

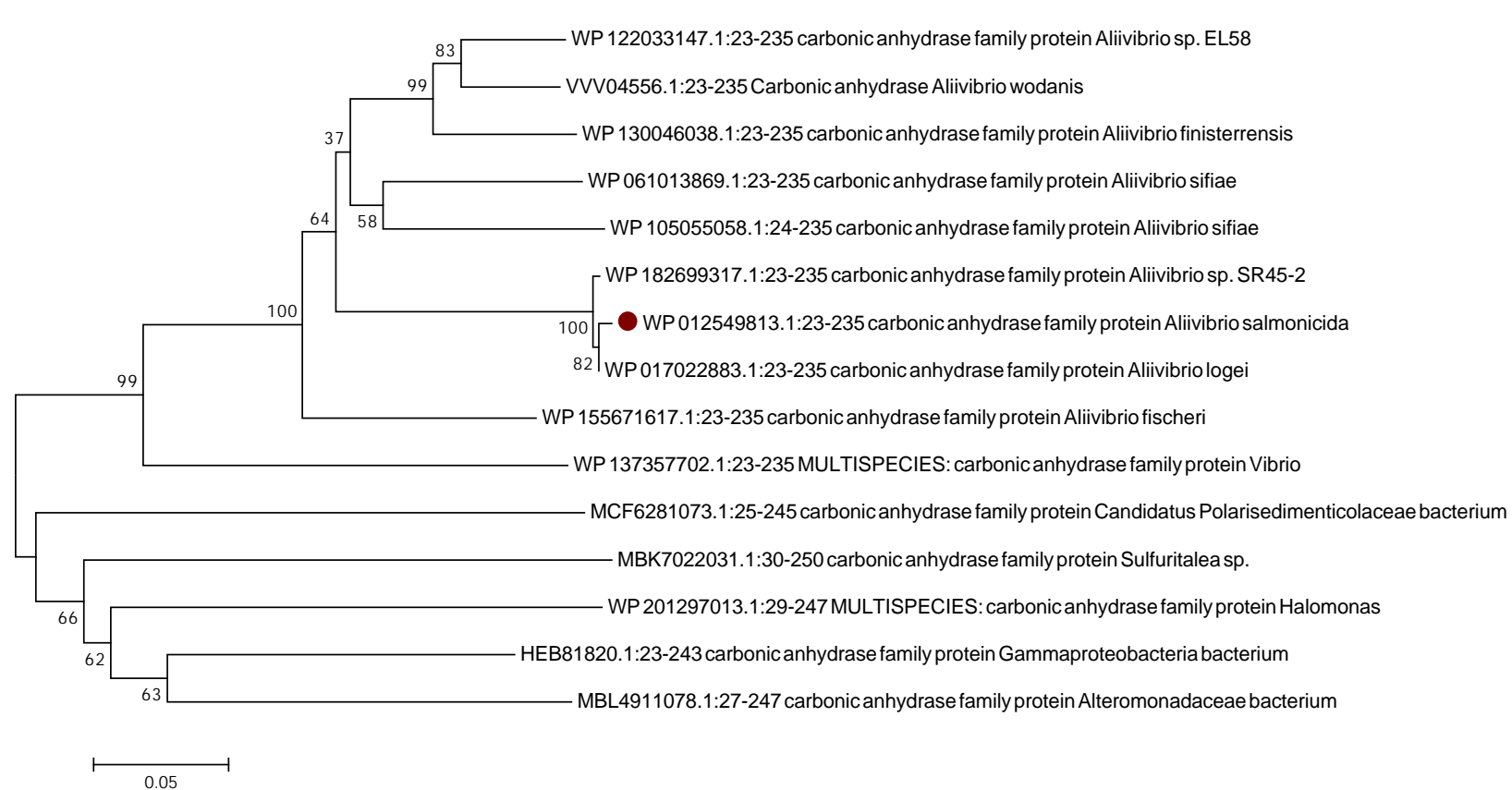
Concentration of Ca <sup>2+</sup> (mg/L)	0	40	80	120	160	200
OD630	0.152	0.158	0.162	0.149	0.189	0.144

