Lateral gene transfer and metal resistance in microbes from the FRC

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Why study plasmid ecology in the subsurface?

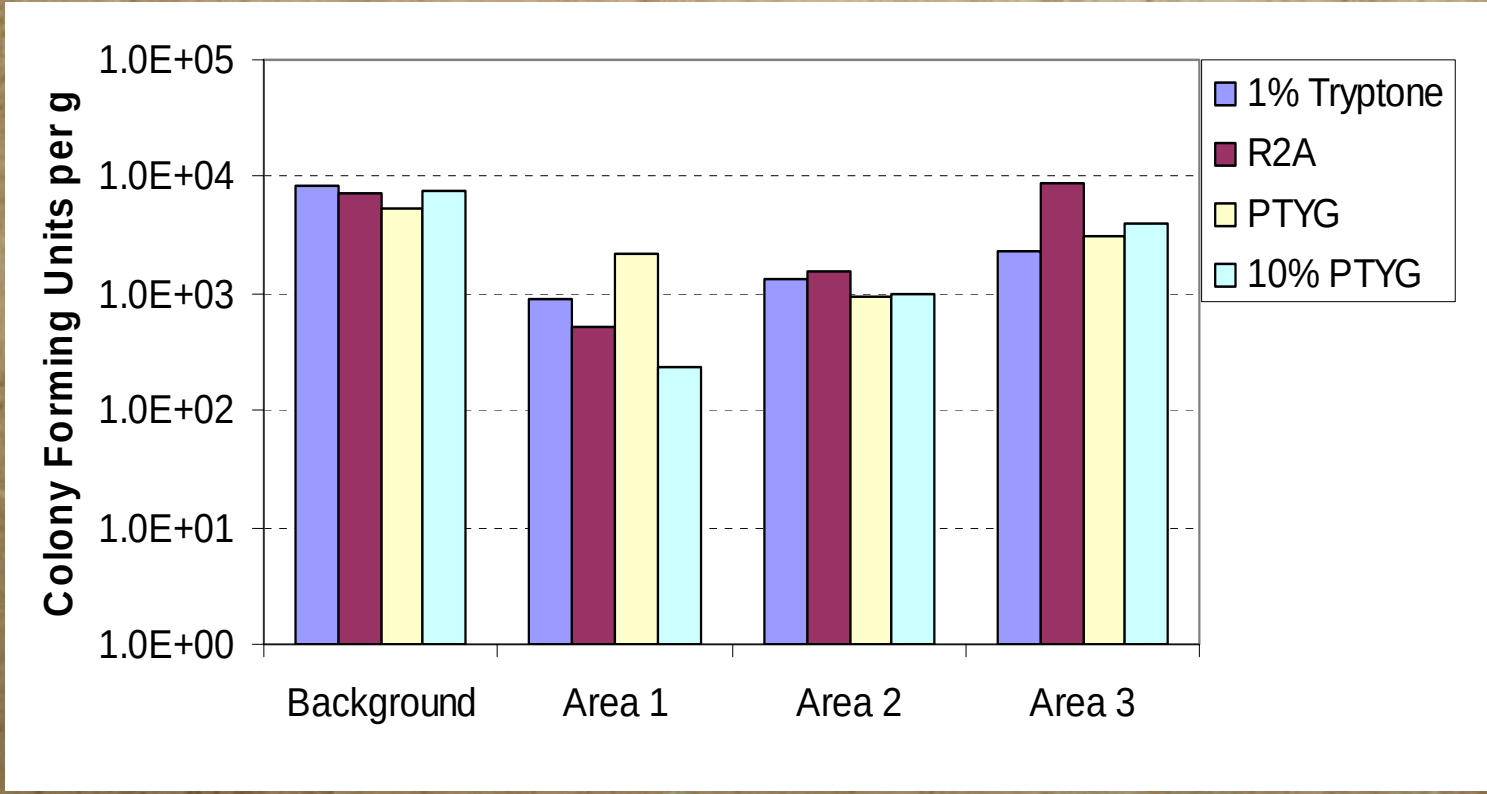
Plasmids represent a **mobile genetic community**, which can transfer genes among different species of bacteria.

Genes which code for resistances to metals (Hg, As, Pb) can be located on plasmids.

