



In vivo Gene Regulation in
Salmonella spp. by a Salicylate-
Dependent Control Circuit

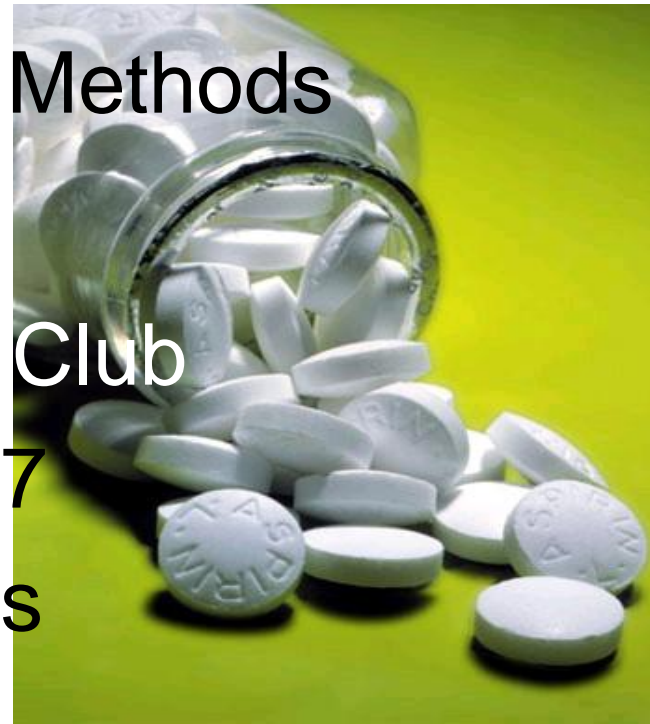
“Pathogens on Aspirin”

Royo et al. 2007. Nature Methods
4:937-942.

Microbiology Journal Club

December 3, 2007

Matthew R. Evans



Agenda

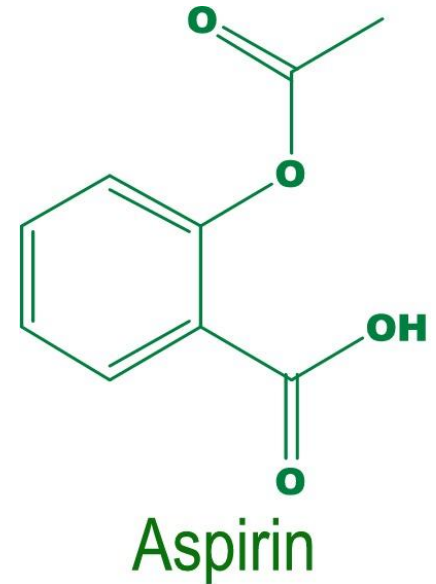
- Introduction
 - *Salmonella*
 - Aspirin
 - Limitations of current expression systems
 - Desirable traits of expression systems
- Objectives
- How the system works
- Results
 - *In vitro* studies
 - Preliminary work with Hela cells
 - Expression in mice tumors
 - Expression of pro-drug converting enzyme in mice tumors
- Summary and conclusions
- Discussion

Salmonella: Quick Facts

- Facultatively anaerobic, Gm⁻, bacillus nonsporeforming, and motile
- At least 4% of *S. Typhimurium* genome required for full virulence in mice (Bowe et al., 1998)
- Sources: water, soil, insects, animal feces, raw meats, factory or kitchen surfaces, etc.
- *S. Typhimurium* LT2: 4,857-kb chromosome and 94-kb virulence plasmid (McClelland et al., 2001)
 - SPI's: 1 & 2 are for invasion and intracellular replication, respectively
- Has been shown to infiltrate and replicate within tumors (Pawelek et al., 1997)

Aspirin (Acetylsalicylic acid)

- Salicylate drug
 - Analgesic
 - Antipyretic
 - Anti-inflammatory
 - Anti-platelet
- Half-life
 - 300-650mg dose: 3.1-3.2 hrs., 1g dose: 5 hrs.
- Dosage
 - Adults: 300-1000mg, 4X's daily (max. 8000mg)
 - Toxic dose: >150mg/kg body mass
 - Lethal dose: >500mg/kg body mass



Current Expression Systems: Limitations

- Studying host-pathogen interactions
 - Toxicity
 - Side effects of inducers
 - Leakiness
 - Cost of inducers
 - Alteration or environmental conditions
 - Not suitable for eukaryotic cells

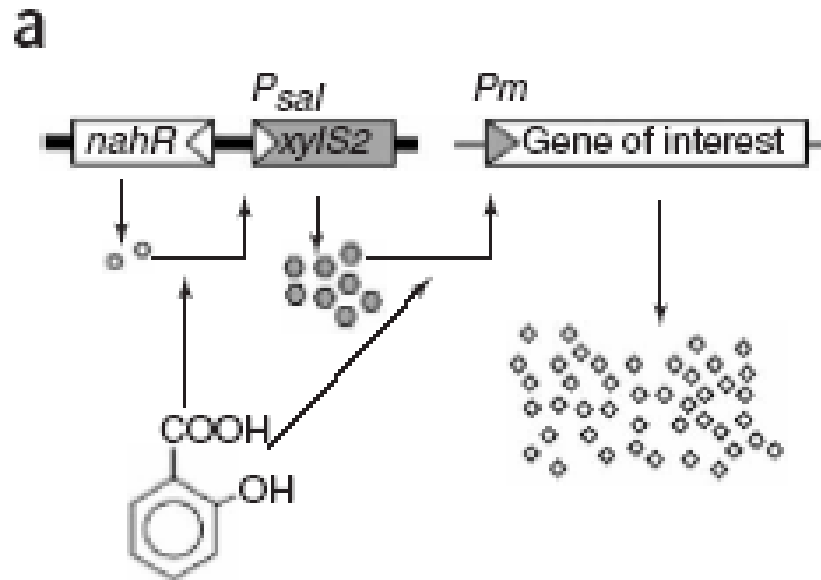
Desirable Expression System

- *In vivo*
- Various stages of infection
- Different environmental conditions
- Negligible basal expression
- High induction ratio
- Non-expensive

