A fast and sensitive bioassay panel to detect antibiotics and new antimicrobials

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MicroLife Solutions

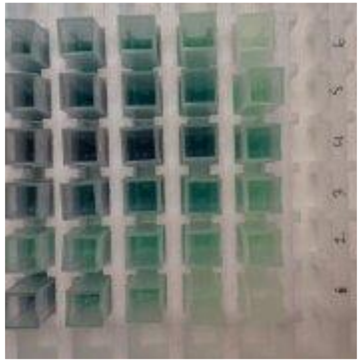
Tjalf de Boer

Explore and exploit nature for valuable microbial activities:

Products

Bioactive compounds

Enzymes

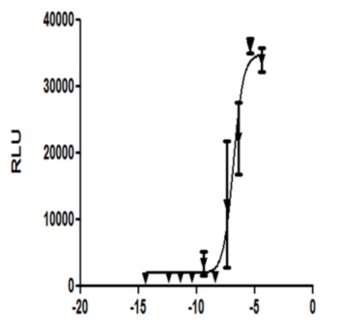


Microorganisms

microlife solutions

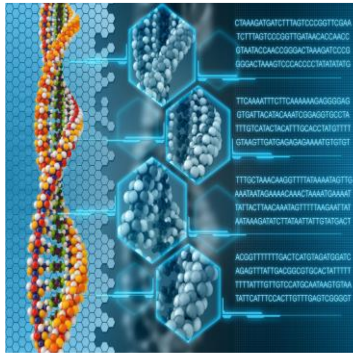
Explore and exploit nature for valuable microbial activities:

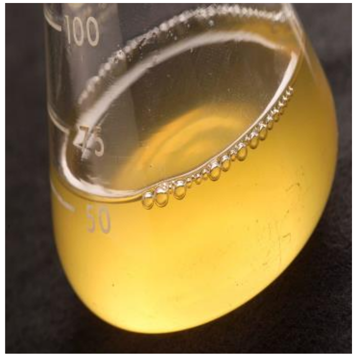
Technology



Functional screens

Sequence analysis

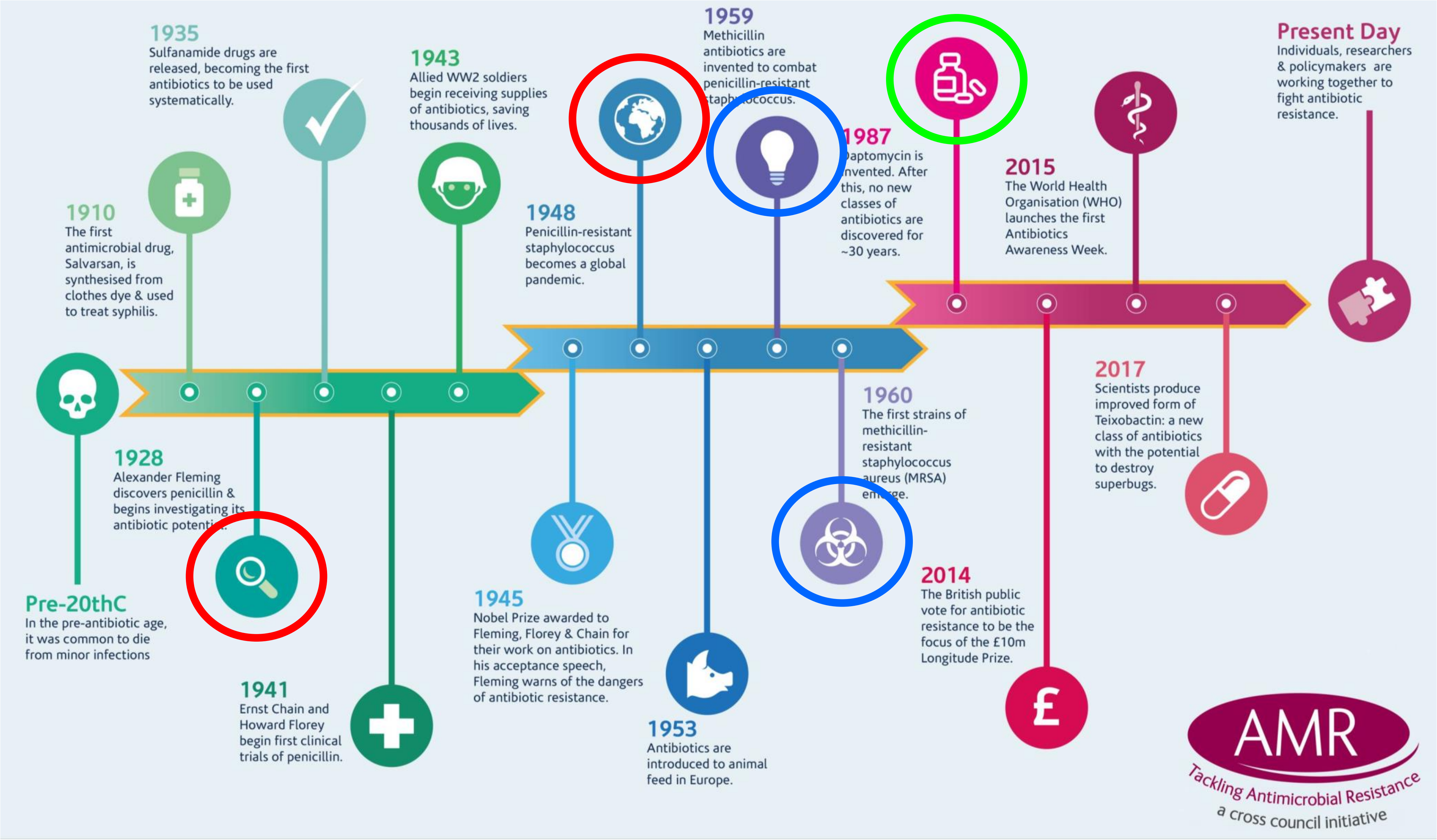




Production

microlife solutions

A (brief) history of antibiotics and antibiotic resistance



Source: UK Research and Innovation (www.ukri.org)

Antibiotic use and water

- Antibiotic use
  - Has increased by 65% worldwide between 2010-2015
  - Increase mainly driven by increased use in low and middle income countries
  - Especially rapid increase in “last-resort” compounds
- Waste water treatment
  - Antibiotics end up in wastewater treatment plants
  - Waste removal often includes biological treatment (bacteria)
  - Old, un-upgraded facilities do not remove antibiotics completely
- Antibiotics in surface water
  - Leads to increased numbers of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG)
  - Measure antibiotic concentrations to link to ARB and ARG

Klein et al, PNAS, 2018

Bacterial reporter bioassays (MicroGLO™)

