

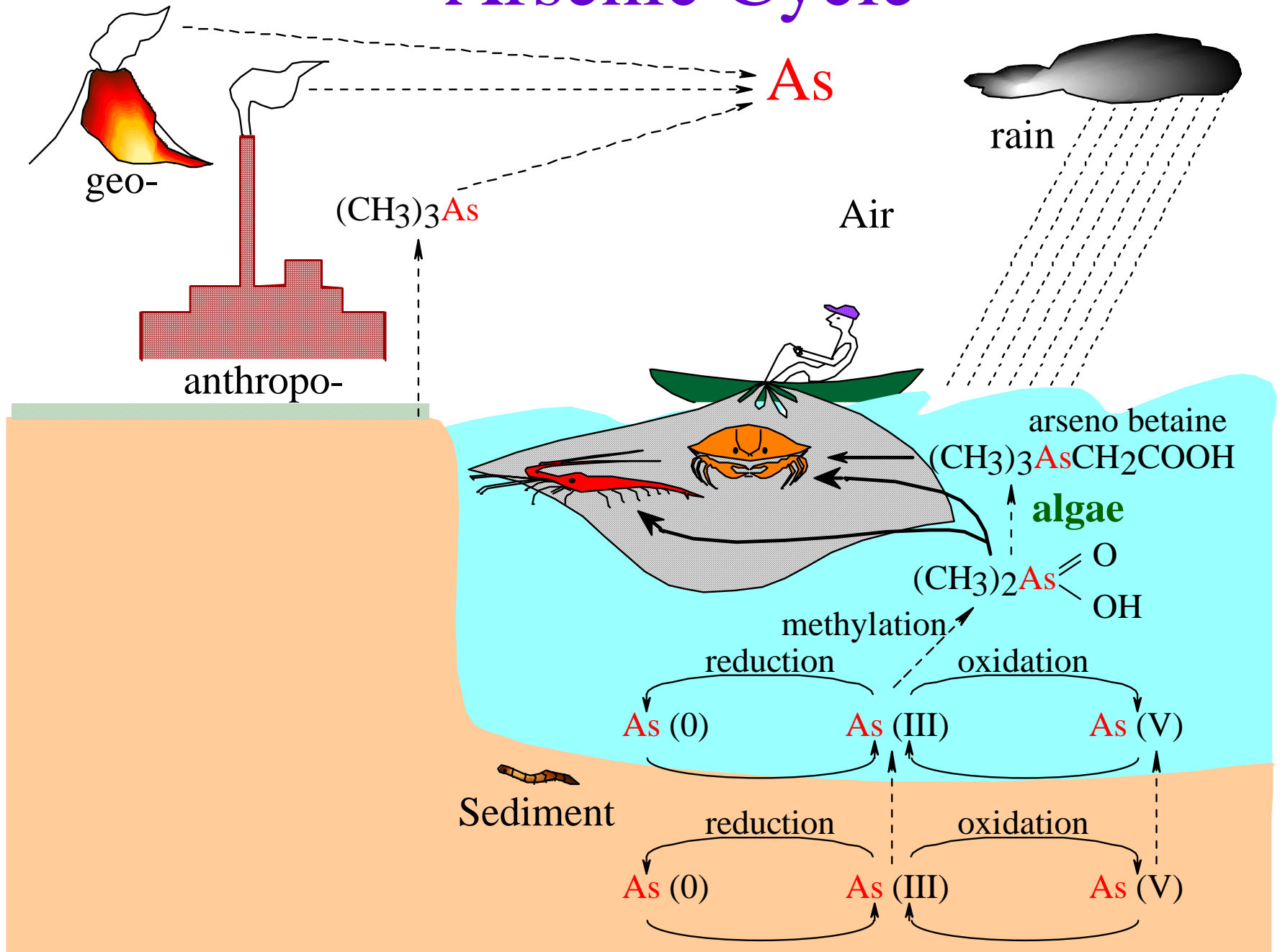
Arsenic Resistance in *Halobacterium* sp. NRC-1 Examined Using an Improved Gene Knockout System

- Halophilic archaea
- Unusual arrangement of *ars* operon
- Contains putative As(III)-methyltransferase

