Vibrio fischeri as a Biosensor in Marine Aquaculture

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Humboldt SU UH Manoa

γ.coli

E. coli doesn't work in marine environments

Vibrio (salt tolerant, forms biofilms, etc.) could be an alternative marine "*E. coli*" for synth-bio applications.

Vibrio includes many important pathogen species to both humans (e.g., cholera, *V. vulnificus*) and other species (coral, *V. coralliilyticus*, fish, crustaceans, *etc*.) and some genetic tools are already developed.

Possible directions we were thinking about:
-**Biosensor**,
-Produce vaccines for marine organisms,
-Carbon neutral protein source for fish food (Vibrio biofilm concentrate) in aquaculture.
-Rich range of Vibriophage exist in sea water for novel promoter development...
-Suggestions?

Vibrio fischeri is a safe alternative.

Symbiotic with squid.

The source of lux operon used in e. coli.

(Things work from Vibrio in E. coli!)

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Beginning—Easy to perform; requires no experience in microbiology.

Light up your students' understanding of bioluminescent organisms. Students transfer *Vibrio fischeri* onto photobacterium agar, incubate the cultures in the dark at room temperature, and observe the bioluminescence the next day.

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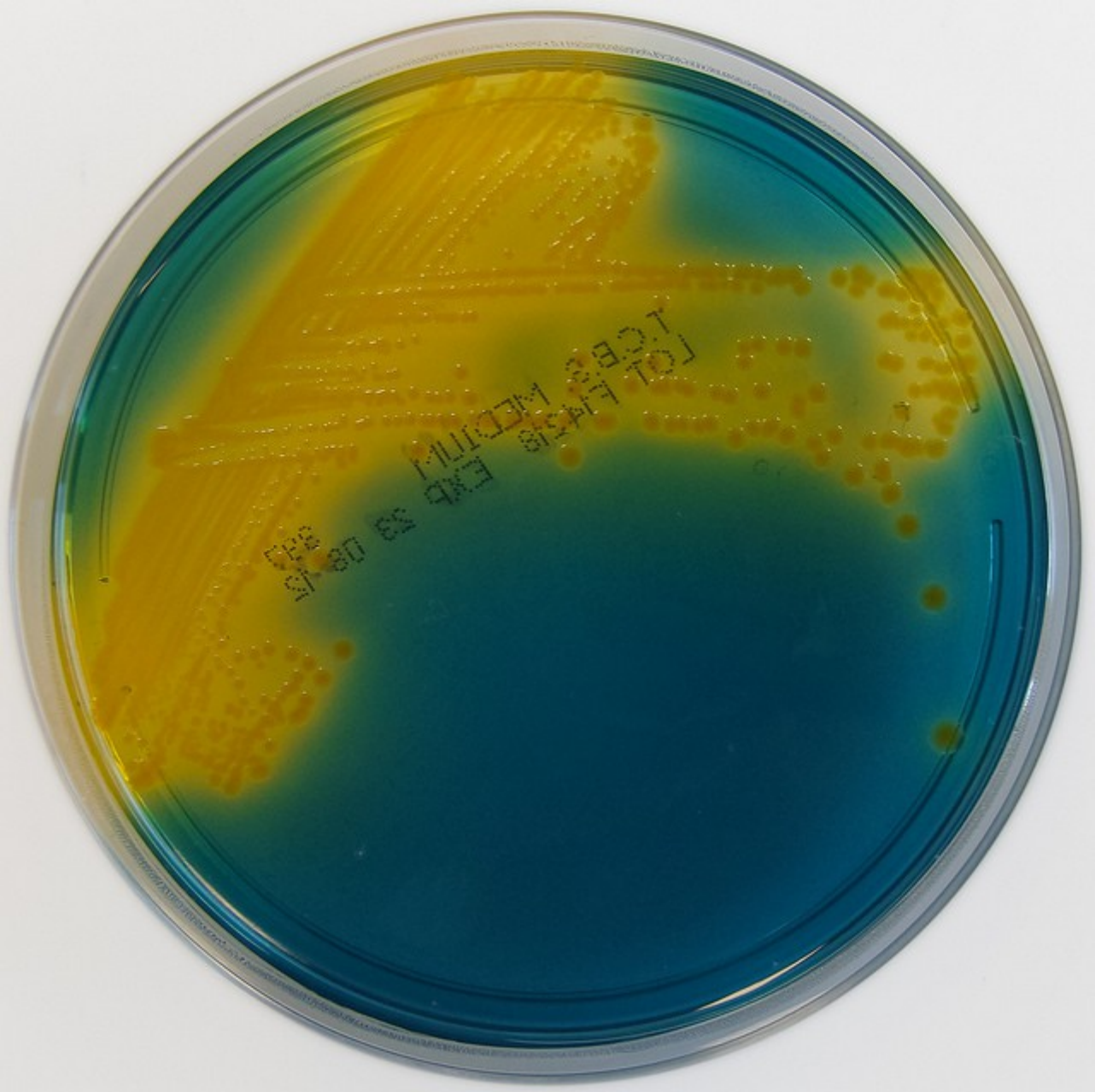
V. fischeri is commercially available and safe to work with in the lab.



