spirochete.** *Science* 281:375-88.

# *Treponema pallidum* repeat (*tpr*) Genes

- Homologous to the *msp* gene of *T. denticola* (porin and adhesin)
- 12 paralogs (*tprA-tprL*)
- Divided into three subfamilies (I, II, and III) according to amino acid homology
- Account for ~2% of the small (1.138 Mbp) *T. pallidum* genome

# Predicted Tpr Cellular Location (using PSORTb)\*

Subfamily I		Subfamily II		Subfamily III	
TprC	OM	TprE	?	TprA	OM
TprD	OM	TprG	?	TprB	?
TprF	OM	TprJ	OM	TprH	?
TprI	OM			TprK	OM
				TprL	?

\*<http://www.psort.org/psortb/>

# Tpr Antigens Architecture

NH<sub>2</sub>-  
conserved  
region

Unique  
central  
region

COOH-  
conserved  
region

Subfamily I  
Tpr C, D, F, I



Subfamily II  
Tpr E, G, J



Subfamily III  
Tpr A, B, H, K, L



# Aims of My Research

- Analyze *tpr* gene transcription in different *T. pallidum* strains

# Aims of My Research

- Analyze *tpr* gene transcription in different *T. pallidum* strains
- Identify mechanisms of transcription regulation for the *tpr* genes

# Why Study *tpr* Transcription?

- To identify if the *tpr* genes are differentially expressed
- To understand which stimuli can influence *tpr* expression

# Experimental Design for *tpr* Gene Transcriptional Analysis

# Experimental Design for *tpr* Gene Transcriptional Analysis

- Infect rabbits intratesticularly with  $10^7$  *T. pallidum* cells/testicle

# Experimental Design for *tpr* Gene Transcriptional Analysis

- Infect rabbits intratesticularly with  $10^7$  *T. pallidum* cells/testicle



- Harvest treponemes ~10 days postinfection (before onset of immune clearance)

# Experimental Design for *tpr* Gene Transcriptional Analysis

- Infect rabbits intratesticularly with  $10^7$  *T. pallidum* cells/testicle

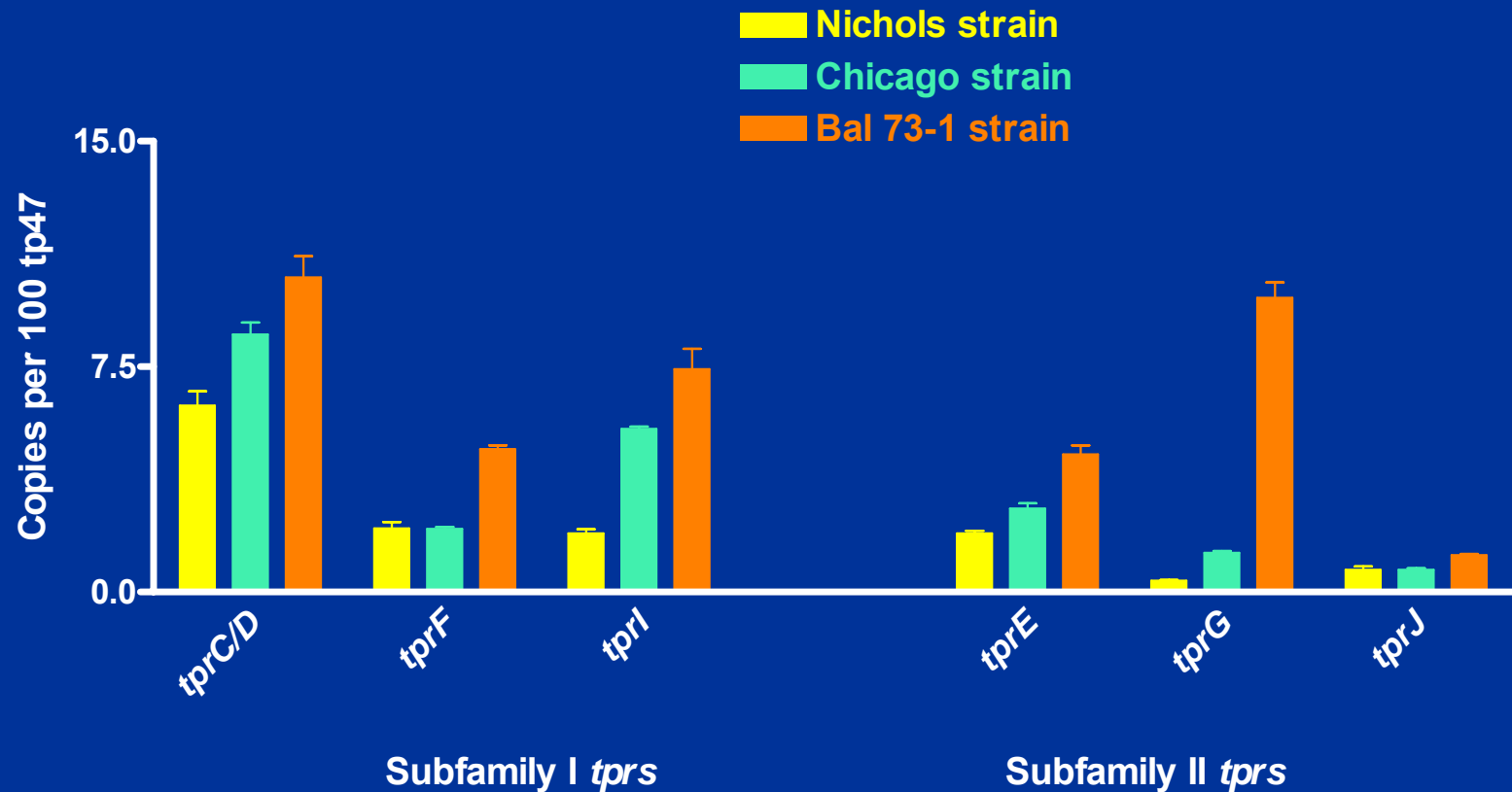


- Harvest treponemes ~10 days postinfection (before onset of immune clearance)