Antimicrobials

Redox cycling

Anti-oxidants

Structural recognition of specific groups of antibiotics

Inhibition of

Quorum sensing

Replication

Transcription

Translation

Cell wall synthesis

Fatty acid synthesis

…

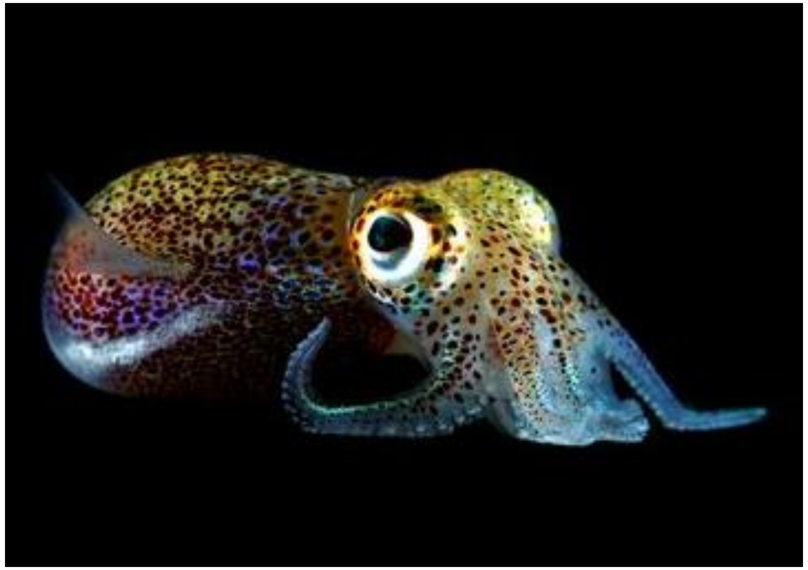
Biomass conversion

Lignocellulose degradation

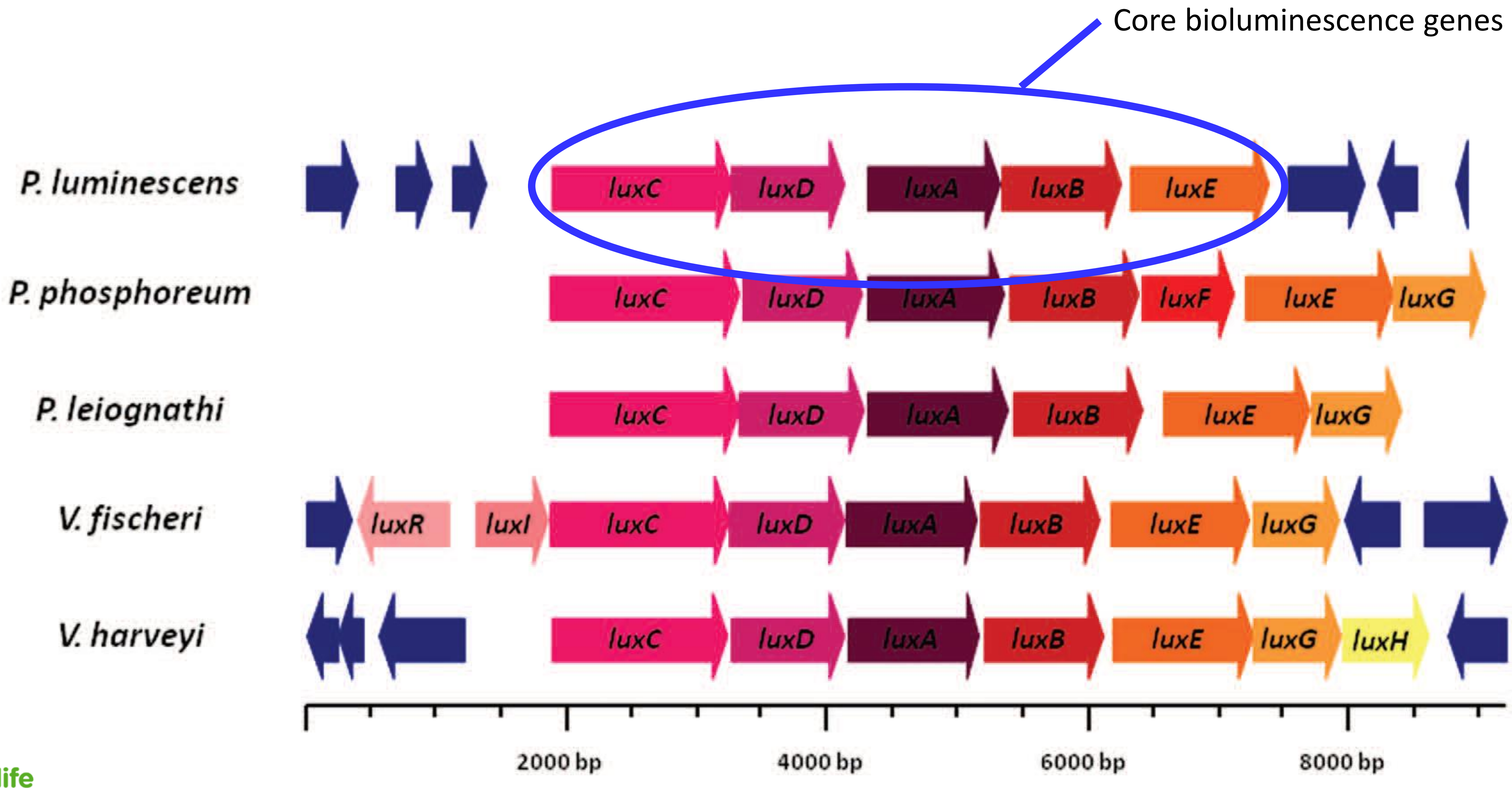