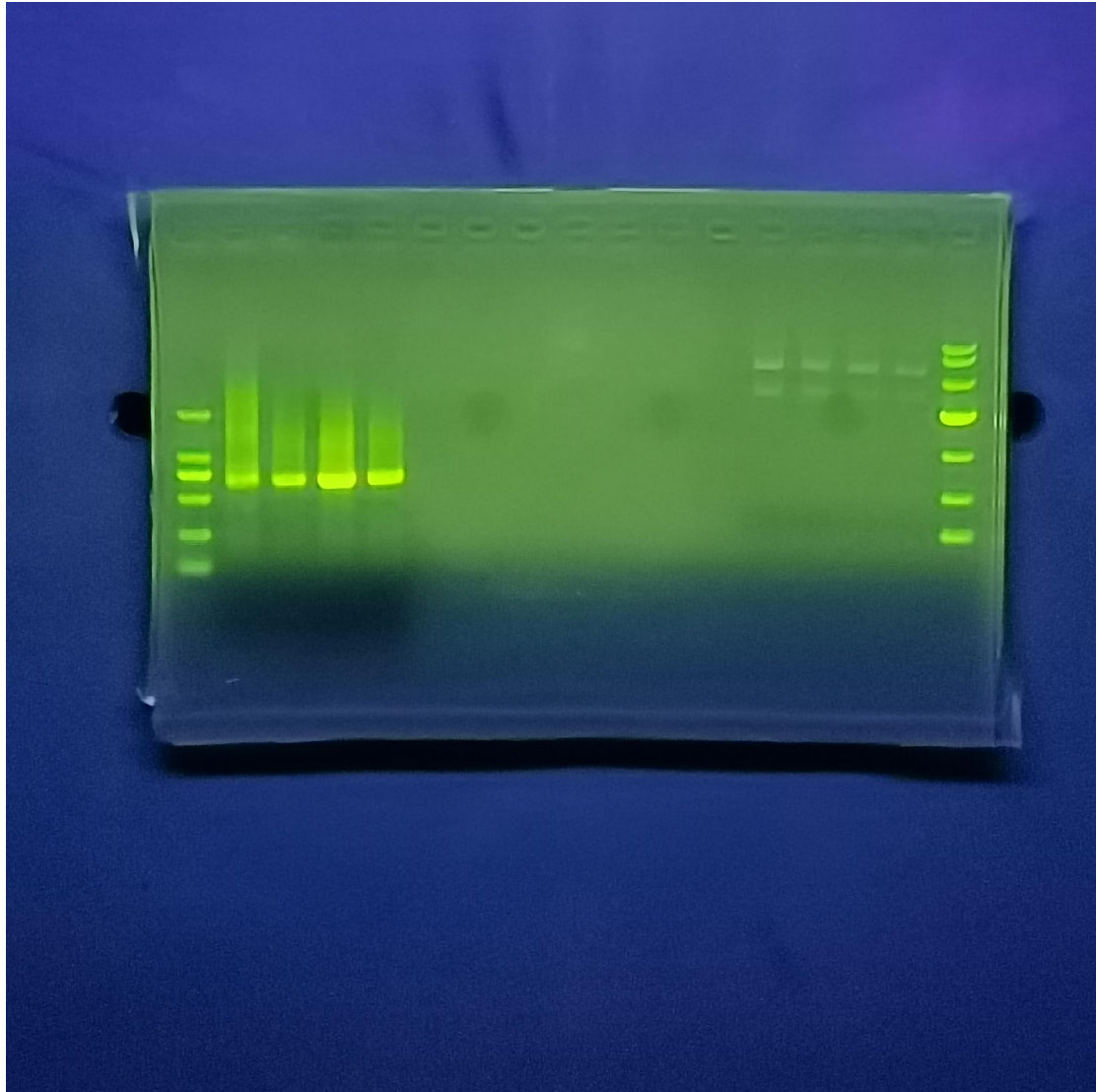
Concentration of Ca <sup>2+</sup> (mg/L)	50	60	70	80	90	100
Specific enzyme activity	0.117	0.072	0.086	0.071	0.047	0.048

