Clare O’Connor

HW3 April 12th 2018

1.

*Probe* ***A****:  5’ – GTGCTCCCCCGCCAATTCCT-3’* ***(Archaea)***

*Probe* ***B****:  5’-GCTGCCTCCCGTAGGAGT   -3’* ***(non-probe)***

*Probe* ***C****:  5’-TCACGCGGCGTTGCTC -3'* ***(Firmicutes)***

*Probe* ***D****:  5'- ACTCCTACGGGAGGCAGC -3'* ***(Bacteria)***

*Probe* ***E****: 5’-GACGCAAGCCCCTCACCAAGCACCT -3’* ***(Dehalococcoides)***

2.

a.      Hha GCG.C

b.      Msp C.CGG

c.      Taq T.CGA

>AJ012094.1 Methanosarcina barkeri 16S rRNA gene, strain DSM 800 (T)

GCTATCGGTGTTCGCCTAAGCCATGCGAGTCATATGTTCTTCGTGAACATGGCGTACTGCTCAGTAACAC

GTGGATAACCTGCCCTTGGGTCTGGCATAACCCCGGGAAACTGGGGATAATTCCGGATAACGCATATATG

CTGGAATGCTTTATGCGTAAAATGGATTCGTCCGCCCAAGGATGGGTCTGCGGCCTATCAGGTAGTAGTG

GGTGTAATGTACCTACTAGCCTACAACGGGTACGGGTTGTGAGAGCAAGAGCCCGGAGATGGATTCTGAG

ACATGAATCCAGGCCCTACGGGGCGCANNAGGCGCGAAAACTTTACAATGCGGGAAACCGTGATAAGGGG

ACACCGAGTGCTAGCATCATATGCTGGCTGTCCAGGTGTGTAAACTACACCTGTTAGCAAGGGCCGGGCA

AGACCGGTGCCAGCCGCCGCGGTAACACCGGCGGCCCGAGTGGTGATCGTGATTATTGGGTCTAAAGGGT

CCGTAGCCGGTTTGGTCAGTCCTCCGGGAAATCTGATAGCTCAACTATTAGGCTTTCGGGGGATACTGCC

AGACTTGGAACCGGGAGAGGTAAGAGGTACTACAGGGGTAGGAGTGAAATCTTGTAATCCCTGTGGGACC

ACCTGTGGCGAAGGCGTCTTACCAGAACGGGTTCGACGGTGAGGGACGAAAGCTGGGGGCACGAACCGGA

TTAGATACCCGGGTAGTCCCAGCCGTAAACGATGCTCGCTAGGTGTCAGGCATGGCGCGACCGTGTCTGG

TGCCGCAGGGAAGCCGTGAAGCGAGCCACCTGGGAAGTACGGCCGCAAGGCTGAAACTTAAAGGAATTGG

CGGGGGAGCACAACAACGGGTGGAGCCTGCGGTTTAATTGGACTCAACGCCGGACAACTCACCGGGGACG

ACAGCAATATGTAGGTCAGGCTGAAGACCTTACCTGAATCGCTGAGAGGAGGTGCATGGCCGTCGCCAGT

TCGTACTGTGAAGCATCCTGTTAAGTCAGGCAACGAGCGAGACCCGTGCCCACTGTTACCAGCATATCCT

CCGGGATGATGGGTACTCTGTGGGGACCGCCGGTGTTAAATCGGAGGAAGGTGCGGGCCACGGTAGGTCA

GTATGCCCCGAATTTCCCGGGCTACACGCGGGCTACAATGAATGGGACAATGGGTCCCTCCCCTGAAAAG

GGCTGGTAATCTCACAAACCCATCCTTAGTTCGGATCGAGGGCTGTAACTCGCCCTCGTGAAGCTGGAAT