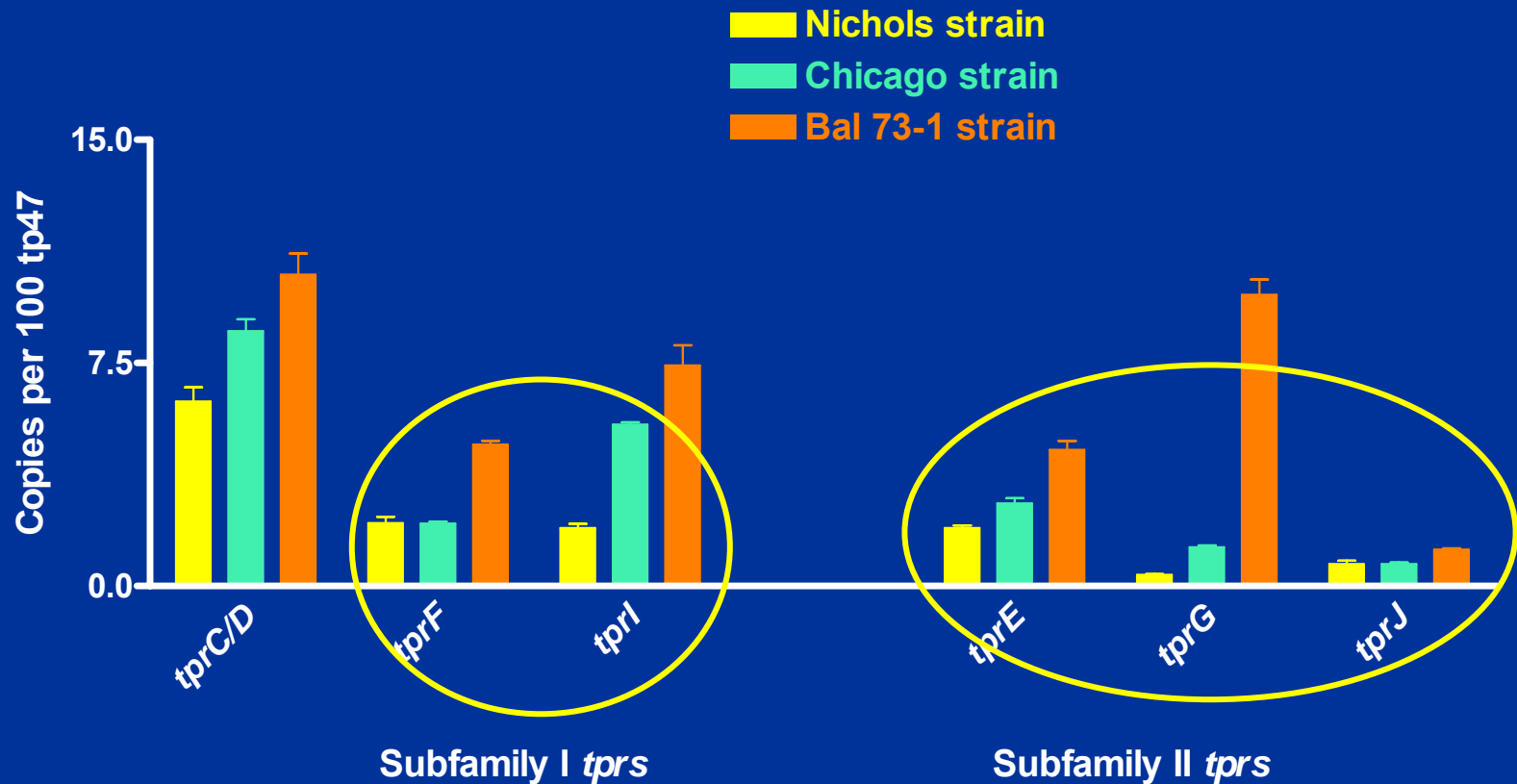
- RNA extraction, retrotranscription into cDNA and message quantification with Real-Time qPCR

# *tprs* Are Differentially Expressed Within and Among Strains



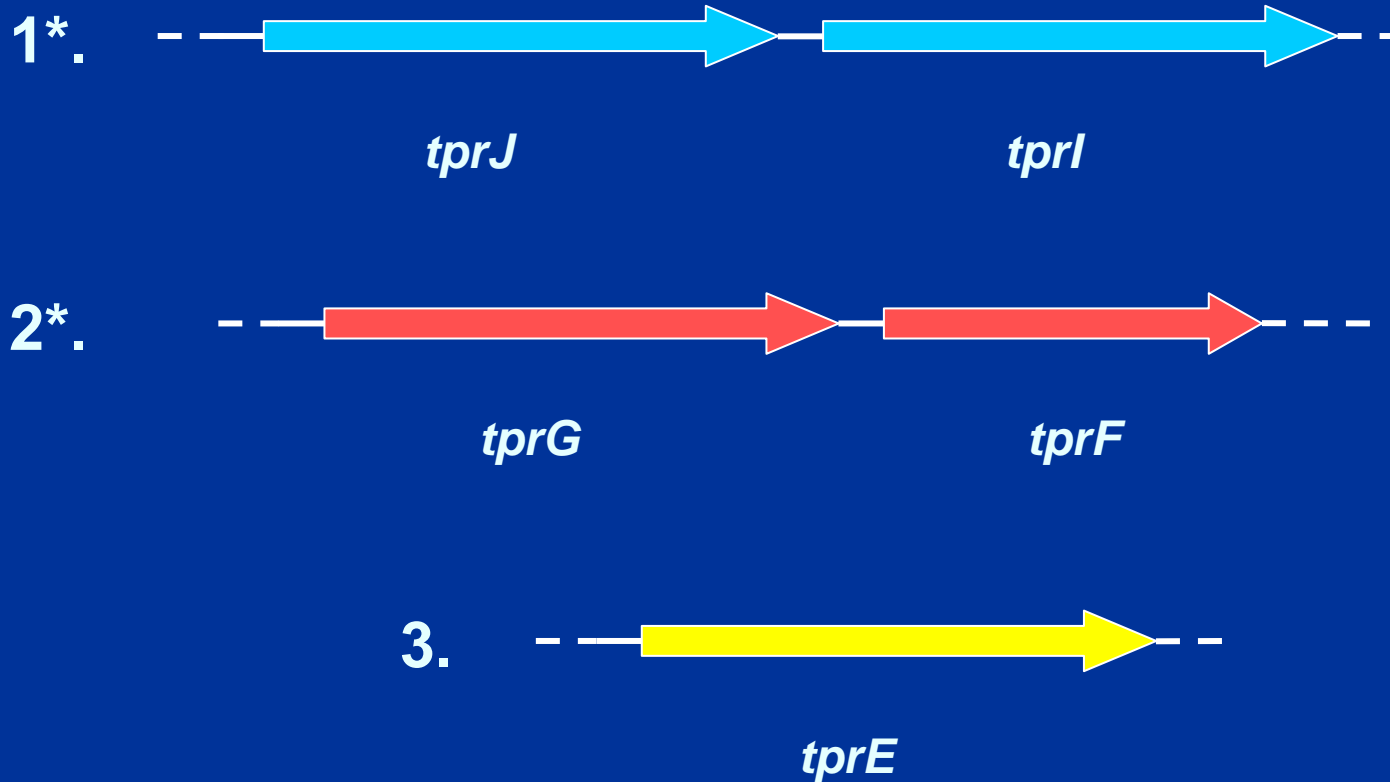
- Giacani, L. et al., 2006.