110	120	130	140	150
0.126	0.086	0.065	0.05	0.085

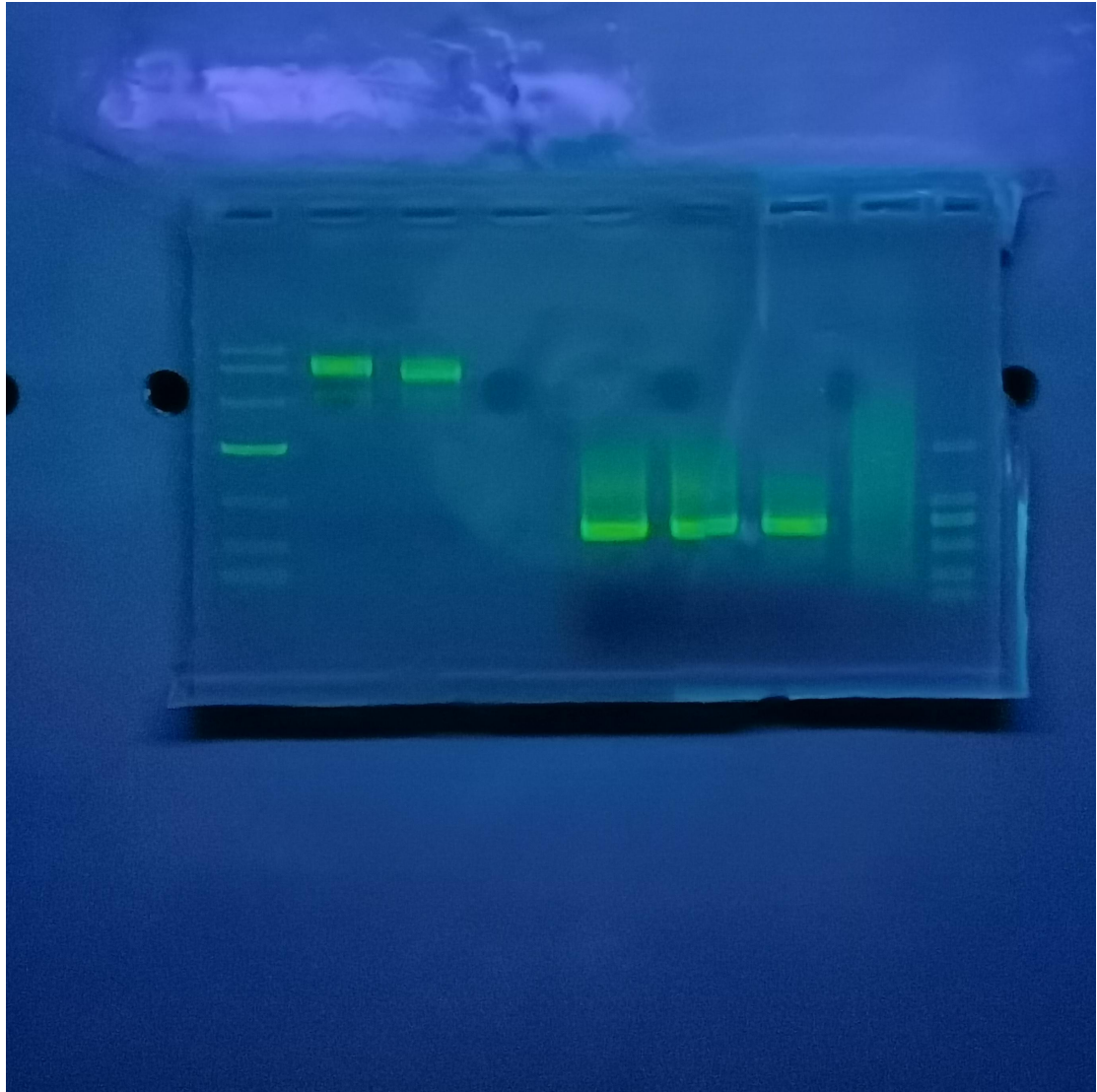
bacterial strains	0	1	2	3	4	5
OD348(3min)–OD348(0min)	0.043	0.098	0.281	0.085	0.087	0.068

