Plasmic (pUC57-Sulfur-3Metallic) Gene Probe to Identify Hydrocarbons

Prairie View A&M University
IGEM Team 2006

Background

- Cation porphyrins, Zn, Cu, Fe enhance mRNA expression in fungi (Cuero et al 2003, Cuero and Ouellet, 2005)
- Cation porphyrins are found in petroleum oil, so they can be used as a markers.
- Cation porphyrins mediate redox potential reactions, they produce reactive (radicals) hydroxides that oxidize DNA. (Byrnes, 1996)
- Iron-sulfur clusters [3Fe4S],in their oxidized form will induce repression of gene, while the reduced form for FdI; [4Fe4S] will derepress the gene expression (A.J Thomson)
- Genes are made of hundreds of atoms.....

Metals Bound to the Negative Charged Sugar-Phosphate of DNA

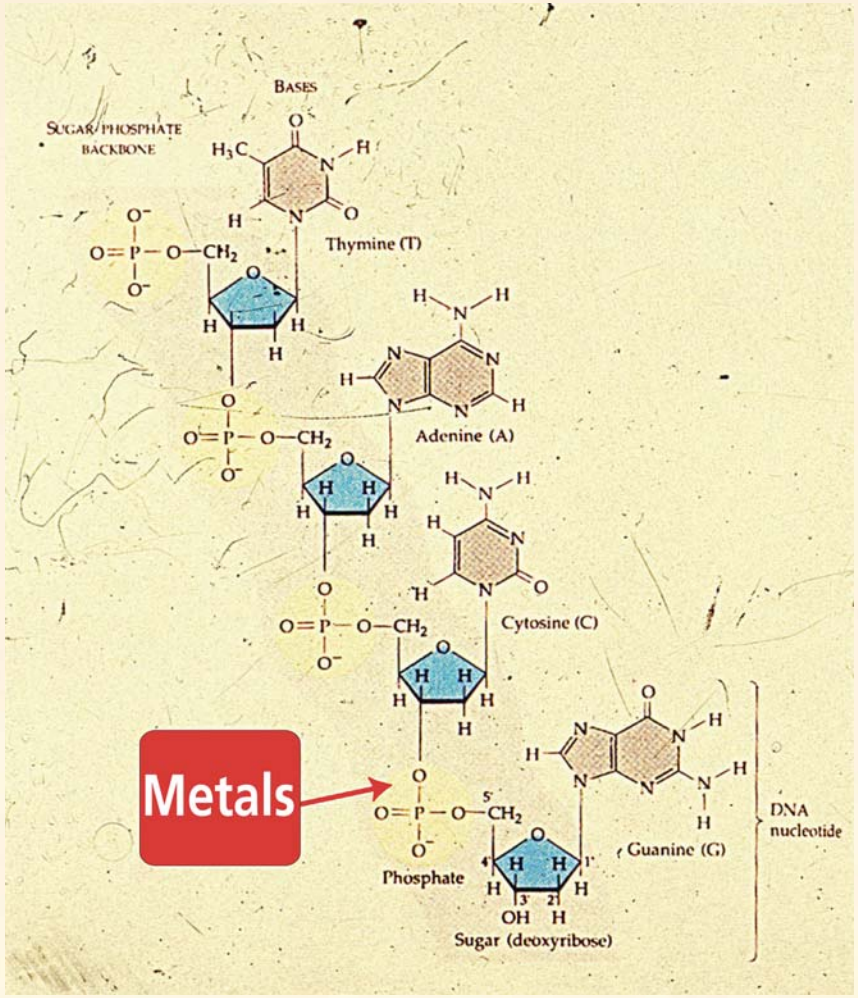


Figure 1

Objective

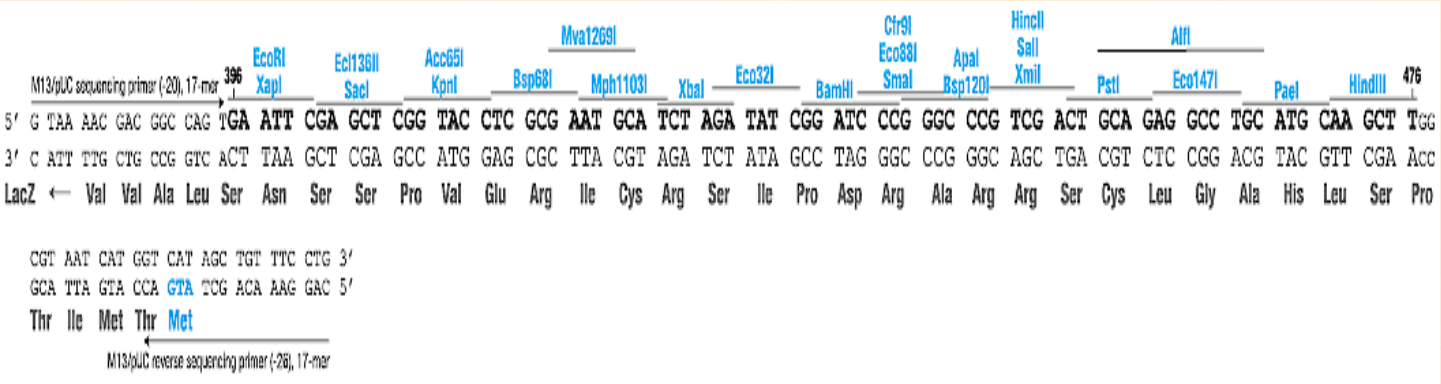
To develop standardized Metallo-genes for sensing Hydrocarbons by using metal ions [Fe (II), Ni(II), V(VI)] as a marker.

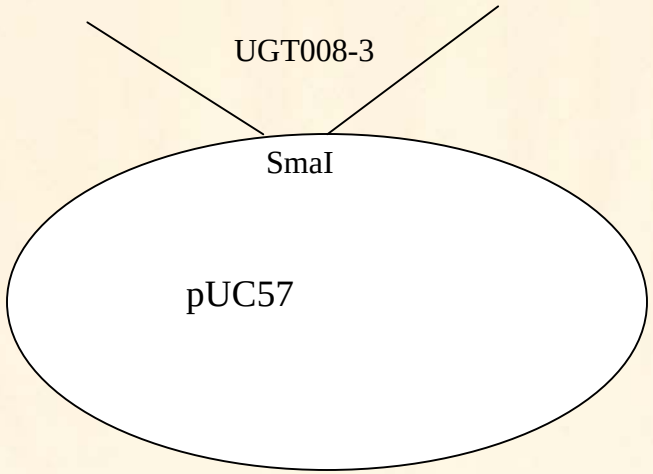
Hypothesis

Developing gene probe sensoribility of metal ions to detect hydrocarbons using standard DNA components (BioBricks).