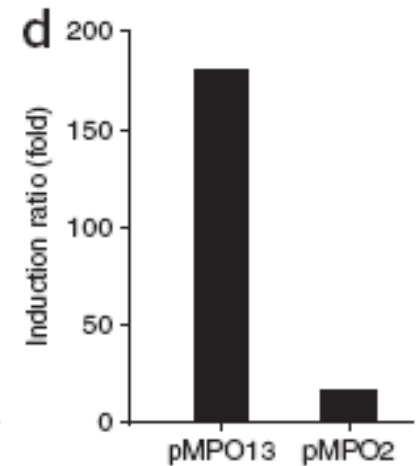
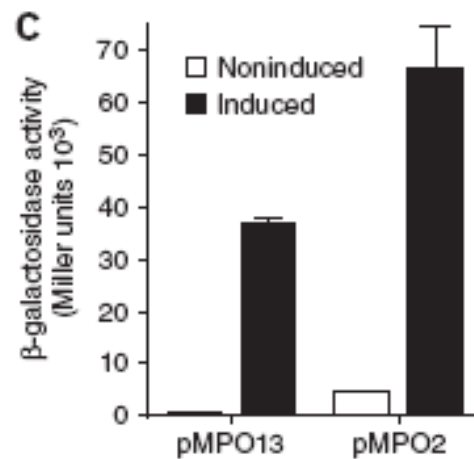
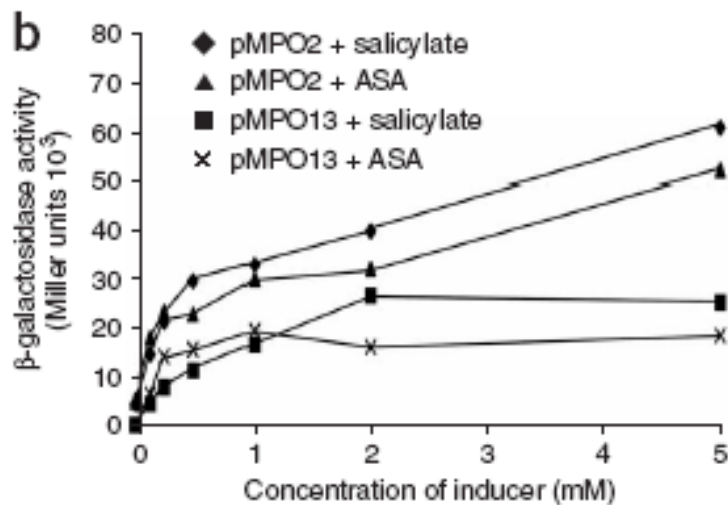
Objectives

- Implement and validate a salicylate-inducible cascade expression system to tightly regulate the *in vitro* expression of a heterologous gene carried by *Salmonella* spp.
- Validate the same system in mice challenged with a fibrosarcoma.

How the system works



Salmonella spp. gene expression in culture medium



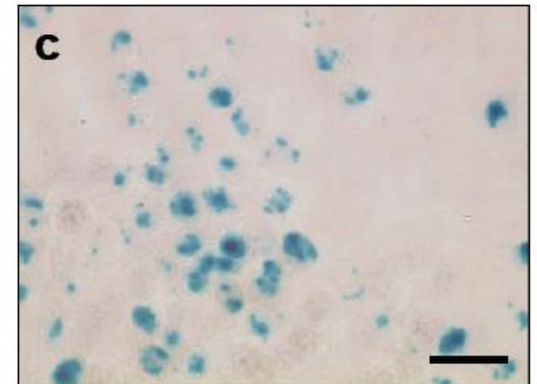
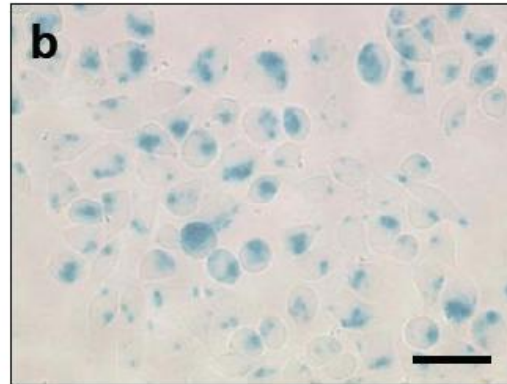
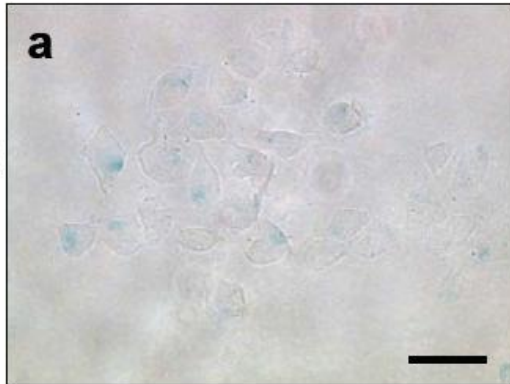
ASA & salicylate induced gene expression in infected cells

Noninduced

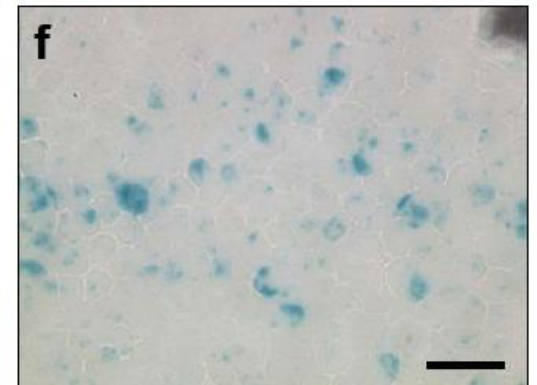
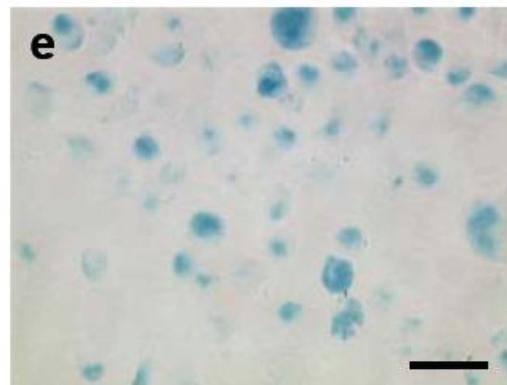
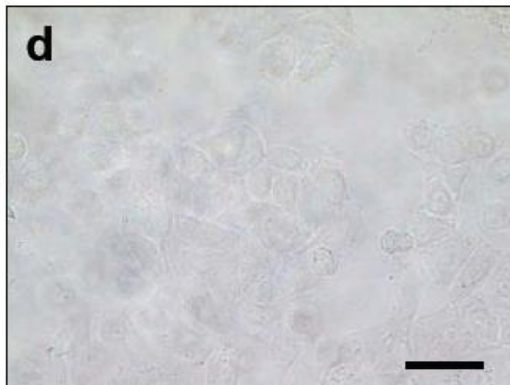
Salicylate