# *tprs* Are Differentially Expressed Within and Among Strains



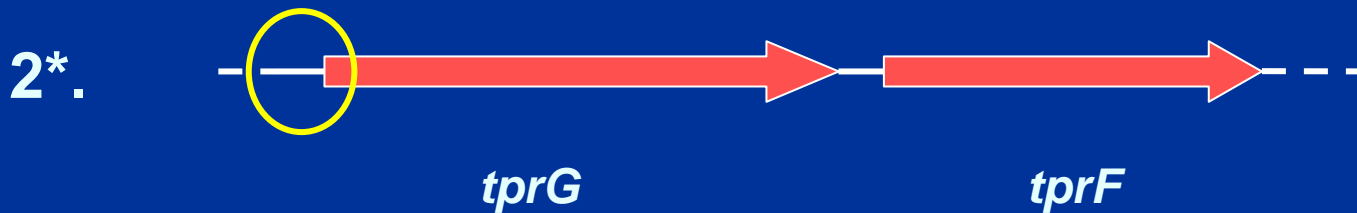
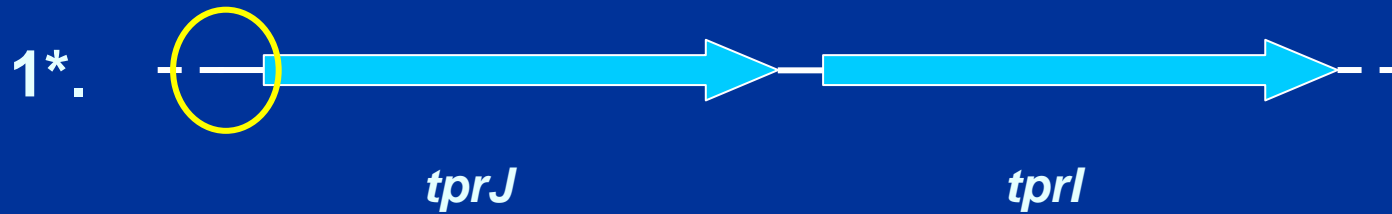
- Giacani, L. et al., 2006.

# Potential Transcription Regulatory Regions



\*Giacani, L. et al., 2004

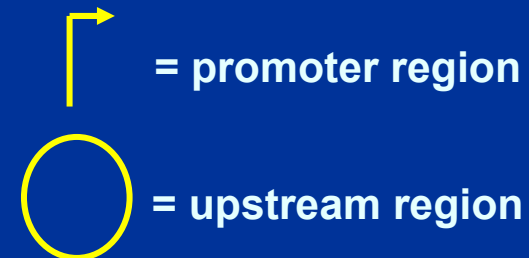
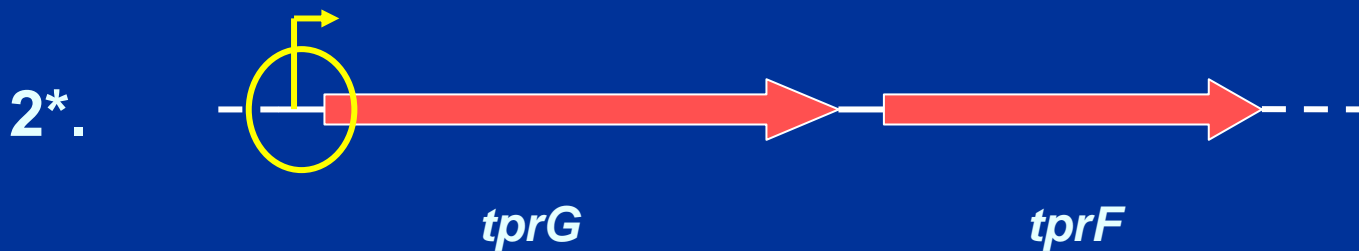
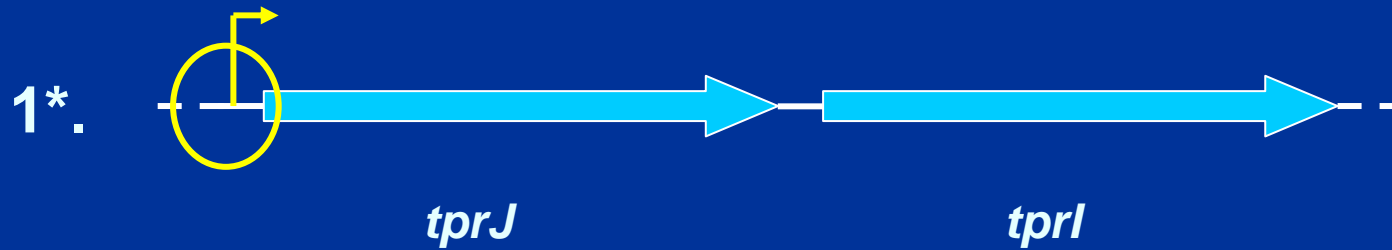
# Potential Transcription Regulatory Regions