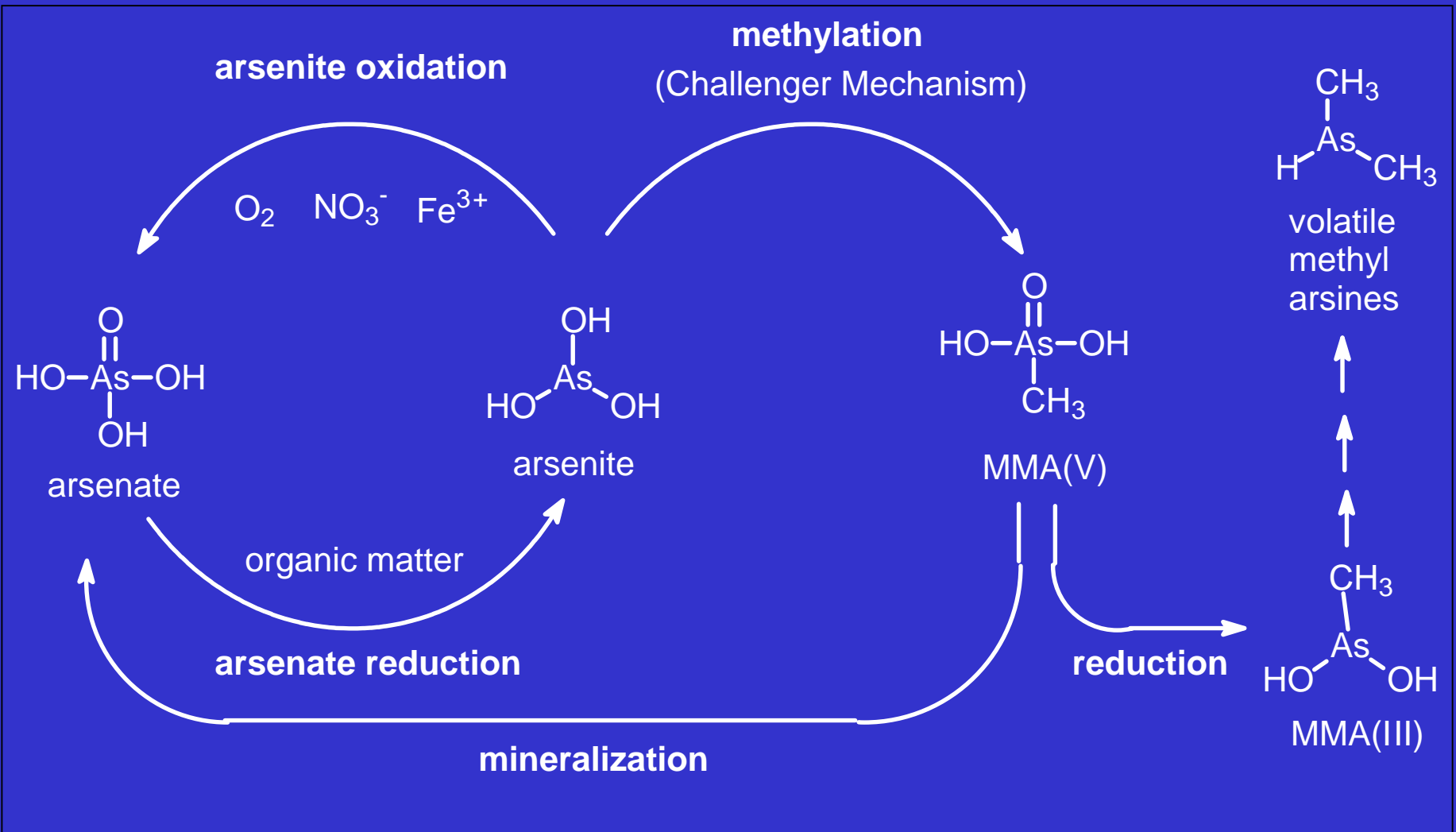


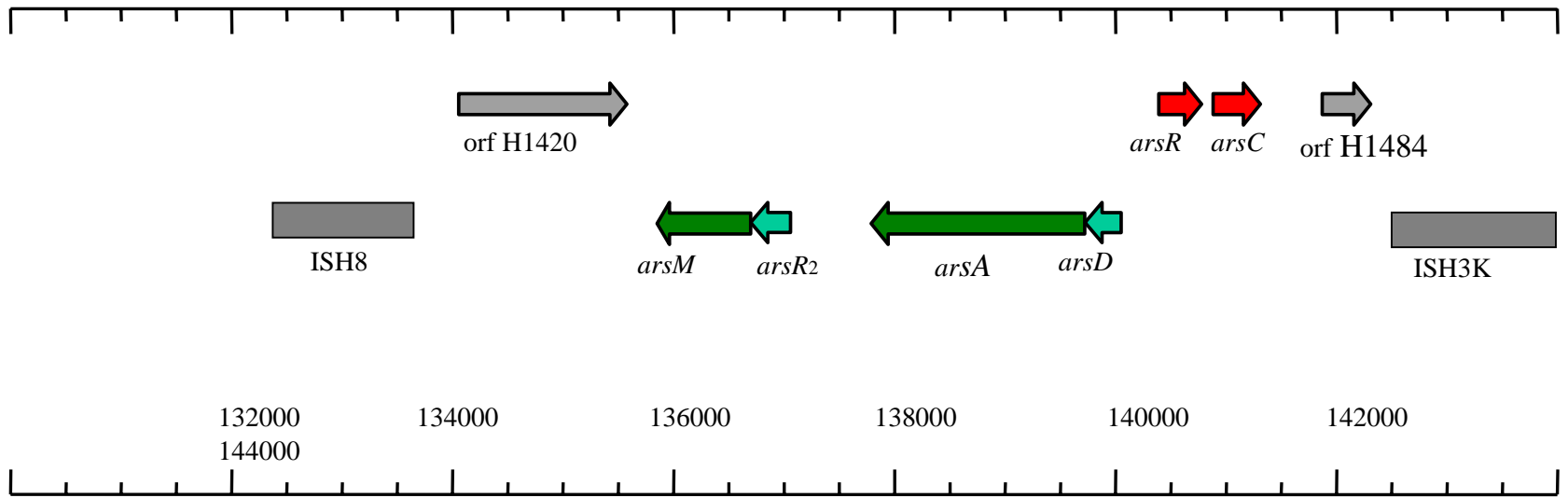
(b)

Arsenic Cycle



Biological Arsenic Cycle





(14000 bps)

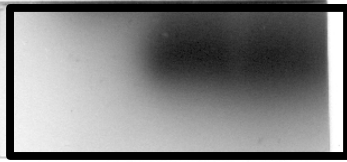
| | | | | | | |
|------------|------------|------------|------------|-------------|-------------|------------|
| mousecyt19 | maa | srdaei hkd | vqnyygnvlk | tsad..... | lqtn...acv | |
| ratcyt19 | maa | prda.eihkd | vqnyygnvlk | tsad..... | lqtn...acv | |
| humancyt19 | maa | lrda.eiqkd | vqtyyggvkl | rsad..... | lqtn...gcv | |
| halo5177 | msndnetmva | drdpeetrem | vreryagiat | sgqdccgdvg | ldvsgdggcc | |
| ** * | * | * | * | * | | |
| | 51 | | | | 100 | |
| mousecyt19 | ... | trakpvp | s..yireslq | nvhedvssry | ygcg..ltvp | erlencrild |
| ratcyt19 | ... | tpakgvp | e..yirkslq | nvheervisry | ygcg..lvvp | ehlencrild |
| humancyt19 | ... | ttarpvp | k..hirealq | nvheevalry | ygcg..lvip | ehlencwild |
| halo5177 | sdeteasgse | rlgydaddva | svadgadlg. | lgcgnpkafa | amapgetvld | |
| | * | | * | * * | ** | |
| | 101 | | | | 150 | |
| mousecyt19 | lgsgsgrdcy | vlsqvlgekg | hvtgidmtev | qvevaktyle | hhmekfgfqa | |
| ratcyt19 | lgsgsgrdcy | vlsqvlggkg | hitgidmtkv | qvevakayle | yhtekfgfqt | |
| humancyt19 | lgsgsgrdcy | vlsqvlgekg | hvtgidmtkg | qvevaekyld | yhmekygfqa | |
| halo5177 | lgsgagfdcf | laaqevgpdg | hvigvdmtp | miskarenva | kn.....da | |
| | **** * | ** | * * * | * * * | | |
| | 151 | | | | 200 | |
| mousecyt19 | pnvtflhgri | eklaeagiqs | esygivisnc | vinlvpdkqq | vlqevyrvlk | |
| ratcyt19 | pnvtflhgqi | emlaeagiqk | esydivisnc | vinlvpdkqk | vlrevyqvlk | |
| humancyt19 | snvtfhgni | eklaeagikn | eshdivvsnc | vinlvpdkqq | vlqeyayrvlk | |
| halo5177 | envefrlgei | ghlp...vad | esvnvvisnc | vvnlapkqr | vfdtdtyrvlr | |
| | ** * * | * | ** * * | * * * * | * * * | |
| | 201 | | | | 250 | |
| mousecyt19 | hggelyfsdv | yaslevpedi | kshkvlwgec | lggalywkdl | aiiaqkigfc | |
| ratcyt19 | yggelyfsdv | yaslevsedi | kshkvlwgec | lggalywkdl | aviakkigfc | |
| humancyt19 | hggelyfsdv | ytslelpeei | rthkvlwgec | lggalywkel | avlaqkigfc | |
| halo5177 | pggrvaisdv | vqtapfpddv | qmdpdsltgc | vagastvddl | kamldeagfe | |
| | ** *** * | | | * * | | |
| | 251 | | | | 300 | |
| mousecyt19 | pprlvtadii | t..venkele | gvlgdcrfvs | atfrvfkplk | tepaercrvv | |
| ratcyt19 | pprlvtanii | t..vgnkele | rvlgdcrfvs | atfrlfkplk | tepagrcqv | |
| humancyt19 | pprlvtanli | t..iqnkele | rvigdcrfvs | atfrlfkhs | tgptkrcqvi | |
| halo5177 | aveiapkdes | tefisdwad | rdlge.ylvs | atiearkpar | dd | |
| | * | | * ** | ** * | | |
| | 301 | | | | 350 | |
| mousecyt19 | ynggikghek | elifdanftf | kegeavavde | etaavlknsr | fapdfllftpv | |
| ratcyt19 | ynggimghek | elifdanftf | kegeavevde | etaailrnsr | fahdfllftpv | |
| humancyt19 | ynggitghek | elmfdanftf | kegeivevde | etaailknsr | faqdfllirpi | |
| | 351 | | | | 395 | |
| mousecyt19 | daslpapggp | f | | | | |
| ratcyt19 | eastlapqtk | viirdpflka | eesdkmkprc | apegtggccg | krksc | |
| humancyt19 | gkllptsgav | llws | | | | |