Vibrio grows on TCBS agar selectable media in standard lab conditions.

Only rule seems to be they can be frozen but don't like to be cold.

P15A isolated from E. coli.
(Chang, A. C. Y., and S. N. Cohen. 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. 134:1141-1156.)

PES213 isolated from V. fisheri
(Dunn, A. K., M. O. Martin, and E. V. Stabb. 2005. Characterization of pES213, a small mobilizable plasmid from Vibrio fischeri. Plasmid 54:114-134.)

Both are functional in both species and derivative selectible plasmids have been constructed but PES213 replicates poorly in E. coli and is much more stable than p15A in V. fischeri.
(Dunn, A. K., Millikan, D. S., Adin, D. M., Bose, J. L., & Stabb, E. V. (2006). New rfp-and pES213-derived tools for analyzing symbiotic Vibrio fischeri reveal patterns of infection and lux expression in situ. Applied and environmental microbiology, 72(1), 802-810.)

Chitin (marine crustaceans) induces transformation competence, just the dissaceride is sufficient: Chitin disaccharide $(GlcNAc)_2$.

Meibom, K. L., Blokesch, M., Dolganov, N. A., Wu, C. Y., & Schoolnik, G. K. (2005). Chitin induces natural competence in Vibrio cholerae. Science, 310(5755), 1824-1827.
Yamamoto, S., Morita, M., Izumiya, H., & Watanabe, H. (2010). Chitin disaccharide (GlcNAc)< sub> 2</sub> induces natural competence in Vibrio cholerae</i> through transcriptional and translational activation of a positive regulatory gene< i> tfoX</i>< sup> VC</sup>. Gene, 457(1), 42-49.