Figure 2.

pUC57-S-3M Map





Courtesy GeneMen Synthesis Inc, CA,2006

<u>Insertion sites for BioBricks</u>

EcoRI

XbaI

BamHI

PstI

Table 1. Met32p Gene Information

Gene No.		Gene Name	Similar to Met32p
Length (bp)	588		
Vector	pUC57		
Plasmid No.	A63403-3		
Cloning site*	SmaI	Host	DH5a

A unique BamH1 and EcoRV sites at 5' and 3' end, respectively was added .

Table 1a. Assembled Parts in Registry

-?-	Name	Type	Description	Length
	BBa_J48108	Measurement	pUC57-Sulfur-3M	10106

-?-	Name	Type	Description	Length
	BBa_J48103	Regulatory	Iron promoter	140
	BBa_J48104	Regulatory	NikR promoter, a protein of the ribbon helix-helix family of trancription factors that repress expre	40
	BBa_J48106	Regulatory	vnfH	891
	BBa_J48107	Regulatory	UGT008-3 Promoter/Met32p	588
	BBa_J48109	Signalling	lux I	4791
	BBa_J48110	Regulatory	Fe Promoter+ mRFP1	1009
	BBa_J48111	Regulatory	E. coli NikR	926
	BBa_J48112	Regulatory	vnfH: vanadium promoter	1816

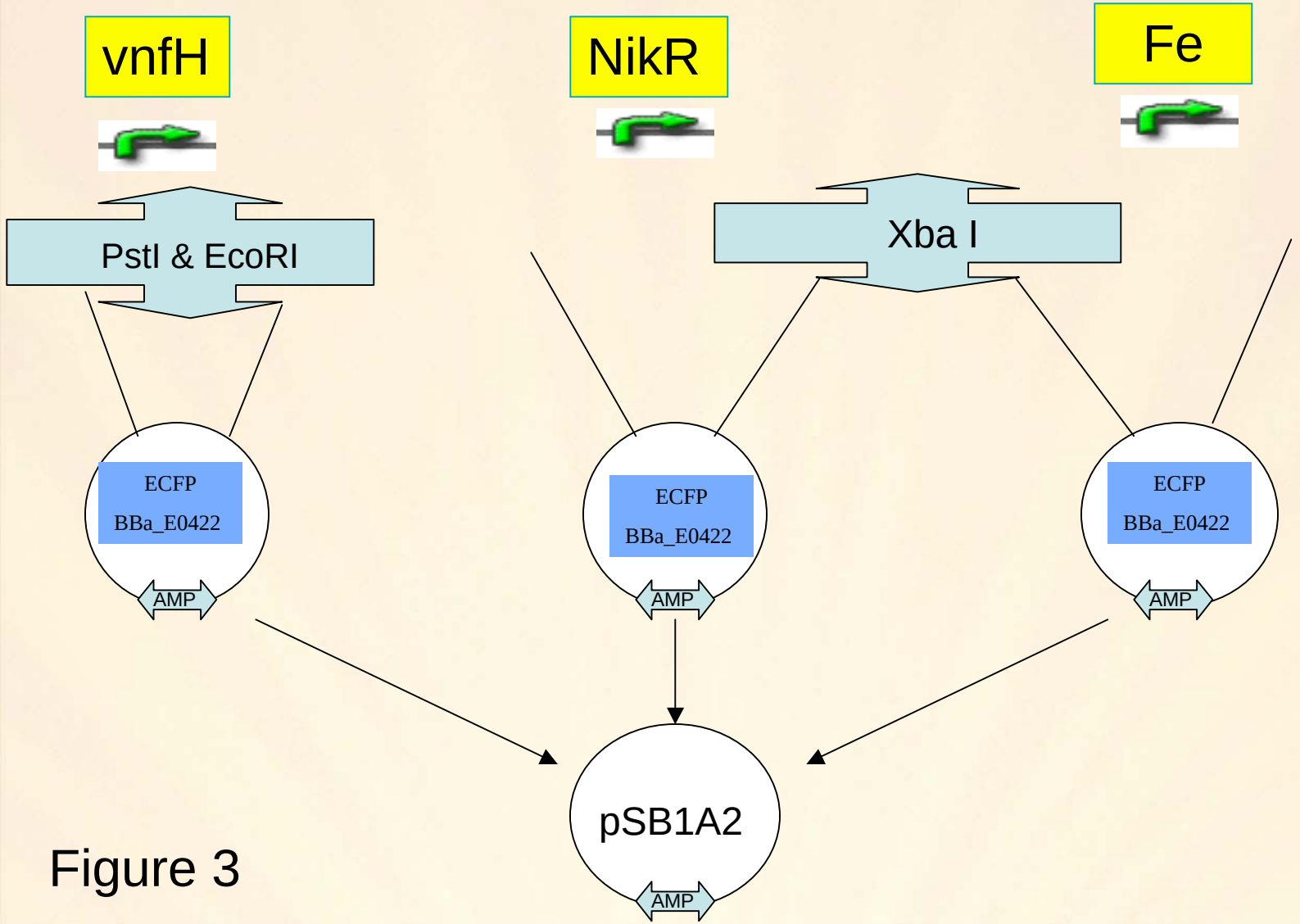

vnfH
NikR
Fe
PstI & EcoRI
Xba I
ECFP
BBa_E0422
AMP
ECFP
BBa_E0422
AMP
ECFP
BBa_E0422
AMP
pSB1A2
AMP