Question:

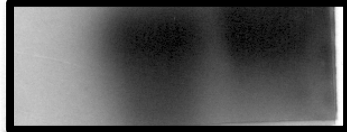
Is ArsM from *Halobacterium* NRC-1
involved in As(III) resistance

1 2 3

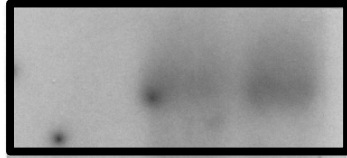
arsB



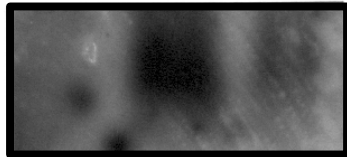
arsR



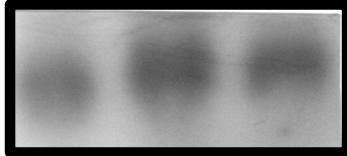
arsD



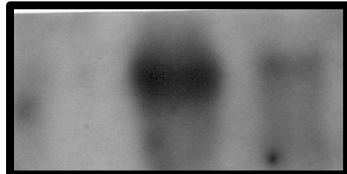
arsA



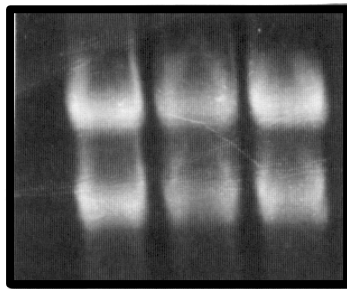
arsR2



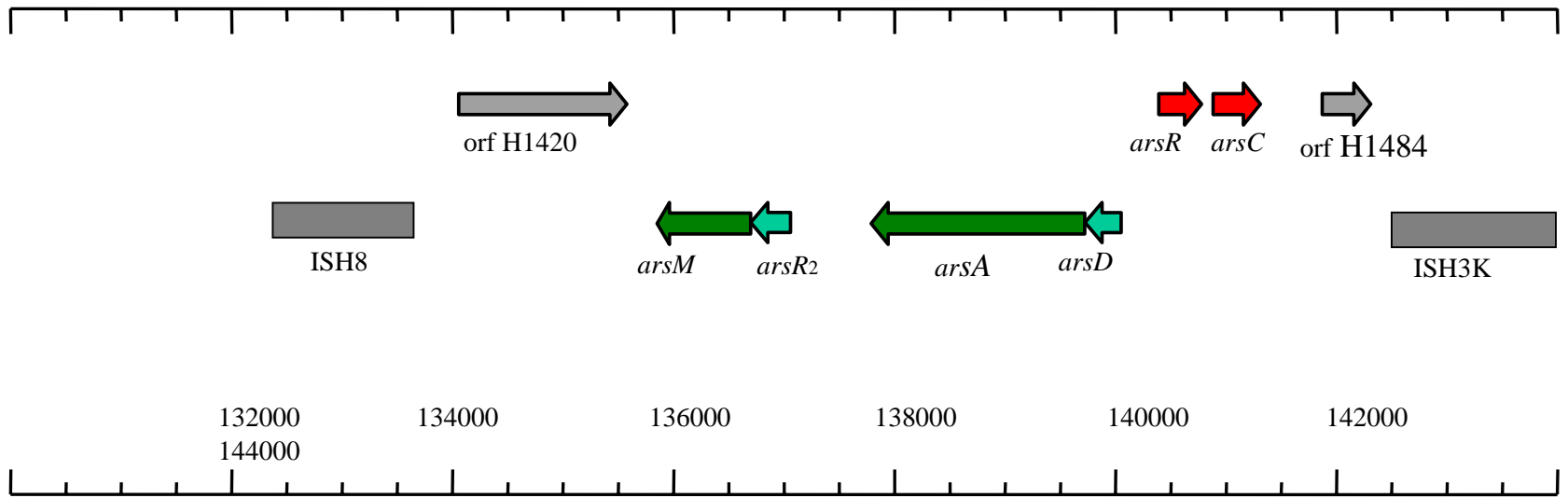
arsM



rRNA

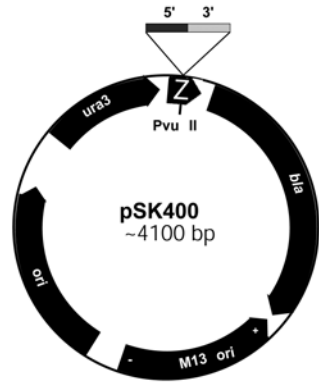


Induction of *ars* genes by As(III)
And Sb(III)



(14000 bps)

A



Transform *Halobacterium*
sp. SK400 or SK402 ($\Delta ura3$)

B

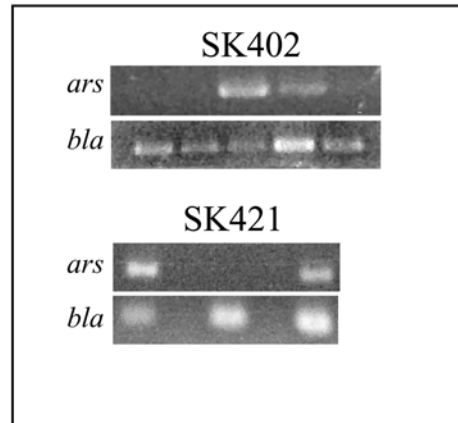


Plate on uracil dropout medium

Pick and screen for integraion
(*bla* and deletion region)