Fermentation inhibitors

Bioluminescence in nature

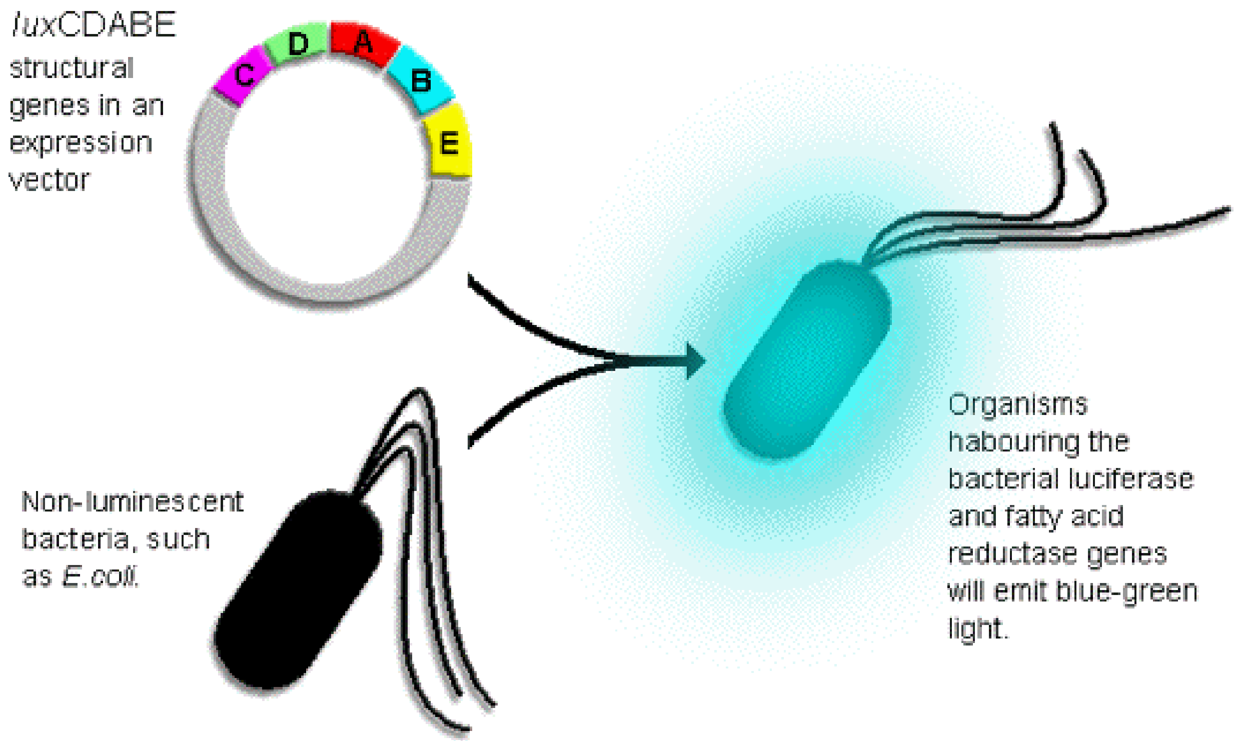





Different bacterial luciferase operons

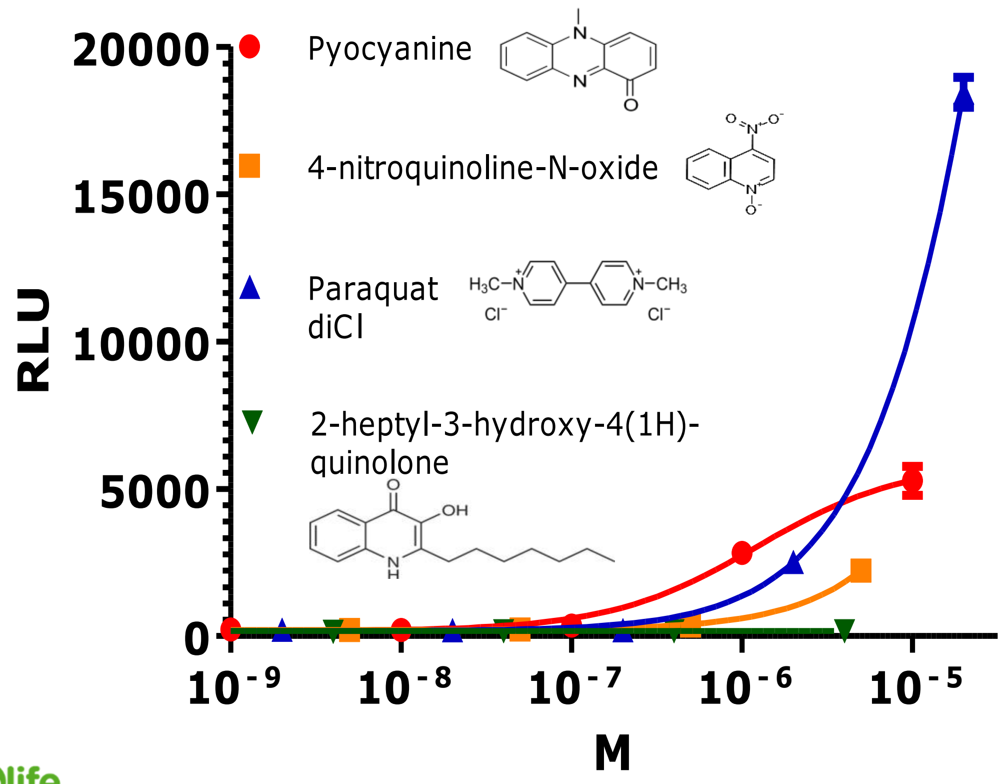


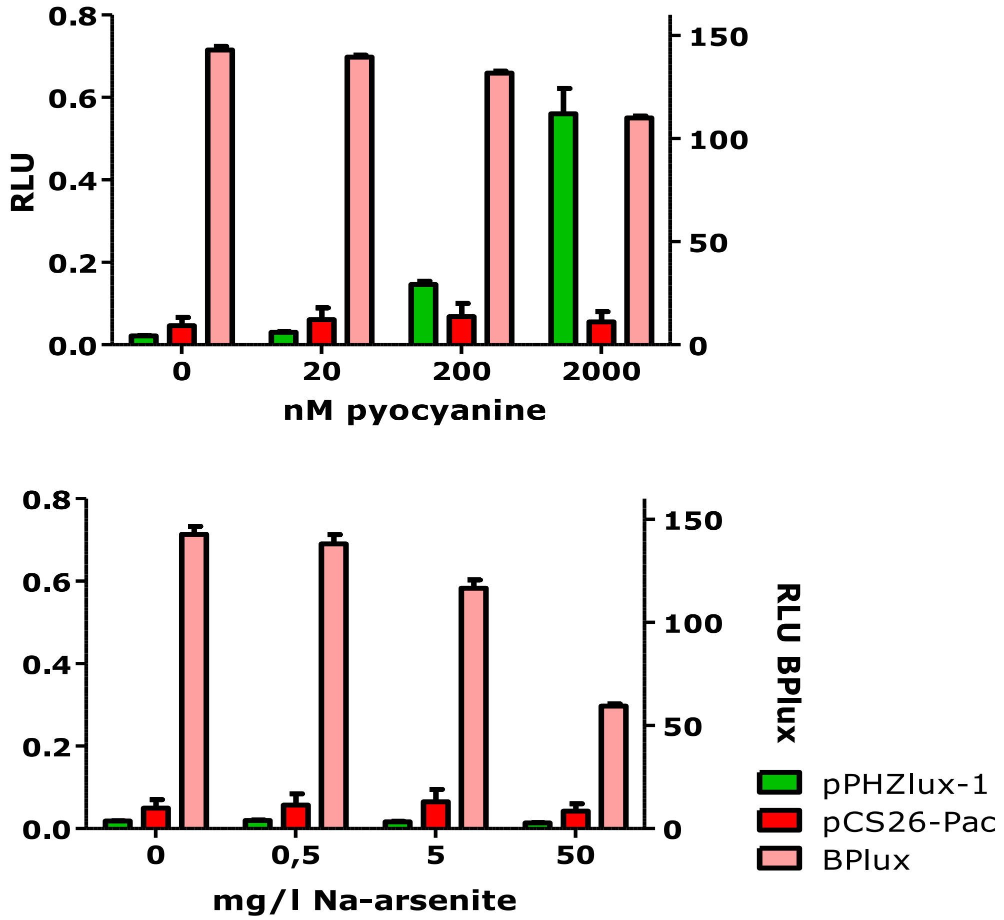
Prokaryote luciferase reporter assay



Microbial reporter assays

Example: pPHZlux reporter (oxidative stress)