6	7	8	9	10	11
0.072	0.03	0.033	0.05	0.057	0.037



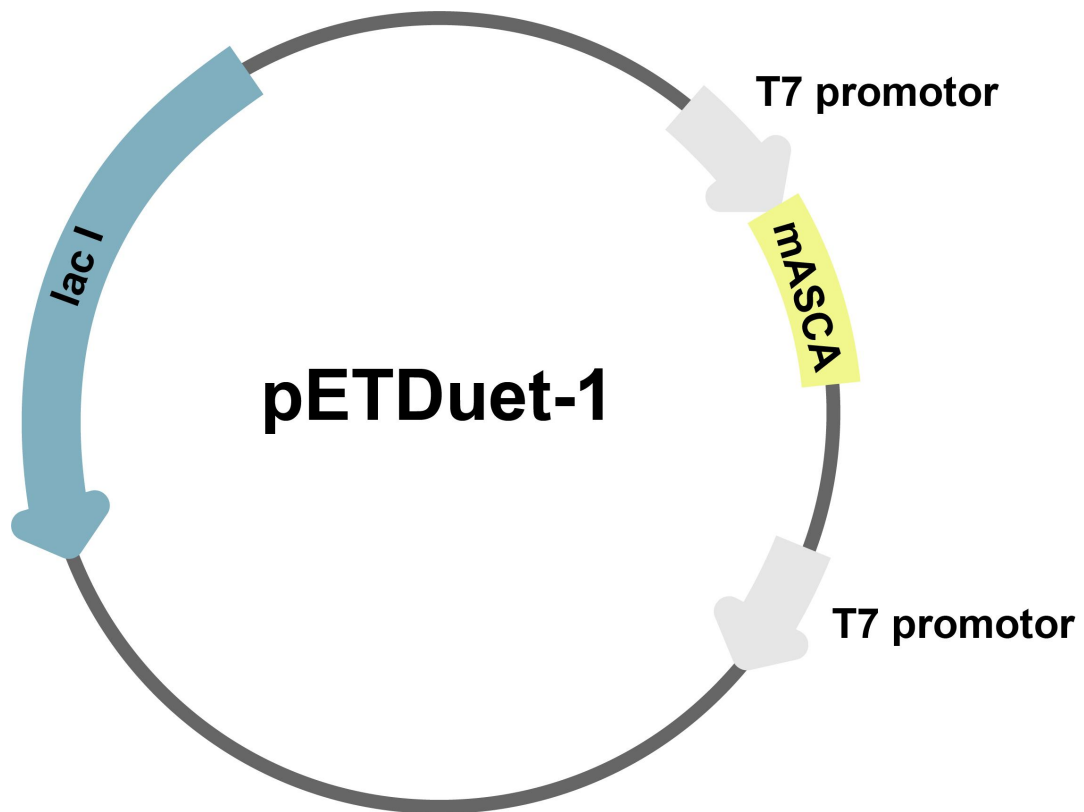


*Fig.2.3.1b* Product electrophoresis results (35 circles)