ASA

SL7207-4S2
[pMPO2]

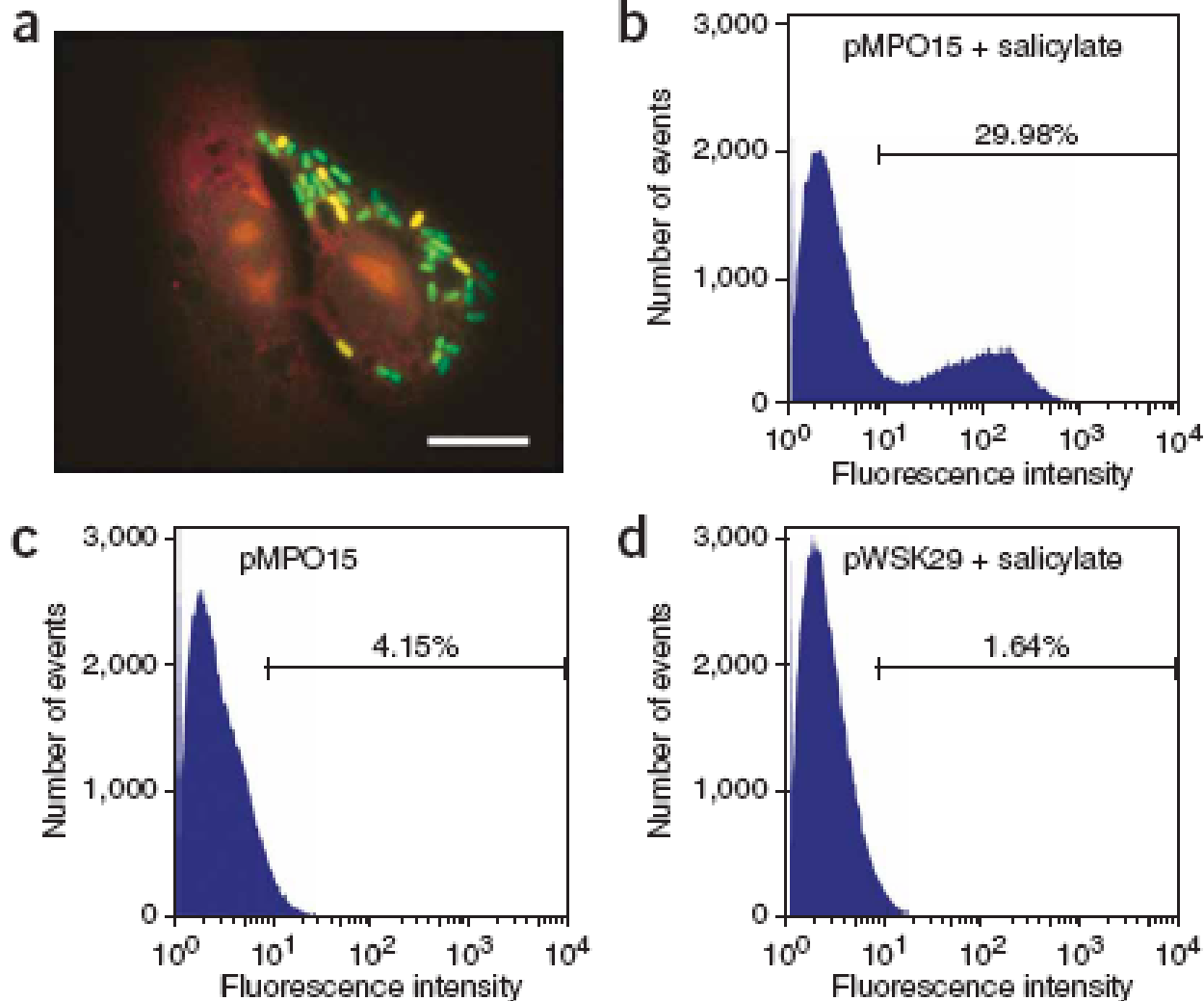


SL7207-4S2
[pMPO13]



