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Production and regulation of  
functional amyloid curli fimbriae by  
Shiga toxin-producing *Escherichia*  
*coli*.

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Eastern Regional Research Center,  
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Wyndmoor, PA

# Shiga Toxin-Producing *E. coli* (STEC)

- Food borne; Ruminant reservoir
- Associated with HC and HUS in humans
- Produce Shiga-like toxin(s) (I, II, variants) – encoded on lambdoid prophage
- Serotype O157:H7 & Serogroups O111, O103, O121, O26, O45, O145, O113
- Attachment and persistence factors
  - Reservoir (ruminants), lairage
  - Food and manufacturing surfaces
  - Human host and disease

# Why are curli important in STEC-associated food safety?

## Host - 37 °C

Curli deletion results in **marked reductions in adhesion to Caco-2 cells at 37 °C** (Rosser *et al.*, 2008, *Infect. Immun.* **76**:5598-5607).

Deletion of curli/cellulose genes significantly **reduced adhesion to HT-29 colonic cells at 37 °C** (Saldaña *et al.*, 2009, *Environ. Microbiol.* **11**:992- 1006).

Curli-expressing serotype O157:H7 variants were **more invasive** for HeLa and HEP-2 cells, and **more virulent** in a mouse model than their non-curliated parents (Uhlich *et al.*, 2002, *Infect. Immun.* **70**:395-399).

## Environment - 30 °C

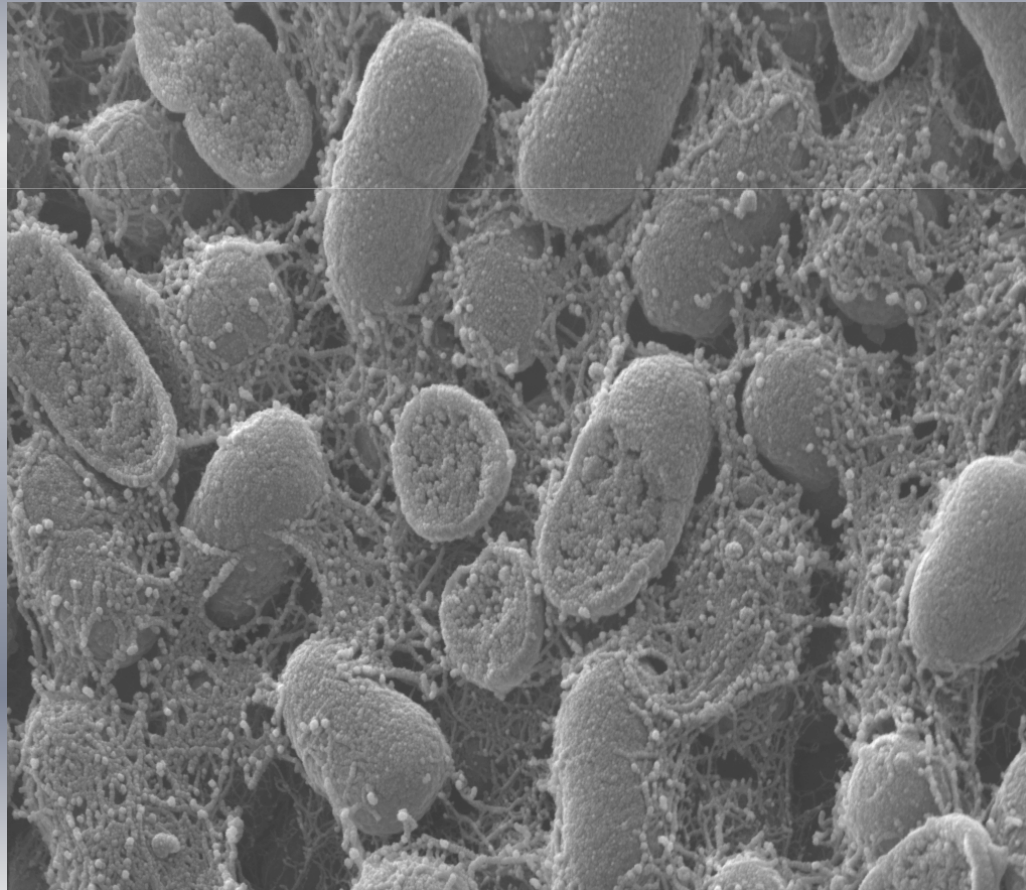
Strong **correlation between biofilm formation and curli expression** of in serotype O157:H7 (Tafarello *et al.*, 2011, *Appl. Environ. Microbiol.* **77**:2201-2208).

In serotype O157:H7 **robust biofilm formation was dependent on curli expression**; in non-O157 STEC, **cellulose and curli both affect biofilm formation** (Uhlich, *et al.*, 2014, *FEMS Microbiol. Lett.* **354**:133-141).

**Curli expression has a critical role in STEC biofilm formation and resistance to sanitizers** (Wang *et al.*, 2012, *J. Food Protect.* **75**:1418-1428).

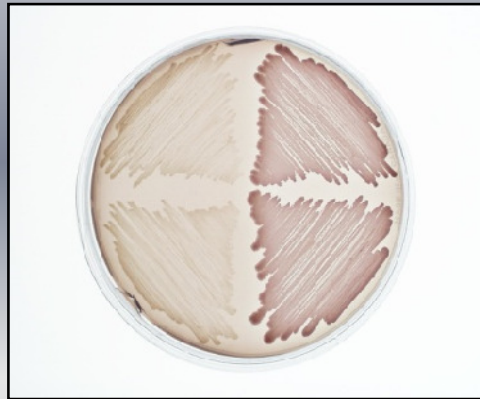
# Biofilm

Multicellular behavior on surfaces characterized by expression of **curli fibers** and polysaccharides, such as **cellulose**, that encase bacteria in a protective matrix