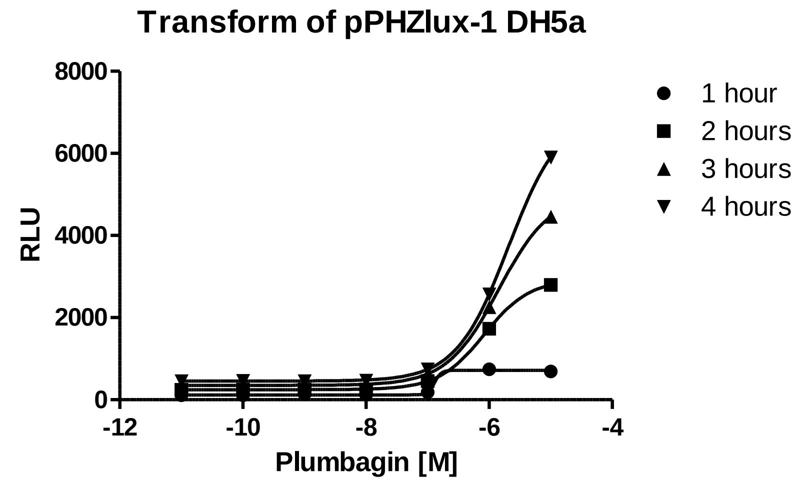


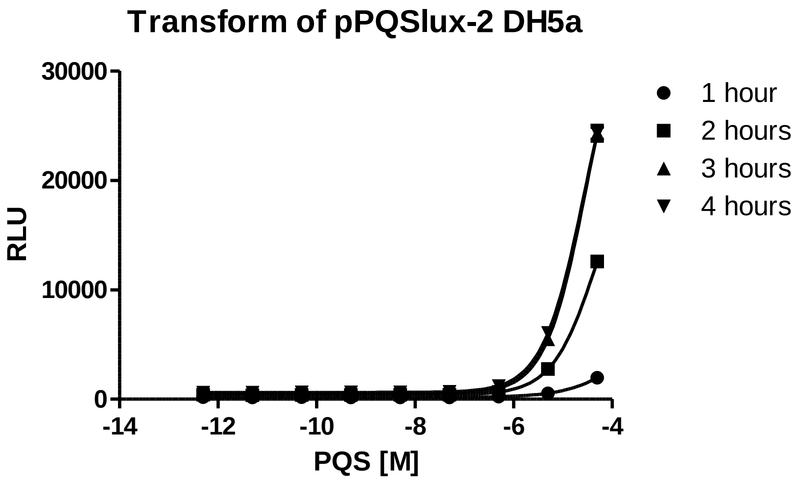


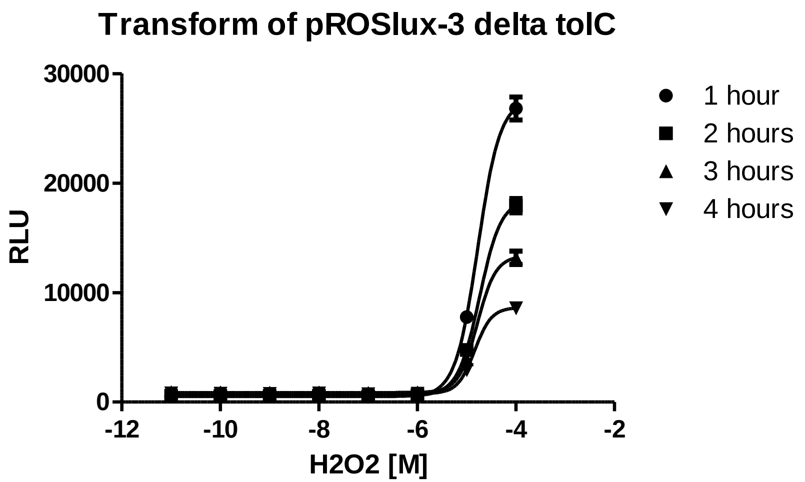
Microbial reporter assay panel (antimicrobial)
Usable antimicrobial reporters and their function

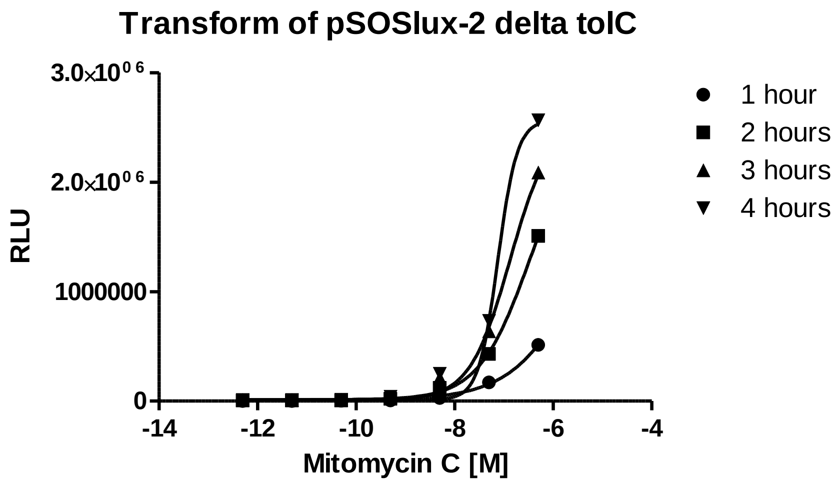
reporter	Measured effects
pTETlux	Protein synthesis inhibition (detects tetracycline)
pSOSlux-2ΔtolC	Reporter that detects DNA damage
pROSlux-3	Reporter for the detection of oxidative stress (agonism) and anti-oxidant compounds (antagonism)
pPQSlux-2	Detects alkyl-quinolones
pAHLlux-1	Detects AHL quorum sensing
pAHLlux-2	Detects AHL quorum sensing
pAHLlux-3	Detects AHL quorum sensing
pBLAlux-2ΔampD	Cell wall synthesis inhibition (detects beta-lactams)
pMAClux-3ΔtolC	Protein synthesis inhibition (detects macrolides)
pPHZlux	Detects phenazines

Microbial reporter assay panel (MicroGLO™)
induction time









Antimicrobialreporterassaypanel
Modelcompounds

Compound	Tetracycline	Doxocycline	Ciprofloxacin	MitomycinC	H_2O_2	PQS	N-butyryl-L-HSL(BHL)	N-3-oxohexanoyl-L-HSL	N-3-oxododecanoyl-L-HSL	PenicillinG	Ampicillin	Erythromycin	Spiramycin	Pyocyanine	Plumbagin
pTETlux															
pSOSlux-2ΔtolC															
pROSlux-3															
pPQSlux-2															
pAHLlux-1															
pAHLlux-2															
pAHLlux-3															
pBLAlux-2ΔampD															
pMAClux-3ΔtolC															
pPHZlux															

Microbial reporter assay panel (anti-microbial)

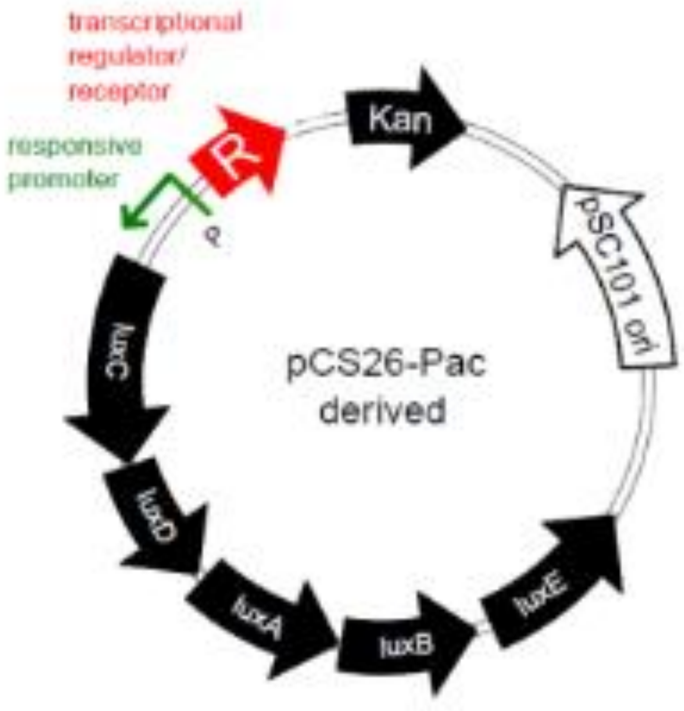
Cross validation (all compounds on all reporters)