Figure 3

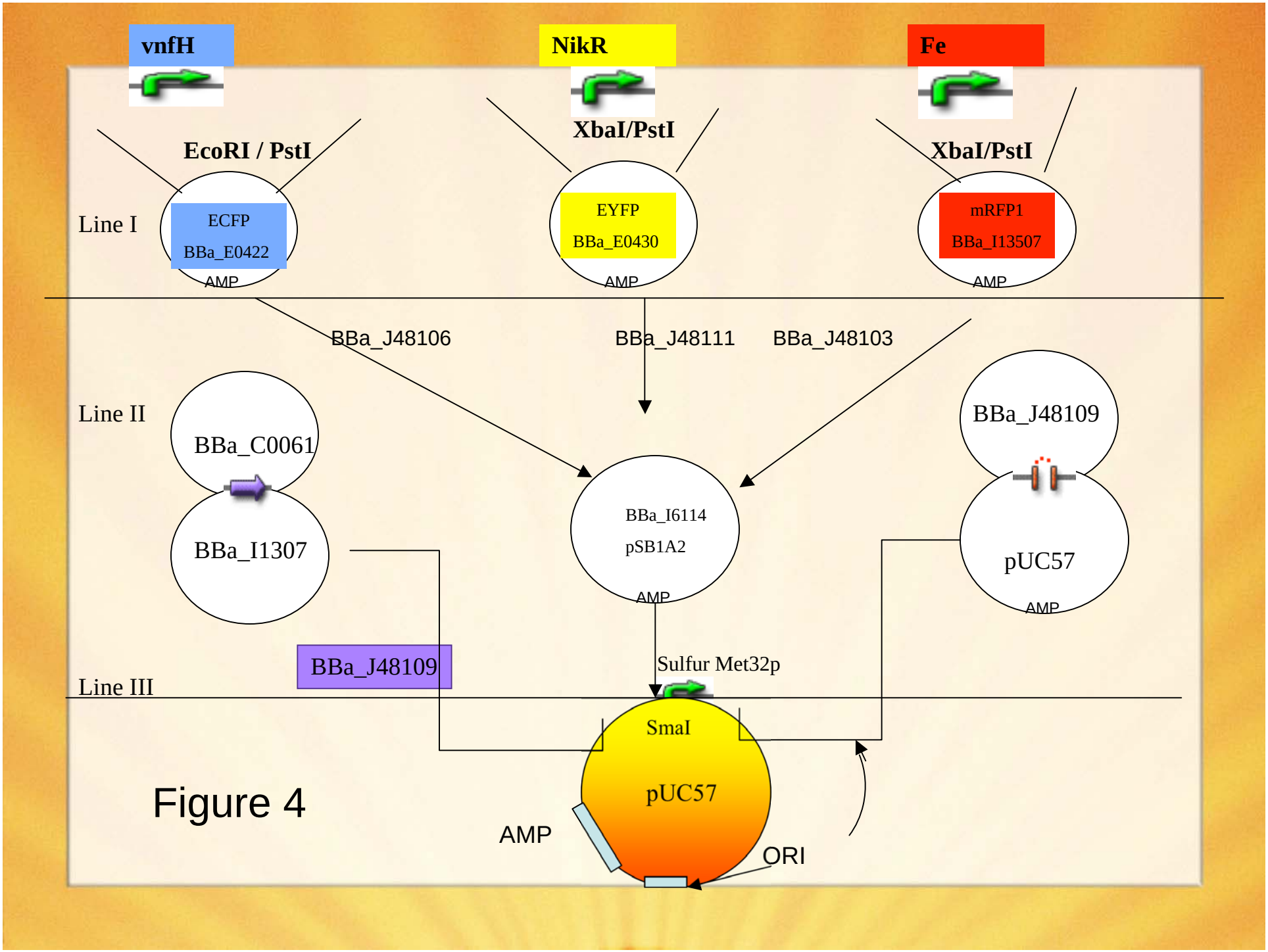
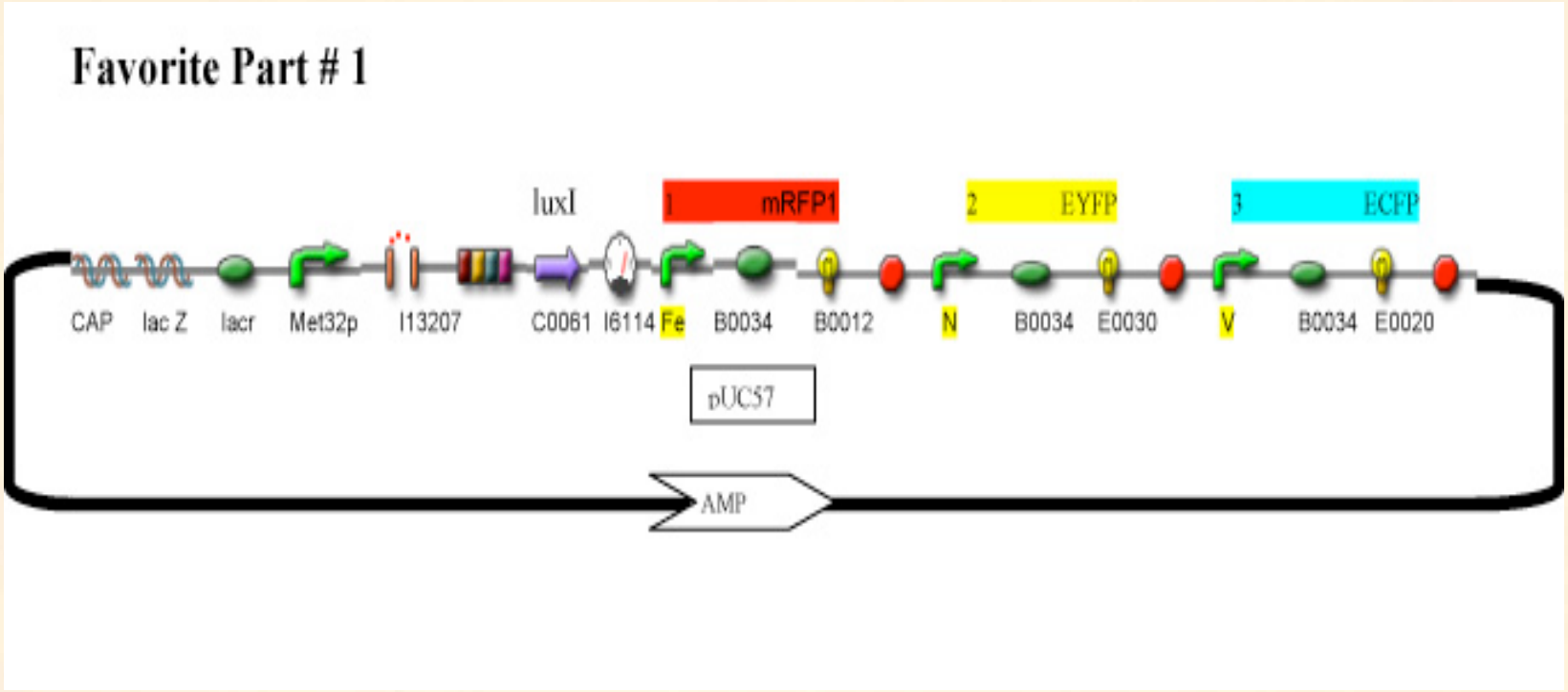

vnfH
NikR
Fe
EcoRI / PstI
XbaI/PstI
XbaI/PstI
Line I
ECFP
BBa_E0422
AMP
EYFP
BBa_E0430
AMP
mRFP1
BBa_I13507
AMP
BBa_J48106
BBa_J48111
BBa_J48103
Line II
BBa_C0061
BBa_I1307
BBa_I6114
pSB1A2
AMP
BBa_J48109
pUC57
AMP
BBa_J48109
Sulfur Met32p
Line III
SmaI
pUC57
AMP
ORI