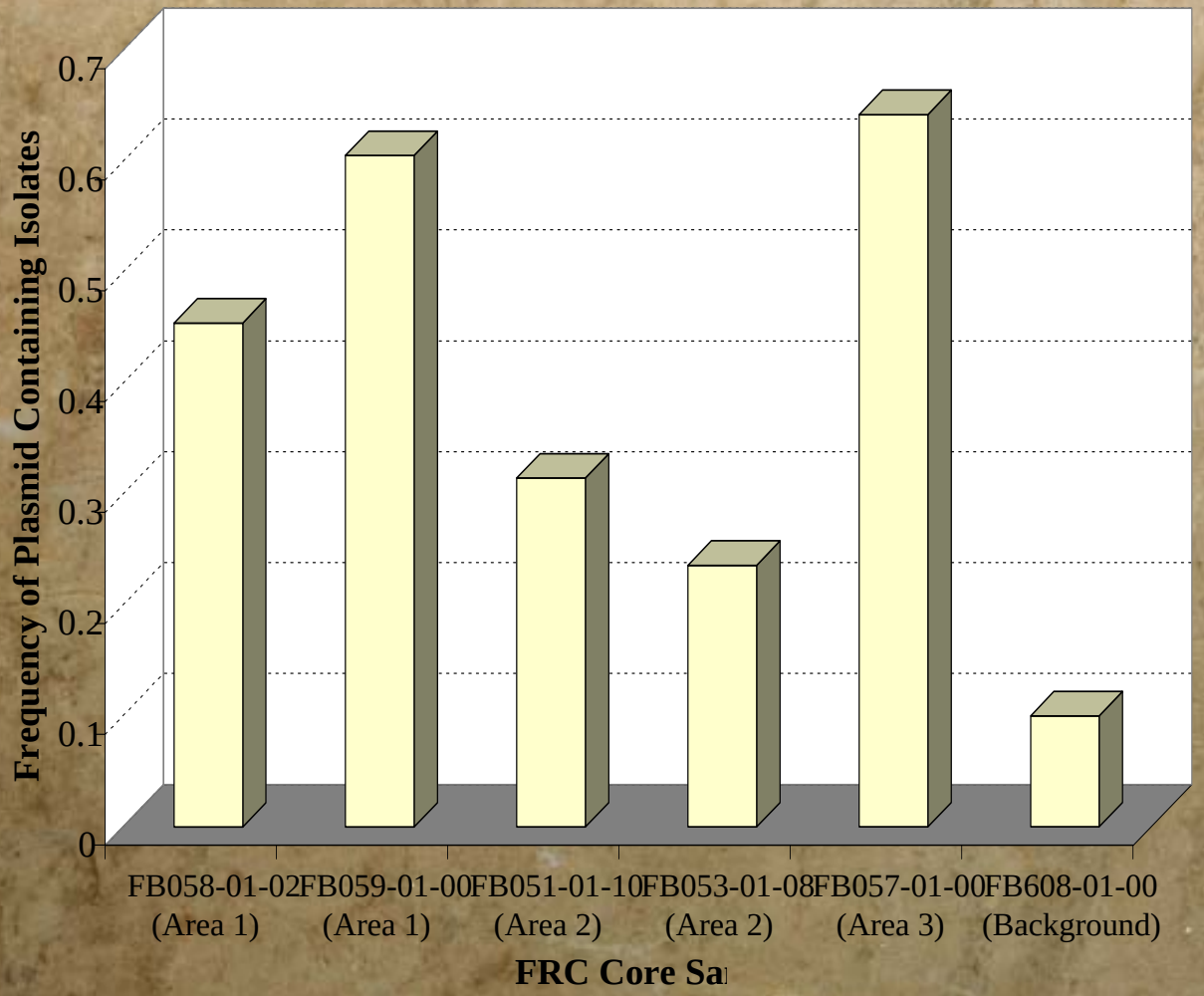
Organisms may need these genes to survive in metal contaminated environments.

Through LGT, they may be able to acquire these resistances from other members of the community.