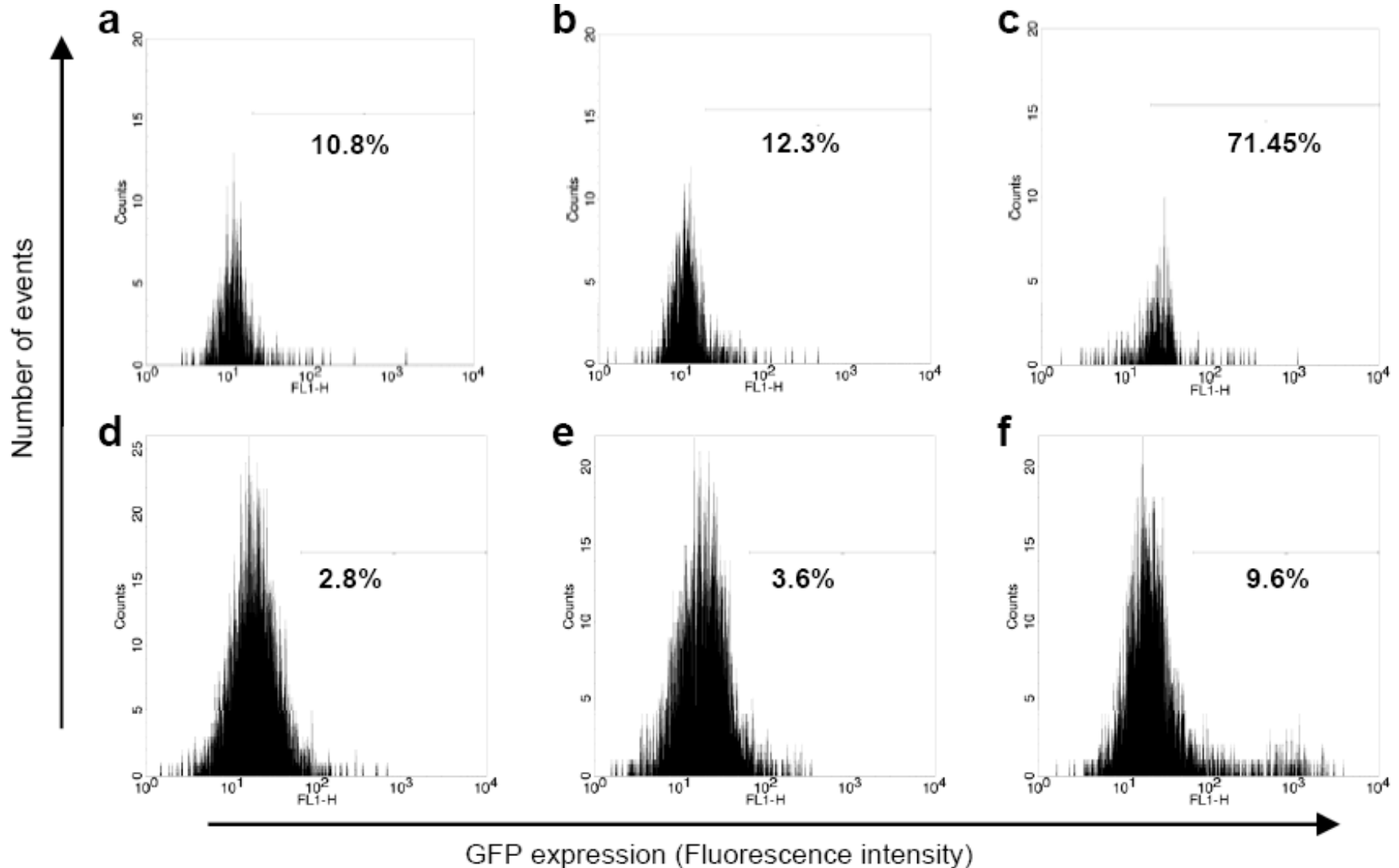
Each scale bar represents 50 μ m.

Regulated *Salmonella* spp. gene expression in infected cell lines



The scale bar in picture (a.) represents 10 μ m.

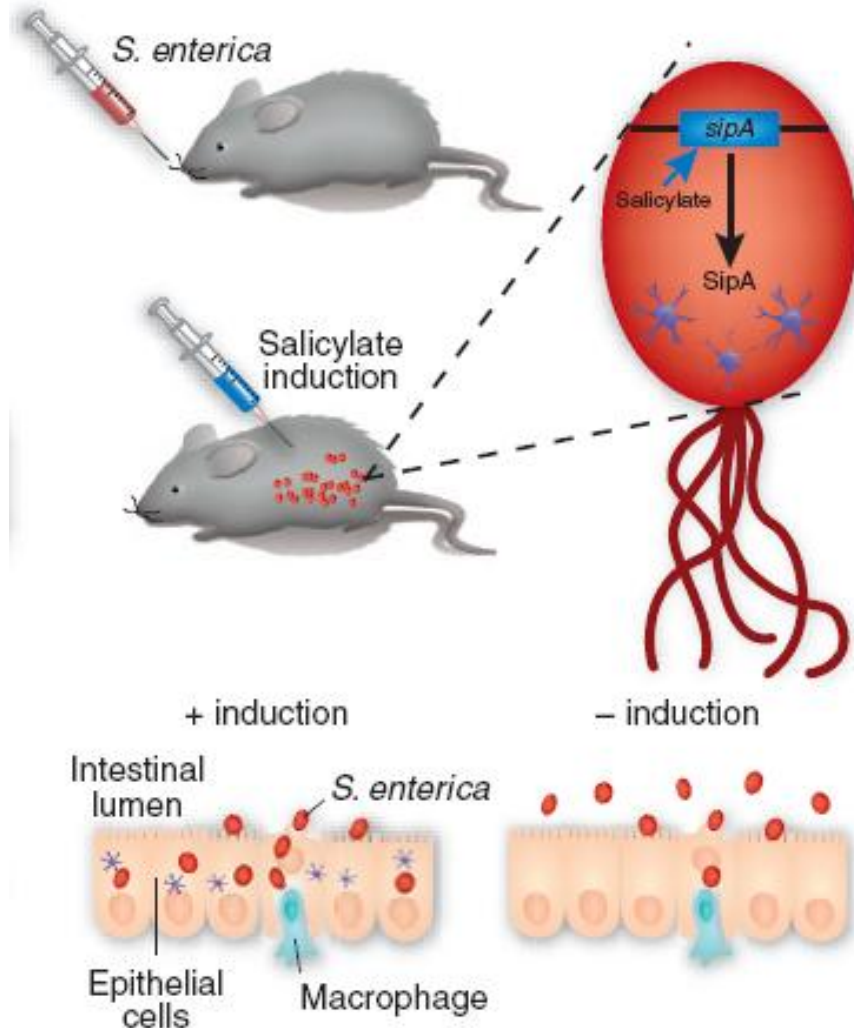
Flow cytometric analysis of cells from the spleen (a to c) and the mesenteric lymph nodes (d to f) from mice infected intraperitoneally with recombinant *Salmonella* carrying either pWSK29 (a and d) or pMPO15 (b, c, e, and f) under control of the regulatory cassette after induction.