C

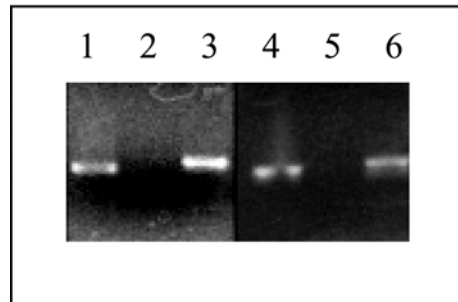
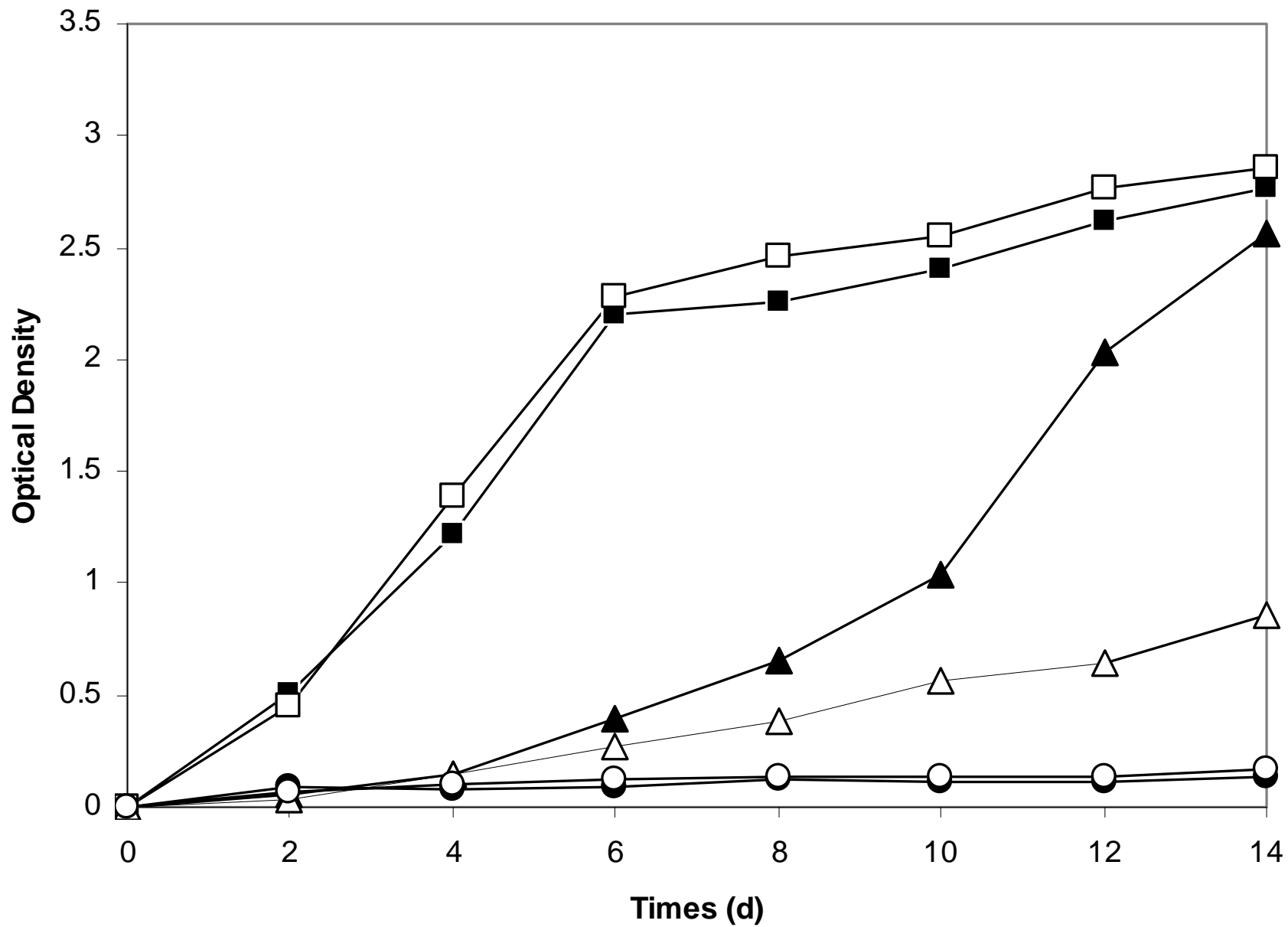
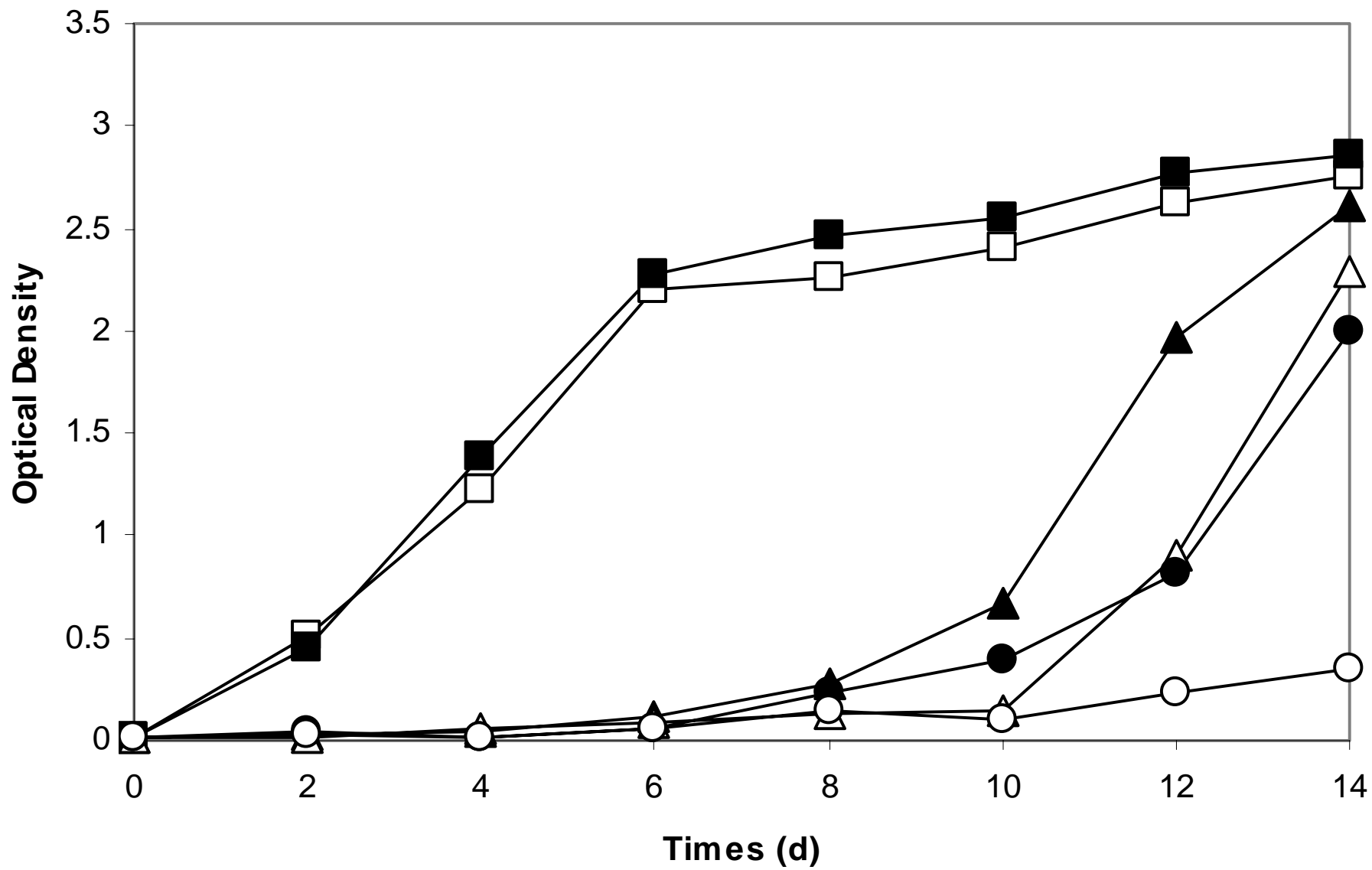
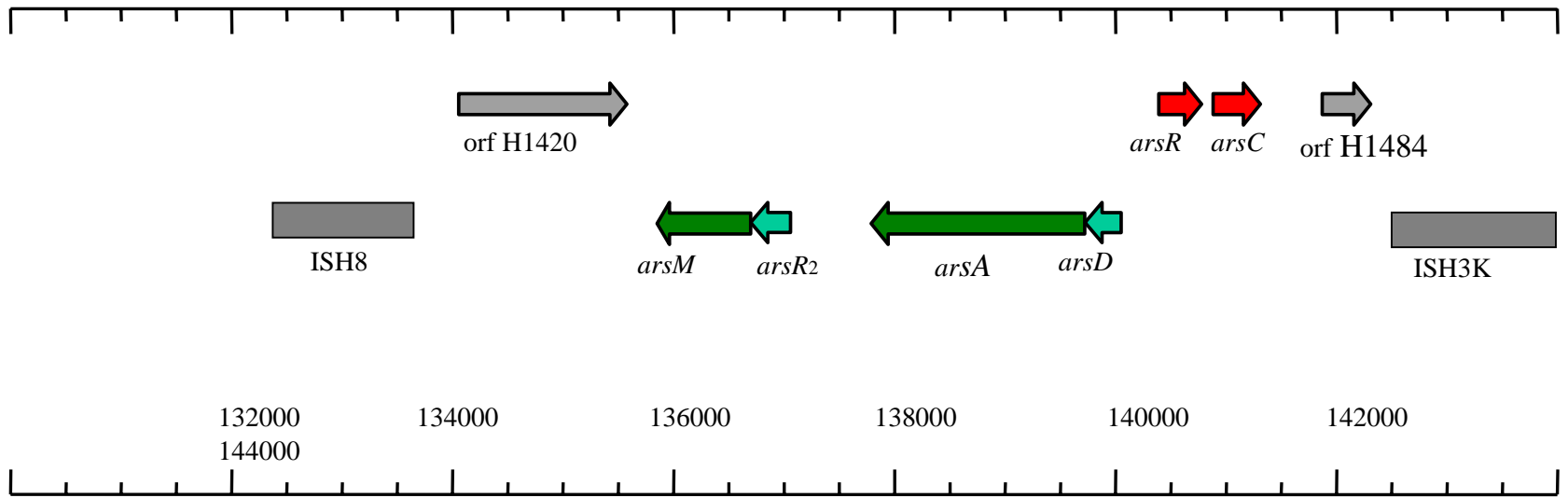