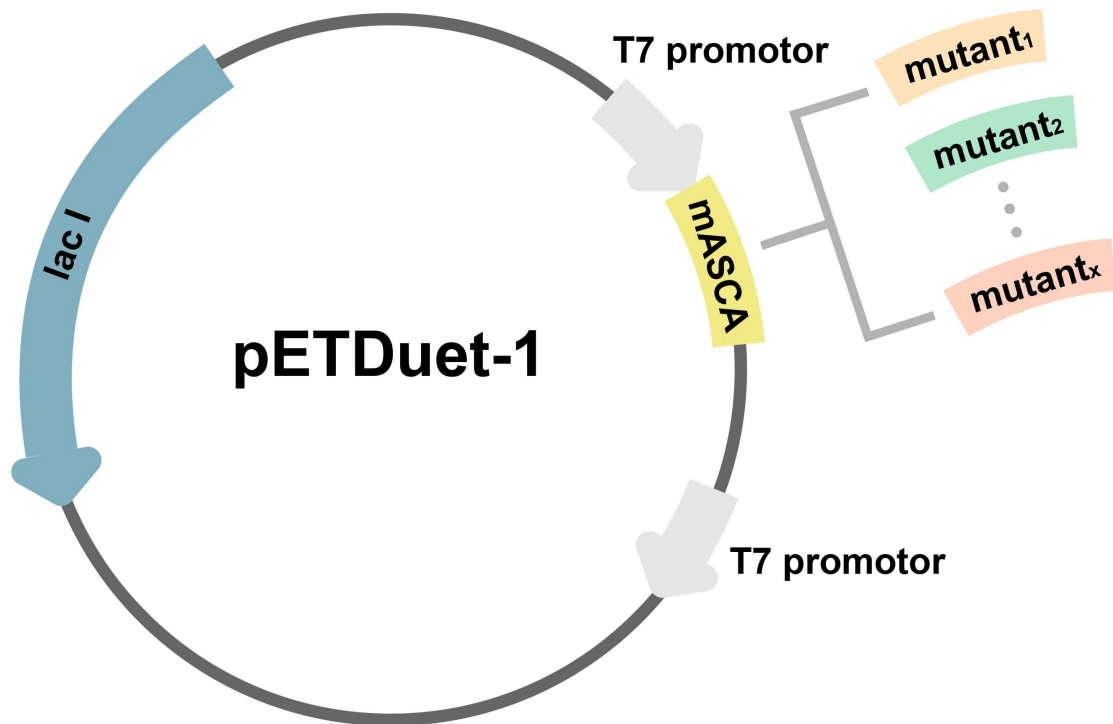


*Fig.2.3.1a* Product electrophoresis results (45 circles)





*Fig.2.1.4* pETD-mASCA



*Fig.2.3.1c* Plasmids for mutant constructs

>mASCA

GAATTCATGTCTGAATGGTCTTACTCTGGTGAAACCGCTCCGACCAACTGGCACGGTA  
CCTGCCAGTCTGGTGTTAACCAGTCTCCGATCGACATCACCCACGCTATCGAATCTAC  
CCTGGACCCGATCATCTTCAACTACTCTAAAGCTGGTAAAAACGTTGTTAACAACGGTC  
ACACCGTTCAGGTTAACTTCGACGCTAAACAGTCTATCCAGATCGAAGGTAAAACCTTC  
ACCCTGCTGCAGCTGCACTTCCACGCTCCGTCTGAAAACCTGATCGAAGGTCACCTCTT  
TCCCGCTGGAAATGCACCTGGTTCACGCTGACAAAGACGGTAACCTGGCTGTTATCG  
GTGTTATGCTGAAAGAAGGTAAACCGAACTCTGAACTGACCAAATCTGGGCTAAAAT  
GCCGCAGTCTGGTCAGGTTGACCTGGAAAACCGCTGGACCTGACCAAATGCTGCC  
GCGTGACCAGGCTTACTACCGTTTCAACGGTTCTCTGACCACCCCGCCGTGCTCTGAA  
GGTGTTACCTGGCTGGTTATGAAAAACCCGATCGAAATCTCTTCTGAACAGCTGGCTC  
AGTTCACCGCTCTGTACAACGGTAACAACCTGCCCGACCCAGGCTATCAACGCTCGTC  
CGATCCTGAAATGAAAGCTT

>WP\_012549813.1:23-235 carbonic anhydrase family protein [Aliivibrio salmonicida]  
SEWSYSGETAPTNWHGTCQSGVNSPIDITHAIESTLDPIIFNYSKAGKNVVNNGHTVQVN  
FDAKQSIQIEGKTFTLLQL

HFHAPSENIEGHSFPLEMHLVHADKDGNLAVIGVMLKEGKPNSELTKIWAKMPQSGQVD  
LENQLDLTKLLPRDQAYYRF

NGSLTTPPCSEGVTWLVLMKNPIEISSEQLAQFTALYNGNNCPTQAINARPILK

>WP\_017022883.1:23-235 carbonic anhydrase family protein [Aliivibrio logei]  
SEWSYSGETAPTNWHGTCQSGVNSPIDITHAIESTLDPIIFNYSKAGKNVVNNGHTVQVN  
FDAKQSIQIEGKTFTLLQL