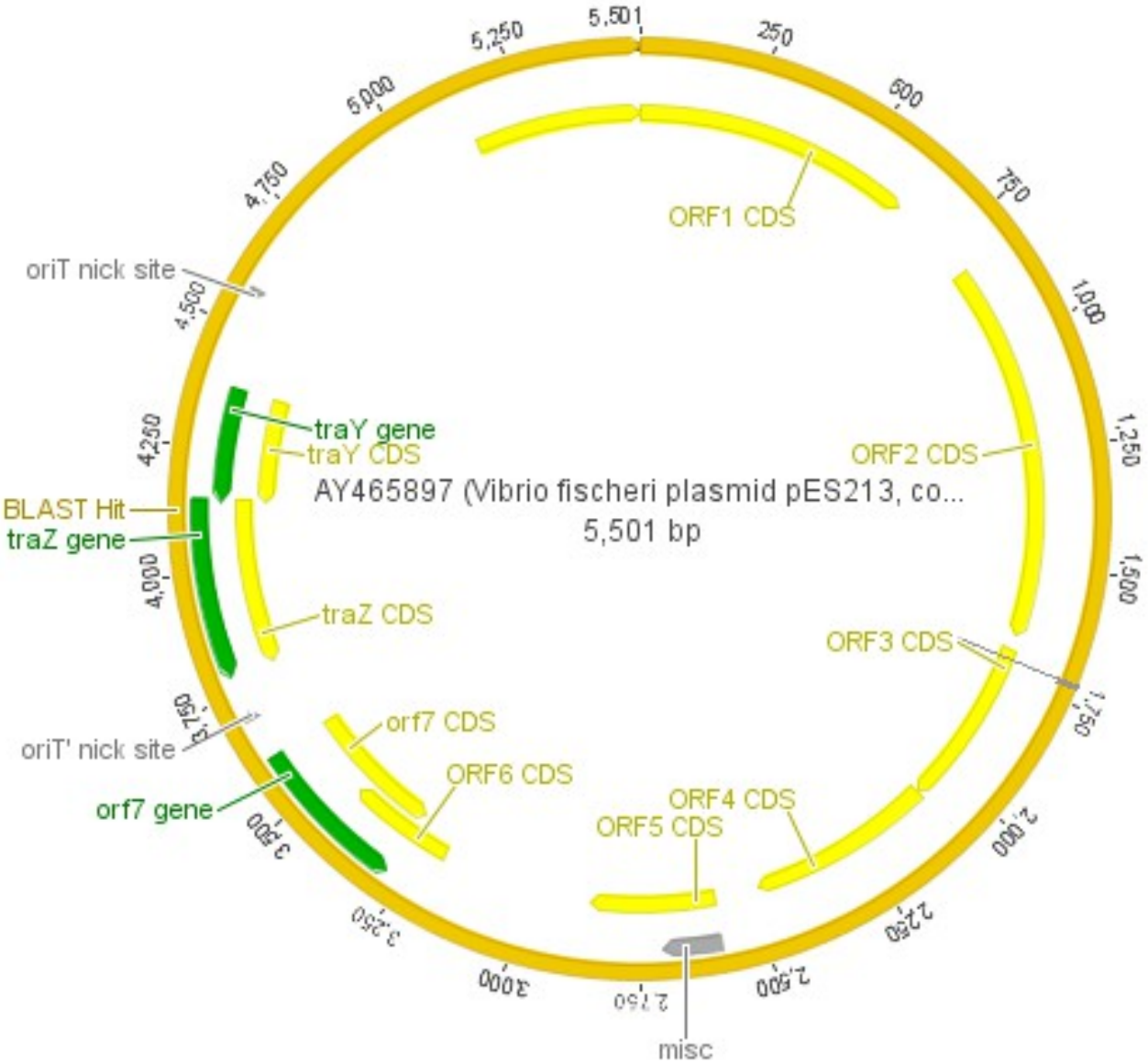
Can be transformed just by mixing bacteria!?!
(Paul, J. H., Thurmond, J. M., Frischer, M. E., & Cannon, J. P. (1992). Intergeneric natural plasmid transformation between E. coli and a marine Vibrio species. Molecular ecology, 1(1), 37-46.)

Several people have worked with plasmids that are portable between Vibrio and *E. coli*

---bottom line what works in one has a good chance of working in the other!

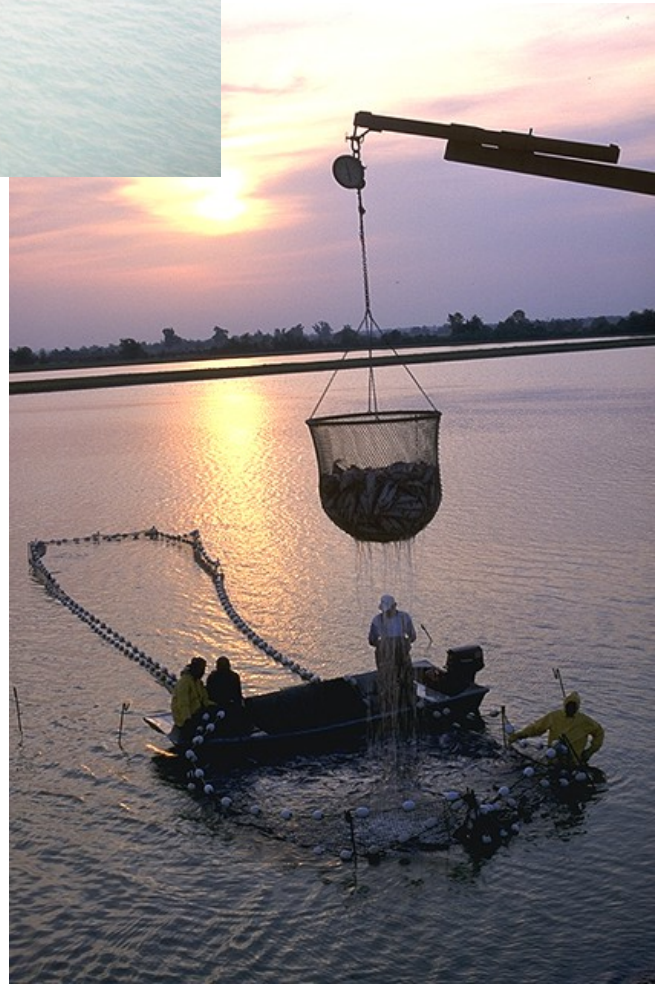
We can do all the manipulation in E. coli and transform V. fischeri with E. coli plasmids.