 = upstream region

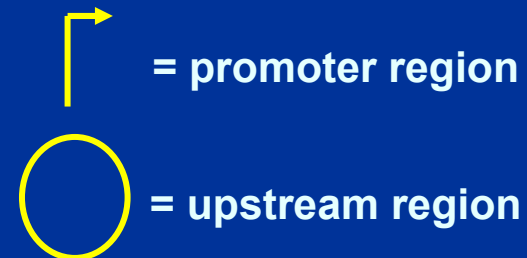
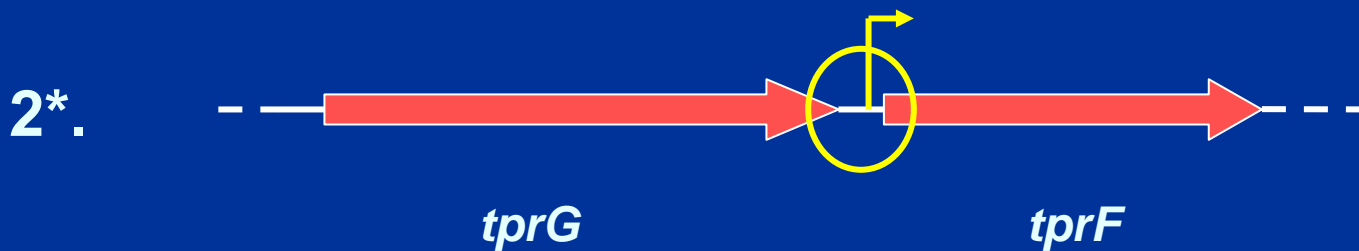
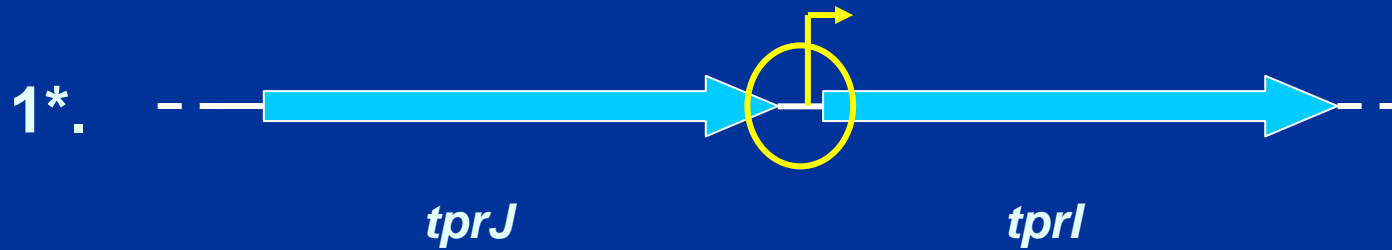
\*Giacani, L. et al., 2004

# Potential Transcription Regulatory Regions



\*Giacani, L. et al., 2004

# Potential Transcription Regulatory Regions



\*Giacani, L. et al., 2004

# Potential Regulatory Elements

*tprE, tprG, and tprJ*

TTAGAAAAGAAGGGTGAGGGGGCTACTAGACAGGGCCGGGGGGGGGGGGTGA TSS(+1)

*tprI and tprF*

GGCGTCACCCTCTCCTGGTAGTCACACGCGGCTAGACGGGTGGGGGGGGGGTGA TSS(+1)

TSS(+1): Transcriptional Start Site, experimentally determined for *tprG, J, F, and I*

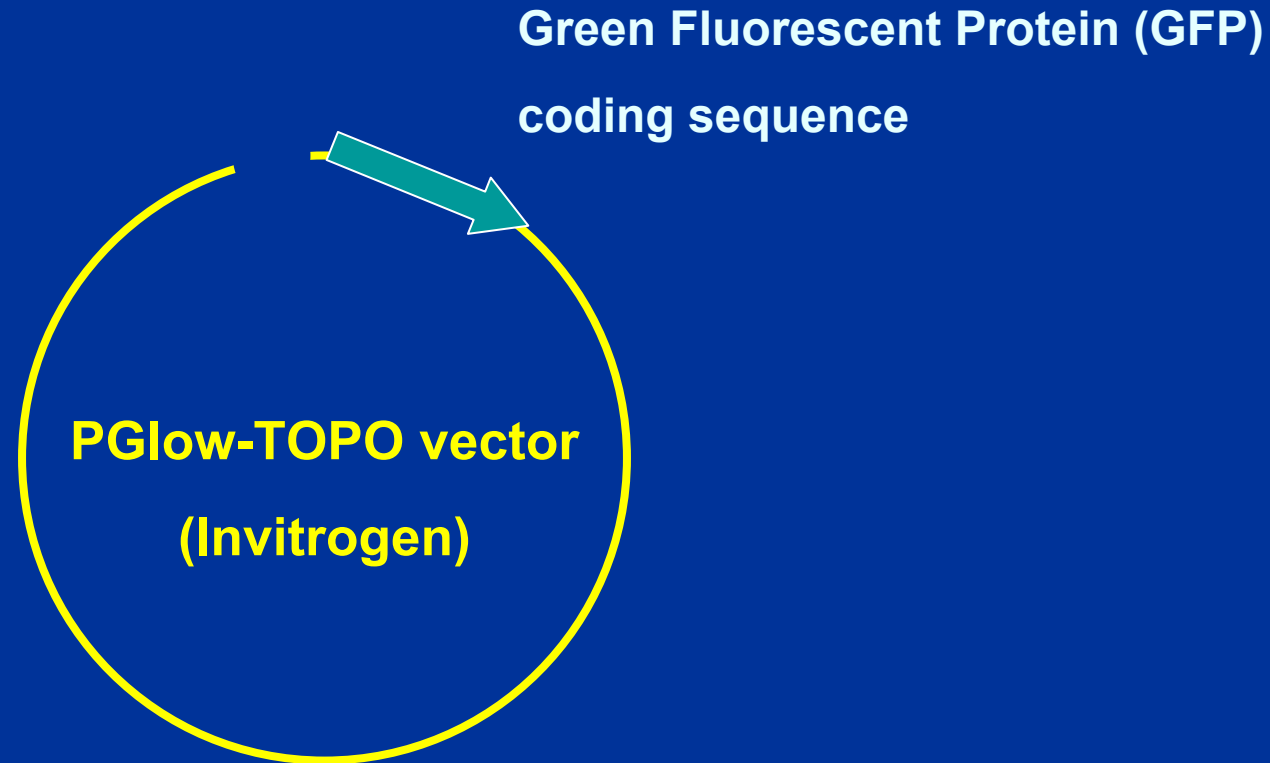
# These Poly-G Tracts Vary in Length

			Nichols	Chicago	Bal 73-1
<i>tprE</i>					
		+1			
8G's	ACAGGGCCGGGGGGGGG	TGAGGTAGCA		10.0	5.0
9G's	ACAGGGCCGGGGGGGGG	TGAGGTAGCA	15.0	35.0	10.0
10G's	ACAGGGCCGGGGGGGGGG	TGAGGTAGCA	50.0	50.0	50.0
11G's	ACAGGGCCGGGGGGGGGGG	TGAGGTAGCA	35.0	5.0	30.0
12G's	ACAGGGCCGGGGGGGGGGGG	TGAGGTAGCA			5.0
<i>tprG</i>					
		+1			
8G's	ACAGGGCCGGGGGGGGG	TGAGGTAGCA		15.0	15.0
9G's	ACAGGGCCGGGGGGGGG	TGAGGTAGCA	55.0	60.0	75.0
10G's	ACAGGGCCGGGGGGGGGG	TGAGGTAGCA	30.0	25.0	10.0
11G's	ACAGGGCCGGGGGGGGGGG	TGAGGTAGCA	15.0		
<i>tprJ</i>					
		+1			
7G's	ACAGGGCCGGGGGGG	TGAGGTAGCA			
8G's	ACAGGGCCGGGGGGGGG	TGAGGTAGCA			
9G's	ACAGGGCCGGGGGGGGG	TGAGGTAGCA	35.0	25.0	35.0
10G's	ACAGGGCCGGGGGGGGGG	TGAGGTAGCA	60.0	75.0	65.0
11G's	ACAGGGCCGGGGGGGGGGG	TGAGGTAGCA	5.0		