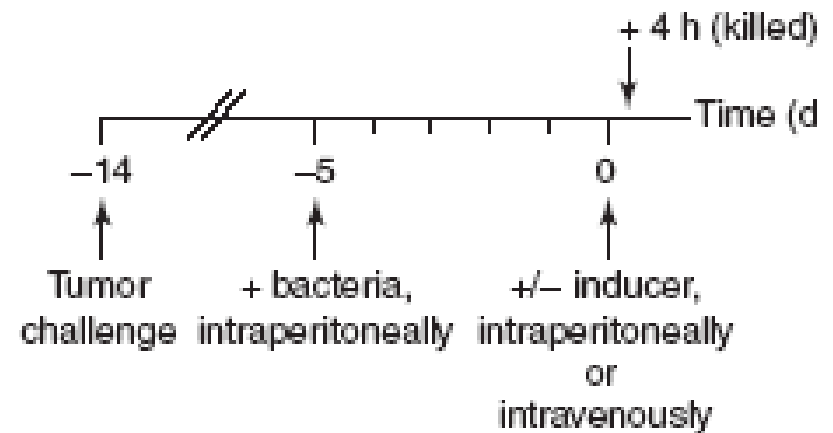
Prokaryotic niche-specific expression in mice

Schematic and time-line of the experimental design

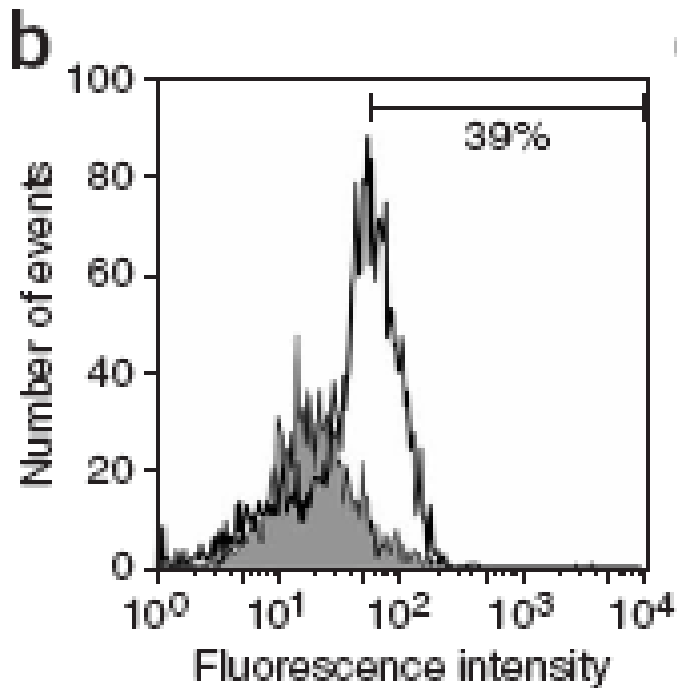
b



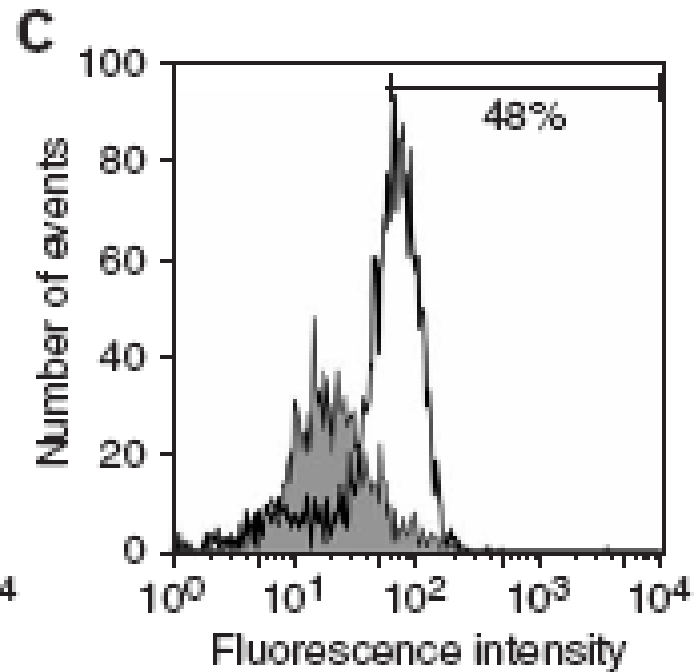
a



GFP expression in tumor cells recovered from mice



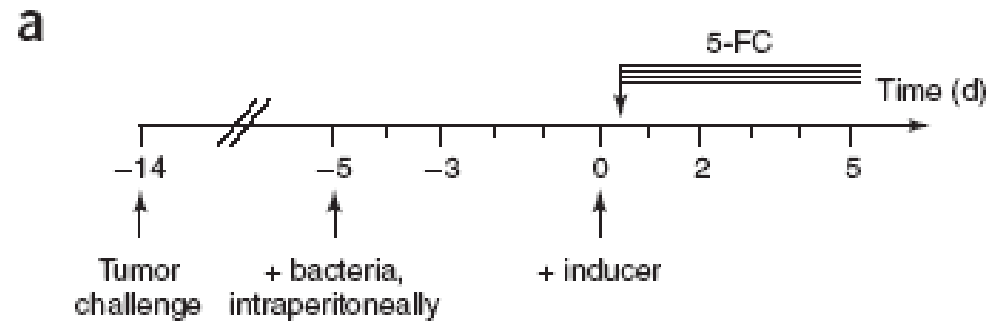
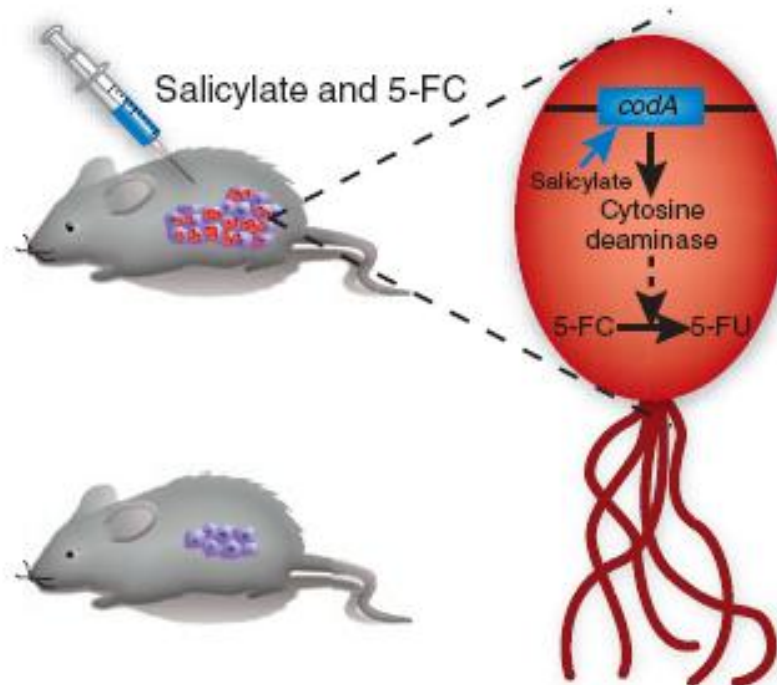
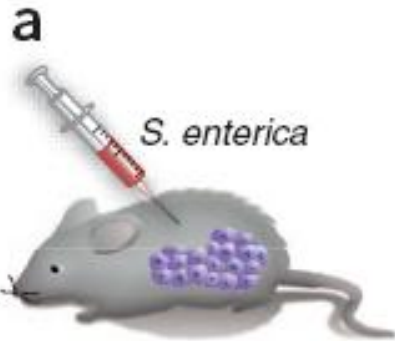
Intraperitoneally