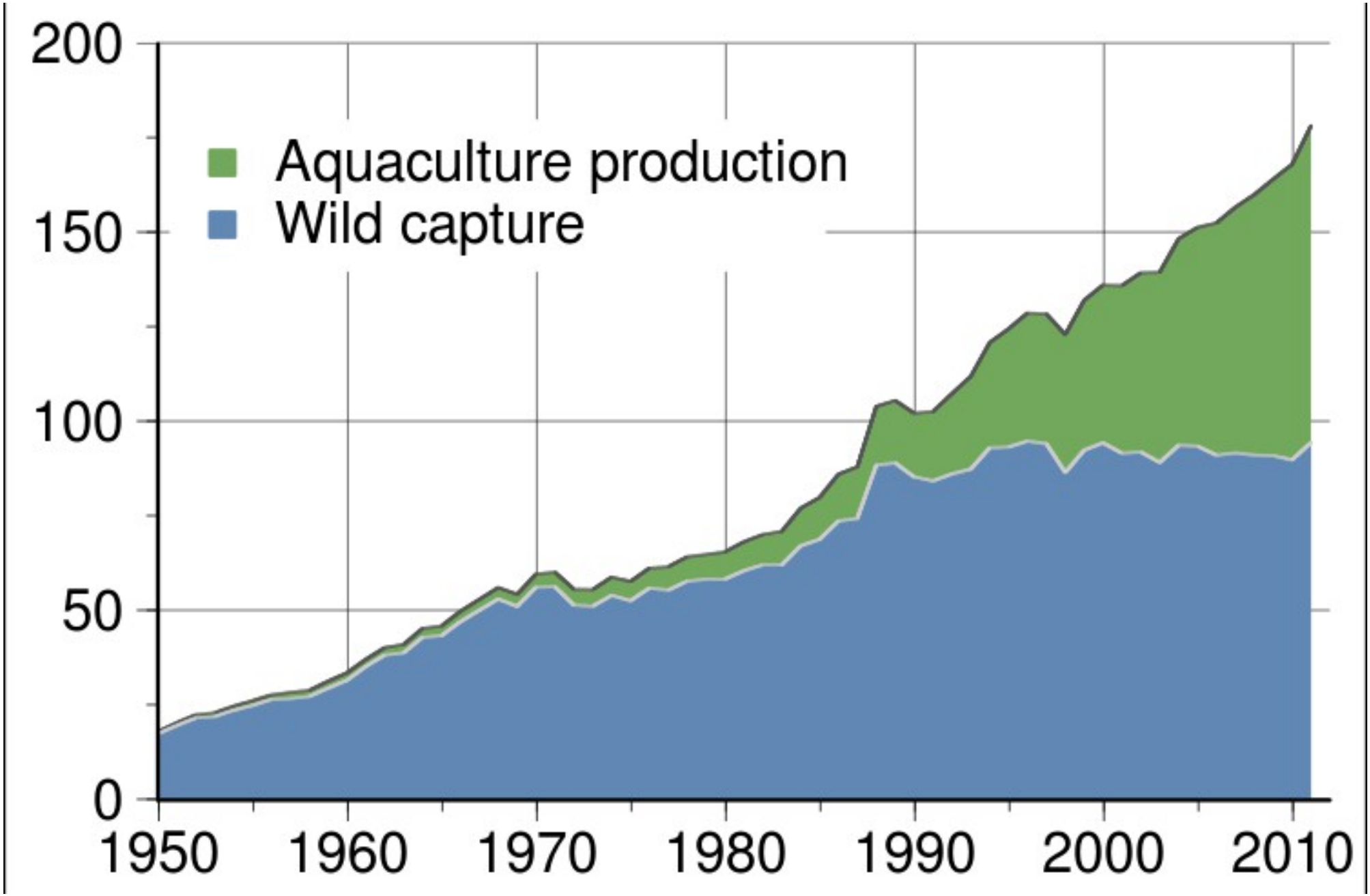
If necessary we can modify and use Vibrio derived plasmids at the last step.