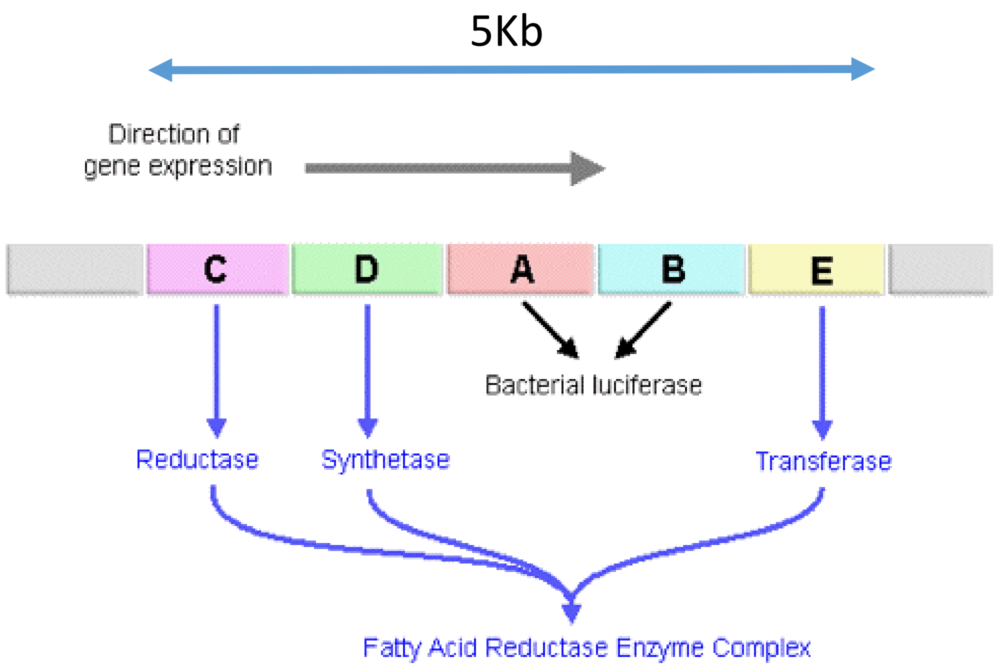
Compound	Tetracycline	Doxocycline	Ciprofloxacin	MitomycinC	H2O2	PQS	N-butyryl-L-HSL(BHL)	N-3-oxohexanoyl-L-HSL	N-3-oxododecanoyl-L-HSL	PenicillinG	Ampicillin	Erythromycin	Spiramycin	Pyocyanine	Plumbagin
pTETlux	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
pSOSlux-2ΔtolC	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
pROSlux-3	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
pPQSlux-2	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
pAHLlux-1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
pAHLlux-2	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
pAHLlux-3	?	?	-	?	-	-	+	-	-	?	?	-	?	?	?
pBLAlux-2ΔampD	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-
pMAClux-3ΔtolC	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-
pPHZlux	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+

Model compound

High background at all concentrations

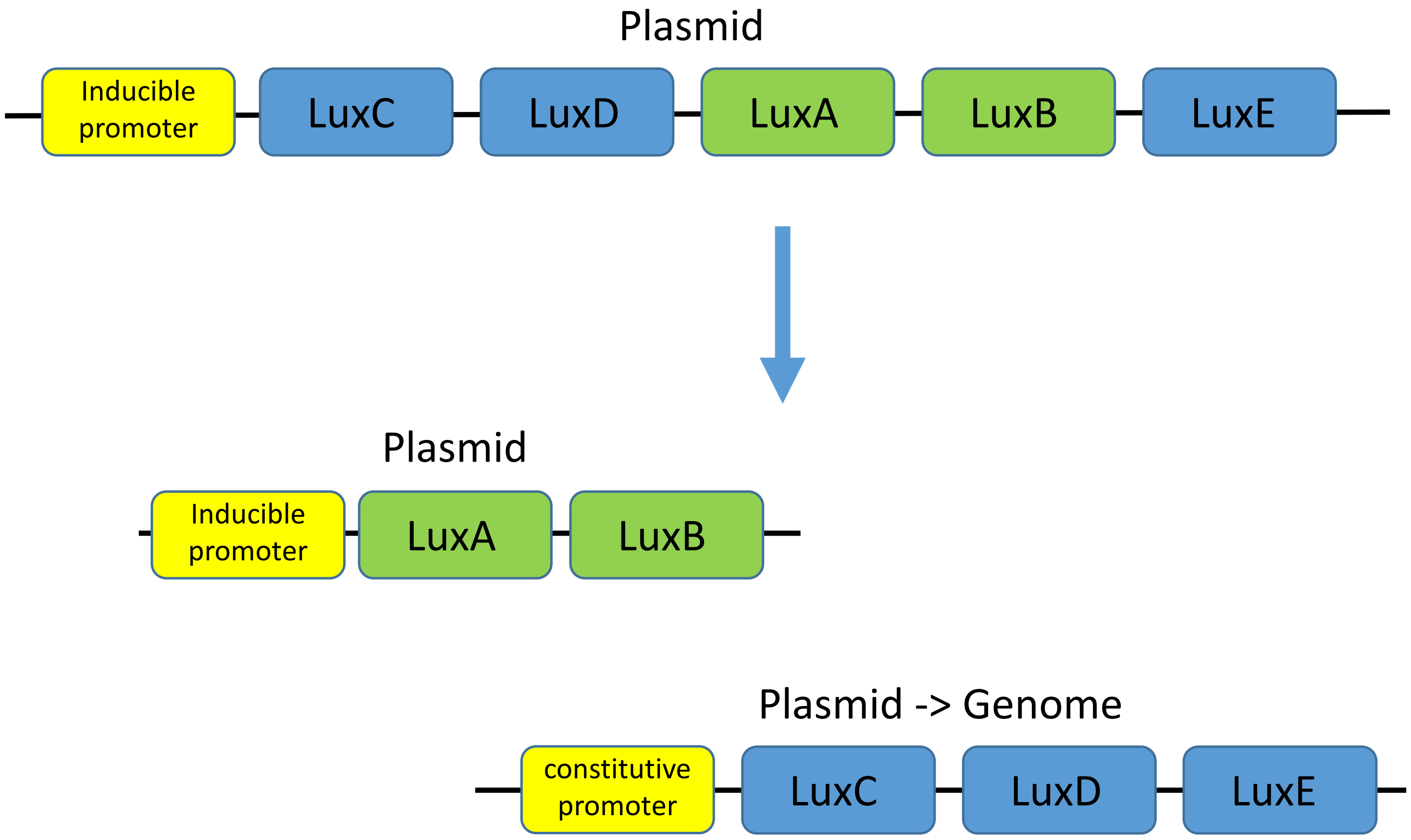
Microbial reporter assay panel (anti-microbial)
Not all reporters are sensitive enough: New reporter system