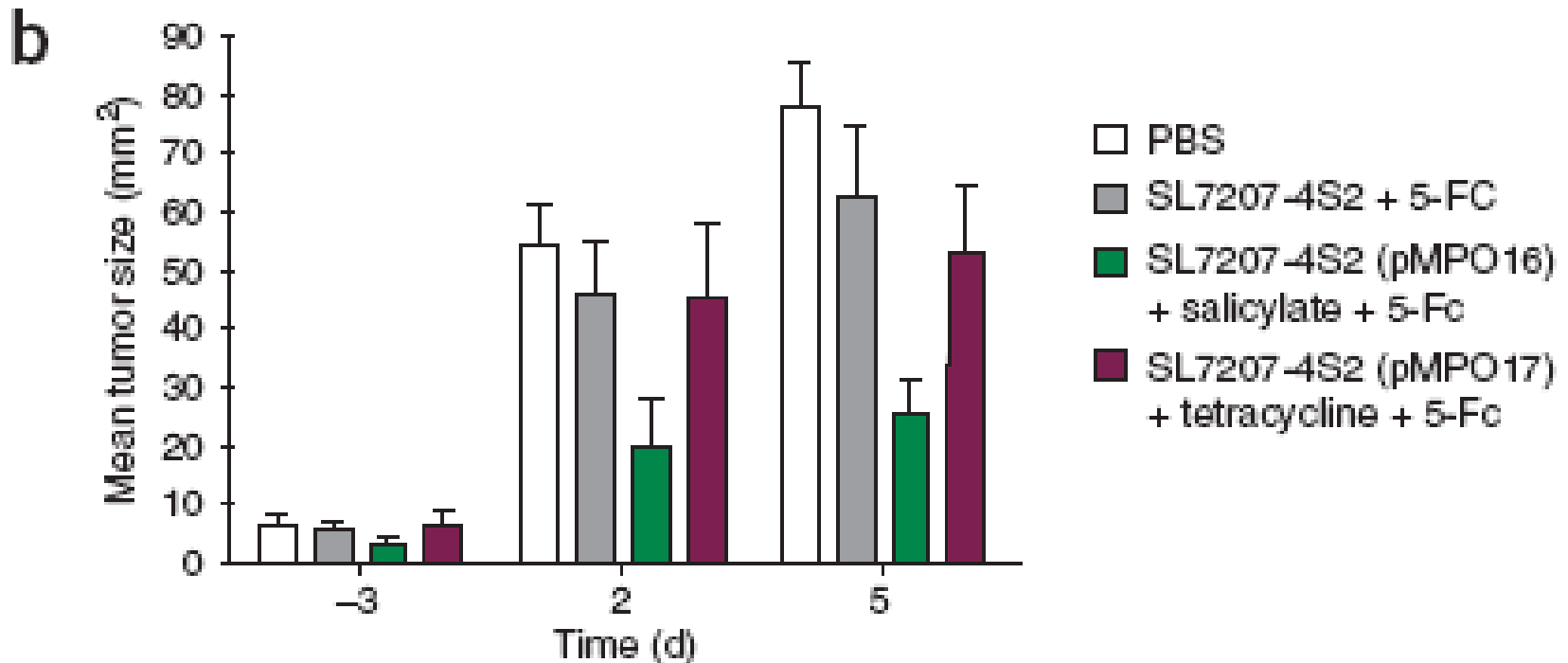
Intravenously

Expression of a Pro-drug Converting Enzyme in Tumor Cells

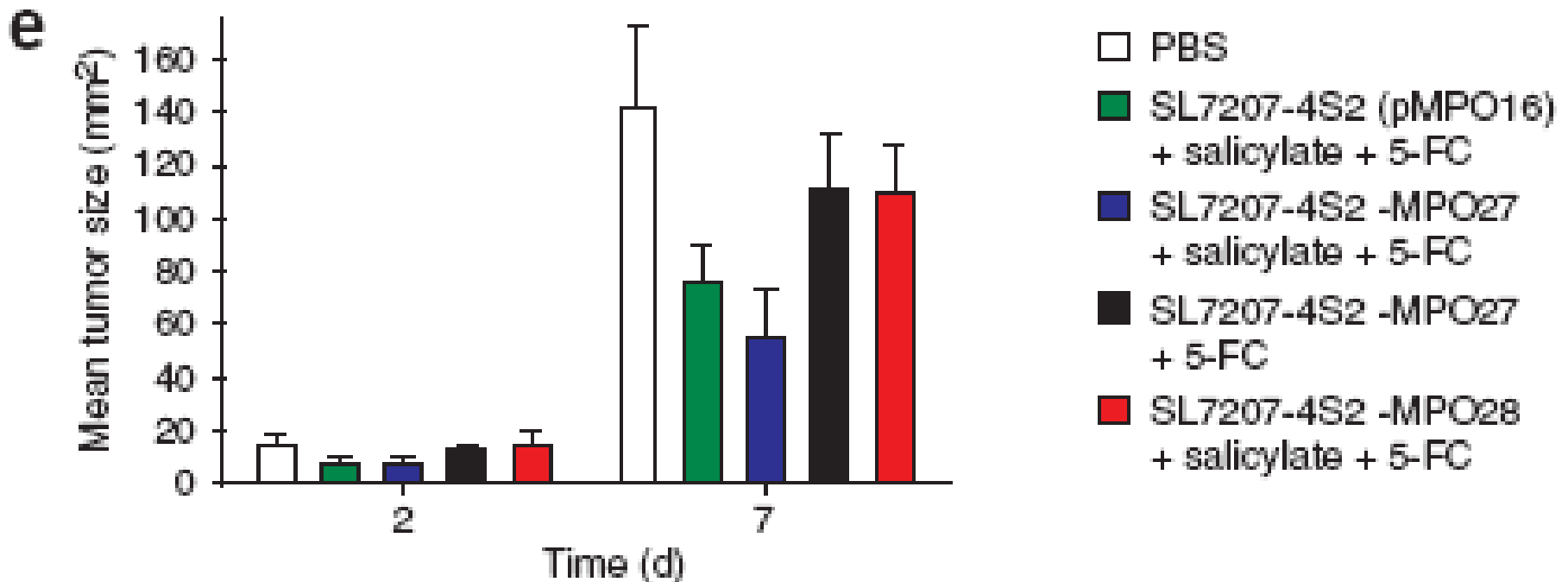
Schematic and time-line of the experimental design