Vibrio: PES213

Various forms of aquaculture.
http://en.wikipedia.org/wiki/Aquaculture

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Aquaculture production
Wild capture
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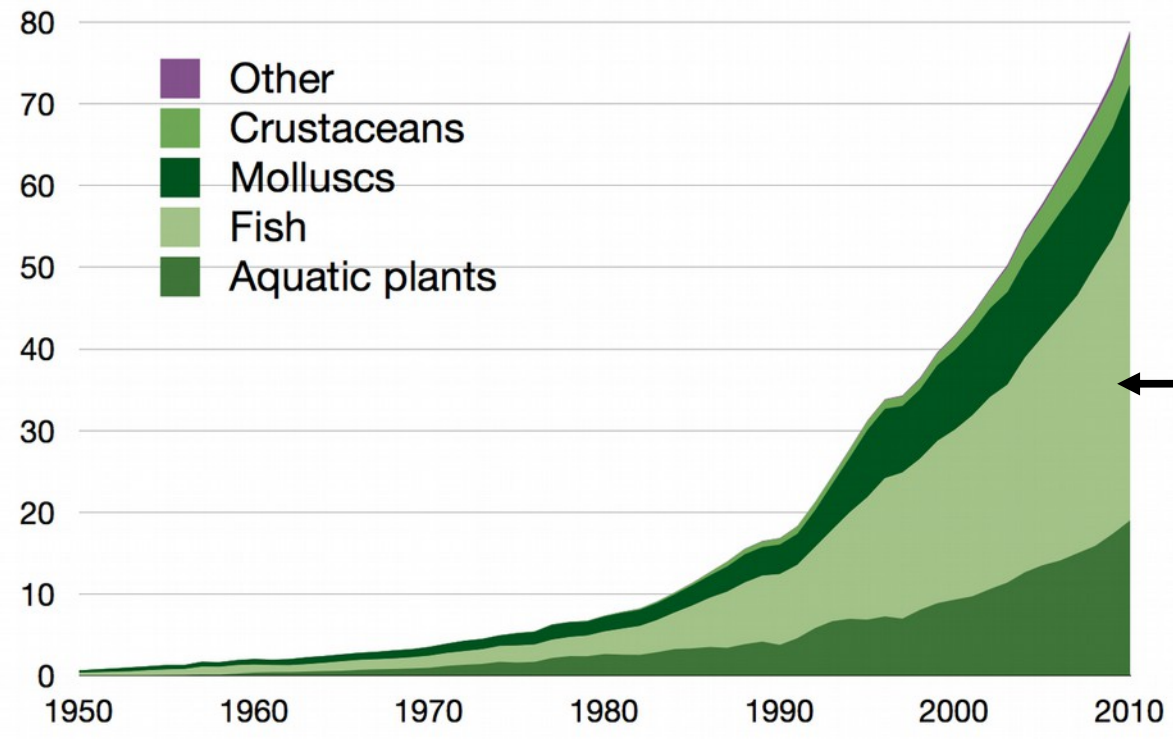
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Fish
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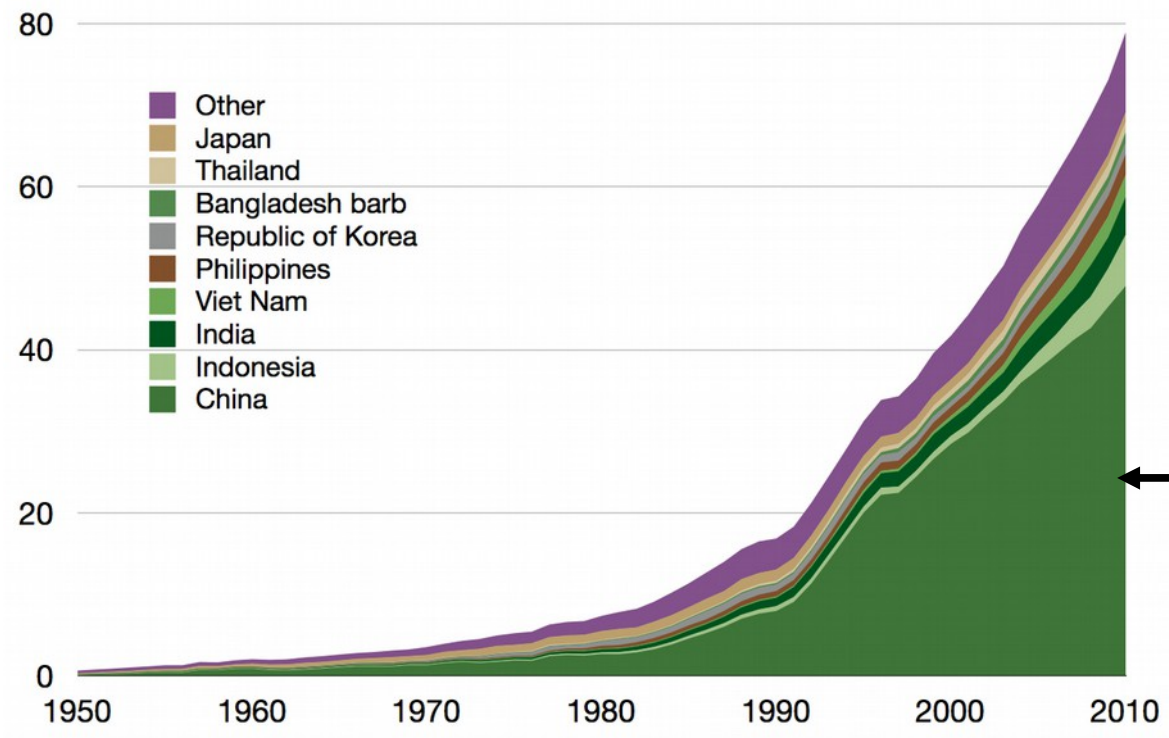
Fish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Other
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Republic of Korea
Philippines
Viet Nam
India
Indonesia
China
1950
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2010