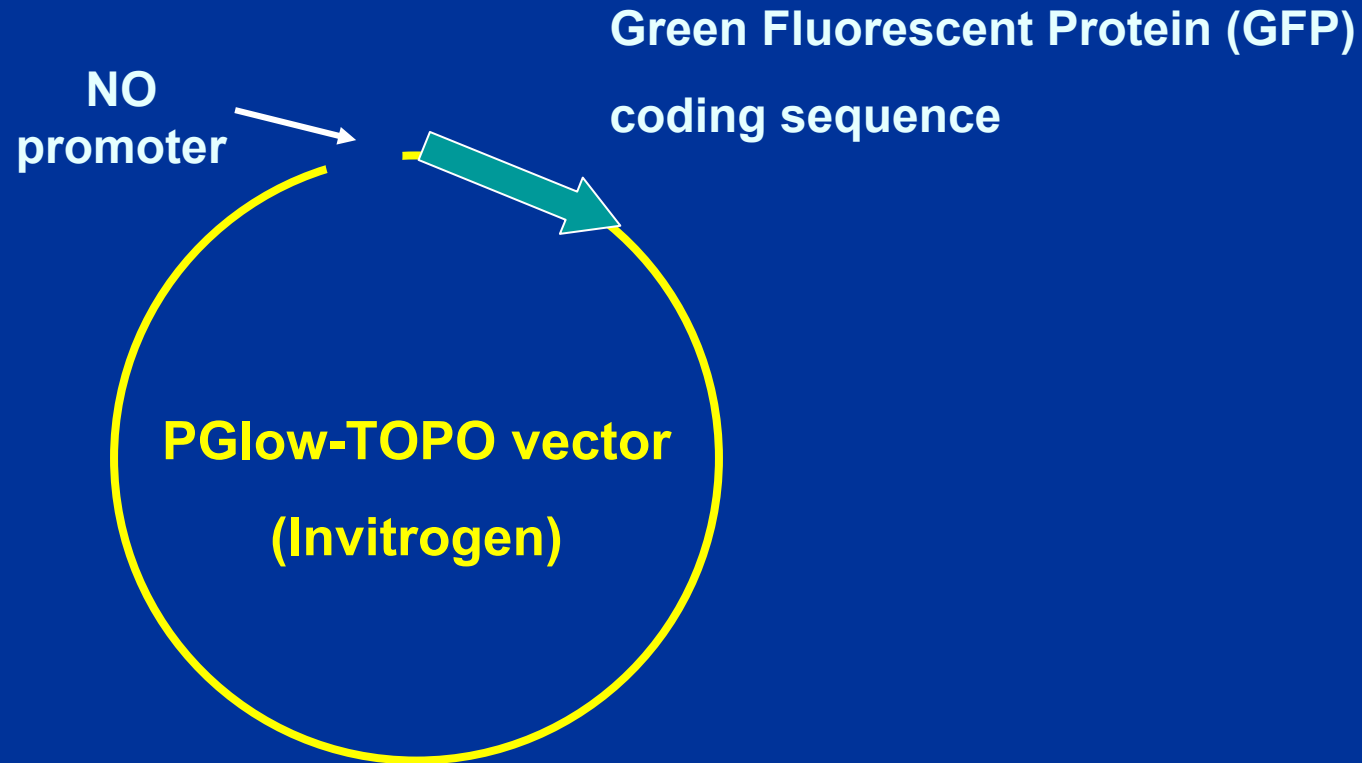
Numbers are % out of 20 clones sequenced



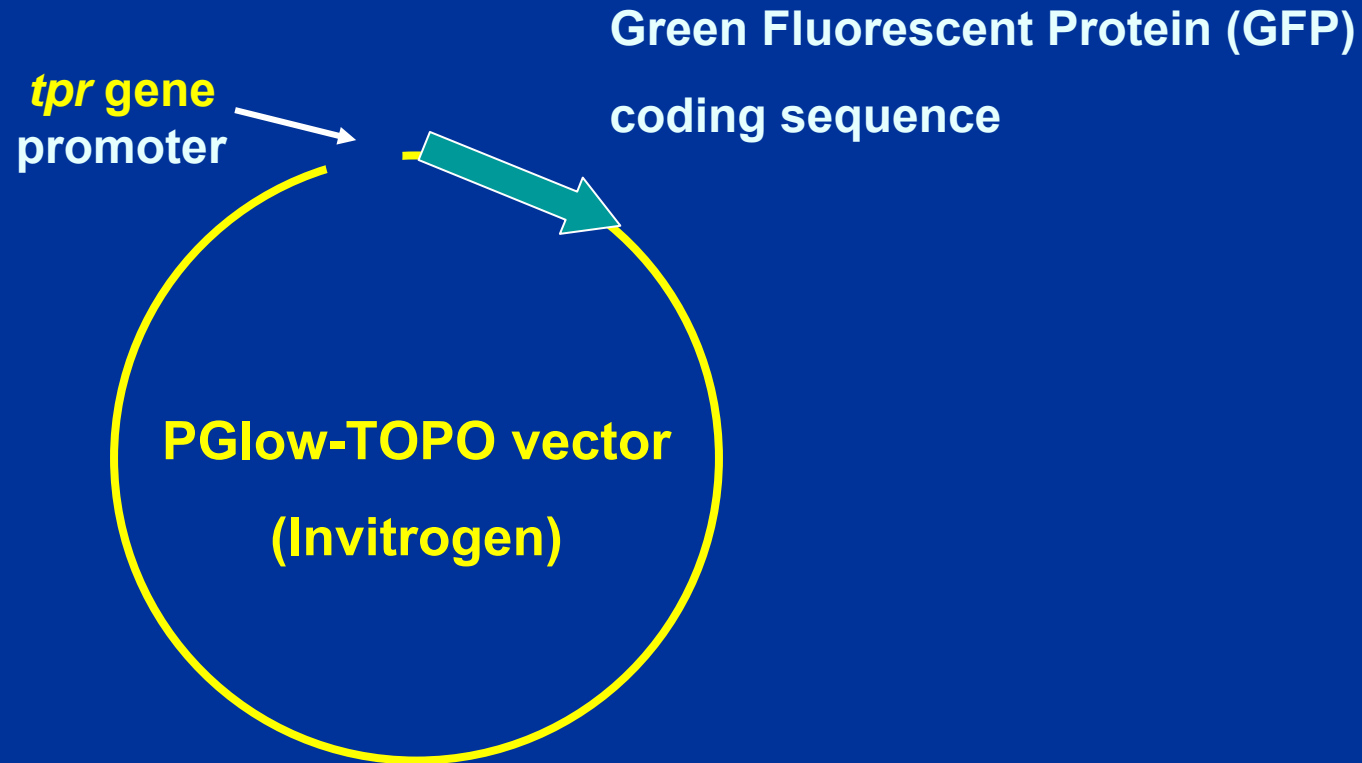
# How to Study Influence of These Poly-G's on *tpr* Transcription?