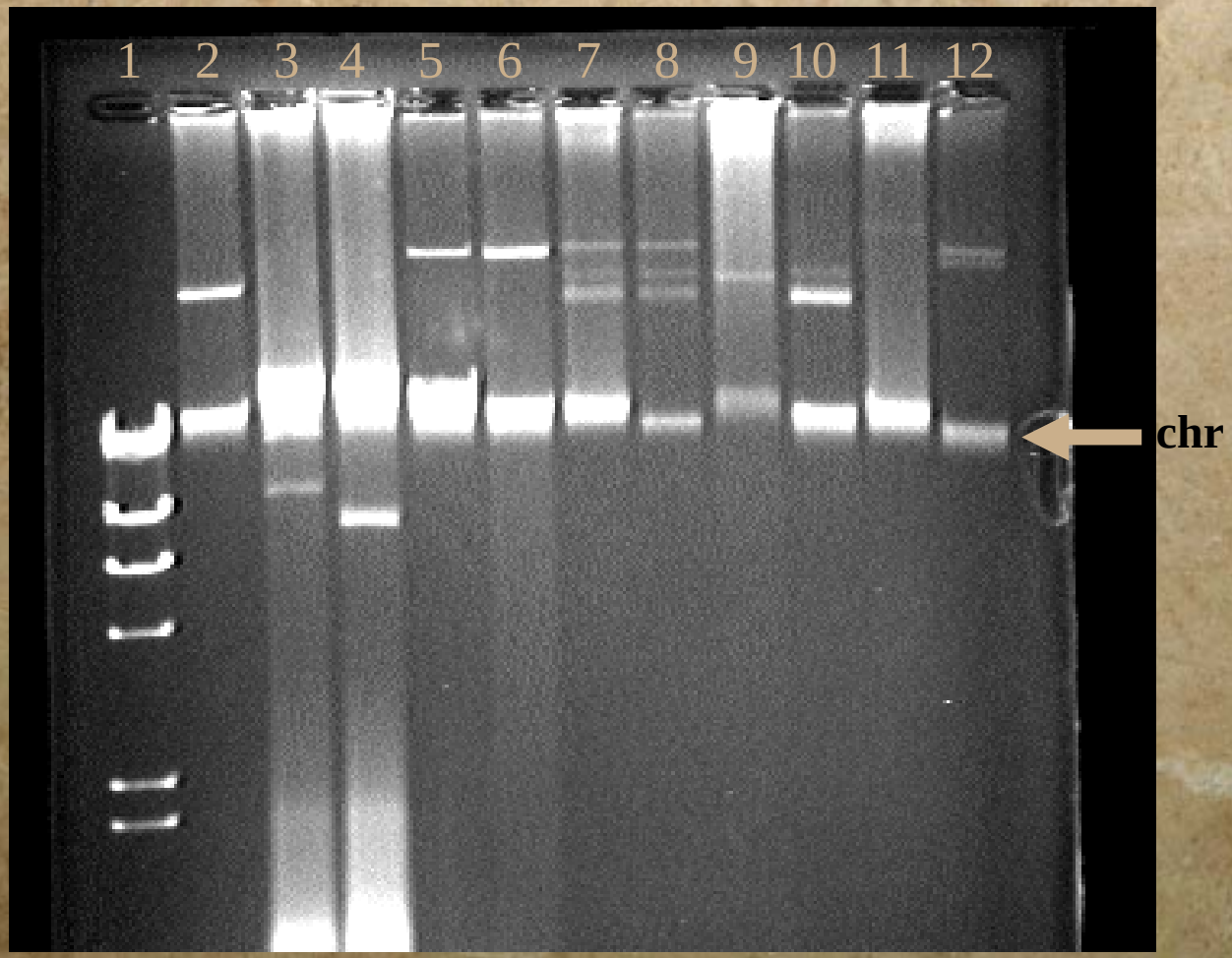
VIABLE CELL COUNTS OBTAINED FROM FRC SEDIMENTS COLLECED IN FEB 2003