Figure 4

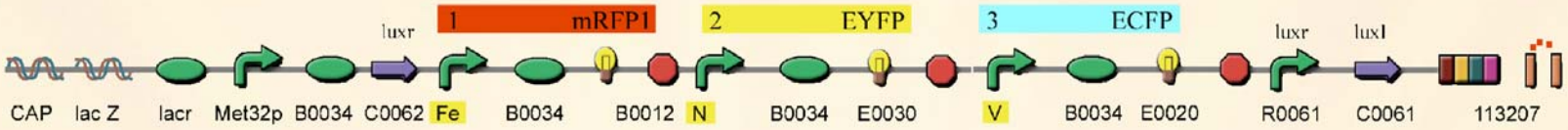
Constructed PV-Trimetallic-gene probe: pUC57-S-3M



Constructed PV-Trimetallic-gene probe: pUC57-S-3M

Favorite Part 2

(Planning)



Growth of the pUC57-S-3M Transformed Micrococcus luteus Cells in Comparison with Non-transformed M. luteus (ATCC #4698)

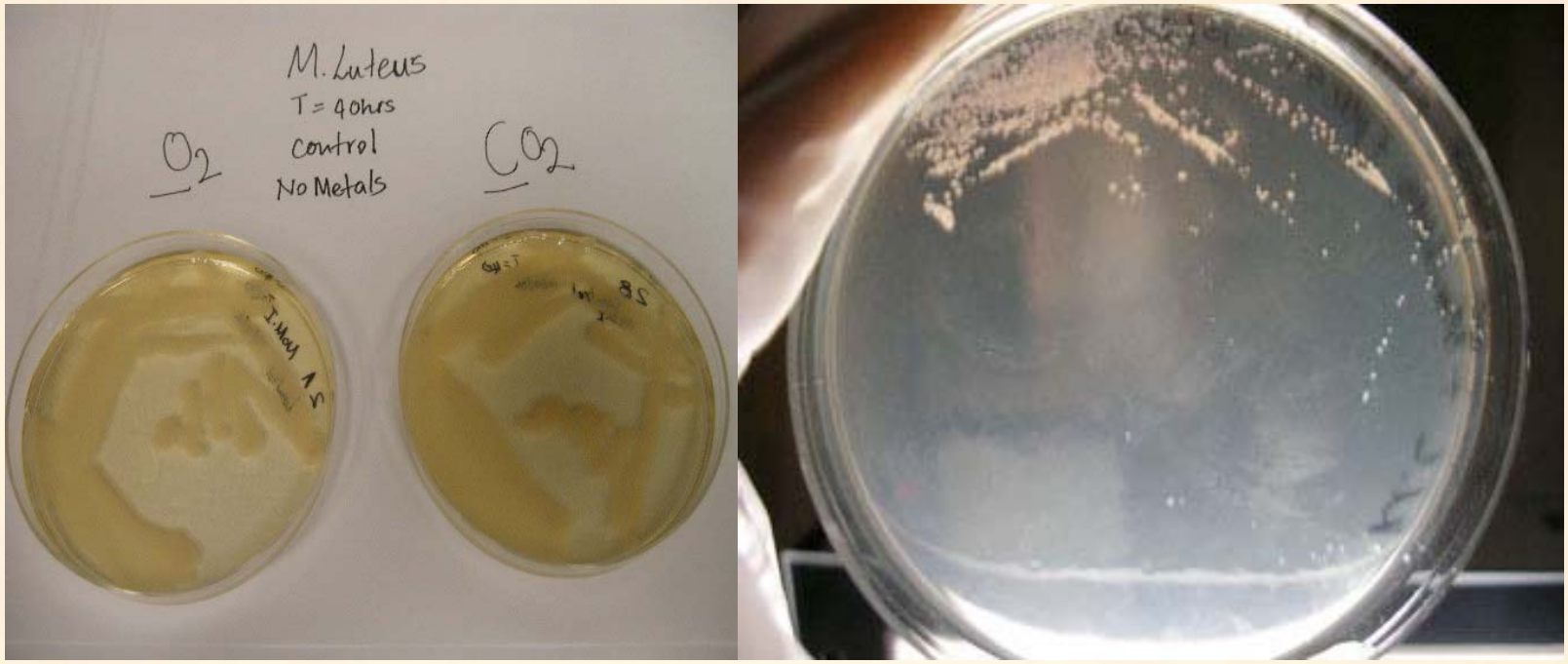


Figure 5.

