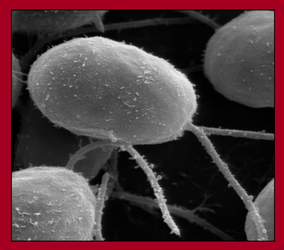
Cloning the MTP1 gene from *Chlamydomonas reinhardtii*



Alis Hart, Allison Rust, David Gingrich, Department of Chemistry
SUNY Potsdam, Potsdam, NY

Introduction

Zinc can function as a ligand or signaling molecule in some processes. Changes in zinc concentrations have been linked to diseases such as diabetes mellitus or cancer. Zinc transporters found in cell membranes play an important role in controlling zinc concentration in cells and can can be categorized into two groups, ZIP and CDF[1]. The crMTP1 transporter from the green algae *Chlamydomonas reinhardtii*. is a member of the CDF family. The goal of these studies is to incorporate the crMTP1 gene to the pET11a plasmid vector to form a recombinant plasmid. The recombinant plasmid can be place into *E.coli* bacterial cells so the crMTP1 protein can be produced for study.

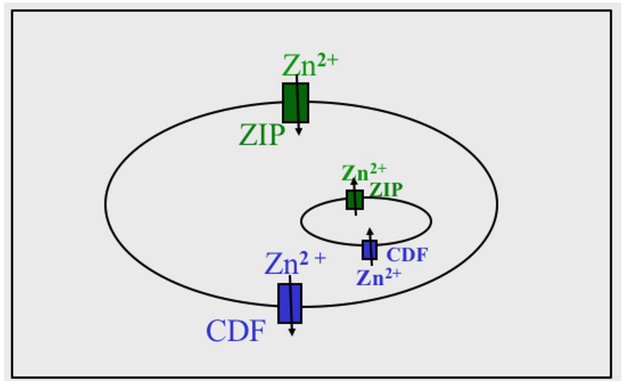


Figure 1: An illustration of the two zinc transporter families ZIP and CDF. crMTP1 is a member of the CDF family.

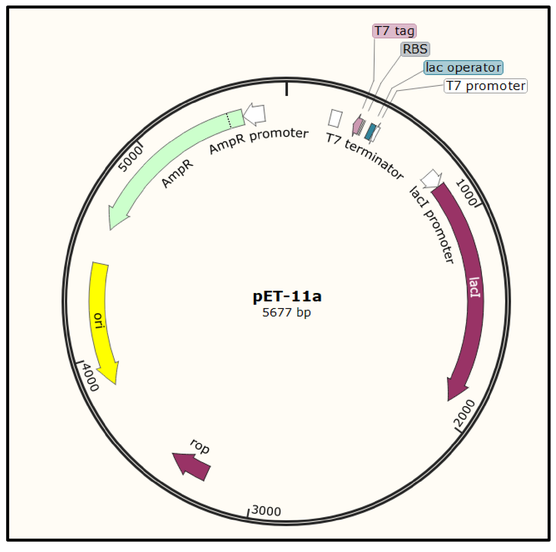


Figure 2: A diagram of the pET11a vector that was used for the insertion of the MTP1 gene between the T7 tag and T7 terminator.

Assembly Primers Design

Name	Primer 5′ (overlap/spacer/ANNEAL) 3′	Len.	%GC	3′ %GC	3′ Tm	3′ Ta
pET11a_fwd	ACCCATTTGCTGTCCACCAG	20	55	55	68.0	69.0
pET11a_rev	CGCGGATCCGGCTGCTAA	18	67	67	71.1	69.0
MTP1_fwd	tgttagcagccggatccgcgCTAGACCTGTGCGTCGCC	38	66	67	68.6	65.5
MTP1_rev	ctggtggacagcaaatgggtTCAGAAAGAACGCCTCTTCTTG	42	50	46	64.5	65.5

Figure 3: The pET11a_fwd and pET11a_rev primers were used to linearize the pET11a vector so the MTP1 gene could be inserted properly. The MTP1_fwd and MTP1_rev primers were used to prepare the crMTP1 gene for combination with the linearized pET11a vector in the NEBuilder Assembly reaction. The primers were designed using the NEBuilder Assembly Tool[2].