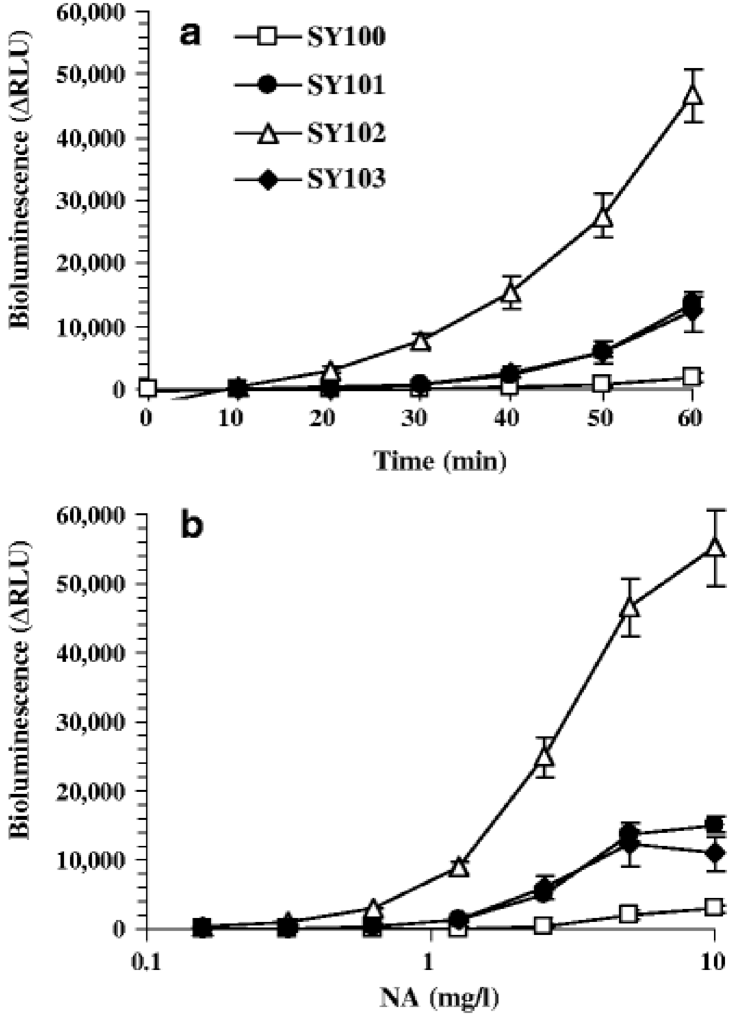


- Not clear if all 5 lux genes are expressed and translated at the same level
- No substrate present at operon induction -> response lag

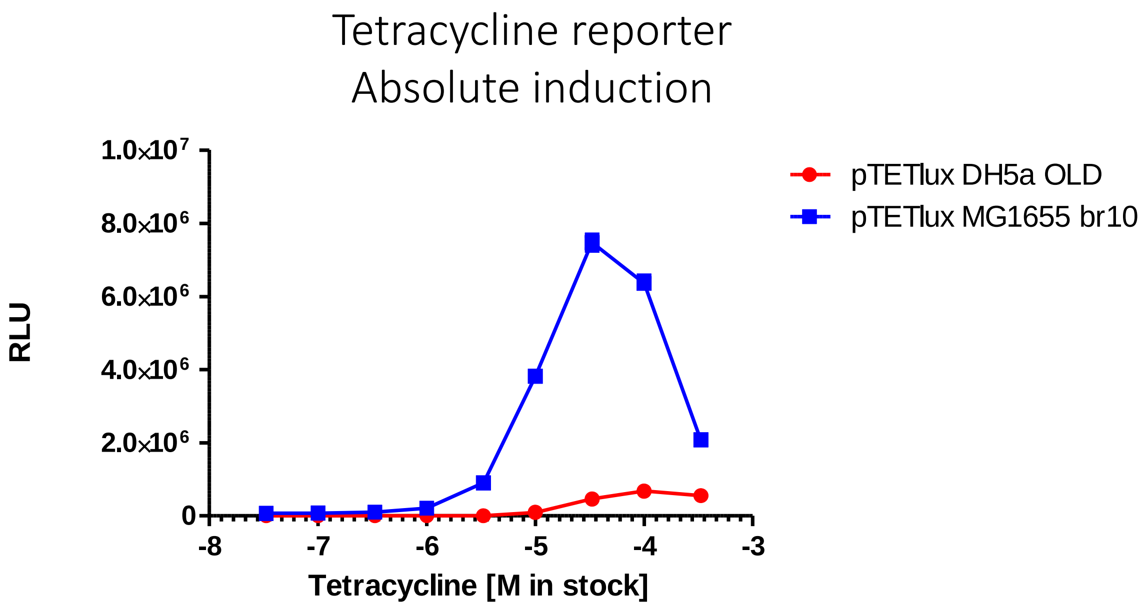
Microbial reporter assay panel (anti-microbial)
splitting the CDABE-lux operon