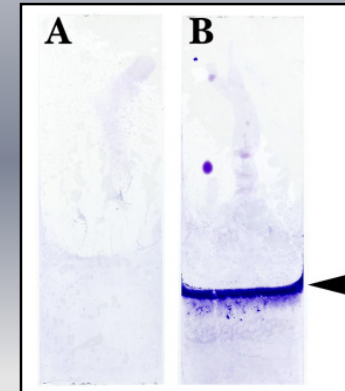


# Measuring Curli / Biofilm in the Lab

Curli

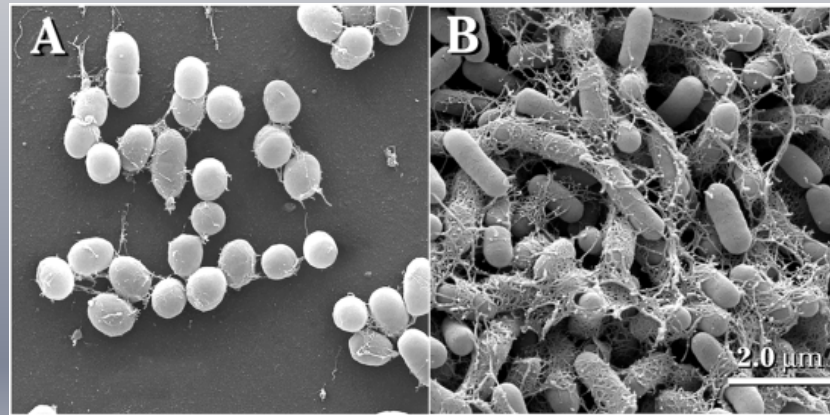


Biofilm



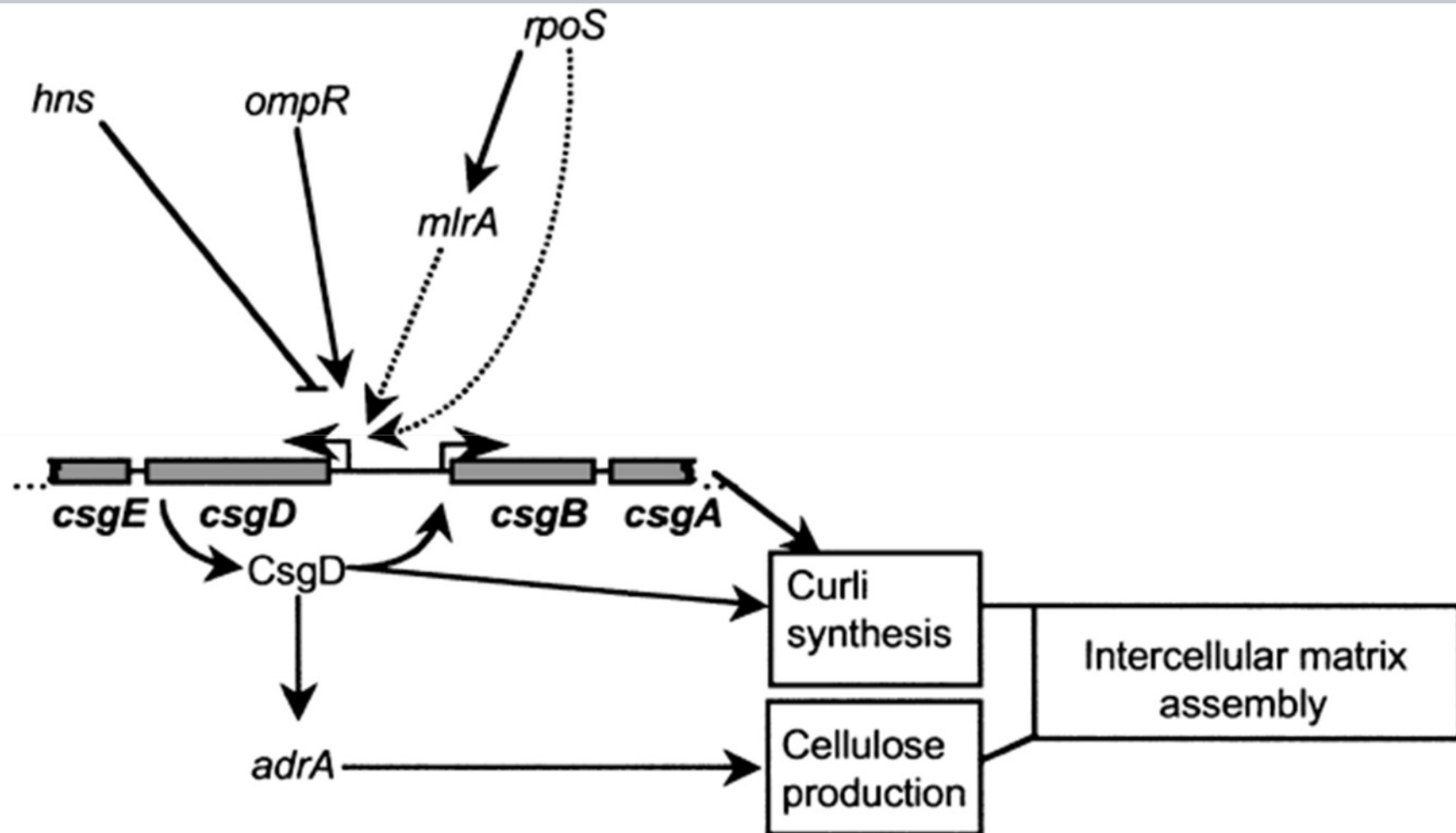
Congo red Indicator Plates

Crystal Violet Staining

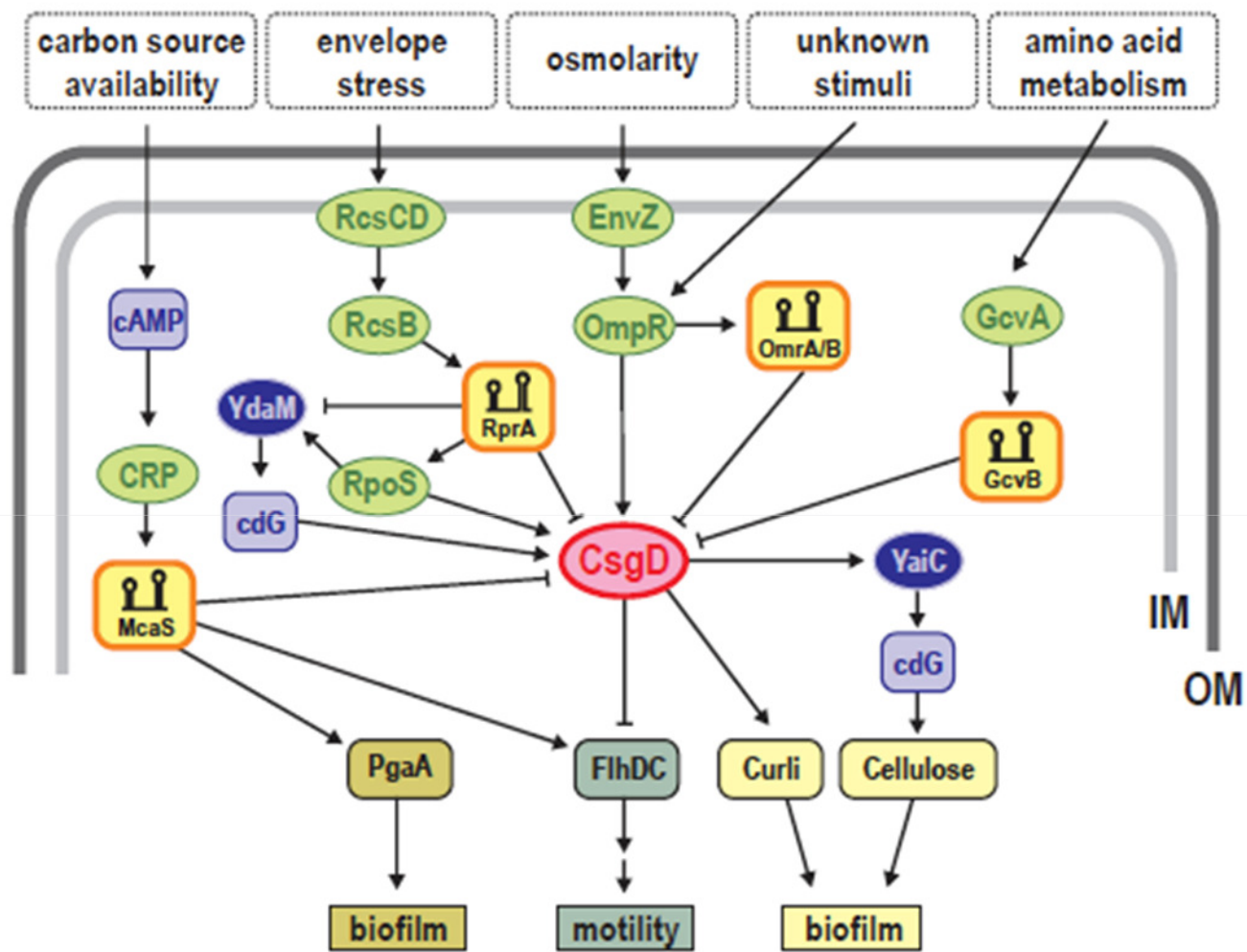


Electron Microscopy









**Fig. 1.** Regulatory network controlling *csgD*.

# CsgD regulation

Sigma factors (2): RpoS, RpoD