CCGTAGTAATCGCGTTTCAATATAGCGCGGTGAATACGTCCCTGCTCCTTGCACACACCGCCCGTCAAAC

CACCCGAGTGAGGTATGGGTGAGGGCACGAACATCGTGTCGTGTTCGAACCTGTGCTTTGCAAGGGGGGT

TAAGTCGTAAC

>AF005049.1 Methanococcus maripaludis 16S ribosomal RNA gene, complete sequence

CTGCTATTGGGATTCGACTAAGCCATGCGAGTCTATGGTTTCGGCCATGGCGGACGGCTCATTAACACGT

GGTTAACATACCCTCAGGTGGGGGATAACCTTGGGAAACTGAGGATAATACCCCATAGAAAAAGCAGTCT

GGAACGATTCTTTTTCGAAAGCATATGCGCCTGAGGATTGGACTGCGCTCGATTAGGTAGTTGGTGGGGT

AATGGCCCACCAAGCCTACGATCGGTACGGGCCTTGAGAGAGGAAGCCCGGAGATGGGGACTGAGACACG

GCCCCAGGCCCTACGGGGCGCAGCAGGCGCGAAACCTCCGCAATGCACGAAAGTGCGACGGGGGGATCCC

AAGTGCTCATGCACAGCATGGGCTTTTATCAAGTGTAAACAGCTTGAGGAATAAGGGCTGGGCAAGTTCG

GTGCCAGCAGCCGCGGTAACACCGACGGCCCGAGTGGTGGCCACTTTTATTGGGCCTAAAGCGTCCGTAG

CCGGTCCAGTAAGTCCTTGTTTAAATCCTGCGGCTTAACCGCAGGACTGGCAGGGATACTGCTGGACTTG

GGACCGGGAGAGGACAAGGGTACTTCAGGGGTAGCGGTGAAATGTGTTGATCCTTGAAGGACCACCTATG

GCGAAGGCACTTGTCTGGAACGGGTCCGACGGTGAGGGACGAAAGCCAGGGGCGCGAACCGGATTAGATA

CCCGGGTAGTCCTGGCCGTAAACTTTGCGAACTAGGTGTTAGGTGGGCCCCGTGCCCATCTAGTGCCGAA

GGGAAGCCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTTAAAGGAATTGGCGGGGGA

GCACCACAACGGGTGGAGCCTGCGGTTTAATTGGATTCAACGCCGGGCATCTCACCAGGAGCGACAGCAT

GATGACGGCCAGGTTGACGACCTTGCCTGAAGCGCTGAGAGGTGGTGCATGGCCATCGTCAGCTCGTACC

GCGAGGCGTCCTGTTAAGTCAGGTAACGAGCGAGACCCGTGCCCTATGTTGCTACTTTTTCCTCCGGGAG

AAAGGCACTCATAGGGGACCGCTGGCGCTAAGTCAGAGGAAGGAGCGGGCAACGATAGGTCCGCATGCCC

CGAATCTCCTGGGCTACACGCGGGCTACAATGGCTAGGACAATGGGCCGCAACCCTGAAAAGGGACGCAA

ATCTCCTAAACCTAGTCGTAGTTCGGATCGTGGGCTGTAACTCGCCCACGTGAAGCTGGAATCCGTAGTA

ATCGCAGTTCATAATACTGCGGTGAATGTGTCCCTGCTCCTTGCACACACCGCCCGTCACACCACCCGAG

TTGGGTTGAAGTGAGGCCTTGGCCTTTGGCTAGGGTCGAACTTGGGCTCAGCGAGGGGGGTG

>AB679168.1 Methanosaeta concilii gene for 16S rRNA, partial sequence, strain: NBRC 103675