Plate integrants on 5-FOA media

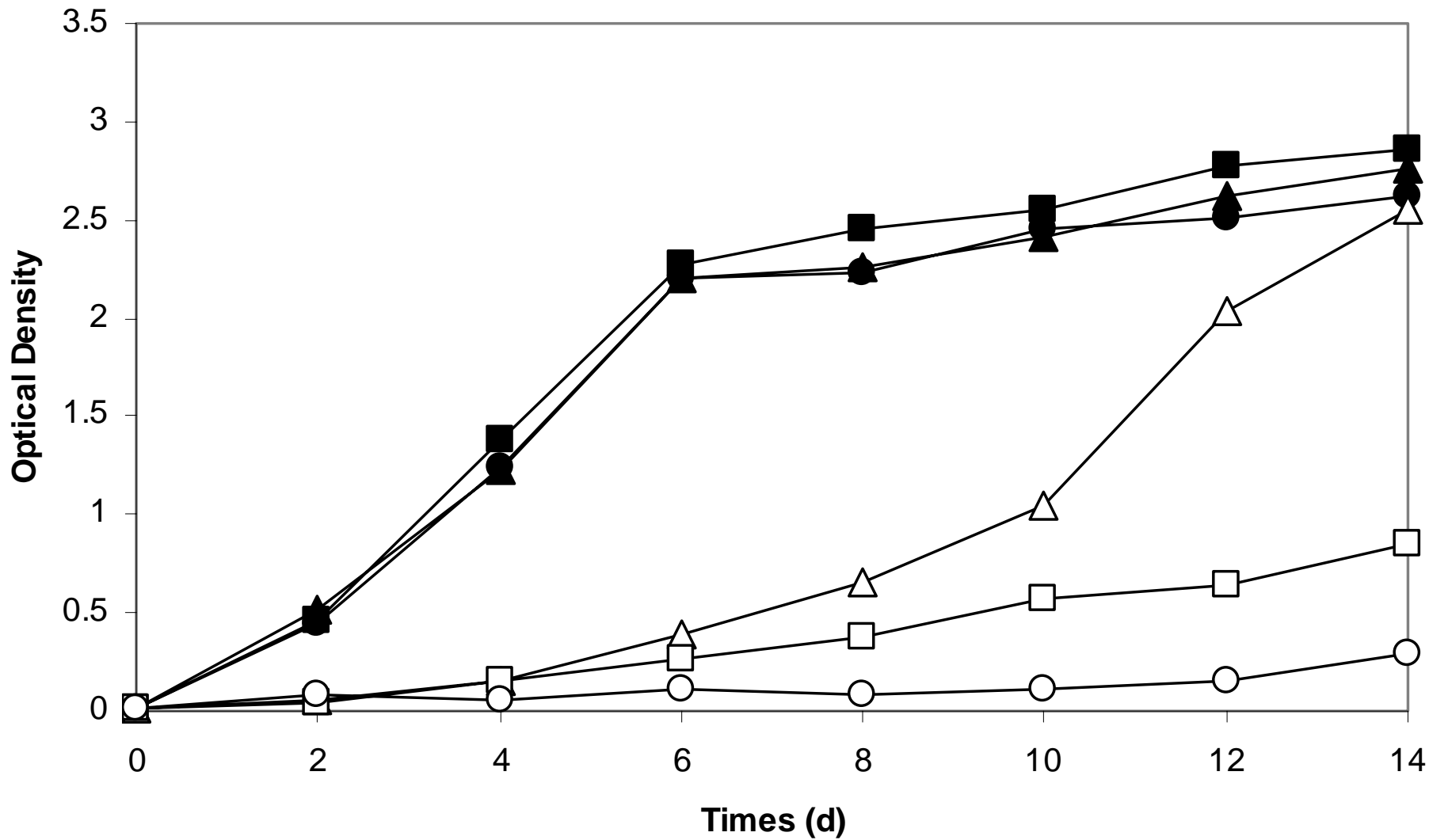
Pick and screen for excision
and deletion