Protein transcription factors:

two-component systems (4): EnvZ/OmpR, RstB/A,  
RcsC/B, CpxA/R

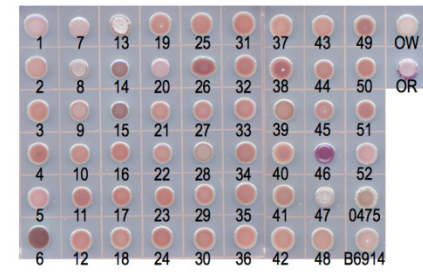
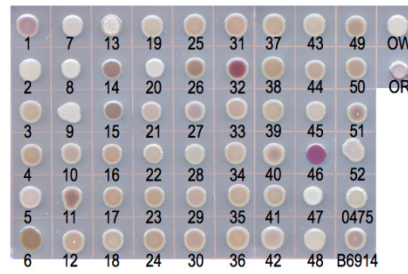
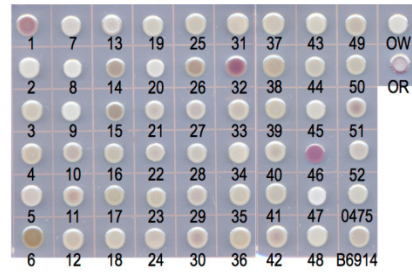
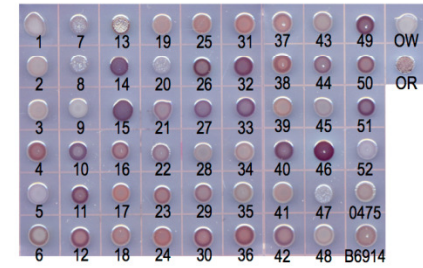
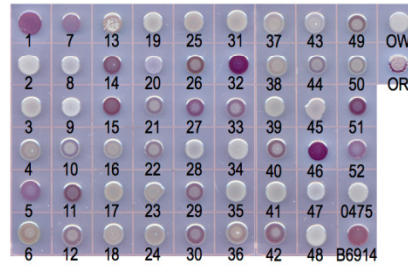
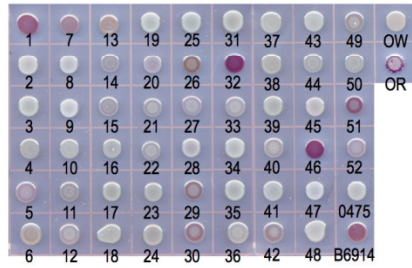
single-component systems (2): MlrA, Crp