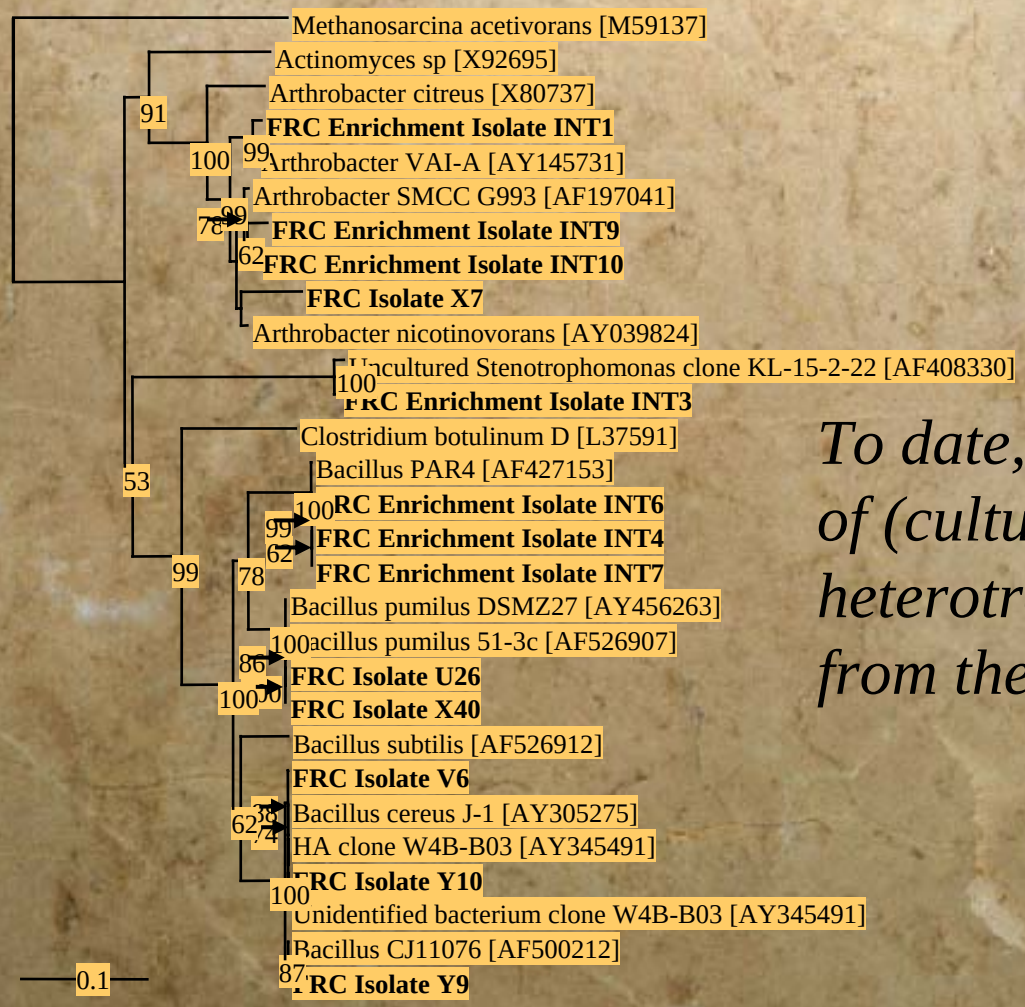


Frequency of plasmids in isolates from February 2003 sample



Plasmid diversity from culturable isolates





To date, the vast majority of (culturable) aerobic heterotrophs obtained from the FRC are Gram positives

Preliminary Data from FRC sediment samples collected in July 2004

Area	Sample ID	Viable cell counts	Plasmid Frequency[b]
1	FB068-03-24	ND[a]	ND[a]
2	FB073-07-12	1.0×10^5	37%
3	FB069-03-36	ND[a]	ND[a]

ND[a]-none detected. Cfu$\tilde{o}$s are detectable following soil washing. However, cfu$\tilde{o}$s are only detectable after a two-week incubation.

Detailed data of sediment samples from **Area 2** FB073-07-12

Media Types	Viable Cell Counts	Plasmid Frequency[b]
A	1.2×10^5	41%
B	1.2×10^5	39%
C	7.3×10^4	42%
D	3.2×10^3	11%

Work in Progress in the Sobecky laboratory …

1) Metal resistance profiling
2) Determination of Plasmid (Molecular) Diversity
3) Additional Sediment and Groundwater Sampling
4) Genetic analysis of Metal Resistance Determinants
5) Isolation of plasmid containing anaerobic microorganisms

Development of a functional Gene array for metal resistance plasmids

Jonna Coombs and Tamar Barkay

How a Functional Gene Array can be used:

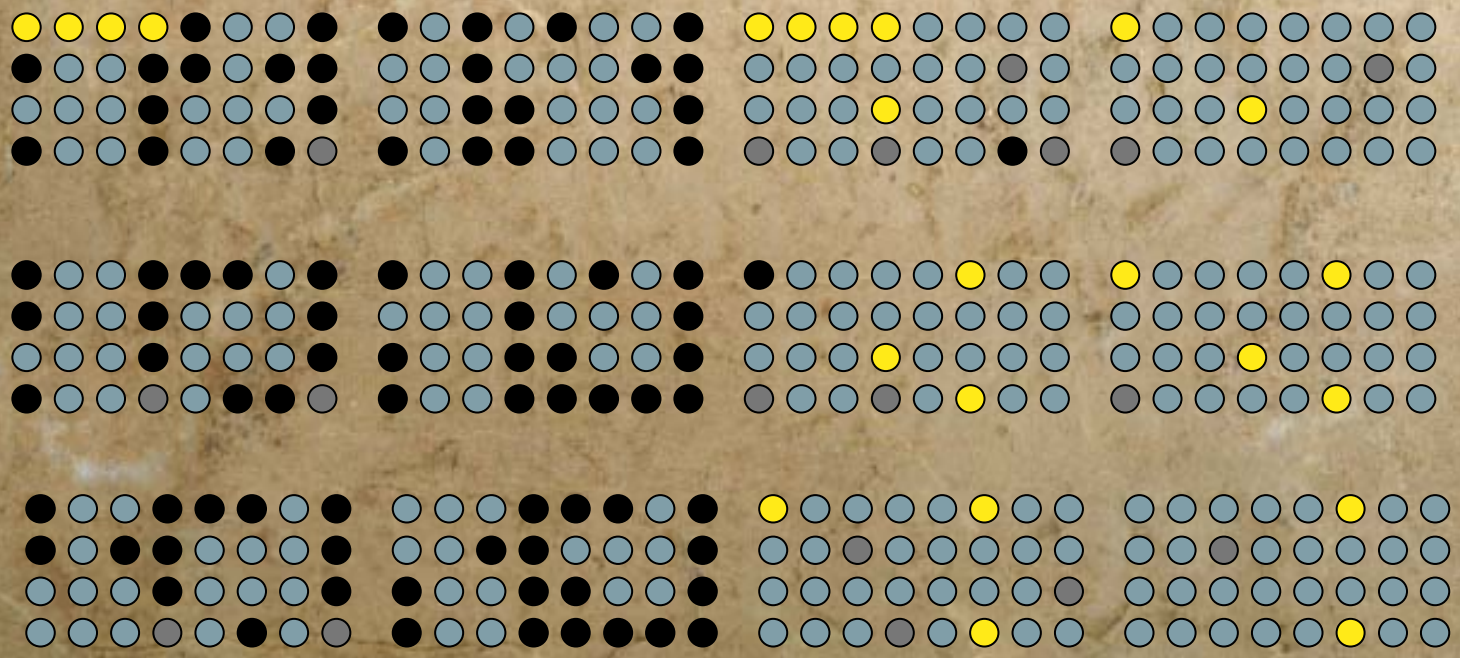
- can rapidly determine plasmid types
- can rapidly screen for several metal resistance genes at once
- can be used on pure cultures, consortia, or DNA extracted from environmental samples.

Genes targeted by the functional gene array

Table 1. Genes targeted by the functional gene array.