China

Bull Environ Contam Toxicol (2009) 82:75–79
DOI 10.1007/s00128-008-9561-z

Heavy Metals in the Farming Environment and in some Selected Aquaculture Species in the Van Phong Bay and Nha Trang Bay of the Khanh Hoa Province in Vietnam

Ngo Dang Nghia · Bjørn Tore Lunestad · Trang Si Trung · Nguyen Thanh Son · Amund Maage

Aquaculture is currently **one of the most rapidly growing production sectors in Vietnam.** This publication describes the concentrations of heavy metals in the farming environment and some aquaculture species in the Khanh Hoa Province in Vietnam. The concentration of total **As** in the sediments ranged from 0.07 to 0.64 mg/kg, whereas the concentration of **Hg** varied from <0.0005 to 0.56 mg/kg. The corresponding concentration span for Cd and **Pb**, were 0.001–0.069 and 0.016–0.078 mg/kg, respectively. The concentrations of As in the aquaculture organisms spanned from 0.14 to 1.03 mg/kg. For Hg the concentrations varied from 0.1 to 0.45 mg/kg, for Cd from 0.02 to 0.10 mg/kg and for Pb from 0.07 to 0.37 mg/kg.

Setting: Undergrad Genetics Teaching Lab

Goals: Teach general mol. bio. procedures.
Our goal is to integrate student directed synthetic biology.

Relevance to Hawai'i / California and the Pacific is a plus.

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Part:BBa K346003 - parts.igem.org
partsregistry.org/Part:BBa_K346003
Oct 14, 2010 - RBS(B0032)+MBP(**mercury** metal binding peptide engineered from MerR). This part is a translational unit for expression of **mercury** metal ...

Part:BBa K346002 - parts.igem.org
partsregistry.org/Part:BBa_K346002
Oct 12, 2010 - PmerT promoter (**mercury**-responsive). This part, PmerT, is a promoter from Tn21 **mercury** resistance (mer) operon. The regulatory region of ...

Part:BBa K346004 - parts.igem.org
partsregistry.org/Part:BBa_K346004
Oct 14, 2010 - Function test of **mercury** MBP showed that our whole-cell bioabsorbents can absorb more than 50% of 10-6 M Hg (II) in 120 minutes, indicating ...