DNA-bending nucleoid proteins (2): IHF, H-NS

Small proteins affecting RpoS (3): Crl, IraP, FliZ

Small regulatory RNAs (sRNA) (5): OmrA, OmrB, RprA, GcvB,  
McaS

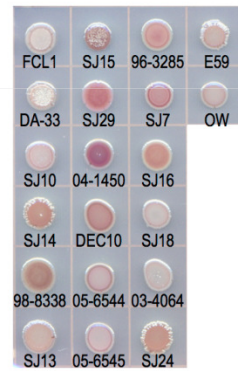
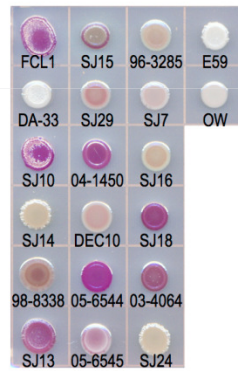
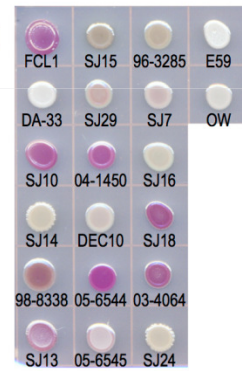
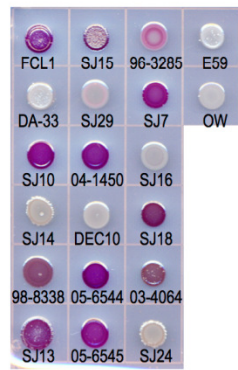
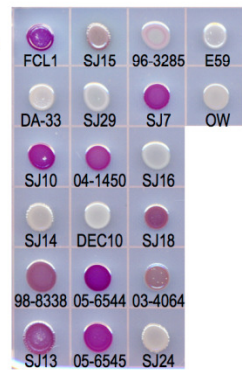
GGDEF/EAL proteins controlling c-di-GMP (6): YdaM/YciR, YeaP  
YegE/YhjH, YhdA

**A CRI****TA**

25°C

30°C

37°C

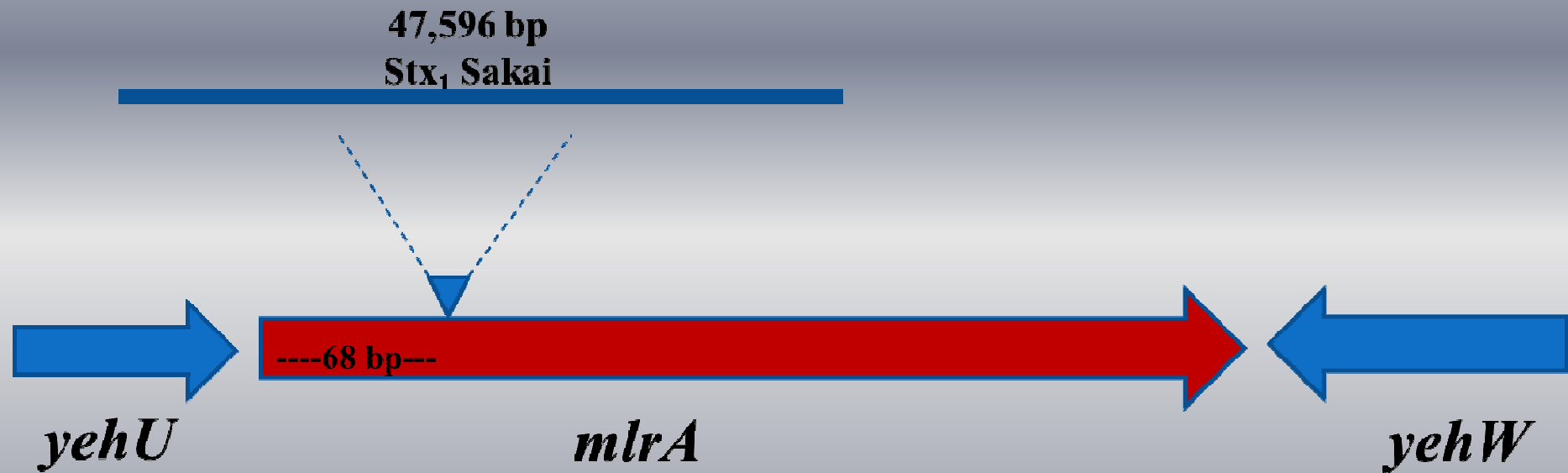
**B CRI****TA**

25°C

30°C

37°C

*mlrA* encodes a prophage insertion site



# Survey of O157:H7 clinical isolates











## Serotype O157:H7 – biofilm barriers

- >96% carried a prophage ( $\pm stx_1$ ) insertion in *mlrA*

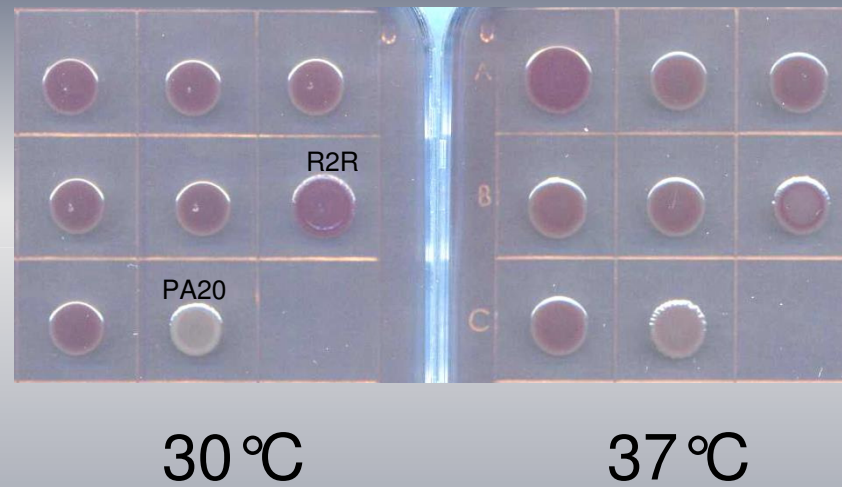
## Non-O157:H7 STEC – biofilm barriers

- <22% carry prophage insertions in *mlrA*

# MlrA complementation

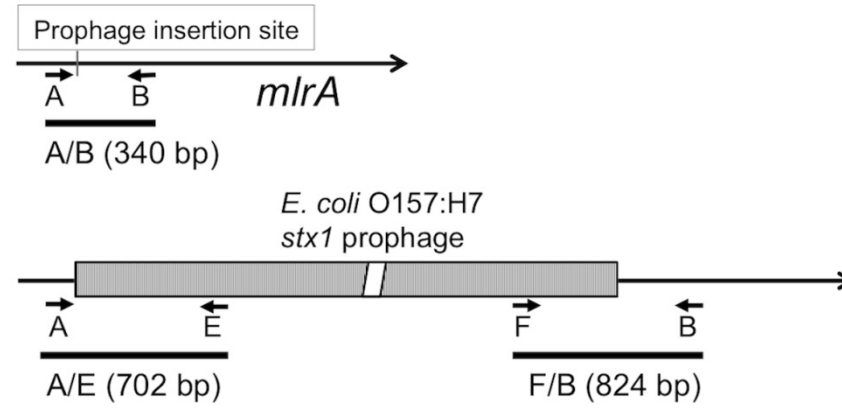
	phage	<i>rpoS</i>	WT	+ <i>mlrA</i>
<b>3</b>	Φ	T		
<b>23</b>	Φ	T		
<b>1</b>	Φ	WT		
<b>46</b>	Φ	WT		
<b>43895</b>	Φ	WT		