CFU of the Transformed Cells Showing the Expression of the Fluorescent Protein

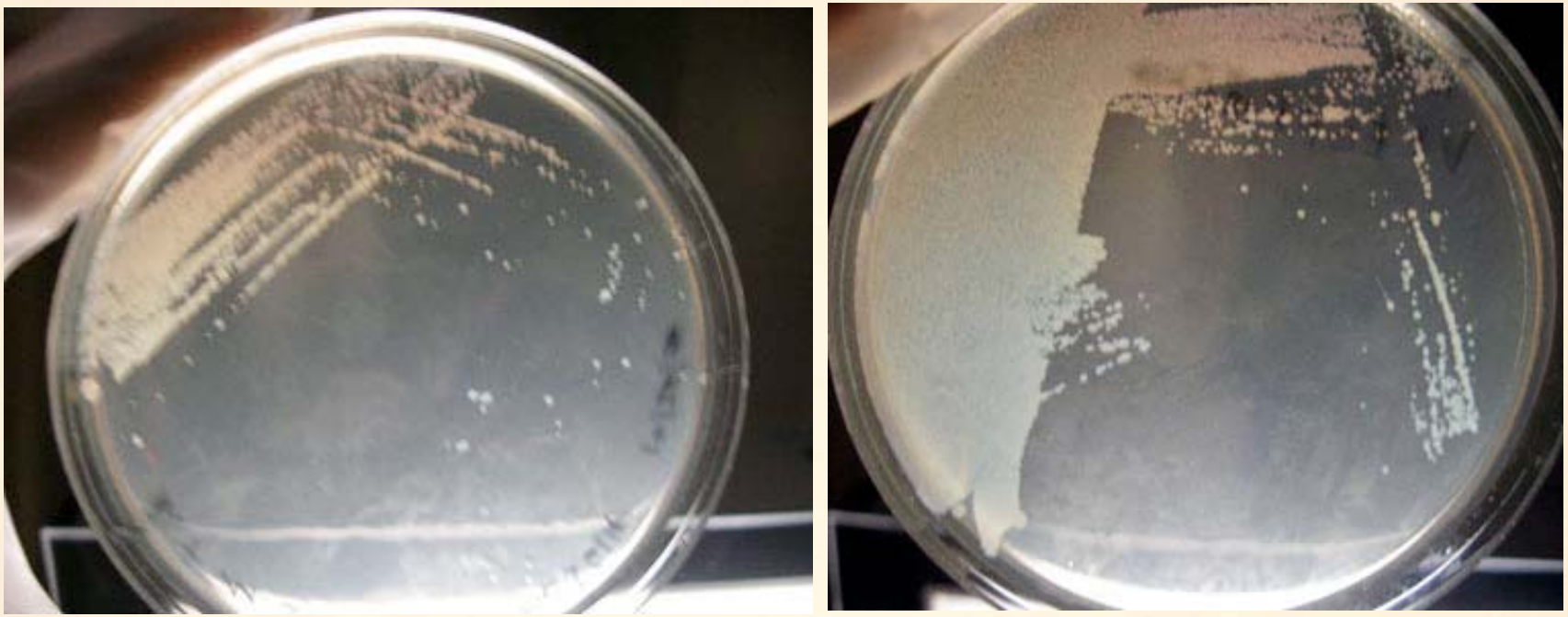


Figure 6

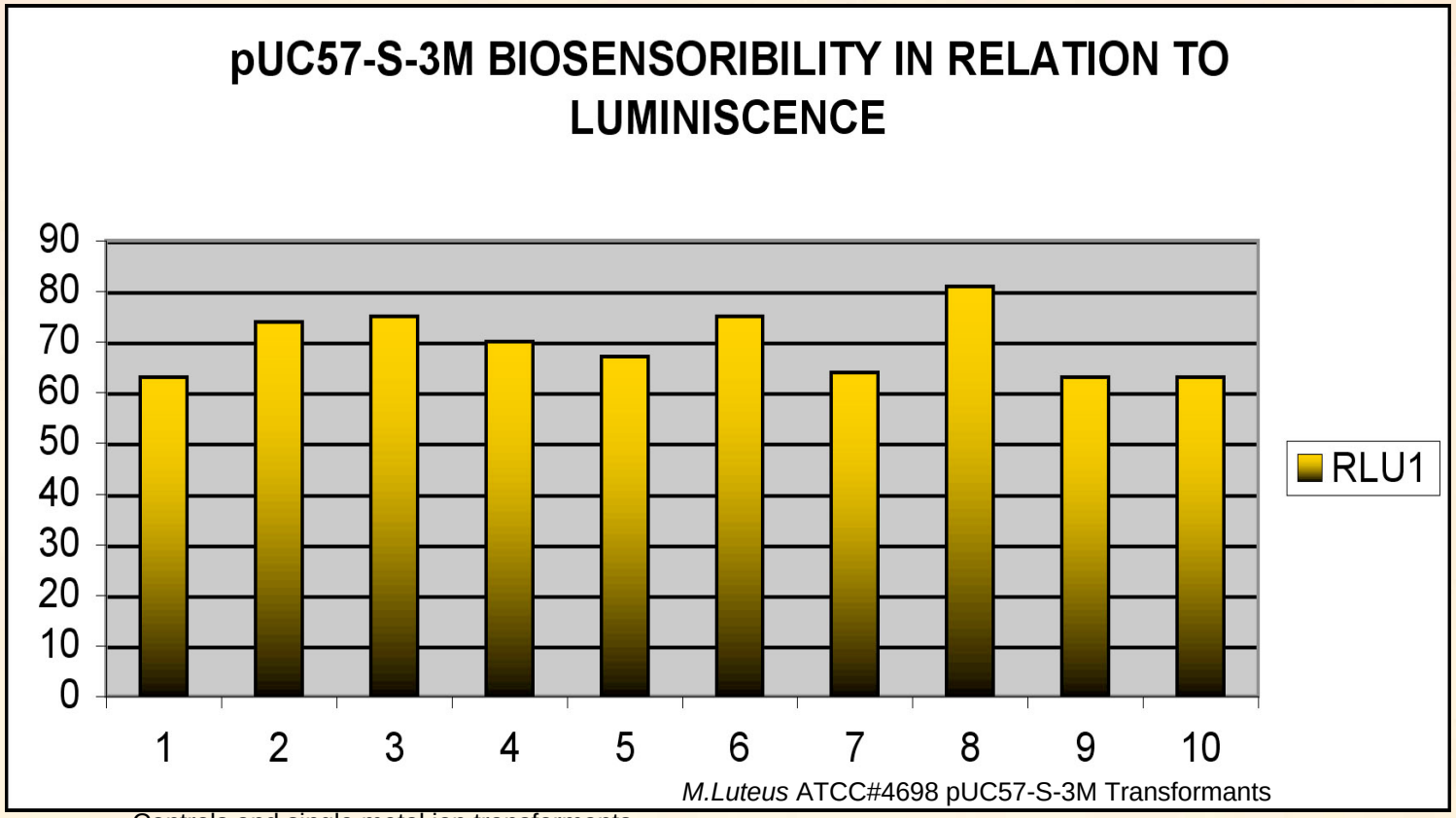

pUC57-S-3M BIOSENSORIBILITY IN RELATION TO LUMINISCENCE
RLU
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
RLU1
M.Luteus ATCC#4698 pUC57-S-3M Transformants
Controls and single metal ion transformants

Figure 7

Table 2. Biosensoribility of the Trimetallic Gene Probe in Relation to DNA Expression under Different Metal Concentration, as Compared to Non-Transformed

Cell Treatment	DNA Concentration (ng/ml)
Transformed (Gene Probe)	
CONTROL (Micrococcus luteus)	30.6
Tr ML-O_2, Fe+Ni+V+S(0.2ppm)	11.9
Tr MLO_2+C6H5SH, Fe+Ni+V+S (2ppm)	18.3
Tr $MLCO_2$ + C6H5SH, Fe+Ni+V+S(0.2ppm)	12.2
Tr O_2+C6H5SH No Metals	5.15
Non Transformed	
M.L -O_2 (2ppm)	3.95
M.L -O_2+C6H5SH(2ppm)	1.15
M.L -$C0_2$+C6H5SH(2ppm)	0.95
M.L-$C0_2$ (2ppm)	4.5