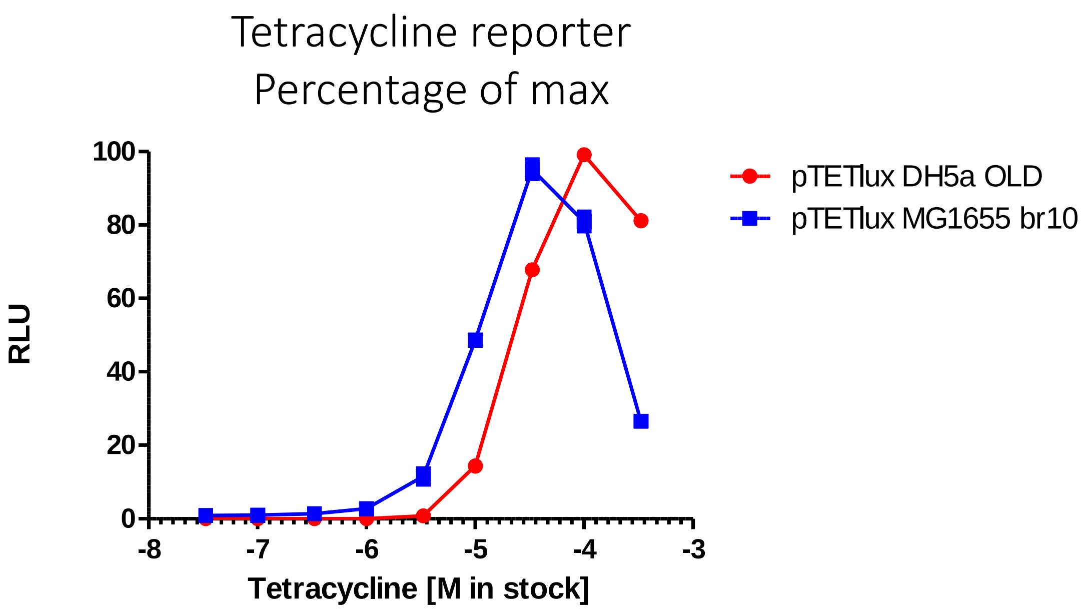


Belkinetal, 2010



New vs old reporter system
absolute induction and percentages

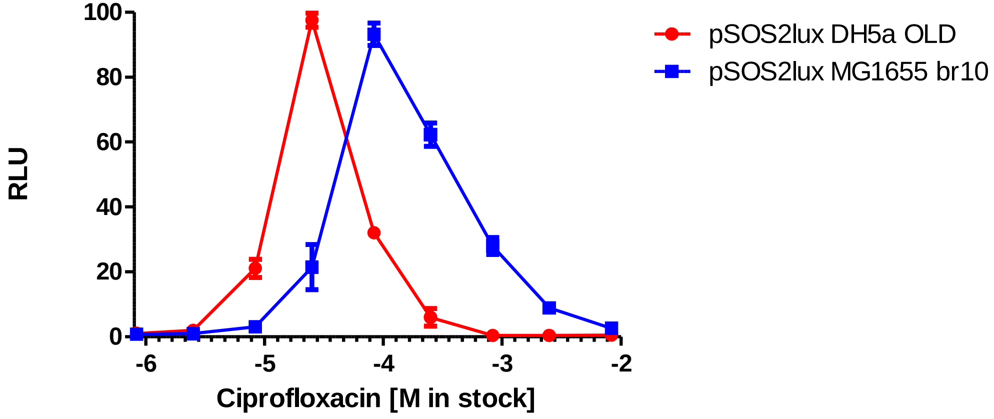




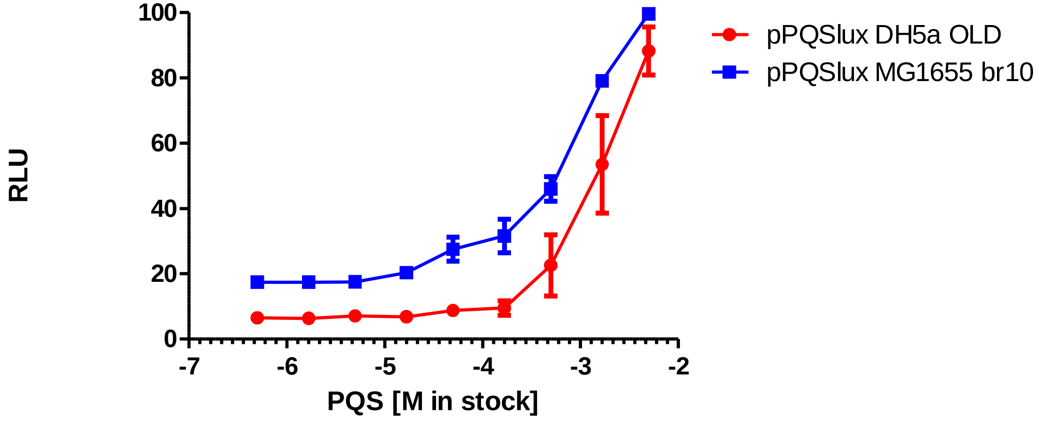
Overview new reporter system

some of the new reporters

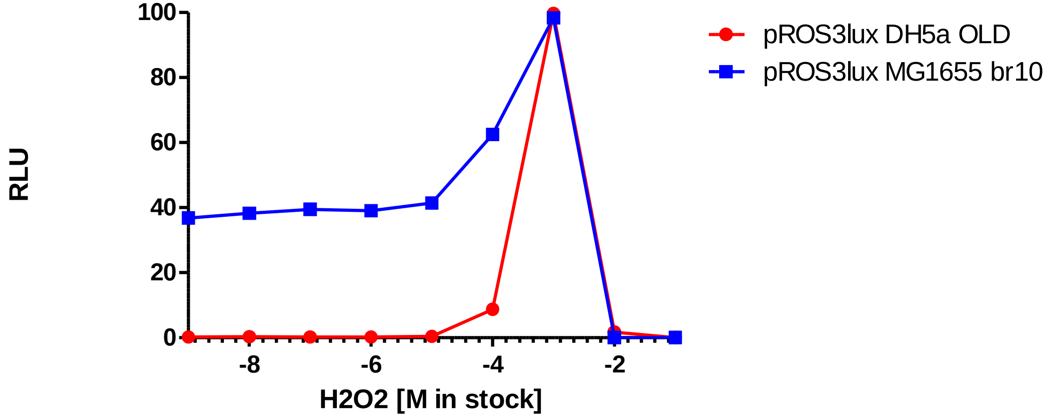
DNA damage reporter



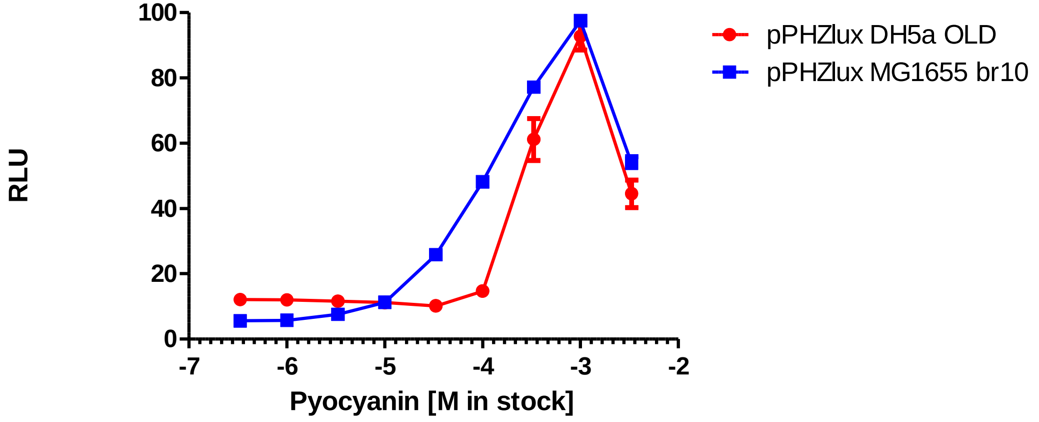
quinolone reporter



alkyl-quinolone reporter

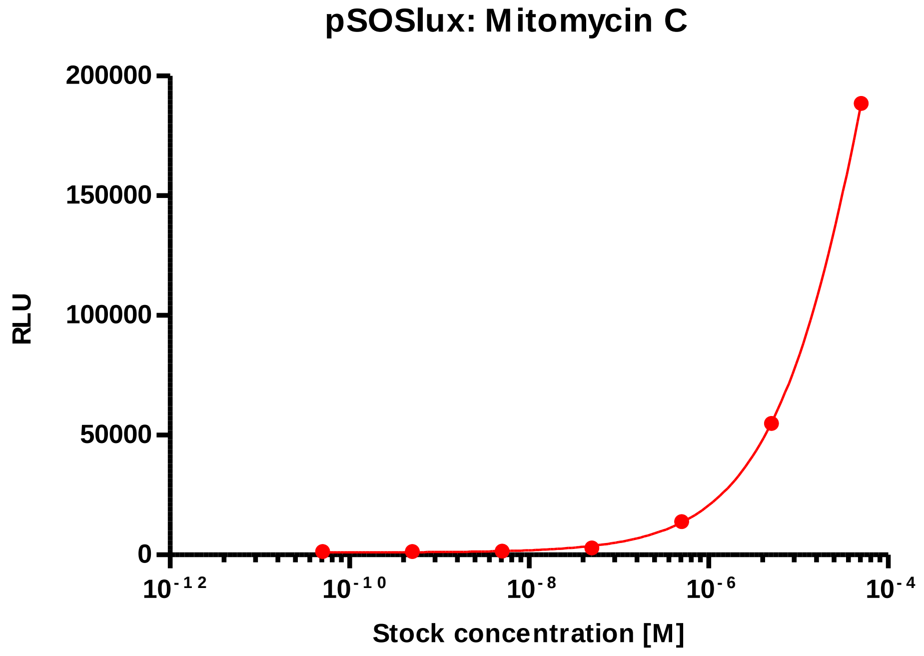


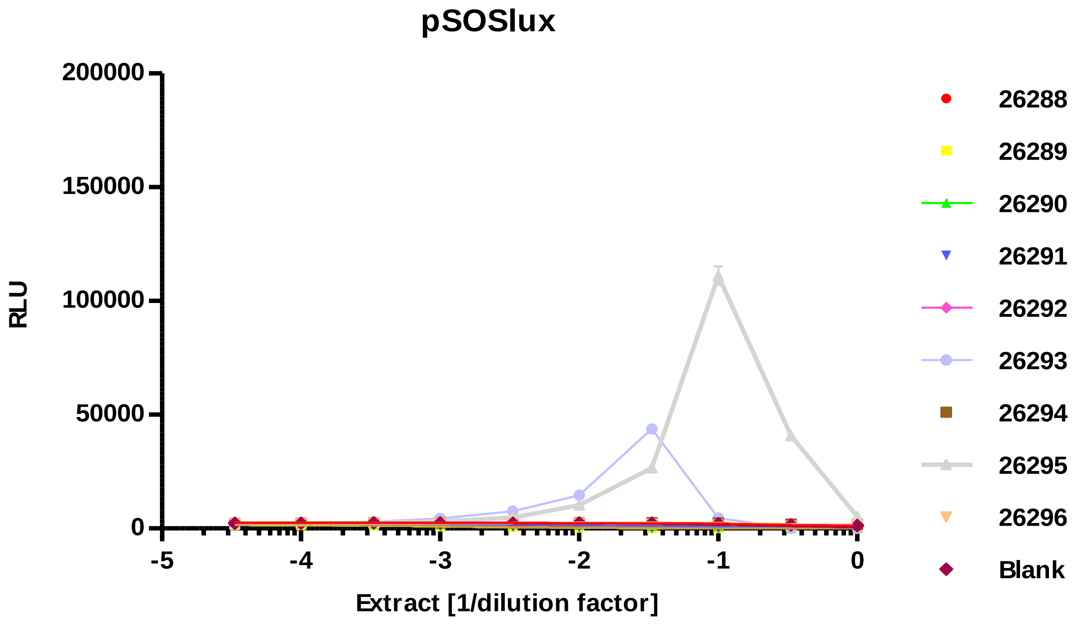
alkyl-quinolone reporter



Microbial reporter assay panel (anti-microbial)
Case studies: KWR medical waste and water samples

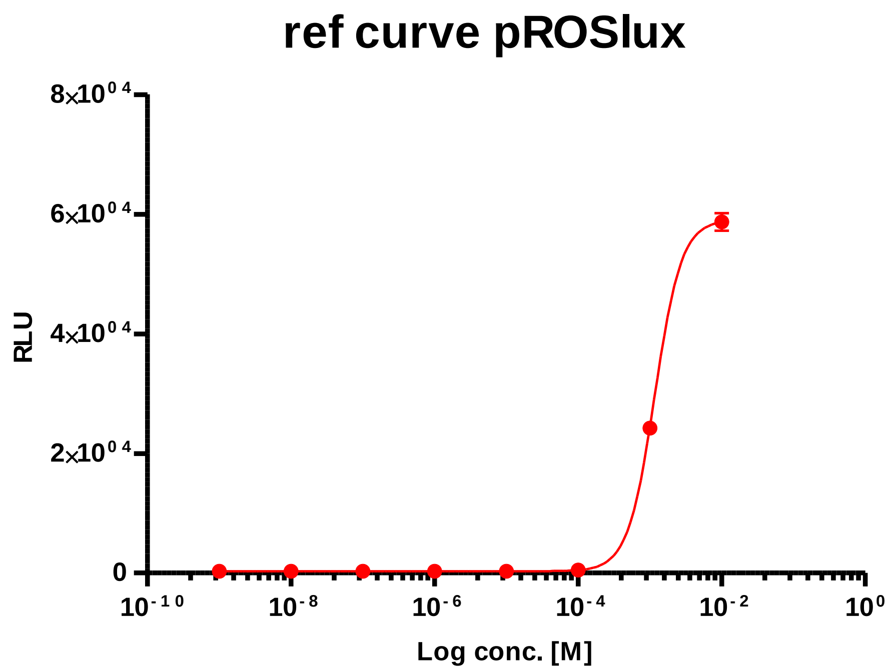
- Several samples from medical origin both pharma filtered and not