Methods

PCR was used to amplify and purify the crMTP1 gene from a *Chlamydomonas reinhardtii* cDNA library. Then two sets of primers were designed for the NEB Hi-Fidelity assembly reaction. One set was for the crMTP1 gene and one set for the pET11a vector (shown in Figure 5), Two PCR reactions were performed, one reaction prepared the crMPT1 gene for use in the assembly reaction and used primers MTP1_fwd and crMTP1_rev. The second reaction linearized the plasmid for use in the assembly reaction using the pET11a_fwd and pET11a_rev primers. The two PCR products were run on an agarose gel to see if the reactions worked correctly. The bands of the reaction products were then extracted from the gel and the DNA was purified by removing it from the agarose. The NEB HI-Fidelity Assembly reaction was performed to splice the crMTP1 gene DNA and the plasmid DNA together. The new recombinant plasmid was then transformed in *E. coli* bacteria cells and the transformed bacteria were plated on agarose. Colony PCR was performed on several resulting bacterial colonies using the primers indicated in Fig 7 to check for the presence of the crMTP1 DNA in the bacteria. Two colonies showed the correct size bands, so the plasmid DNA from each was purified, and after endonuclease digestion, the isolated plasmid DNA was analyzed on an agarose gel.

MTP1 Gene Sequence

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   1 acttacttcc tgtctaggct ttcgtcgcgc actcgtaacg caagtttcca cagcttggag
  61 ccgccgagcg acagctagaa gtagttttag gctgtaactg tcatagttcc agaaagaacg
 121 cctcttcttg cagcagatca tggacatgca gctgctgggc ctcctgagcg gaaggcgtcc
 181 gactgccctt tgcacaagga gacctgcttc ttgtcggatg gctctacaaa gaaggatgac
 241 gctgagcacc ggaaggtcca gcgcaagctg ctcatagcct gcatcctctg tgttctgttc
 301 atgctcgttg aggtggtcgg aggctatgtt gcgcacagta ttgccatcct ggccgatgcc
 361 gcacacatgc tgtctgacgt cgcgggcttt gcggtgtcgt tgtttgcggc gtgggcggtg
 421 actcgcaaga gccacaagtc ctactcgttc ggctaccacc gcattgagat cctgggcgcg
 481 ctggcctcgg tgctgaccat ctgggcggtc accggcgcgc tggtgtacga ggccgtcctg
 541 cgtgtcatgt accccgagcc cgtcaacgga aaacttatgt tcattgtggc ctgcgccggc
 601 atcggcttca acctgatcat tgcggcggtg cttggcgagc accacgtgca cggcatcagc
 661 ggccactgcc acggccacga cgacgagggc ggcgacgggcc atgggcacgg ccacggccat
 721 gggcatgggc acggccactc ccacgggcac tcgcatggcc acggccacaa ccacggtcag
 781 cggcagcaca gccacggcca cggccacggc catgacggca gcgagcacgg ccacgggcac
 841 agcagtgacg aggccggtgc ggccgccggc ggcagtggct gcaagcacgc cggtcacgcg
 901 cacgagggag gcagctgcat catcatcaac gtgccgctgg cggacggggc ggcggcggcg
 961 gcggcggcgg gtgccgcggc gcctgtgtcg gccatcatca gggagggcgg ccacgaggtg
1021 cctgtgcggc tggacgtgca gcagatgctg gctgccggca aggagcccgc ttcgcgcagc
1081 gaccacgacc ataagcacgg gcaaggagag ggcacgaacg gccacgctgg acacagcagc
1141 gatgaggagg ggggccatga ccaccacgac cacagccacg aacacggcgc cggcagctgc
1201 tgtggcggcc atggccaggc cgcggcggca gctggcgcgg gcgccgcggc ggcgggccct
1261 tctgctcctg acggccatgg ccacggccat gaccacgggcc atgaccacga ccacaagcac
1321 ggcggtggcg gtgacggcca tggccacagc cgtgaggtcg tgccgcacgc gcacagccac
1381 gaccacggcg ccggcggatg cgggcatgcc cattcggact cggaccagcc gggccacagc
1441 catggacacg gcctgggcgg atgcggccac ggccactcgc acggccacgg ccactcgcac
1501 ggccacggcc acggtcacgg cggtaacatc aacctgcgtt cggcggtgct gcacgtggtc
1561 ggcgacctgc tgcagagcat tggcgttgcc attgccggcg cgctcatctg gtggaagcag
1621 gacgacccgc gctggcagct ggctgacccg gtgtgcacct tcggcttcgc catcctggtg
1681 ctgctgacca ctcgcagcat cattgccgac atcacgcaca cgctcatgga gcgcacgccg
1741 caccacgtgg acctgcagca ggtcacacag gcctggcagg ggatggacgg cgtgctggac
1801 gtgcacgacc tgcacgtgtg gaacctgtcc gtgggcctgc ccatcctcac cgcgcacgtg
1861 cacatcgcgc gggcagcgga cgctgacgcc gtgctgcgcg ggctggaggg ctacgtgcgg
1921 cgcacgctgg gcatcacgca cagcacgatc cagatctgca attaccaggg cgccgccgtt
1981 gctgtgacgc cgtctgccgc cggcgccgct ggcgacgcac aggtcaagc acctgggatg
2041 cagctgggat gtagcgccgg tccccccttg ggcgcgacag cacgggcgag cgcagcgttg
2101 ctcaagccgg gtgttagctt gtgagagagt gcatgttcga cggcggccct gcggccgtct
2161 tagcgactgg agccaggaat gtacgcacgg tcagaggttg cgcaacattg taggcagcct
2221 ggacgggagc tcggatacca gttcctgcct tggccgggcg cattgcgagt gcaaggagcg
2281 atggcgtctg cgatgtcgtg cagccacttg actcccgggc aataacggtg aactgtacaa
2341 atggttgggg gcgttgtgtc caccaatatg atgcgtgtta gggtgacgag ttccaccgct
2401 acatgtggcc gcgttagggt gacgagtcac acggctagtc gcaaccgcgc ctgggtgcta
2461 agctgtgcgg gcgccaaggc aggatgcggt ttgatgtgga tggcaagggt gttgagagcc
2521 agtcatgttg tccgtgggta tggcccggca gccttgtcaa ggagtggagt ggtggagaac
2581 tgctggcgcg gcattacaag aatgtctgcg cgcatatctt ttgtaagctt gaagccttga
2641 a
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Figure 4: The crMTP1 gene (mRNA) seuence. The codon high lighted in green is the start codon and the codon highlighted in red is the stop codon.