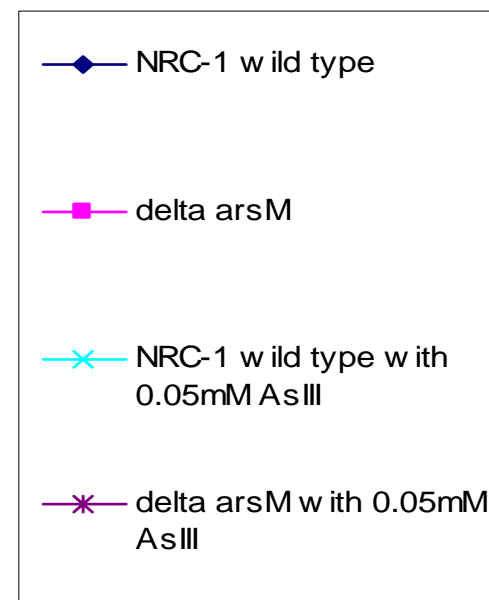
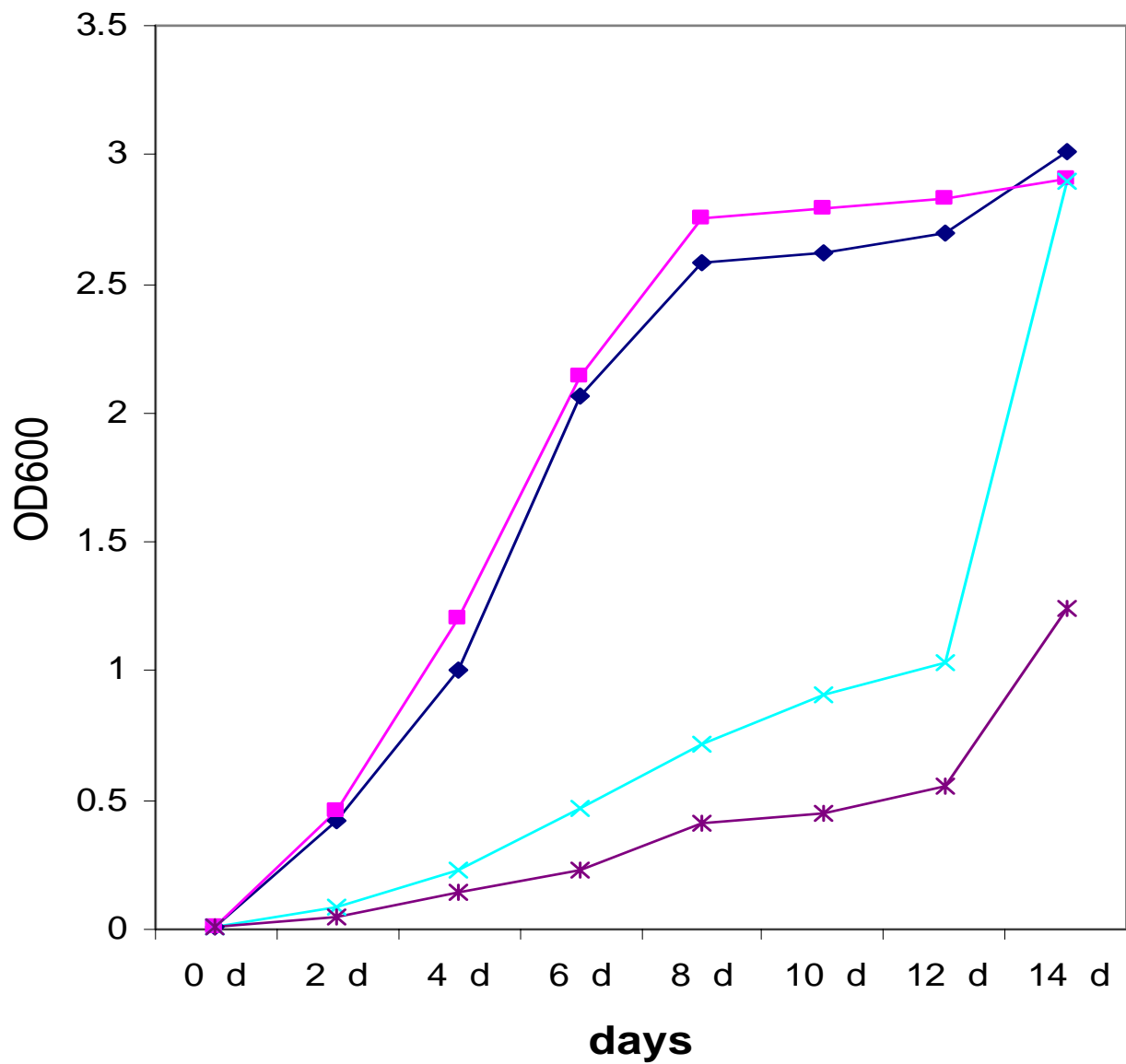