# How to Study Influence of These Poly-G's on *tpr* Transcription?



# How to Study Influence of These Poly-G's on *tpr* Transcription?



# Experimental Design

Amplify specific *tpr* promoters with a known number of G residues

# Experimental Design

Amplify specific *tpr* promoters with a known number of G residues



Clone the promoters into pGlow-TOPO vector

# Experimental Design

Amplify specific *tpr* promoters with a known number of G residues



Clone the promoters into pGlow-TOPO vector



Transform *E. coli* cells with the various *tpr*/pGlow-TOPO clones

# Experimental Design

Amplify specific *tpr* promoters with a known number of G residues



Clone the promoters into pGlow-TOPO vector



Transform *E. coli* cells with the various *tpr*/pGlow-TOPO clones



Grow liquid cultures, harvest samples at different time points  
and measure GFP fluorescence

# Inventory of *tpr*/pGlow-TOPO Clones

*tprE*

8-12 G's

*tprG*

8-11 G's

*tprJ*

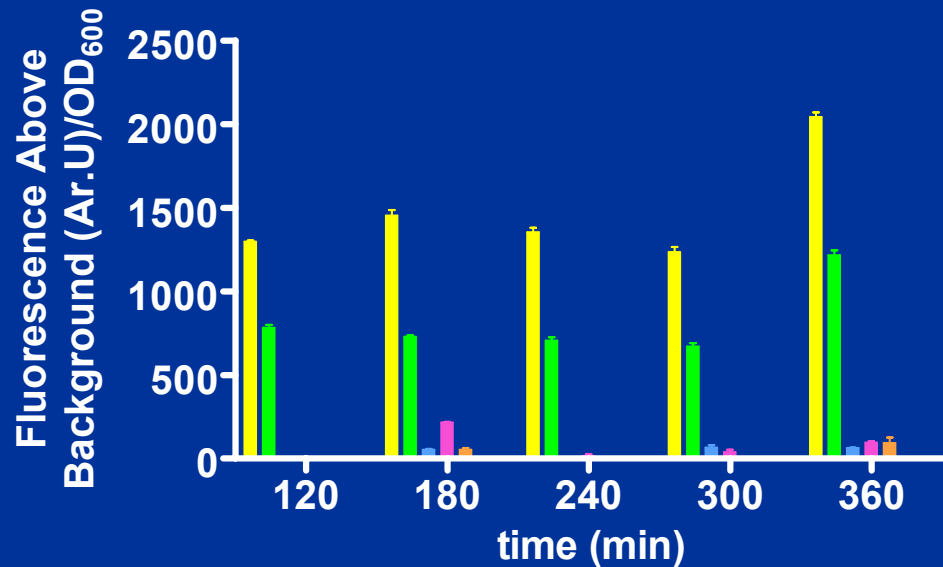
7-11 G's

*tprF/I*

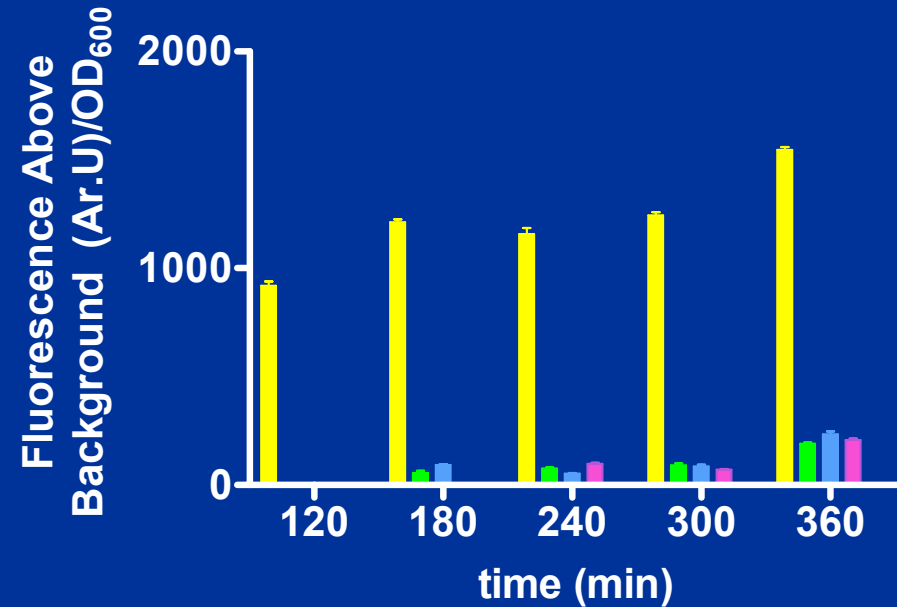
8-10 G's

# *tprJ* and *tprG* Promoters-GFP

*tprJ* promoter