AGAGGTTACTGCTATCGAGGTTCGACTAAGCCATGCGAGTCGAATGTAGCAATACATGGCGAACTGCTCA

GTAACACGTGGACAACCTGCCCTTAGGACGGGTATAAACCCGGGAAACTGGGTATAATACCCGATAGGTC

TCGATTGCTGGAATGCATCGAGATTTAAAGCTCCGGCGCCTAAGGATGGGTCTGCGGCCTATCAGGTAGT

AGTGGGTGTAGCGTACCTACTAGCCTACGACGGGTACGGGTTGTGAGAGCAAGAGCCCGGAGATGGATTC

TGAGACACGAATCCAGGCCCTACGGGGTGCAGCAGGCGCGAAAACTTTACAATGCTGGCAACAGCGATAA

GGGAACCTCGAGTGCCAGGTTACAAATCTGGCTGTCGAGATGCCTAAAAAGCATTTCATAGCAAGGGCCG

GGCAAGACCGGTGCCAGCCGCCGCGGTAACACCGGCGGCTCGAGTGGTAACCGTTATTATTGGGTCTAAA

GGGTCTGTAGCCGGCCGGATAAGTCTCTTGAGAAATCTGGCAGCTTAACTGTCAGGCTTTCAGGAGATAC

TGTCTGGCTCGAGGCCGGGAGAGGTGAGAGGTACTTCAGGGGTAGGGGTGAAATCTTGTAATCCTTGAAG

GACCACCAGTGGCGAAGGCGTCTCACCAGAACGGACCTGACGGCAAGGGACGAAAGCTAGGGGCACGAAC

CGGATTAGATACCCGGGTAGTCCTAGCCGTAAACGATACTCGCTAGGTGTCGGCCACGGTGCGACCGTTG

TCGGTGCCGTAGGGAAGCCGTGAAGCGAGCCACCTGGGAAGTACGGCCGCAAGGCTGAAACTTAAAGGAA

TTGGCGGGGGAGCACCACAACGGGTGGAGCTTGCGGTTTAATTGGATTCAACGCCGGAAATCTTACCGGG

ACCGACAGCAATATGAAGGCCAGGCTGAAGACTTTGCCGGATTAGCTGAGAGGTGGTGCATGGCCGTCGT

CAGTTCGTACTGTGAAGCATCCTGTTAAGTCAGGCAACGAGCGAGACCCACGCCCACAGTTGCCAGCGTA

CTCTCTGGAGTGACGGGTACACTGTGGGGACCGCCGCTGCTAAAGCGGAGGAAGGAATGGGCAACGGTAG

GTCAGTATGCCCCGAATATCCCGGGCTACACGCGAGCTACAATGGTTGGTACAATGGGTATCTACCCCGA

AAGGGGACGGGAATCTCCTAAAACCAATCTTAGTTCGGATTGAGGGCTGCAACTCGCCCTCATGAAGCTG

GAATCCGTAGTAATCGCGTTTCAACAGAACGCGGTGAATACGTCCCTGCTCCTTGCACACACCGCCCGTC

AAACCACCCGAGTAGGGTCTGAATGAGAGCGCTTTCTTTGGAGGCGTTCGAATTTGGGCTTTGCAAGGGG

GGTTAAG

>M60880.1 M.hungatei 16S ribosomal RNA

ATTCTGNTTGATCCTGCCAGAGGCCACTGCTATCGGGGTTTGACTAAGCCATGCGAGTCGAGAGGTGCAA

GACCTCGGCGTACTGCTCAGTAACACGTGGACAATCTGCCCTGAAGAGGAGGATAATCCCGGGAAACTGG

GGGTAATACTCCATAGTTCGTGCTGACTGGAATGTTATGCGAACGAAAGATCCGTCGCTTCAGGATGAGT

CTGCGGCCGATTAGGTAGTTGTTGGGGTAACGGCCCAACAAGCCTGTCATCGGTACGGGTTGTGGGAGCA

AGAGCCCGGAGATGGATTCTGAGACACGAATCCAGGCCCTACGGGGCGCAGCAGGCGCGAAAACTTTACC

ATGCGGGCAACCGTGATAAGGAAACCCCGAGTGCCAGCACAGGCTGGCTGTCCACCAGTGTAAATAACTG

GTGAAGAAAGGGCCGGGCAAGACCGGTGCCAGCCGCCGCGGTAATACCGGCGGCTCGAGTGGTGGCCGCT

ATTACTGGGCTTAAAGGGTCCGTAGCTGGATATACAAGTCCCTTGAGAAATCCGCCGGCTTAACCGGTGG

GCGTTCAGGGGAAACTGTATTTCTAGGGACCGGGAGAGGTGAGAGGTACTGCCGGGGTAGGAGTGAAATC

CTGTAATCCCGGTGGGACCACCTATGGCGAAGGCATCTCACCAGAACGGGTCCGACAGTGAGGGACGAAA

GCTGGGGGAGCAAACCGGATTAGATACCCGGGTAGTCCCAGCTGTAAACGATGCGCGTTAGGTGTGTCAG