Tr- Transformed cells by pUC57S-m3

Figure 8

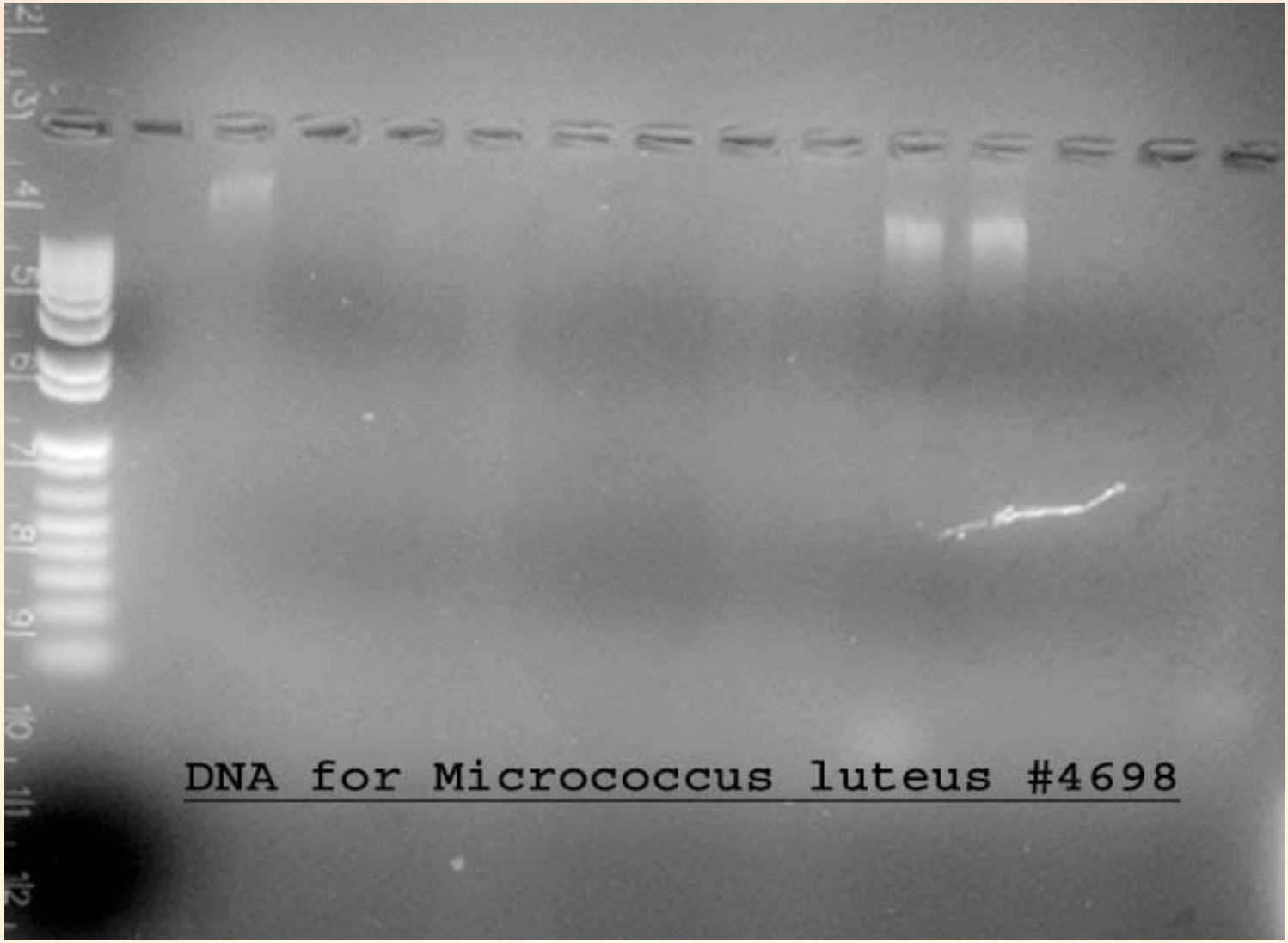


Table 3. Biosensoribility of trimetallic gene probe (pUC57 SM-3) in relation to cell growth (CFU) as Compared to Cells Transformed with Single Metal Genes (before subjection in metal ion media)

	BACTERIAL STRAINS				
Time	**M.L. ATCC#4698**	**Fe +mRFP1**	**V+ECFP**	**N+ EYFP**	**pUC57-S-3m probe**
Day1	2.56 x 10^5	1.5x10^4	1.2x10^4	2.1x10^4	9x10^3
Day2	1.79x10^5	1.50x10^5	3.00x10^5	1.55x10^5	2.50x10^5

CFU=Colony Forming Units
Fe+mRFP1 Iron promoter + red flourescence protein
V+ECFP Vanadium + Enhanced Cyan Fluoresence Protein
N+ EYFP Nickel+ Enhanced Yellow Fluorescent Protein

Table 4. Sensorability of M. luteus Strain (ATCC4698) Using in Relation to Metal Ion Concentration, pH, O_2, and Redox Potential at Different Times

		M.luteus		*M.luteus+metal*				
Metal(ppm)		2	50	2		50		
Time (hr)		INITIAL		INITIAL				
0								
	O.D.	0.007	0.03	0.16	0.5	0.06	0.3	0.3
	pH	7.1	7.1	6	6.5	6	7	7
	mV	20	27	60	94	87	90	90
		O2	CO2	O2	CO2	O2	CO2	
18								
	O.D.	0.4	0.2	0.3	0.32	0.17	0.21	0.21
	pH	7.7	7.5	6.6	6	2	1.4	1.4
	mV	-13	34	50	94	309	336	336
		O2	CO2	O2	CO2	O2	CO2	
40								
	O.D.	1.1	1.6	0.2	0.33	0.4	0.1	0.1
	pH	7.8	7	5.7	6	1.4	2	2
	mV	-16	42	103	86	332	305	305

FOR CO2 INCUBATION; CULTURES WERE KEPT AT 20% CO2 ATMOSHPEHE

METAL IONS WERE USED @ 0.2,2,50ppm

O.D.=OPTICAL DENSITY

M.Lhas the ability to grow in both CO_2 and O_2 atmosphere. However the presence of thiophenol enables the transfomed micrococcal cells to grow under CO_2 .atmosphere.