# PA20-R2R: *mlrA* restored

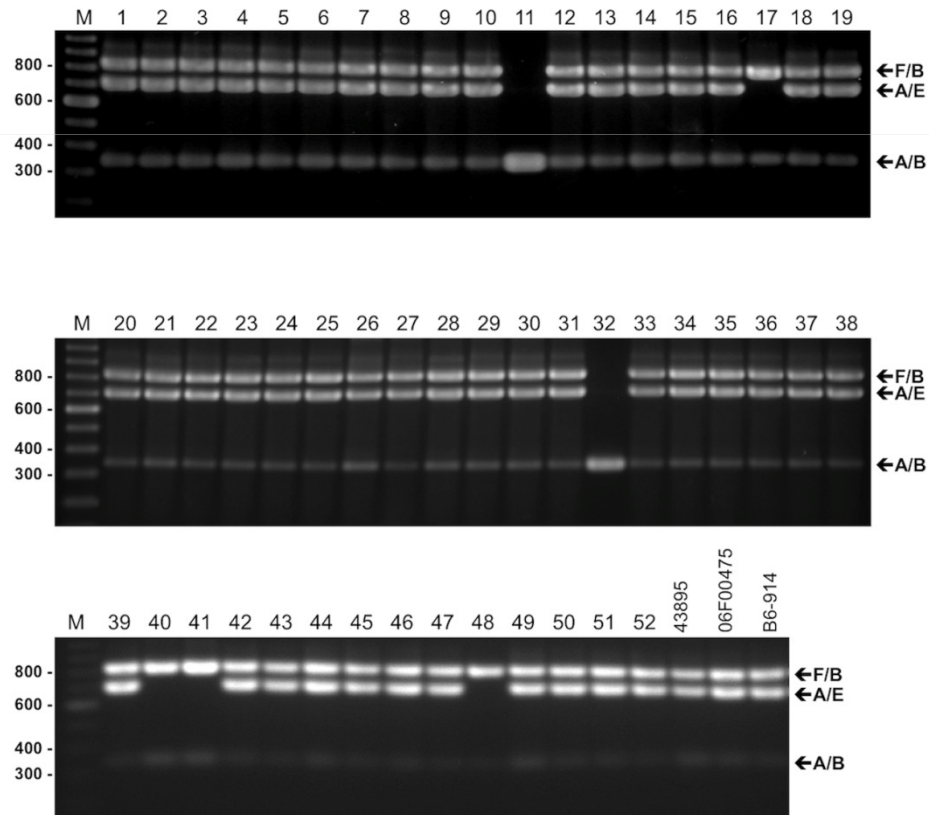


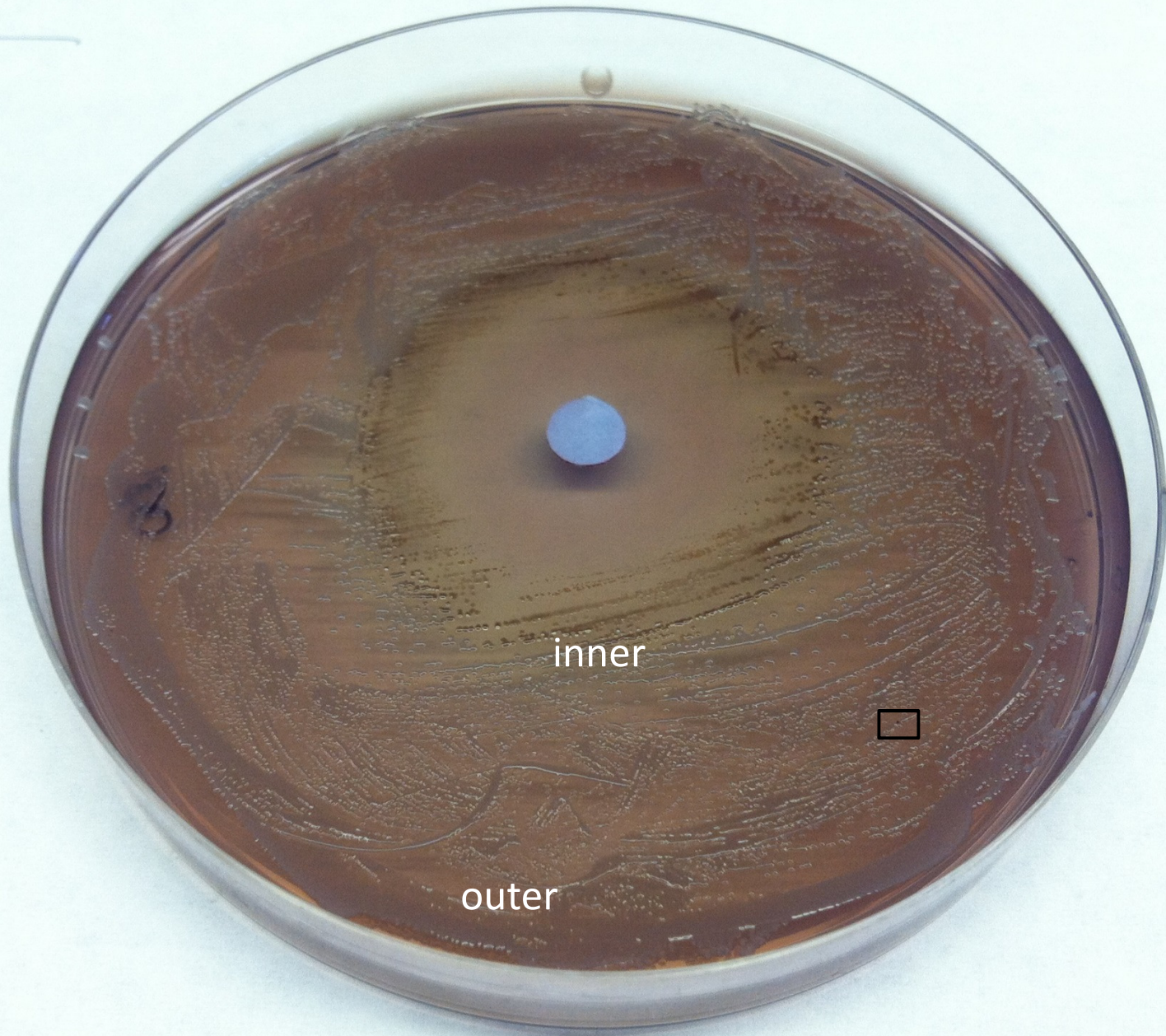


A



B



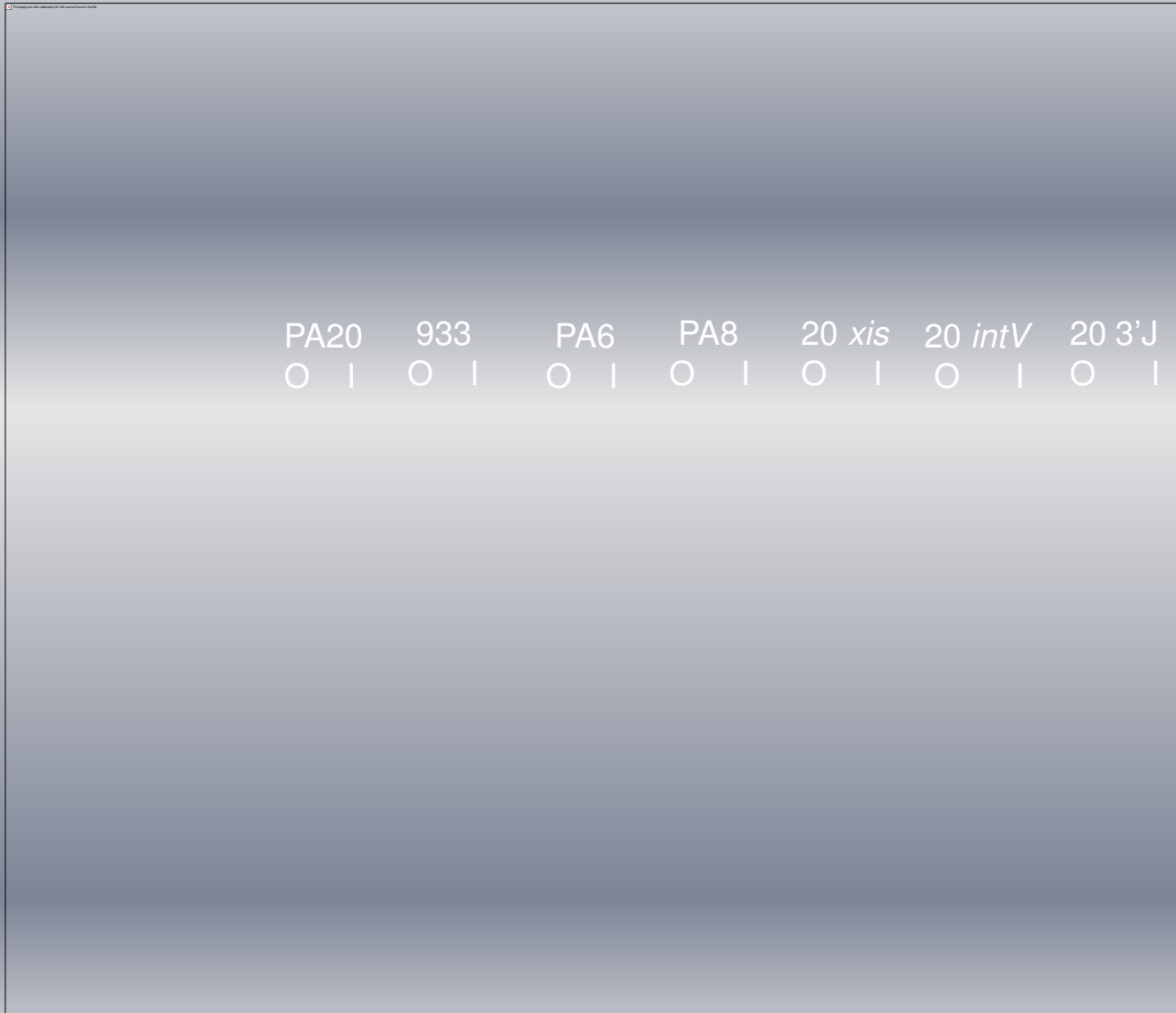


inner

outer

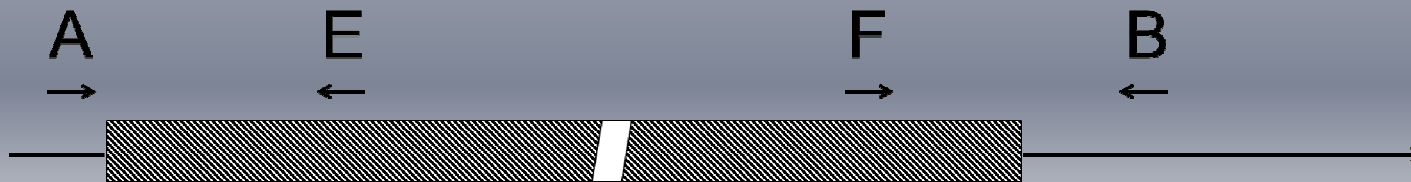


# SMX/TM increases ancestral *mlrA*



# qRT-PCR

	PA20		PA20 control	
	inner	Outer	inner	outer
% AB	21.80	3.48	5.41	2.75
I/O (% AB)	6.26		1.97	