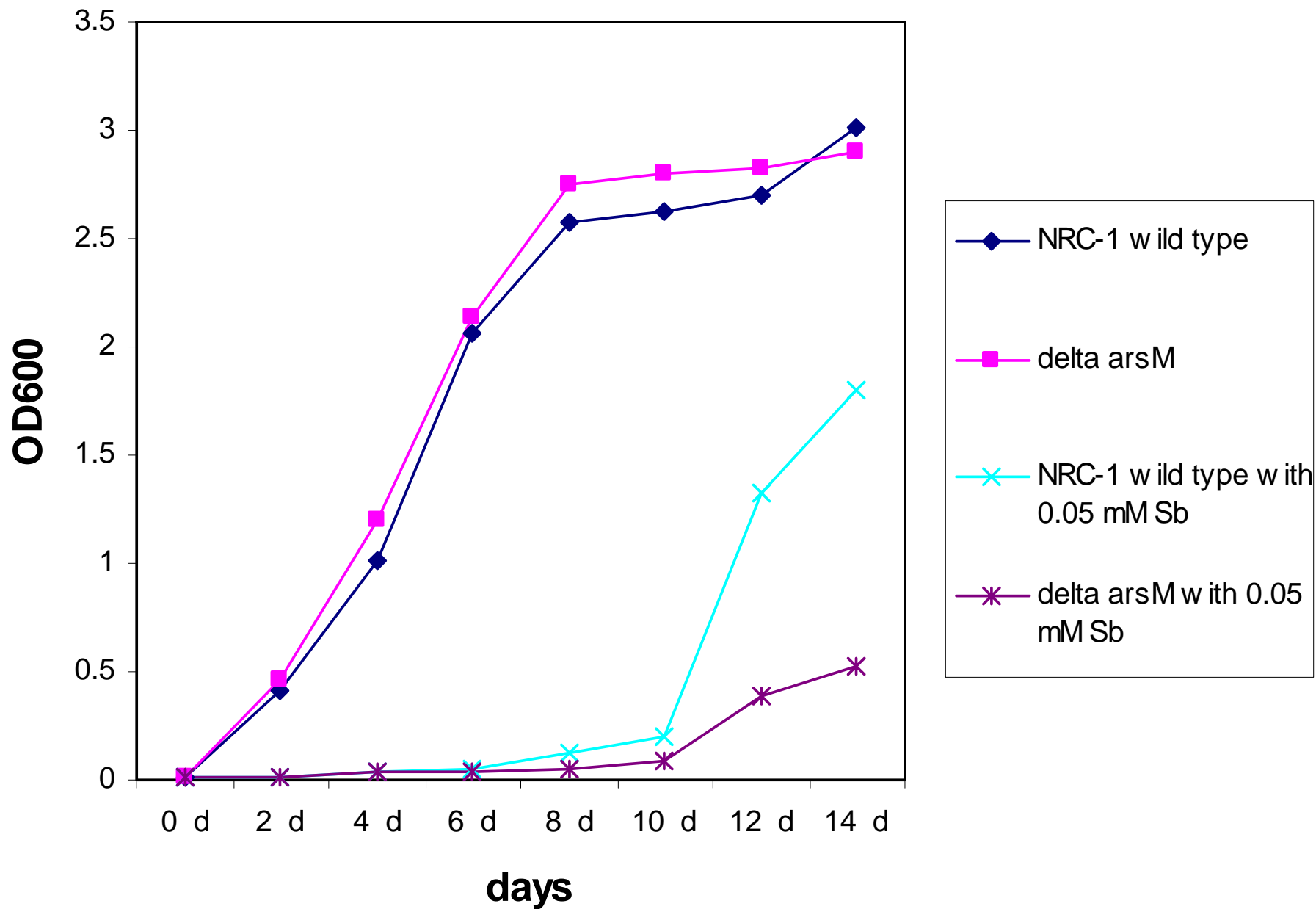




(14000 bps)



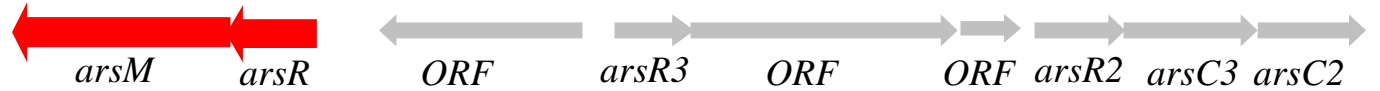




Problem:

ArsM from *Halobacterium* NRC-1 is
a halophilic enzyme

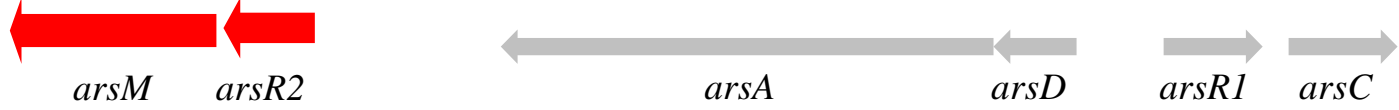
R. palustris CGA009



M. magnetotactium



Halobacterium sp. NRC-1



M. mazei Gö1



C. hutchinsonii



M. acetivorans str. C2A



MM0661 ----MDANEKKEVIKKRFQEIAMSGG-----TCCSGGGCCGDSSSSDI SRAIG---YSEADIEAVPD 55
MA3783 ----MDAAEKKEVIKKKYQEIATLGG-----SCCSGGGCCGDL SAADLSRSLG---YSEADVQAVPD 55
VNG7121 MVADRDP EETREVMVRERYAGIATSGQDCCGDVGLDVSGDGGCCSDETEASG SERLG---YDADDVASVD 67
Magn029446 -----MG---YDESDLAAAGD 13
RPA3562 --MPTDMQDVKDIVREKYASAA-----LKVATGGASCCGSSALPGASPITSNLYDAAEQOGLPA 57
Chut02001955 --MKNNNQDVKEMVQKQYSEIANQ-----SKVQNETSCCGAGSCGTYTIMS---EDYSGLDGYNP 55

: . .

MM0661 -ANMG-LGCGNPASIAELKPGDIVL DLGSGAGFDCFLAAQKVGNSGKVI GVDMTPEMVEKVQANARKYGY 123
MA3783 -ANLG-LGCGNP TAF AELKPGDIVL DLGSGAGFDSFLAAQRVGSLGKVI GVDMTQEMVKAQDNARKYGY 123
VNG7121 GADLG-LGCGNP KAF AAMAPGETVLDL GSGAGFDCFLAAQEVGPDGHVI GVDMTPEMI SKARENVAKNDA 136
Magn029446 GANLG-LGCGNP QAIAAMRPGEVVVDL GSGAGFDAFLAVRQVGESGRVI GVDMTHEMLAKARANA AKLGL 82
RPA3562 EAMLASLGCGNP TALAQLSPGETVLDL GSGGGIDVLLSARRVGPTGKAYGLDMTDEMLALARDNQRKAGL 127
Chut02001955 DADLG-LGCGLP TEYAKIKKGD TVVDL GSGAGNDAFVARTLTGEEGEVIGIDMTEAMIQKAKGNTEKLG F 124
* :. **** * * : * : * :*****. * * ::. . * *.. *:*** * : . : * * .

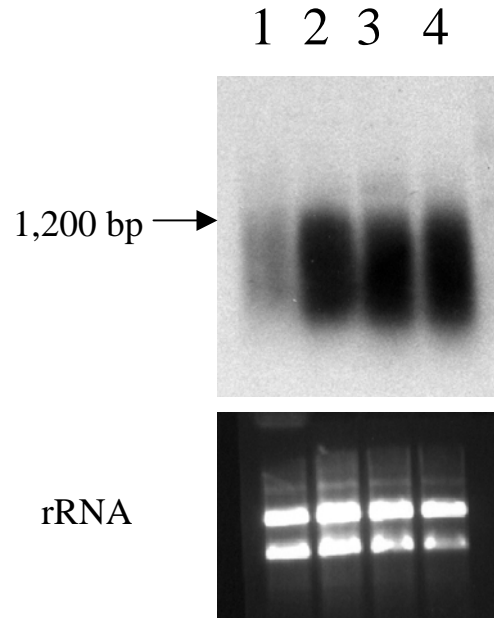
MM0661 SNVEFRQGDIEALPVEDSSVDV IISNCVINLAPDKEKVFREAFRVLKLGGRLYISDLVLLDELPEDLKND 193
MA3783 SNVEFRQGDIEALPLDDRSVDV IISNCVINLAPDKEKVFREAFRVLKPGGRMYVSDMVLLLEDLPEDLKND 193
VNG7121 ENVEFRLGEIHL PVADES VNVV IISNCVINLAP EKQRFVDDTYRVL RPPGGRVAISDVVQTAPFPDDVQMD 206
Magn029446 ANVEFRLGEIEHLP IADNTADV IISNCVINLSPDKPAVLNDAFRVLKPGGRVAISDVVMLRPLPELAAM 152
RPA3562 DNVEFLKGEIEA IPLPDHSVDV IISNCVINLSGDKDRVLREAFRVLKPGGRFAVSDVVTRGEIPEALRRD 197
Chut02001955 TNVAFRLGDI EDIPLSSKRADV VVSN CVMNLVPDKAKAFSEVFRILKPLGHFSISDIVLKGDL PDAIKKE 194
** * * : * : * : . . : * : : * : * : * : * : * : * : * : * : * : * : * : * : * : *