- Activity tested on Calux and Antimicrobial panel
- Antimicrobial activity on pre-pharma filtered but not in clean samples

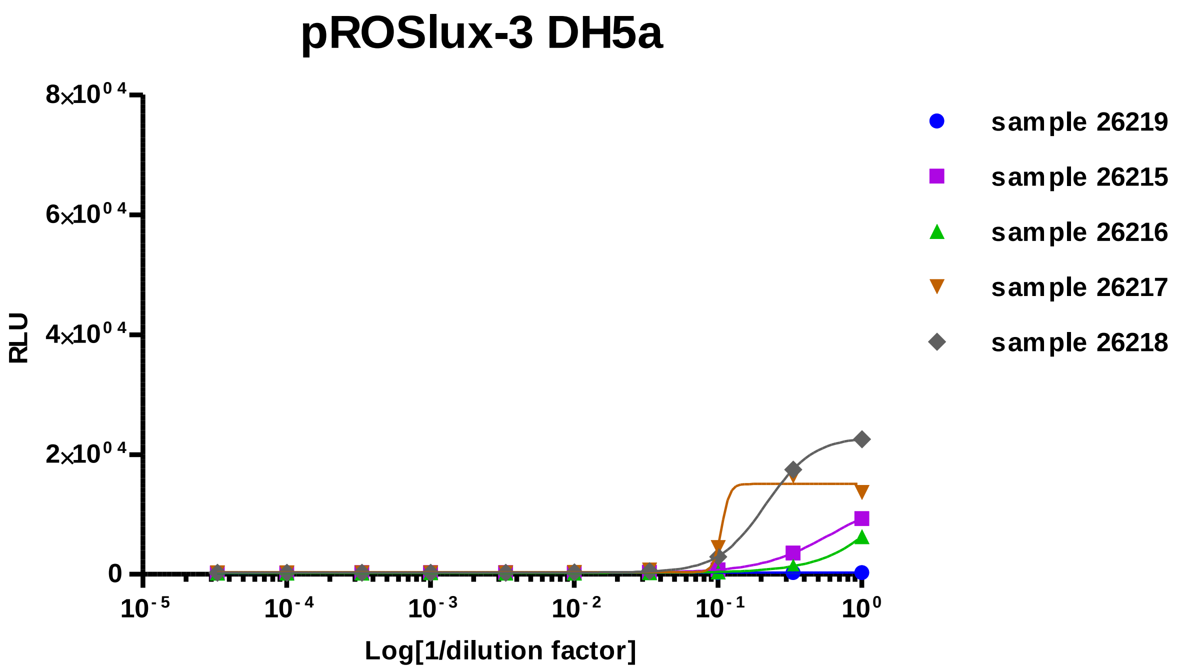




Microbial reporter assay panel (anti-microbial)
Case studies: NIOO plant microbe interactions

- Plant samples treated with microbes
- Differential metabolites detected in treated samples
- Low activity (only oxydative stress) probably due to low extract concentrations





MicroGLO bioassay panel
To conclude…

- We have developed a fast and sensitive bioassay panel to measure antibiotics and/or novel antimicrobial compounds

- Effect based which means a broad range of compounds will be measured and cumulative effects can be determined