# SMX/TM stimulates biofilm formation

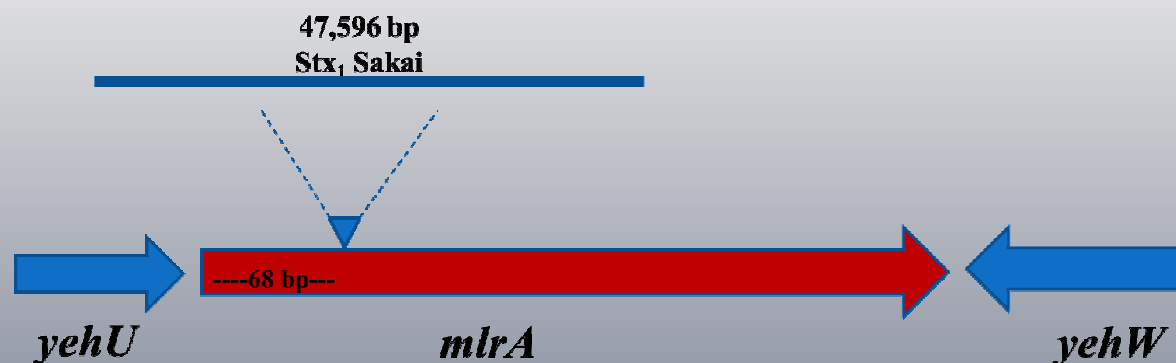
LSD	c	bc	ab	a	bc	b	b	bc	bc
SMX/TM	media	27x	9x	3x	20/4 µg/l	0.333x	0.111x	0.0370x	0
Trial 1	0.0874	0.1124	0.1725	0.2148	0.1193	0.1327	0.1450	0.0927	0.1014
	0.0856	0.0939	0.1020	0.1097	0.1136	0.1617	0.1181	0.0960	0.1509
	0.0817	0.0979	0.1026	0.1071	0.1667	0.1190	0.1077	0.0984	0.0900
	0.0808	0.0873	0.1336	0.1021	0.1065	0.1099	0.1095	0.1071	0.0915
	0.0834	0.0956	0.1043	0.1023	0.1068	0.1131	0.1064	0.1155	0.0933
	0.0882	0.0950	0.1025	0.0940	0.1149	0.1356	0.1083	0.1342	0.0941
Trial 2	0.0760	0.1079	0.1145	0.1484	0.0900	0.1170	0.1283	0.1210	0.1303
	0.0733	0.0946	0.1153	0.1341	0.0909	0.1102	0.1383	0.1047	0.1017
	0.0731	0.0983	0.1152	0.1217	0.0900	0.1063	0.1235	0.1098	0.0934
	0.0726	0.0981	0.1187	0.1239	0.0910	0.1063	0.1196	0.1108	0.0942
	0.0774	0.1034	0.1208	0.1164	0.0893	0.1033	0.1285	0.1056	0.1039
	0.0723	0.1092	0.1488	0.1184	0.0968	0.1103	0.1197	0.1088	0.1006
Trial 3	0.0895	0.1147	0.1227	0.1614	0.1053	0.0999	0.1048	0.1056	0.1126
	0.0844	0.1054	0.1291	0.2049	0.0951	0.1047	0.0929	0.0981	0.0996
	0.0829	0.1149	0.1251	0.1803	0.0963	0.0968	0.0911	0.1130	0.0978
	0.0986	0.1116	0.1328	0.1666	0.1141	0.1174	0.1071	0.1085	0.1048
	0.0838	0.1224	0.1324	0.2130	0.0968	0.1056	0.0999	0.1024	0.0977
	0.0922	0.1123	0.1222	0.2671	0.0979	0.0975	0.0995	0.1264	0.1092



# PA20 expression studies

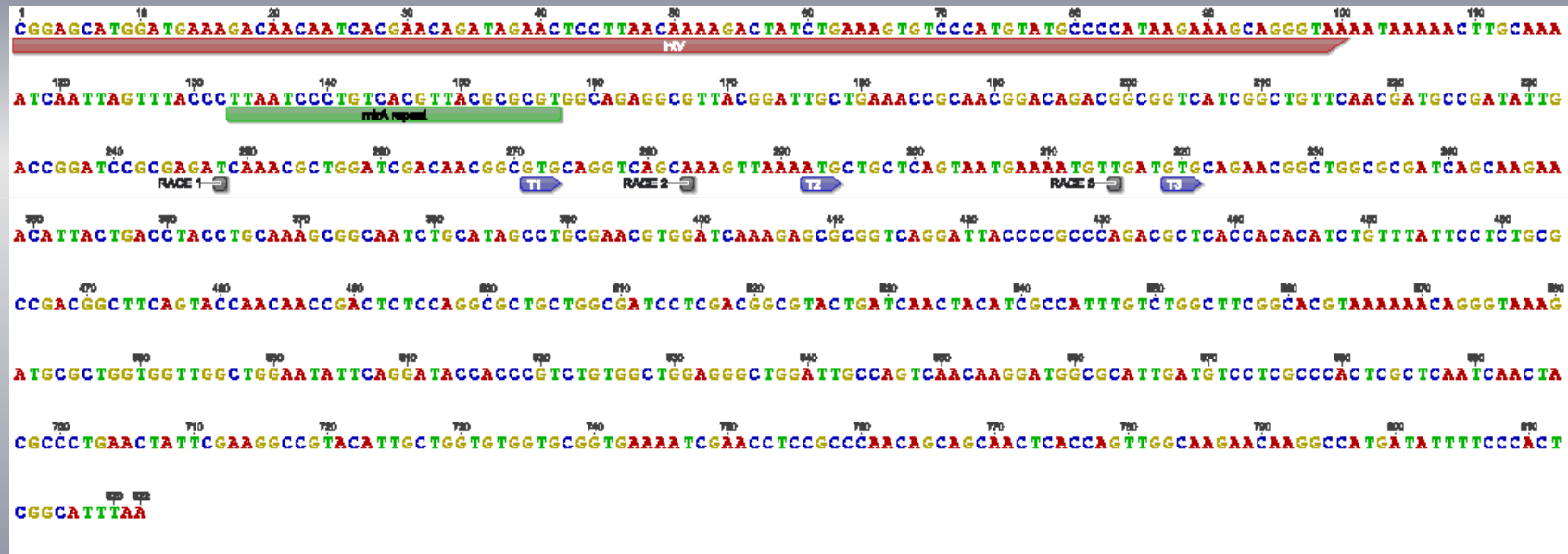
Ancestral *mlrA* (primers A/B): CT = 36.9