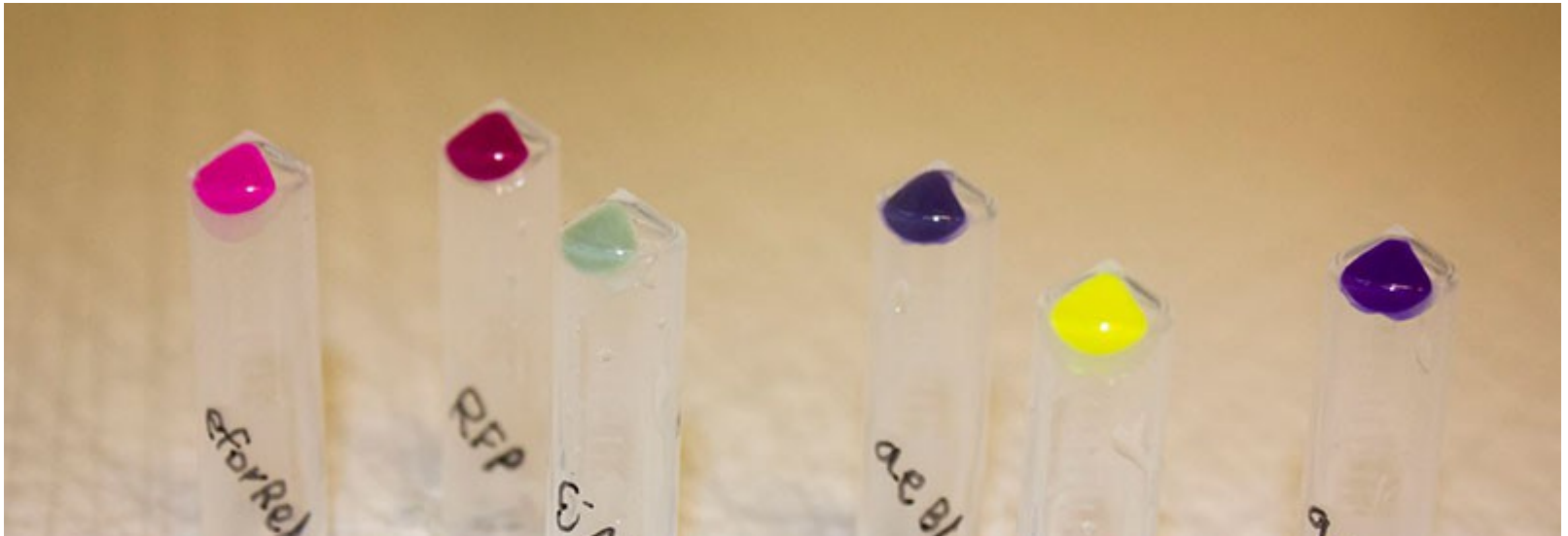
Part:BBa K346005 - parts.igem.org
partsregistry.org/Part:BBa_K346005
Oct 14, 2010 - This part was designed to function as **mercury**(II) ions absorption device in our project. If T7 polymerase is inductively expressed, this device ...

Part:BBa K346001 - parts.igem.org
partsregistry.org/Part:BBa_K346001
Oct 8, 2010 - RBS (B0034) + MerR (**mercury**-responsive transcription factor). This part was designed as a translational unit for MerR expression.