TGACCACGTGTCACTGAGGTGCCGAAGGGAAACCGTGAAACGCGCCGCCTGGGGAGTACGGTCGCAAGGC

TGAAACTTAAAGGAATTGGCGGGGGAGCACCACAACGGGTGGAGCCTGCGGTTTAATCGGACTCAACGCC

GGAAATCTCACCGGATAAGACAGCTGAATGATAGTCGGGATGAAGACTCTACTTGACTAGCTGAGAGGAG

GTGCATGGCCGTCGTCAGTTCGTACTGTGAAGCATCCTGTTTAGTCAGGCAACGAGCGAGACCCACGCGA

GCAGTTGCCAGCTTGACCTTCGGGTTGATGGGGACACTGCTCGGACCGCCTCTGCTAAAGGGGAGGAAGG

AATGGGCAACGGTAGGTCAGCATGCCCCGAATTATCCGGGCTACACGCGGGCTACAATGGACAGGACAAT

GGGTTTCGACACCGAGAGGTGAGGATAATCTCCTAAACCTGTCCGAAGTTCGGATTGCGGGTTGTAACTC

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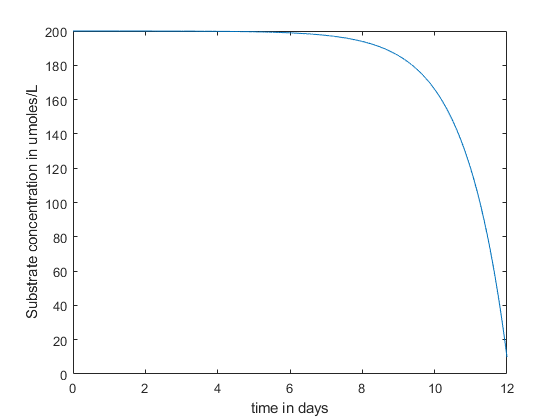
CACACACCGCCCGTCAAACCACCCGAGTGAGGTCTTGATGAGGATGTATCATTGATATGTTCGAATCTGG

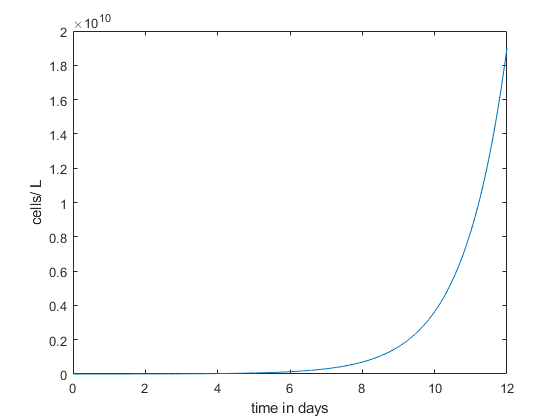
GTTTTGCAAGGGGGGTTAAGTCGTAACAAGGTAGCCGTAGGGGAATCTGCGGCTGGATCACCTCCT

|  |  |  |  |
| --- | --- | --- | --- |
| Organism | Hha | Msp | Taq |
| *Methanosarcina barkeri GenBank# AJ012094.1* | 268 | 123 | 362 |
| *Methanococcus maripaludis GenBank* ***#****AF005049.1* | 74 | 135 | 829 |
| *Methanosaeta concilii*GenBank# AB679168.1 | 711 | 80 | 460 |
| *Methanospirillum hungatei* GenBank # M60880.1 | 230 | 123 | 569 |

Hha and Taq do a good job resolving the four organisms, but Msp does not because the TRF length for *Methanosarcina barkeri* and *Methanospirillum hungatei* are exactly the same, meaning it wouldn’t be apparent which organism was present if Msp was used. Of the other two the TRF lengths using Taq are more different (~100bp between lengths) meaning it might get better resolution the HHa depending on the concentrations present in the sample.

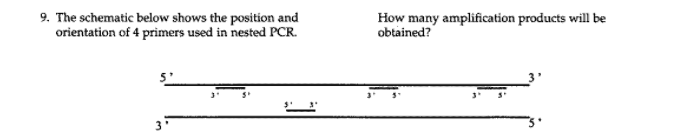
3.

Plot of S over time:

 Plot of X over time:

It takes ~11 days for concentrations to reach near steady state conditions.

4.



Two amplification products will be produced. As the primer on the bottom strand will cause the chain to grow towards the right, while the three on the top will grow towards the right, only the two on the right on the top will cause amplification products with the one on the bottom as the primer will be growing towards each other.

5.

Naphthalene Dioxygenase:

**nahAc naphthalene 1,2-dioxygenase iron sulfur protein component large subunit [ *Pseudomonas putida* ]**

FASTA:

>NC\_004999.1:16051-17400 Pseudomonas putida NCIB 9816-4 plasmid pDTG1, complete sequence