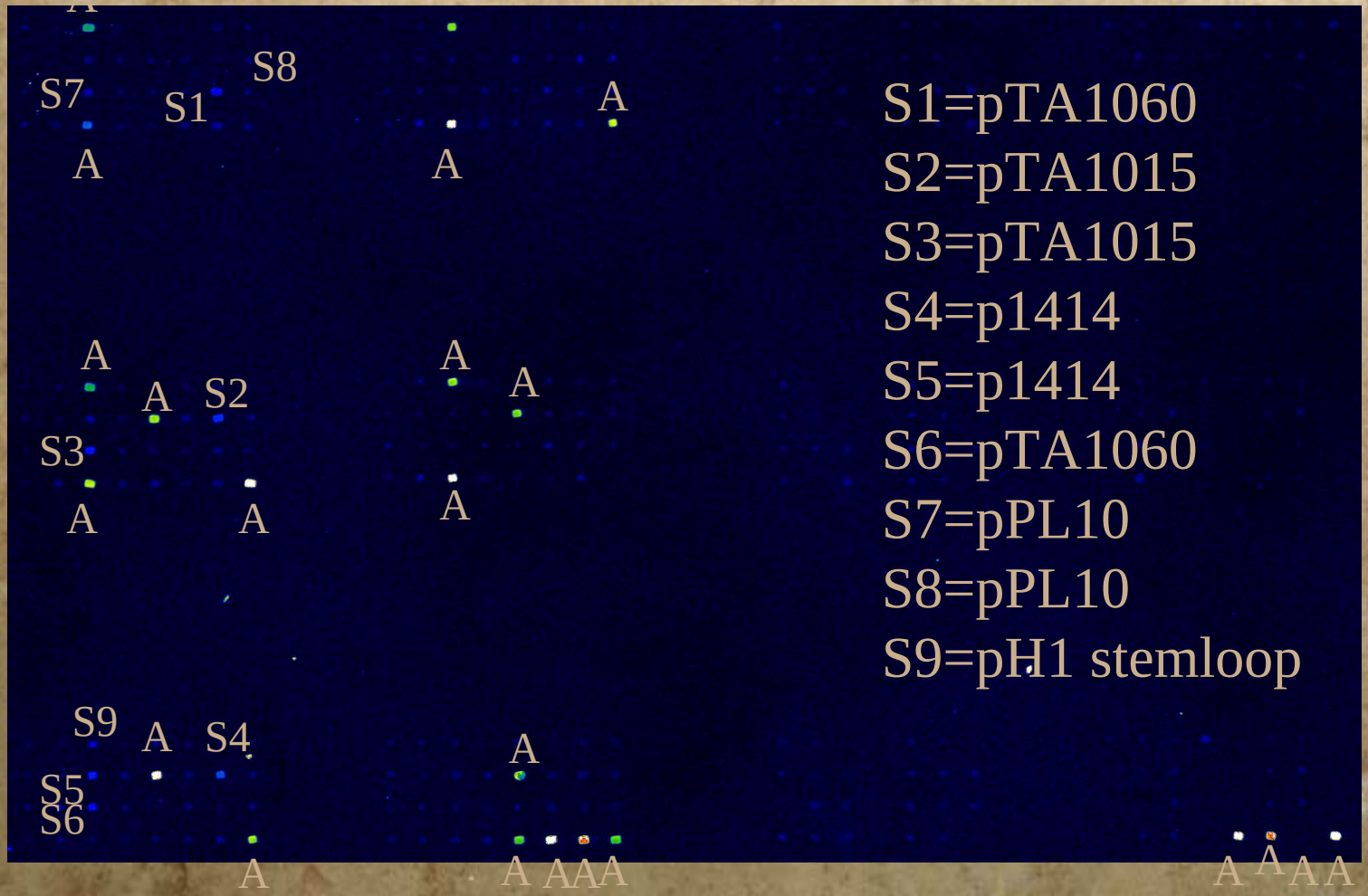
Gene group	Number of probes	control
IncPbeta *rep*	8	pTSA
IncW *rep*	21	pSa
IncQ *rep*	17	pDN1
IncN *rep*	22	R46
IncP *rep*	12	pM3
FIC-like *rep*	15	P307
pEP2-like *rep*	26	PEP2
pCI411-like *rep*	4	PCI411
p121BS-like *rep*	12	p121BS
pH1 repeat region	2	pH1
Tol plasmid *rep*	4	tol
Bacillus plasmid *rep*	8	pTA1060
Marine plasmid *rep*	9	many
arsR	6	R46
arsB	14	R46
czcC-like	10	PMOL28
merR	15	many
merA	17	many
merB	14	many
zntA/copA-like	25	pCGR2
Total number of probes:	261	

Array schematic



- Alien DNA
- *rep* genes
- metal resistance genes
- buffer only

A sample array of pTA1060



S1=pTA1060
S2=pTA1015
S3=pTA1015
S4=p1414
S5=p1414
S6=pTA1060
S7=pPL10
S8=pPL10
S9=pH1 stemloop

HOMOLOGY TO PROBE

S1	pTA1060#1	91.4%
S2	pTA1015#1	100%
S3	pTA1015#2	100%
S4	P1414#1	88.6%
S5	P1414#2	94.3%
S6	pTA1060#2	87.1%
S7	pPL10#1	78.6%
S8	pPL10#2	70%
S9	pH1	47.1%

FRC strains to be tested Using Functional Gene array

	Hg^R (50nmol)	Pb^R (500nmol)	Identification
U5	8mm	1mm	*Bacillus*
U26	10mm	10mm	*Bacillus*
V6	7mm	3mm	*Bacillus*
X40	4mm	1mm	*Bacillus*
Y10	5mm	1mm	*Bacillus*
Z1	9mm	1mm	*Bacillus*
Z29	7mm	6mm	*Arthrobacter*