HFHAPSENIEGHSFPLEMHLVHADKDGNLAVIGVMLKEGKPNSELTKIWAKMPQSGQVD  
LENQLDLTKLLPRDQAYYRF

NGSLTTPPCSEGVTWLVLMKNPIEISSEQLAQFTALYNGNNRPTQAINARPILK

>WP\_182699317.1:23-235 carbonic anhydrase family protein [Aliivibrio sp. SR45-2]  
SEWSYSGETAPTNWHGTCQSGVNSPIDITHAIESTLDPIIFNYSKAGKNVVNNGHTVQVN  
FDVKQSIQIEGKTFTLLQL

HFHAPSENIEGHSFPLEMHLVHADKDGNLAVIGVMLKEGKPNSELTKIWAKMPQSGQVD  
LENQLDLTKLLPRDQAYYRF

NGSLTTPPCSEGVTWLVLMKNPIEISSEQLAQFTALYNGNNRPTQAINARPILK

>WP\_122033147.1:23-235 carbonic anhydrase family protein [Aliivibrio sp. EL58]  
SEWSYAGETAPANWHGNCQTGVNSPIDITNAIESTLDPITFNQAQAGKNIVNNGHTVQVN  
FDGKQSIQVEGKTFSLQL

HFHAPSENLIAGHSYPLEMHLVHANKDGNLAVIGVMFKEGKENTELAKVWAQMPKSGDV  
KLDSKLTLLKLLPGDQAYYRF

NGSLTTPPCTEGVTWLVLMKNPITISAEQLTQFESLYQGNNRPVQAINARPVLK

>WP\_061013869.1:23-235 carbonic anhydrase family protein [Aliivibrio sifiae]  
SEWSYSGETAPAHWHGTCQSGVNSPIDITGAVESELKPITFNQTQAGKNIVNNGHTVQVN  
FDGKQSIKIEGKTFNLLQL

HFHAPSENLIAGHSFPLEMHLVHSDKEGNLAVIGVMFKEGKANTELAKVWKEMPKSGEVD  
LTNKLDLVNLLPADQAYYRF

NGSLTTPPCSEGVTWLFVMKNPISISSQQLTQFKALYDGNNRPTQAINARPVLQ

>VVV04556.1:23-235 Carbonic anhydrase [Aliivibrio wodanis]  
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FDGKQSIQVEGTTFNLLQL

HFHAPSENLIAGHSYPLEMHLVHADKDGNLAVIGVMFKEGKTNTELAKIWAQMPKSGDVN  
LDSKLMLTRLLPSNQAYYRF

NGSLTTPPCTEGVTWLFVMKNPITISAEQLIQFKSLYQGNNRPVQAINARPILK

>WP\_130046038.1:23-235 carbonic anhydrase family protein [Aliivibrio finisterrensis]  
SEWSYAGETAPANWHGTCQSGLNQSPIDITNAIESTLKPITFNQAQAGKNIVNNGHTVQVN  
FDGKQSIQVEGKTFNLLQL