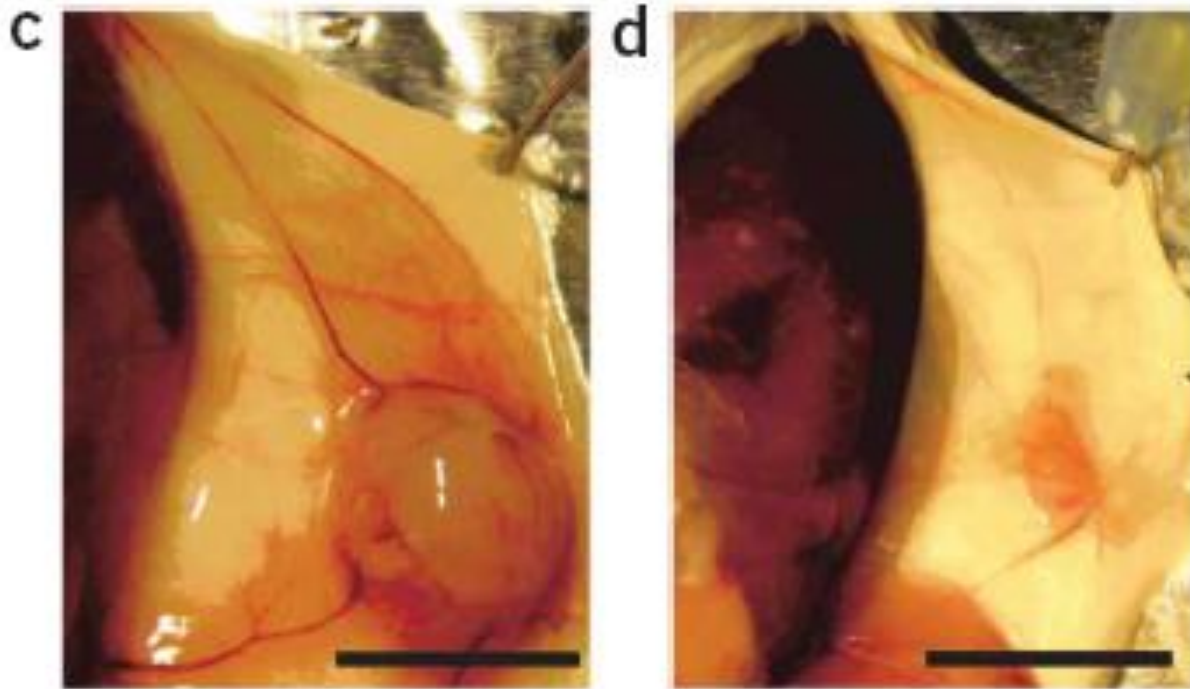
Salicylate-mediated *in vivo* expression of cytosine deaminase (*codA*) in tumor cells using the cascade control circuit



Salicylate-mediated *in vivo* expression of cytosine deaminase (*codA*) in tumor cells using the cascade control circuit