Assembly DNA Cloning

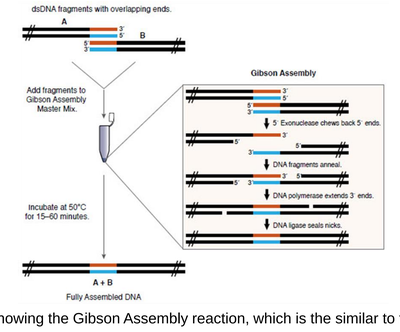

Figure 5: A diagram[3] showing the Gibson Assembly reaction, which is the similar to the NEB Hi-Fidelity Assembly reaction.

Agarose Gels

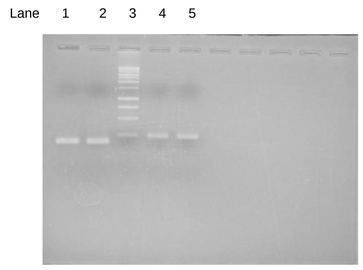

Figure 6: Agarose gel comparing the results of PCR reactions using colony primer primers designed for the crMTP1 gene and crMTP1 DNA as template. Lanes 1and 2 used the ColonyPCR1Plus Minus primer set. Lanes 4 and 5 used ColonyPCR2Plus/Minus set. Lane 3 contains DNA markers. ColonyPCR1Plus and ColonyPCR1Minus were chosen for colony PCR

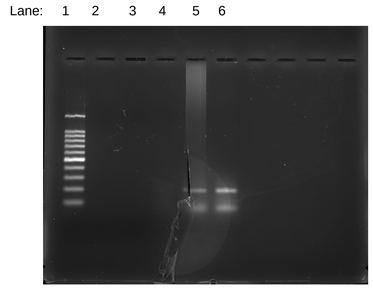

Figure 7: A 2% agarose gel analyzing the success of a colony PCR. Each sample was compared with 100 bp Promega marker in lane 1. There were only two *E. coli* colonies from separate cultures indicate the presence of crMTP1 gene (lanes 5 and 6),

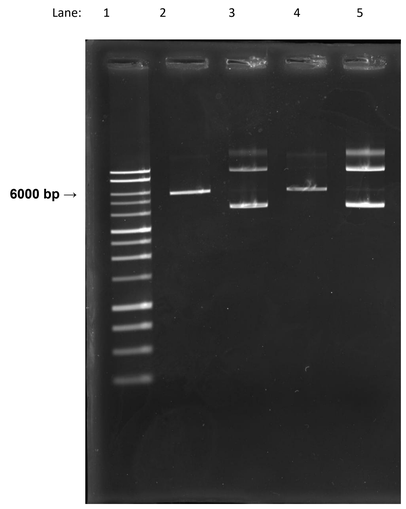

Figure 8: A 1% agarose gel comparing the size of the digested and undigested plasmids isolate from the colonies positive via colony PCR. Lane 1 holds 1kb marker to compare band sizes with the rest of the lanes. The digested plasmids are shown in lanes 2 and 4 and were cut using HindIII. The digested sample show no addition of the MTP1 gene in the vector.

Conclusion

The size of plasmid isolated from colonies after screening several colonies indicates the cloning of the crMTP1 gene into the pET11a vector was unsuccessful so far. Even though it appeared the crMTP1 gene was present in two of the colonies via colony PCR, Figure 8 only presented bands that were the size of the vector, not the vector and the crMTP1 insert.

References

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2. NEBuilder Assembly Tool (https:nebuilder.neb.com)
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Acknowledgements

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