Table 5. Sensorability of M.L. Strain (ATCC4698) Using in Relation to Metal Ion Concentration, pH, O_2, Redox Potential, and Hydrocarbon (Thiophenol)

M.luteus+metal+CHO

Metal(ppm)		2		50	
Time (hr)		INITIAL			
0					
	O.D.	0.3	0.5	0.6	0.3
	pH	6.1	6.3	0.6	0.3
	mV	71	97	88	93
		O2	CO2	O2	CO2
18					
	O.D.	0.23	0.2	0.36	0.24
	pH	1.6	1.43	6.4	5.7
	mV	327	311	63	98
40		O2	CO2	O2	CO2
	O.D.	0.2	0.2	0.1	0.1
	pH	1.4	2	6	1.5
	mV	332	305	335	47

CHO=HYDROCARBON=THEOPHENOL

The hydrocarbon, thiophenol provides the substrate for catalase and oxidase which allows for better growth in low O_2 concentration.

Table 6. Interactive Effect of pH, O_2, CO_2, and CHO on Transformed M. luteus pUC57-S-3M in Relation to Biosensoribility

pUC57-S-3M

Metal(ppm)		0.2		2		no metals	
Time (hr)			INITIAL				
0							
	O.D.	0.021	0.038	0.03	0.023	0.006	0.011
	pH	7.01	6.85	672	653	7.01	7
	mV	28	36	54	53	28	26
		O2	CO2	O2	CO2	O2	CO2
18							
	O.D.	0.986	0.261	0.508	0.314	0.753	0.435
	pH	5.18	5.06	4.96	4.85	5.82	5.14
	mV	127	137	142	149	94	131
		O2	CO2	O2	CO2	O2	CO2
40							
	O.D.	0.99	0.171	0.514	0.282	0.869	0.51
	pH	5.56	5.26	5.71	5.09	6.79	5.66
	mV	108	121	99	130	31	102

CHO=HYDROCARBON=THEOPHENOL

Table 7. Interactive Effect of pH, O_2, CO_2, and CHO on Transformed M. luteus pUC57-S-3M in Relation to Biosensoribility

INCUBATION TIME = 0 hr

M.L. pUC57-S-3M + 0.2 ppm METAL			
CO2	0.038	6.85	36
O2	0.021	7.01	37
C6H5SH +O2	0.177	6.99	27
C6H5SH +CO2	0.179	7.03	33
M.L. pUC57-S-3M + 2 ppm METAL			
CO2	0.022	6.53	53
O2	0.05	6.72	54
C6H5SH +O2	0.043	6.66	46
C6H5SH +CO2	0.17	7.06	47
M.L. pUC57-S-3M _ NO METAL			
CO2	0.011	?	28
O2	0.007	7.01	33
C6H5SH +O2	0.033	6.92	30
C6H5SH +CO2	0.184	6.89	31

Time = 18 hrs

M.L. pUC57-S-3M + 0.2 ppm Metal			
CO2	0.281	5.06	37
O2	0.986	5.18	35
C6H5SH +O2	1.241	6.35	55
C6H5SH +CO2	1.907	6.01	49
M.L.pUC57-S-3M + 2 ppm METAL			
CO2	0.374	4.60	49
O2	0.808	4.96	42
C6H5SH +O2	1.324	5.09	96
C6H5SH +CO2	1.905	5.82	97
M.L.pUC57-S-3M + NO METAL			
CO2	0.435	5.74	47
O2	0.753	5.82	54
C6H5SH +O2	1.97	5.49	47
C6H5SH +CO2	1.89	6.06	90

NOTE: ppm = metal concentration; also types of metals = Ni, V, Fe.
S = sulfur; C6H5SH = PYRIDOXAMINE = THIOPHENOL
M.L. = Micrococcus luteus

Redox potential is high under CO_2 atmosphere- CO_2 is a reductant

There is redox potential threshold around 300mV which indicates sensoribilty for the metals ions.

Sulfur Clusters

- Sulfur is the bridging ligand in the Cu site of cytochrome oxidase
- Important componet of coenyzme A
- Sulfur is used in as H2S and can be used in place of water as an electron donor.

- Iron sulfur clusters-motifs found in metalloproteins e.g Ferrodoxins, NADH dehydrogenase, Coenzyme Q-cytochrome C reductase of the ETS.
- [2Fe2S]cluster-Bridging Ligands by
    - 2Fe-4 cysteine side chains
    - 2Fe-2 cysteine sulfur
    - 2Fe -2 nitrogen atoms of histidine
- [4Fe4S]Bridging Ligand by 4 sulfur of cysteine
- [3Fe4S]cluster