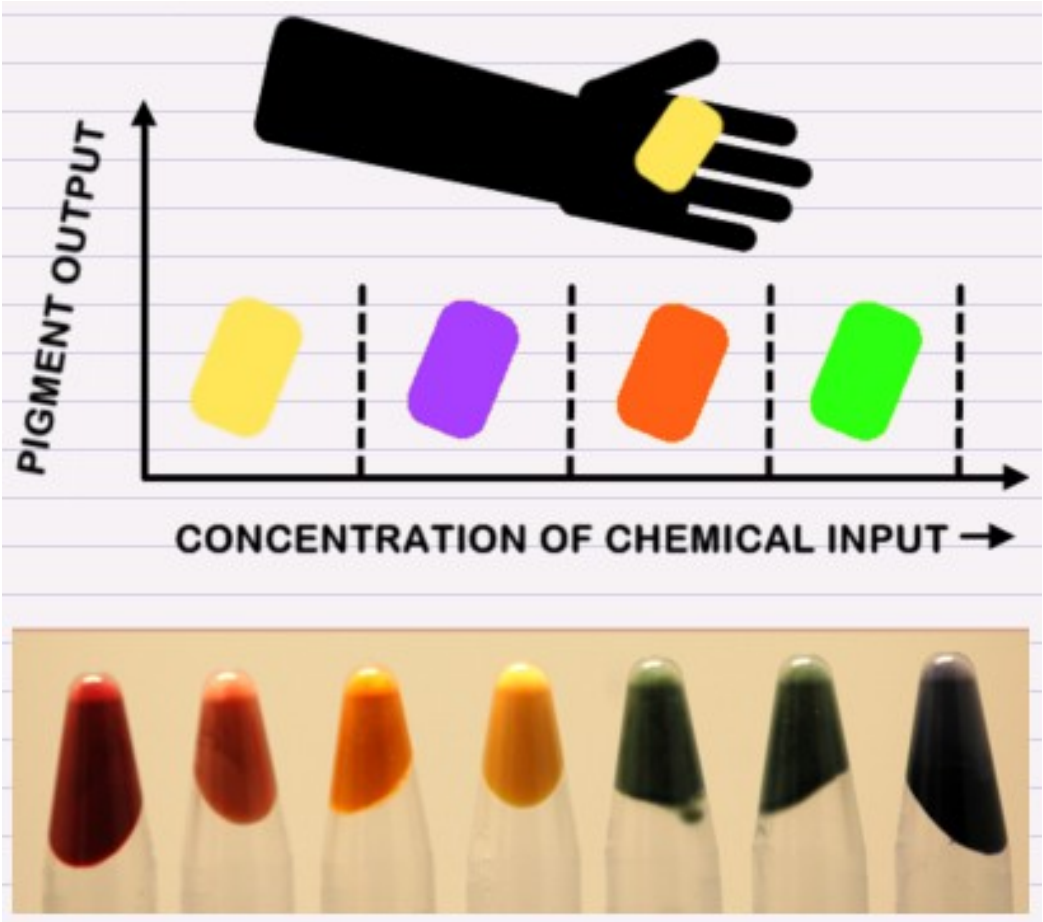
A range of sensors already exist:

Mercury	http://2010.igem.org/Team:Peking/Project/Biosensor
Lead	http://parts.igem.org/Part:BBa_I721001
Arsenic	http://2006.igem.org/University_of_Edinburgh_2006
Nitrites	http://2009.igem.org/Team:Edinburgh
Copper	http://parts.igem.org/Part:BBa_I760005
Toxins found in smoke	http://2011.igem.org/Team:ETH_Zurich
Iron	http://2013.igem.org/Team:Evry/Biology

And a range of reporters (chromoproteins, etc.):
http://parts.igem.org/Protein_coding_sequences/Reporters



Tools already exist to also quantify the concentration of the metal ion being sensed.



http://2009.igem.org/Team:Cambridge

Project length ~ 9/10 weeks; assuming students are already oriented re pipettors, gels, PCR;

Week 1 - Intro to SynBio thinking/techniques (largely drylab)

paper exercise; read discuss synbio paper or iGEM wiki (sensor)

Weeks 2 and 3- (Biobrick assembly); digestion, gel purification, ligation and transformation; students pick project

(plasmid preps? Or instructor does)

Week 4 - oligo design; string design; (student designed)

Vibrio fischeri transformation

Week 5 - oligo design; string design; order oligos or strings

→ Ethical discussion: guided by Katie questions (what is this, how should we treat it, how should it be used, how should it not be used)

optimize Vibrio transformation protocol (student designed projects)

(could spend two weeks here)

|

Week 5- GGA assembly and E. coli transformation

Week 6 - PCR verification of assembly; column purification of PCR products

(pick X successful transformants/unsuccessful controls)

------PCR products sent out for sequencing

Week 6 - Sequence analysis and verification / Plasmid preps

Week 7 - Vibrio transformation - discuss detection schemes

Weeks 8 and 9 – Students construct a model (could just be a flowchart of +/-) to **predict results and then test detection** ability / sensitivity of Vibrio strains carrying plasmids (student designed projects) (natural samples as well as controls?)

Week 10 - presentation of results - posters? Oral? Sharing/archiving of data/resources (submission of biobricks?)

Backup - we have stock of already assembled sensor from registry of parts…they transform and test