HFHAPSENLIAGHSYPLEMHLVHADKDGNLAVVGMFKEGKANPELAKIWAQMPKSGDV  
KLDNKLRLIDLLPSDQAYYRF

NGSLTTPPCTEGVTWLVMMKPPITISTEQLTQFRTLYKGNNRPVQSINARPVLK

>WP\_105055058.1:24-235 carbonic anhydrase family protein [Aliivibrio sifiae]  
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DGKQSIEIEGKIFNLLQLH  
FHTPSENLIAGHSFPLEMHLVHSDKNGNLAVVGVMFKEGKTNLELAKIWSKMPKSGEVDL  
DSKINLTNLLPKEQAYYRFN  
GSLTPPCTEGVTWFMKNPITISSQQLAQFKSLYEGNNRPVQTINARPVLK  
>WP\_155671617.1:23-235 carbonic anhydrase family protein [Aliivibrio fischeri]  
SEWGYTGDTGPNHWHGVCQTGVNQSPIDITNAIESELEPITFNYGQAGKNIVNNGHTVQVN  
IKSPQSIQIEGKTFSLLQL  
HFHAPSENLIAGHSYPLEMHLVHADKDGNLAVVGVMFKEGKANPELAKIWSQMPESGEIT  
LDKKLTLNLLPNDQAYYRF  
NGSLTPPCSEGVTWLVKMNPIEISSEQLSQFKELYNGNNRPTQAINARPILK  
>WP\_137357702.1:23-235 MULTISPECIES: carbonic anhydrase family protein [Vibrio]  
SEWGYSGKQGPEHWHGICQTGKNQSPIDISKGIESELEPIKFNYDVSGENVVNNGHTIQVN  
FNGDQSIEVEGKSYSLLQF  
HFHAPSENLIKGHSYPLEVHLVHADENKNLAVVGVMFEEGATNPELEKVVWDVMPANKGQ  
QDLQHSINLTGLLPKQSDYFR  
FNGSLTPPCSEGVTWLVKMNPIETISKAQLEKFRTLYSGNNRPTQDVNARPVL  
>HEB81820.1:23-243 carbonic anhydrase family protein [Gammaproteobacteria  
bacterium]  
SHWGYSGHEGPENWAKLSADNFACAGRNQSPINLTGFIEAELSPINFNYQPGGREILNNG  
HTVQINYQPGSSMTIDGKVF  
KLLQFHFHAPSENNINGKSFPLEAHLVHADKDGNLAVVAVMFEEGKANGLSKAWQKMP  
EHEGDKNALQTTVSVDDILPA  
DRDYRFRNGSLTPPCTEGVRWLVMKQPVSASAEQLAKFRHALHGPNRPLQAVNARPV  
LK  
>MBL4911078.1:27-247 carbonic anhydrase family protein [Alteromonadaceae  
bacterium]  
ADWGYSGHQGPENWAKLSGDNLSCDGKNQSPINLTGFIEADLTPLIFDYQQGGGEQVINNG  
HTVQVNYQQGSSITIDGKHF  
NLLQFHFHAPSENHINGHSYPLEAHLVHADKNGNLAVVAVMFKEGKSNALLAKAWQSMP  
KHAGEQHKLTSKVNVDKLLPK  
NRDYRFRNGSLTPPCSEGVRWLVMKDAVSASKTQIENFESALHEANNRPIQPLNARSVM  
Q  
>MBK7022031.1:30-250 carbonic anhydrase family protein [Sulfuritalea sp.]  
AHWGYSGDVGPDRWVWKLKPEFSACAGKNQSPIDVSGLIEAQLPPVKFDYKQGGSEVVNN  
GHAIQVNYDAGSGIEVDGTRF  
DLKQFHFHSPENRINGKSFPLEAHLVHADKDGNLAVVAVMFDEGKENS AVGAAWGQMP  
KSAGGKNALPNKVAATGILPA  
DRDYRYRNGSLTPPCSEGVRWIVMKKPLTVSKQIETFQKTLGFANNRPLQAVNARPILK  
>MCF6281073.1:25-245 carbonic anhydrase family protein [Candidatus  
Polarisedimenticolaceae bacterium]  
AHWGYSGSTGPKDWGKIAPSYKICGTGRNQSPIDITGIRAALEPITFNYEAGTTAILNNGH  
TIQVNYEAGNSIVLNGHE  
YELKQFHFHAPSENHIDGKSYPLEAHFVHADKDGHLAVVGVMLESGSNAVIGKLWEHMP  
ANAGDKNELPWKIDGELLLP

NDRSYRFRNGSLTTPPCSEGVTWLVMMKTPVAISSAQLEAFTTVMGEDNNRPLQAINARPIL  
>WP\_201297013.1:29-247 MULTISPECIES: carbonic anhydrase family protein  
[Halomonas]  
WSYSGETGPENWAQLTPEYGACAGSNQSPIDLTGFIDAELEPIDFHYENGGAEILNNGHTV  
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KQFHFHVPSENLIHGESFPMEGHLVHADEDGNLAVVAVMVTEGEANEALARAWAQMPPEE  
GETLALTS DISPLGILPADRD  
YYRFNGSLTTPPCTEGVRWLVLKQPITASQEIQIDQFLAAIDDDHHNNRPVQPVNARPVLQ