Distal *mlrA* (primers 3' to prophage): CT = 26.5



93% of *mlrA* coding sequence is 3' to the prophage

# Distal *mlrA* encodes potential truncated proteins





# Cloning

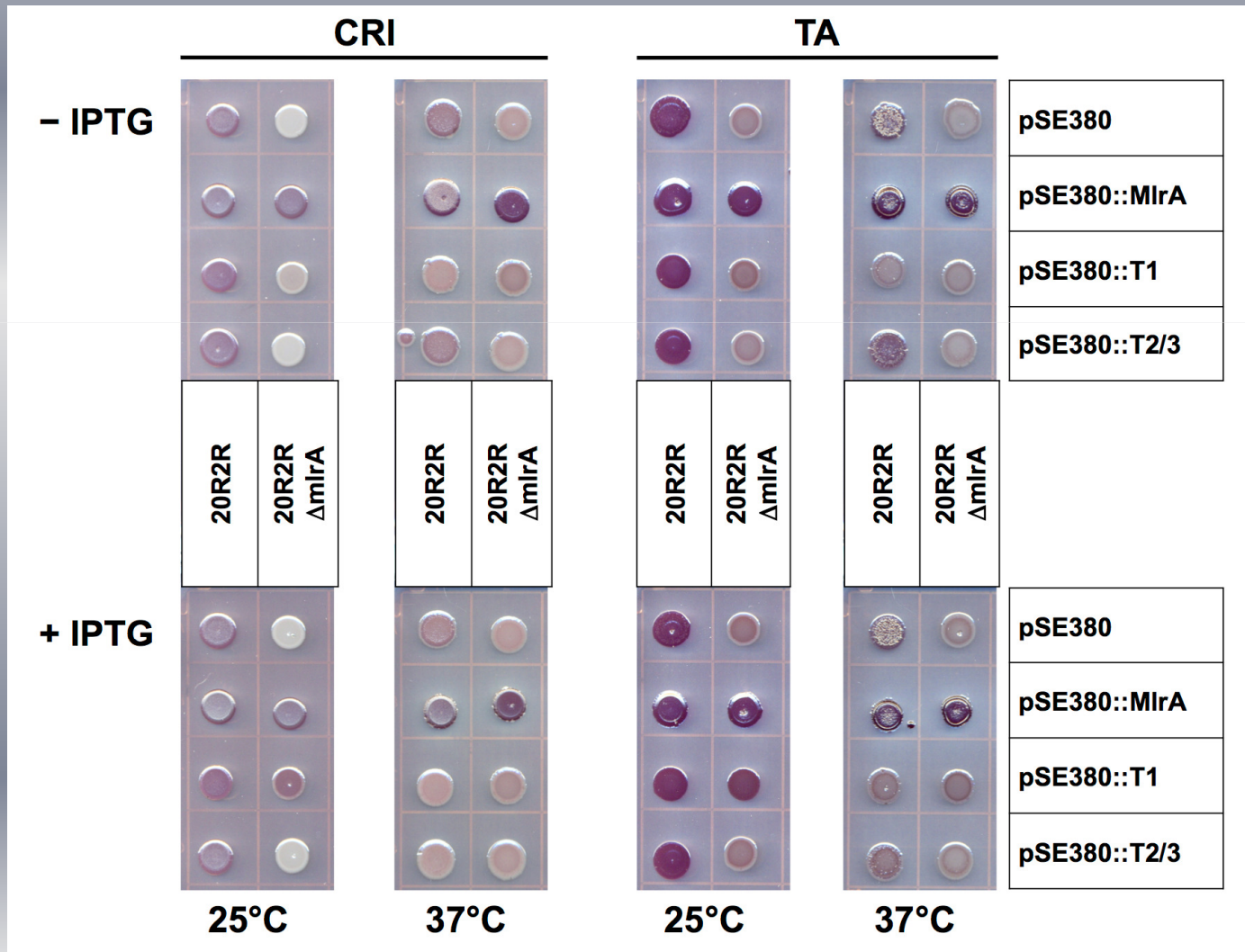
T1, T2, T3 coding region

- GST fusion protein
- Histidine Tag fusion protein

5' RACE Transcripts 1 and 2

- pSE380 - inducible *lacZ* promoter w/o RBS
- Native protein

# Truncated MlrA proteins regulate CR affinity



# DNA binding assays: MlrA and Truncates with the *csgD* promoter

- GST proteins captured on magnetic beads + plasmid cloned target sequences IGS1 and IGS2 (95 nt each)
- Detection by RT-PCR: bound target/input target x 100 = % bound by protein



# MlrA, but not the truncated proteins, bind the *csgD* promoter

	IGS1 % bound	IGS2 % bound
GST	5	≤1
GST-MlrA	5	30
GST	≤1	≤1
GST-T1	≤1	≤1
GST-T2	≤1	≤1
GST-T3	≤1	≤1

# C-di-GMP

- Second messenger signaling molecule
- Controlled by diguanylate cyclase (DGC) and phosphodiesterase (PDE) pairs – 29 proteins in *E. coli*
- Temporal expression or functional sequestration gives specificity

# YdaM/YciR & YegE/YhjH control CsgD by affecting MlrA

- Two DGC/PDE pairs (control modules) control *csgD* transcription by affecting *mlrA*:
  - YegE (DCG) / YhjH (PDE) - ↑*csgD*, ↓motility
  - YdaM (DCG) / YciR (PDE) - ↑*csgD*
- The control modules function in a cascade, connected by the bi-functional trigger enzyme, *yciR*
- YdaM, YciR and MlrA all bind to each other using multiple domain contacts
- YciR inhibitory role is due to direct contact and that inhibition is antagonized by c-di-GMP from module 1
- YdaM activation of MlrA is by direct interaction

# Conclusions

- Curli deficiencies result from prophage insertions in *mlrA* in serotype O157:H7
- SMX/TM will increase ancestral *mlrA* to levels sufficient for biofilm formation
- Transcripts encoding truncated *mlrA* proteins are expressed from the distal prophage / *mlrA*
- Truncated *mlrA* products can modulate CR phenotypes
- Truncated products do not bind the *csgD* promoter and may rely on protein contacts



# Acknowledgments

- Chen-Yi Chen
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- George Paoli
- Peter Irwin



*Questions?*



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