ATGAATTACAATAATAAAATCTTGGTAAGTGAATCTGGTCTGAGCCAAAAGCACCTGATTCATGGCGATG

AAGAACTTTTCCAACATGAACTGAAAACCATTTTTGCGCGGAACTGGCTTTTTCTCACTCATGATAGCCT

GATTCCTGCCCCCGGCGACTATGTTACCGCAAAAATGGGGATTGACGAGGTCATCGTCTCCCGGCAGAAC

GACGGTTCGATTCGTGCTTTTCTGAACGTTTGCCGGCATCGTGGCAAGACGCTGGTGAGCGTGGAAGCCG

GCAATGCCAAAGGTTTTGTTTGCAGCTATCACGGCTGGGGCTTCGGCTCCAACGGTGAACTGCAGAGCGT

TCCATTTGAAAAAGATCTGTACGGCGAGTCGCTCAATAAAAAATGTCTGGGGTTGAAAGAAGTCGCTCGC

GTGGAGAGCTTCCATGGCTTCATCTACGGTTGCTTCGACCAGGAGGCCCCTCCTCTTATGGACTATCTGG

GTGACGCTGCTTGGTACCTGGAACCTATGTTCAAGCATTCCGGCGGTTTAGAACTGGTCGGTCCTCCAGG

CAAGGTTGTGATCAAGGCCAACTGGAAGGCACCCGCGGAAAACTTTGTGGGAGATGCATACCACGTGGGT

TGGACGCACGCGTCTTCGCTTCGCTCGGGGGAGTCTATCTTCTCGTCGCTCGCTGGCAATGCGGCGCTAC

CACCTGAAGGCGCAGGCTTGCAAATGACCTCCAAATACGGCAGCGGCATGGGTGTGTTGTGGGACGGATA

TTCAGGTGTGCATAGCGCAGACTTGGTTCCGGAATTGATGGCATTCGGAGGCGCAAAGCAGGAAAGGCTG

AACAAAGAAATTGGCGATGTTCGCGCTCGGATTTATCGCAGCCACCTCAACTGCACCGTTTTCCCGAACA

ACAGCATGCTGACCTGCTCGGGTGTTTTCAAAGTATGGAACCCGATCGACGCAAACACCACCGAGGTCTG

GACCTACGCCATTGTCGAAAAAGACATGCCTGAGGATCTCAAGCGCCGCTTGGCCGACTCTGTTCAGCGA

ACGTTCGGGCCTGCTGGCTTCTGGGAAAGCGACGACAATGACAATATGGAAACAGCTTCGCAAAACGGCA

AGAAATATCAATCAAGAGATAGTGATCTGCTTTCAAACCTTGGTTTCGGTGAGGACGTATACGGCGACGC

GGTCTATCCAGGCGTCGTCGGCAAATCGGCGATCGGCGAGACCAGTTATCGTGGTTTCTACCGGGCTTAC

CAGGCACACGTCAGCAGCTCCAACTGGGCTGAGTTCGAGCATGCCTCTAGTACTTGGCATACTGAACTTA

CGAAGACTACTGATCGCTAA

Some primers:

F: GTACGGCGAGTCGCTCAATA (not great, lots of things found with it)

R: TCATTGTCGTCGCTTTCCCA

F: AAGCACCTGATTCATGGCGA (this one found many things for naphthalene dioxegynase)

R: ATGGAACGCTCTGCAGTTCA

F: GATAGCCTGATTCCTGCCCC (also pretty good to get things that have naphthalene dioxygenase)

R: TTCTAAACCGCCGGAATGCT

10 Closest “hits” when using the Primer in Blast (I used the third one)

1. Uncultured bacterium clone fosmid A179-233 genomic sequence
2. Pseudomonas frederiksbergensis strain AS1 plasmid, complete sequence
3. Pseudomonas gessardii strain LZ-E clone E3 naphthalene 1,2-dioxygenase subunit alpha gene, complete cds
4. Uncultured bacterium clone nah-N45 naphthalene dioxygenase (nah) gene, partial cds
5. Uncultured bacterium clone nah-N23 naphthalene dioxygenase (nah) gene, partial cds
6. Uncultured bacterium clone nah-N11 naphthalene dioxygenase (nah) gene, partial cds
7. Uncultured bacterium clone N34 naphthalene dioxygenase gene, partial cds
8. Uncultured bacterium clone N29 naphthalene dioxygenase gene, partial cds
9. Pseudomonas putida plasmid pAK5 naphthalene dioxygenase reductase component (nahAa), ferredoxin (nahAb), naphthalene dioxygenase large subunit (nahAc), naphthalene dioxygenase small subunit (nahAd), dihydrodiol dehydrogenase (nahB), and salicylaldehyde dehydrogenase (nahF) genes, complete cds; and 1,2-dihydroxynaphthalene dioxygenase (nahC) gene, partial cds
10. Mutant Pseudomonas chlororaphis strain PS6 plasmid pHL1 PAHs hydroxylase iron sulfur protein gene, complete cds

All of these genes matched 100% with the primer over the range that BLAST looked as did many more from this primer.