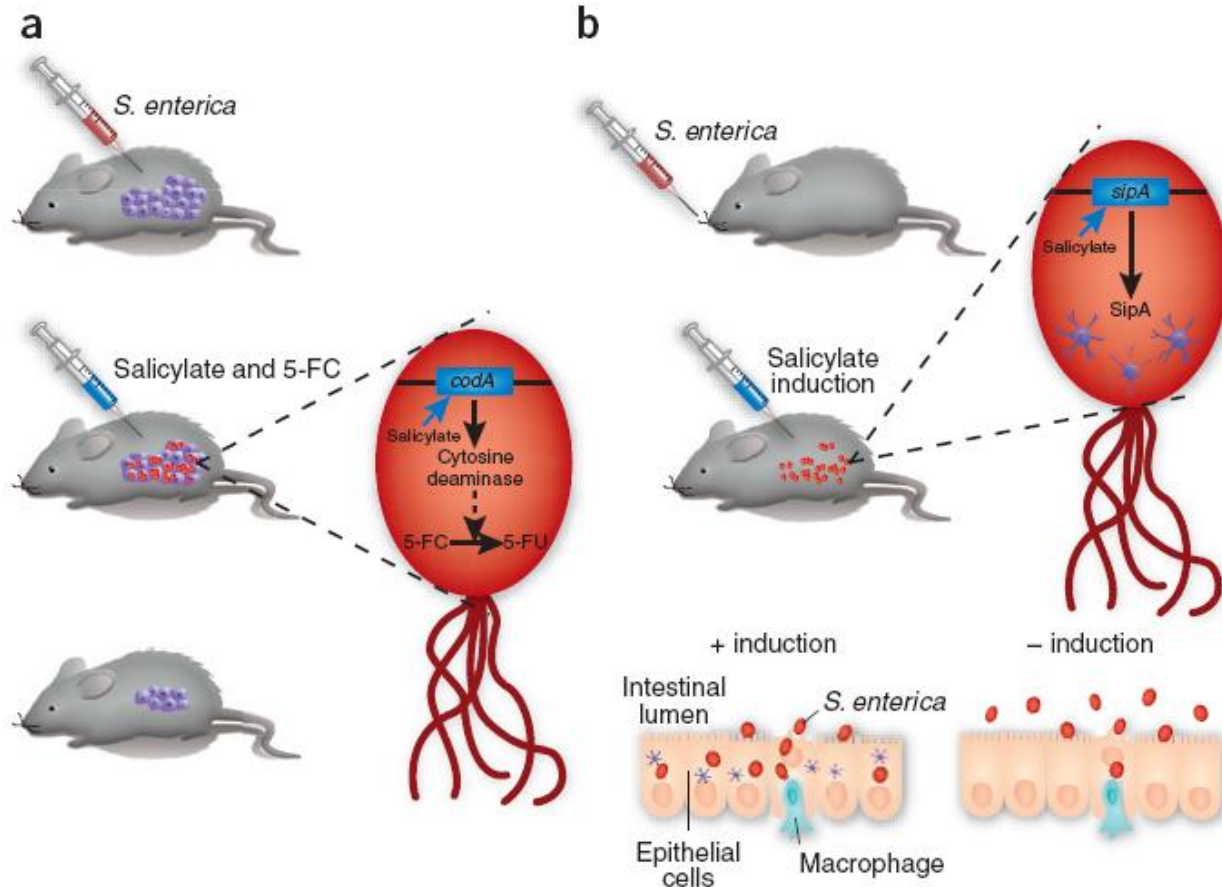


Macroscopic tumor sizes of treated (SL7207-4S2) (pMPO16) and PBS-treated mice



Each scale bar in pictures (c and d) represents 10 mm.

Summary



- Tight regulation of expression of selected genes under the control of Pm *in vivo* and in the presence of an inexpensive inducer led to the activation of expression.

Advantages

- Expression system
 - Integrate additional cassettes under control of Pm
 - Minimal metabolic burden
 - High induction levels
- Salicylate
 - Rapidly absorbed
 - Distribution in different tissues
 - Short half-life
 - Lack of toxicity at therapeutic doses
 - Cheap

Questions to be Addressed

- What is the efficiency of the technique in humans?
- What is the fate of the bacteria within the tumor?
- Are the bacteria subjected to an immune response within the tumor?
- What is the effect of colonizing bacteria and cytosine deaminase expression in systemic sites?

References

- Bowe, F., C. J. Lipps, R. M. Tsois, E. Groisman, F. Heffron, and J. G. Kusters. 1998. *Infect. Immun.* 66:3372-3377.
- Cebolla, A., J. L. Royo, V. de Lorenzo, and E. Santero. 2002. *Appl. Environ. Microbiol.* 68:5034-5041.
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- McClelland, M., et al. 2001. *Nature* 413:852-856.
- Pawelek, J. M., K. B. Low, and D. Bermudes. 1997. *Cancer Res.* 57:4537-4544.
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Happy Holidays!!!

	Relevant genotype or characteristics	Reference or source
Strains		
<i>E. coli</i> DH5 α	<i>deoR</i> , <i>endA1</i> , <i>gyrA96</i> , <i>recA1</i> , <i>supE44</i> .	1
<i>E. coli</i> S17-1 (λ pir)	F' <i>recA</i> , <i>hsdR</i> , RP4-2 (Tc::Mu)(Km::Tn7) lysogenized with λ pir phage.	2
<i>S. enterica aroA</i> SL7207	<i>Salmonella typhimurium</i> 2337-65 (=WRAY) derivative, <i>hisG46</i> DEL407 [<i>aroA</i> ::Tn10 {Tc ^S }].	B. Stocker
<i>S. enterica</i> SL7207-4S2	Rif ^R , Km ^R ; SL7207 derivative with a miniTn5 bearing a <i>nahR/Psal::xylS2</i> fusion inserted in the chromosome.	This study
<i>S. enterica</i> SL7207-4S2-MPO27	Rif ^R , Km ^R , Cm ^R ; SL7207-4S2 derivative carrying the Pm- <i>codA</i> expression module integrated into the <i>aroC</i> locus.	This study
<i>S. enterica</i> SL7207-4S2-MPO28	Rif ^R , Km ^R , Cm ^R ; SL7207-4S2 derivative carrying the Pm control expression module integrated into the <i>aroC</i> locus.	This study
Plasmids		
pCAS	Ap ^R ; expression vector with <i>rrnBT1</i> -Pm::MCS, ColE1 replication origin.	Active Motif, CA.
pCNB4-S2	Ap ^R , Km ^R ; miniTn5 vector with the <i>nahR/Psal::xylS2</i> fusion cloned between the I and O sites.	3
pASK-IBA43	Ap ^R , pUC18-derivative with the <i>tetR/tetO</i> expression system.	IBA GmbH, Göttingen
pMPO2	Ap ^R ; pCAS derivative with a <i>trp'</i> :: <i>lacZ</i> fusion downstream of Pm.	This study
pMPO13	Ap ^R ; pWSK29 derivative with the Pm- <i>trp'</i> :: <i>lacZ</i> fusion of pMPO2.	This study
pMPO15	Ap ^R ; pWSK29 derivative with a Pm- <i>gfp</i> fusion replacing the Pm- <i>trp'</i> :: <i>lacZ</i> fusion of pMPO13.	This study
pMPO16	Ap ^R , pCAS derivative with a Pm- <i>codA</i> fusion.	This study
pMPO17	Ap ^R , pASK-IBA43 derivative with a Ptet- <i>codA</i> fusion.	This study
pWSK29	Ap ^R ; low-copy number vector for cloning and expression, pSC101 replication origin.	4
Cell Lines		
HeLa	Cell line derived from a human cervix adenocarcinoma.	ATCC CCL-2
J774.A1	Macrophage-like cell line derived from BALB/cN mice.	ATCC TIB-67
F1.A11	Cell line derived from a spontaneous murine fibrosarcoma from BALB/c mice.	5