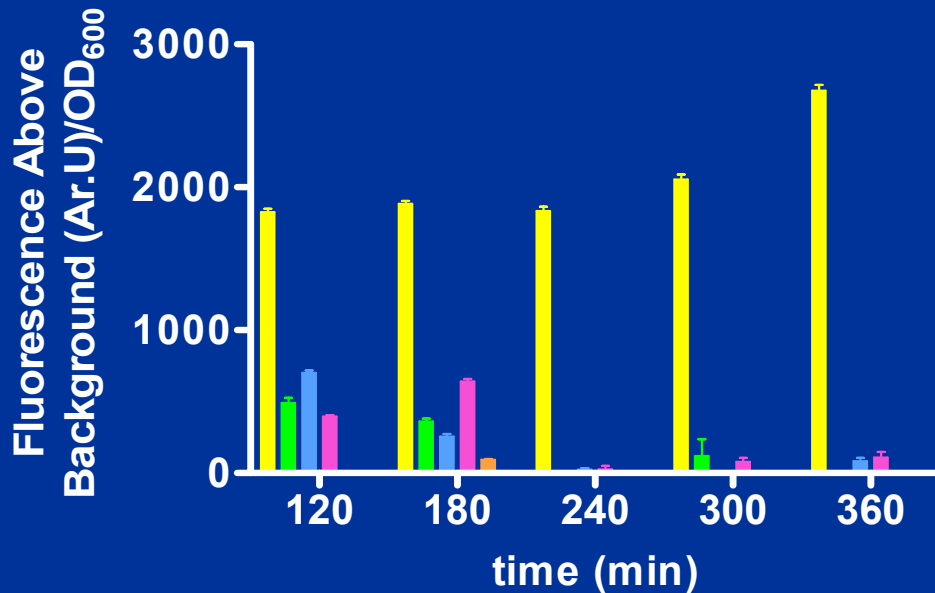


*tprG* promoter

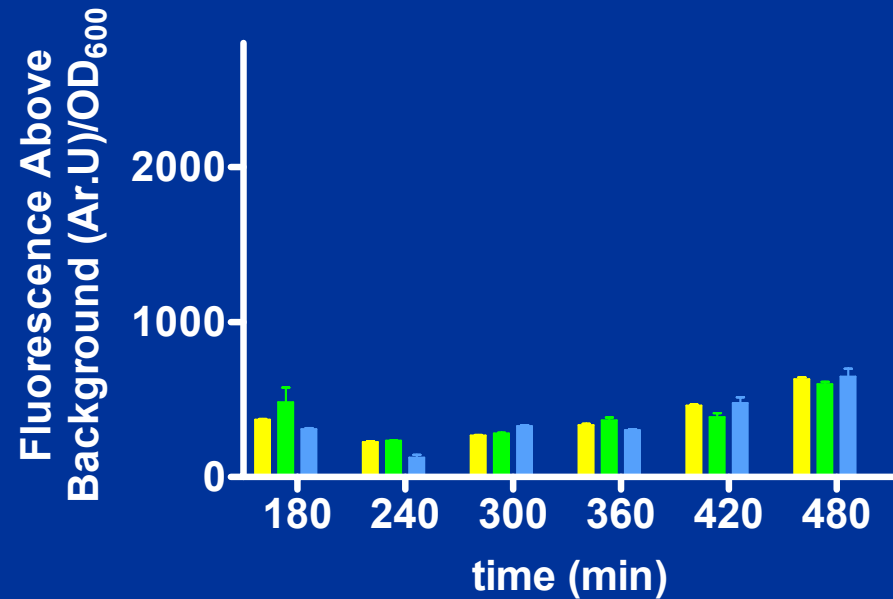


# *tprE* and *tprF//* Promoters-GFP

*tprE* promoter

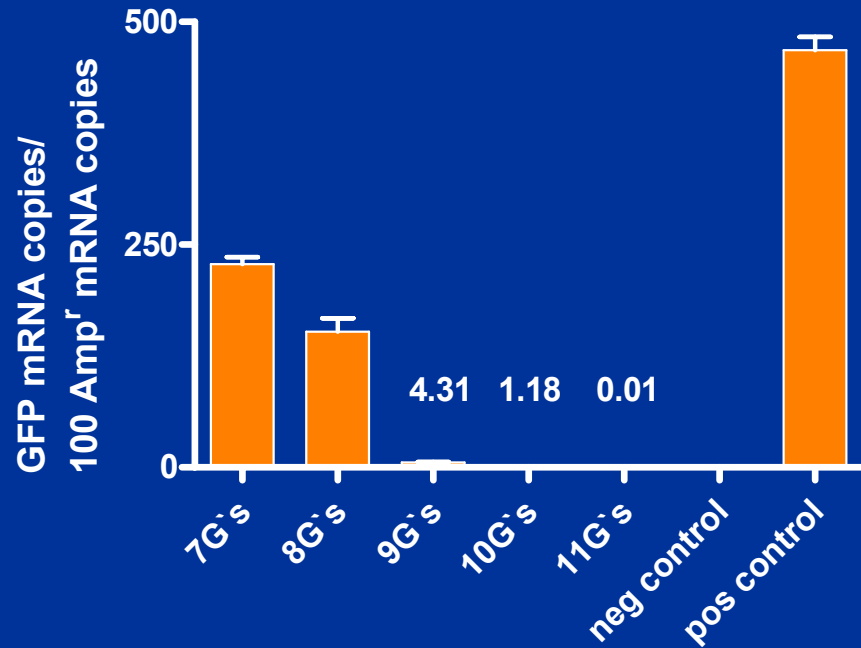


*tprF//* promoters

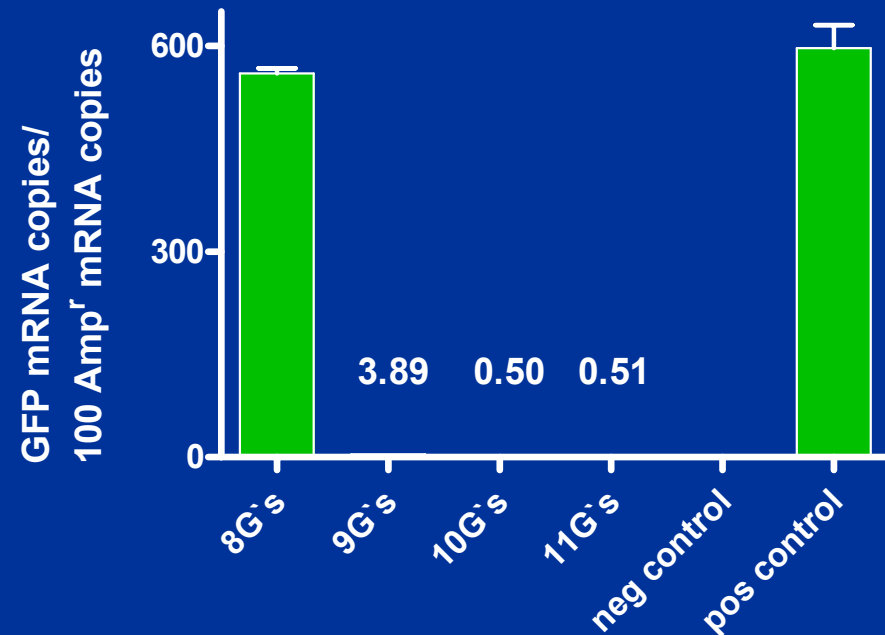


# GFP mRNA Quantification (Real-Time qPCR)

*tprJ* promoter

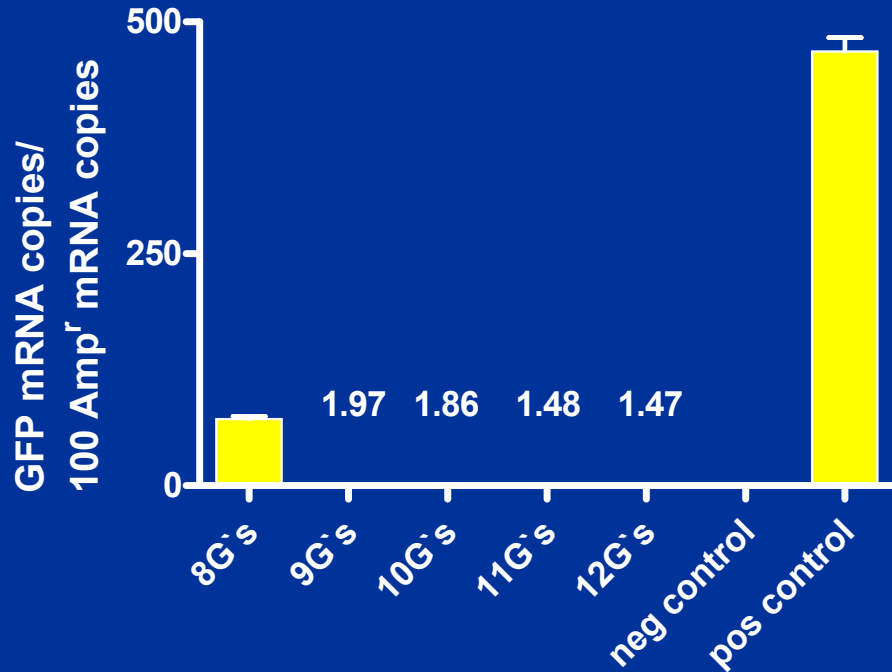


*tprG* promoter

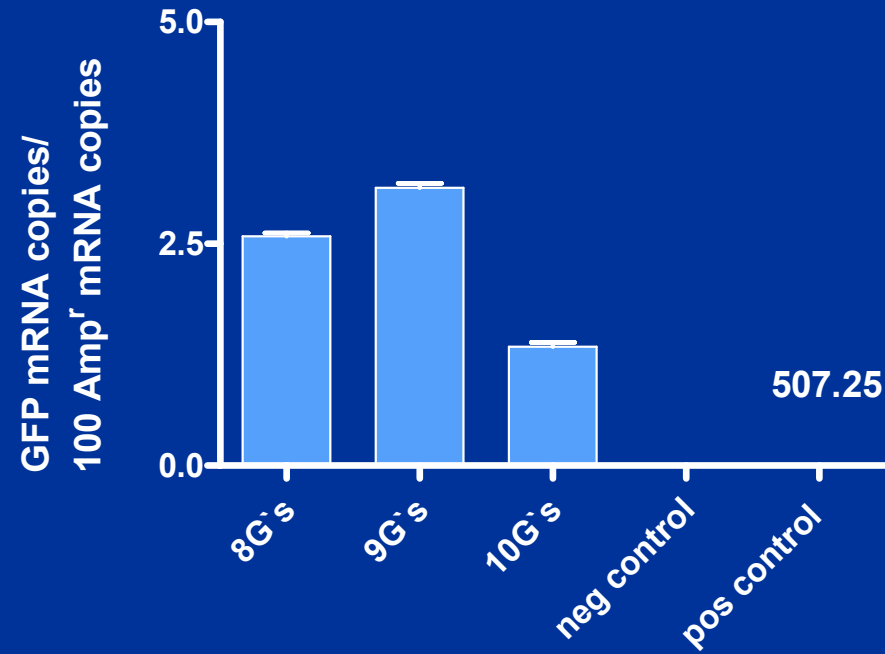


# GFP mRNA Quantification (Real-Time qPCR)

*tprE* promoter



*tprF*//promoters



# Conclusions

- Variation in the poly-G sequence length influences promoter activity of Subfamily II *tprs*

# Conclusions

- Variation in the poly-G sequence length influences promoter activity of Subfamily II *tprs*
- Promoters containing  $\leq 8$  G's induce higher GFP expression than promoters with  $\geq 9$  G's

# Conclusions

- Variation in the poly-G sequence length influences promoter activity of Subfamily II *tprs*
- Promoters containing  $\leq 8$  G's induce higher GFP expression than promoters with  $\geq 9$  G's
- This phenomenon occurs at transcription initiation level

# Conclusions

- Variation in the poly-G sequence length influences promoter activity of Subfamily II *tprs*
- Promoters containing  $\leq 8$  G's induce higher GFP expression than promoters with  $\geq 9$  G's
- This phenomenon occurs at transcription initiation level
- Subfamily I *tprF* and *I* do not share the same mechanism and their promoters appear to be weak

# Conclusions

- Variation in the poly-G sequence length influences promoter activity of Subfamily II *tprs*
- Promoters containing  $\leq 8$  G's induce higher GFP expression than promoters with  $\geq 9$  G's
- This phenomenon occurs at transcription initiation level
- Subfamily I *tprF* and *I* do not share the same mechanism, and their promoters appear to be weak
- Subfamily I *tprF* and *I* transcription is likely to be mostly regulated by the activity of the promoters upstream of *tprG* and *tprJ*, respectively

# THANKS TO

## Sheila Lukehart

Barbara Molini

Charmie Godornes

Troy Leader

Rebecca La Fond\*

Catherine Brissette\*

Heidi Pecoraro

Philip Rodenbough

Linh Le

Paul Brinn

Kristina Bernardi

## Arturo Centurion-Lara

Cristina Guerra

Maritza Puray\*

Karin Hevner\*