MM0661 RDLLAGCVAGAVLKEEY LKLLKRAGFSVEILAED-----PDISKSQYKGLP-----VESLKLKAW 248
MA3783 CDLLAGCVAGALLKEEY LGLLKKAGFSFKILAED-----SDVSKRQYEGLP-----VESLKLKAW 248
VNG7121 PDSL TGCVAGASTVDDL KAMLDEAGFEAVEIAPK-----DESTEFISDWDADR-----LGEYLV SAT 264
Magn029446 KELLTGCAAGAATVAELS N WLEQAGFTDIRIEPK-----PESRELIANWAPGLG-----IEDYVASAT 210
RPA3562 VLLWVGCLAGALDEADYVAKLAAAGFAQISIEPTR--VYDIEDAREFLTGKGIDVDALAPQM QDKFFSGF 265
Chut02001955 GEMYAGCVSGAIKKSEYLGILAEQGFVNITVQKEKEIIIPVEVLSQYLSQEEINSYK-EKGMGIYSITVY 263
. ** : ** : * ** :

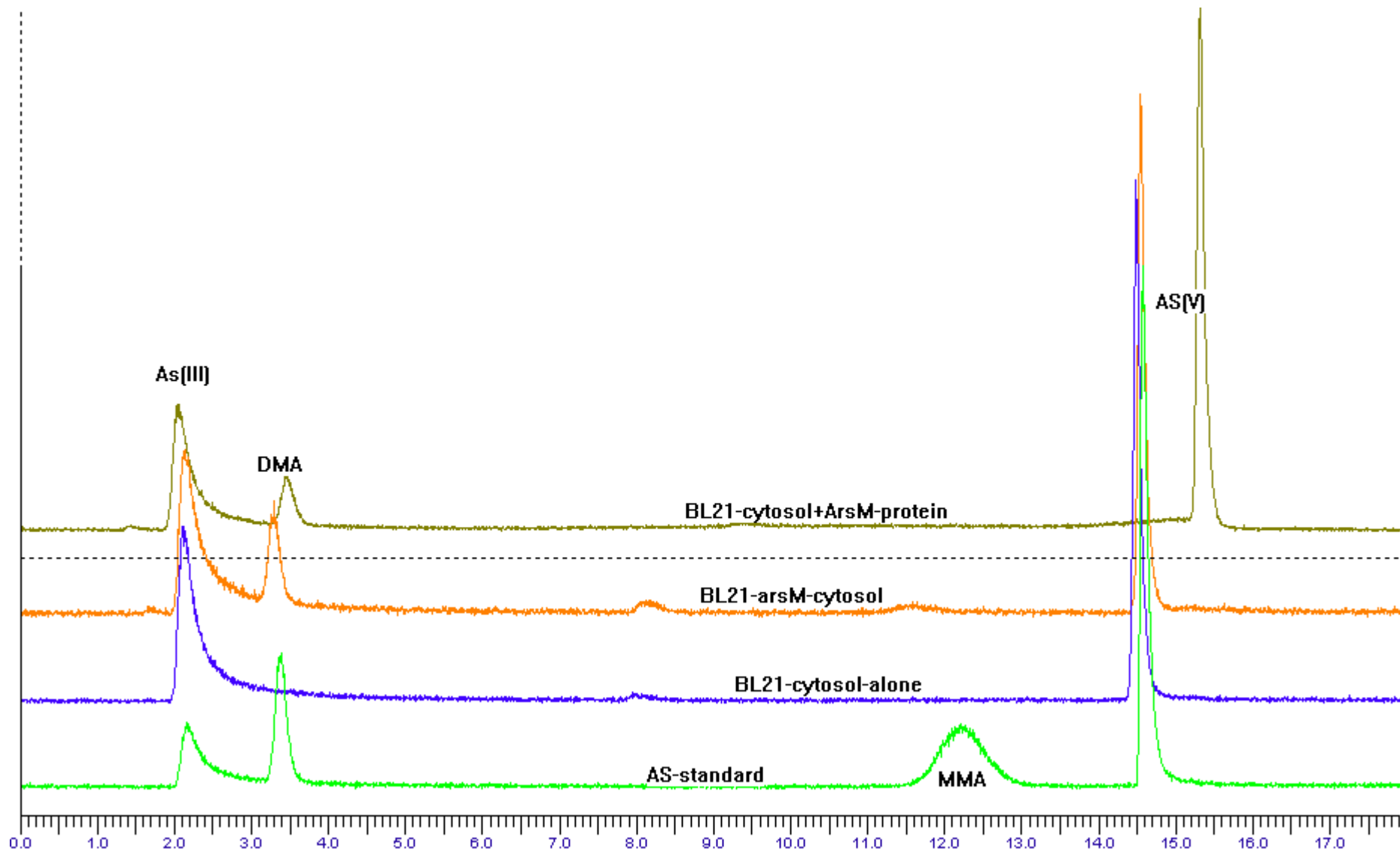
MM0661 V----- 249
MA3783 V----- 249
VNG7121 IEARKPARDD----- 274
Magn029446 IEARRPLV----- 218
RPA3562 VRATKPGADGEV PARCCG 283
Chut02001955 AERP DASC GCDPRATCC- 280

Question:

Is ArsM from *Rhodopseudomonas palustis* an As(III)-methyltransferase



Northern Blot analysis of *arsM* using RNA of *Rhodospseudomonas palustris* after different growth conditions. 1 control RNA; 2. Growth with 1mM As(V); 3. Growth with 0.1 mM As(III); 4. Growth with 0.05 mM Sb(III). Cells were cultured in LB medium at 27°C for 3 days.



Summary

- *ars* operon in *Halobacterium* NRC-1 is involved in conferring arsenic and antimonite resistance
- Several *ars* genes are induced by arsenite and antimonite
- ArsM is involved in arsenite resistance
- Improved method for creating deletions in *Halobacterium*

Future plans

- Transcriptional startpoint determination of *arsM* by primer extension
- Purification and enzymatic characterization of ArsM from different organisms
- Establishing an enzymatic assay
- As(III) methylation in different bacteria by HCLP-ICP-MS

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Barry Rosen

Jie Qin