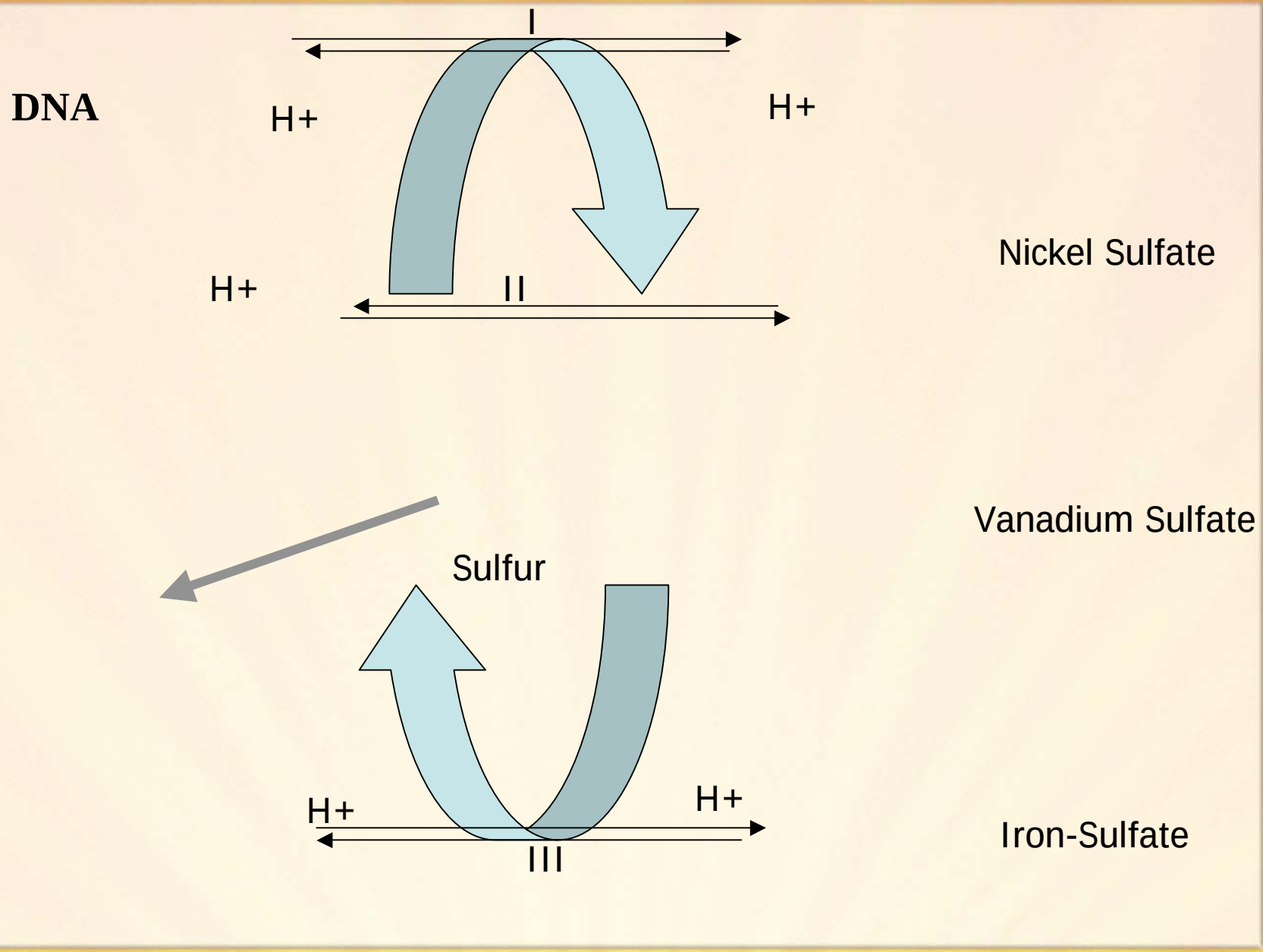


Figure 9. Metallic-sulfate Shuttle

Machine or Circuit?

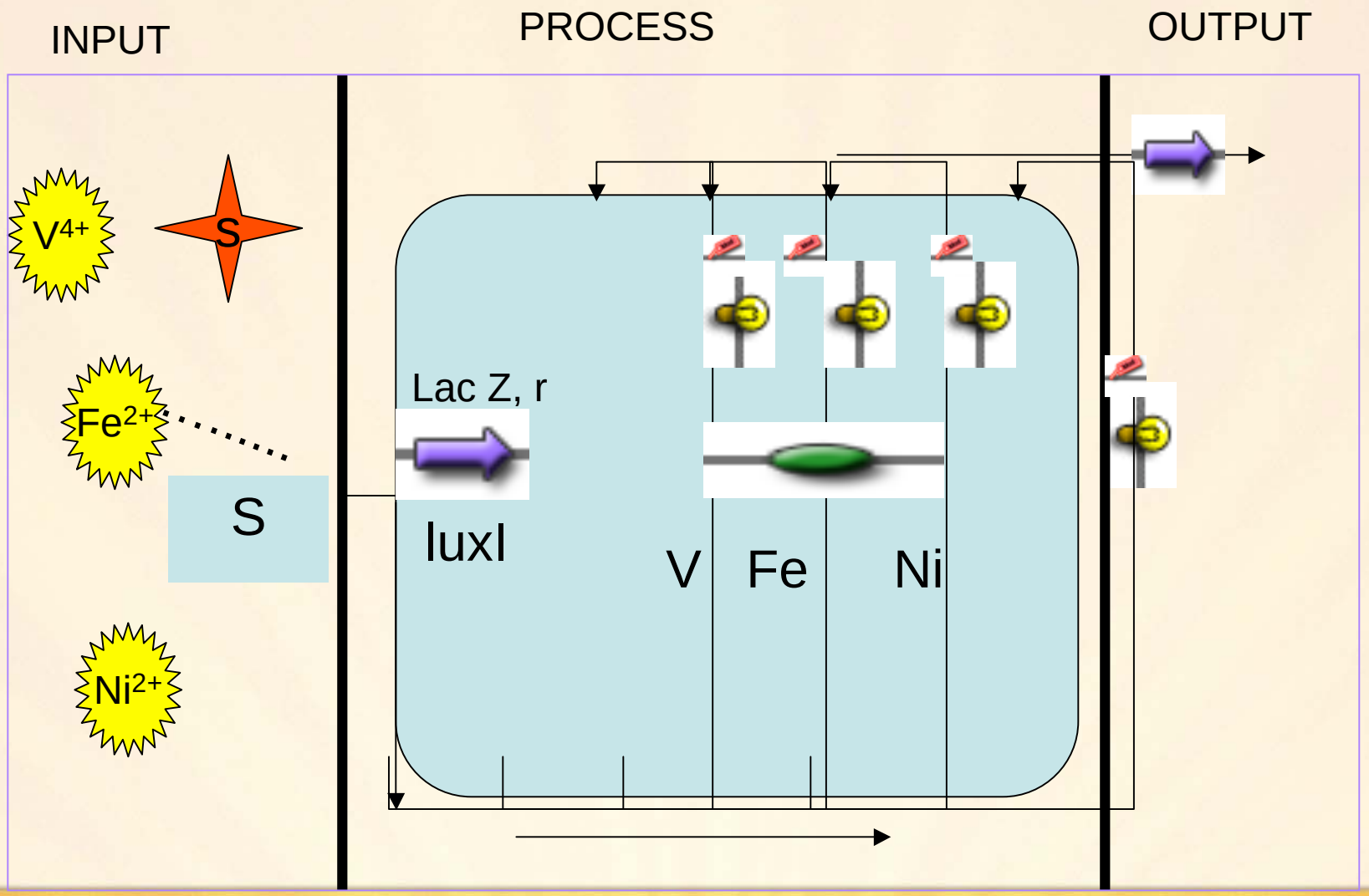


Figure 10

Conclusion

- Our BioBrick Assemblence, resulting in pUC57-S-3M was achieved.

Possiblity of Different assemblages can be configured different sensoribilty.

2. The pUC57-S-3M probe showed biosensoribility to different metal ion concentrations related to hydrocarbons.

- A much cheaper biological initiator in synthetic oil production.
- A possible better alternative to Microdiesel production.
- A biological Device that can be used in industry as a non persistant biological Chelater and complexation agent : a triplet function.

References

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Credits

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- IGEM Planning Committee

The Micro- “Coccus” Team.

The Prairie View A&M University
IGEM Team 2006

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