```
                         54           68
Gster_LDH         1  MKNNGG--ARVVILGA-CFVGASYVFALMNQGIADEIVLIDANESKAIGDAM--DFNHGKVFAP--KPVDI-WHGDYDDC
Tth_LDH           1  -------MKVGILGS-GMVGSATAYALALLGVAREVVLVDLDRKLAQAHAE--DILHATPFA---HPVWVRA-GSYGDL
Blon_LDH          1  MAETTVKPTKLAVIGA-GAVGSTLAFAAAQRGIAREIVLEDIAKERVEAEVL--DMQHGSSFY---PTVSIDGSDDPEIC
Ctep_MDH          1  --------MKITVIGA-CNVGATTAFRLAEKQLARELVLLDVVEGIPQGKAL--MYESGPVGL--FDTKVTGSNDYADT
Nvie_MDH          1  --------MTITVIGS-GKVGASAALNCGLRELDD-ILLDIVQGLPQGEAM--DINHQLSERG--SDSVARGSNNYEDM
Nmar_MDH          1  ---------MITIIGS-GKVGGDAALFSALKRLDDQILLDVAEGLPQGEAM--DINHMLSEQG--IDVEVKGSNNFEDM
Nkor_MDH          1  ---------MITIIGS-GKVGGDAALFSALKRLDDQILLDVAEGLPQGEAM--DINHMLSEQG--IDVEVKGSSNFEDM
NPMRtheta1_MDH    1  ---------MITIIGA-GKVGGGAALFSALRNLDDQILLDIVEGLPQGEAM--DINHALSEQG--IDVEIKGSNDYSDM
NPMRtheta5_MDH    1  ---------MITIIGA-GKVGGGAALFSALRNLDDQILLDIVEGLPQGEAM--DINHALSEQG--IDVEIKGSNDYSDM
NPMRtheta2_MDH    1  ---------VITIIGA-GKVGGGAALFSALRNLDDQILLDIVKGLPQGEAM--DINHALSEQG--IDVEIKGSNDYSDM
YK1312theta_MDH   1  --------MITIIGA-GKVGGEAALFSALKKLDDQILLDIIEGLPQGEAM--DINHMLSEQG--IDVEVKGSNDYSDM
NPMRtheta4_MDH    1  ---------MITIIGA-GKVGGDAALFSALKKLDNQILLDIIEGLPQGEAM--DINHTLSEQG--IDVDIKGSNDYSDM
Nbrev_MDH         1  ---------MITIIGA-GKVGGDAALFSALRRLDDEILLDIAEGLPQGEAM--DLNHMLSEQG--IDVNVKGSNNYEDM
NPMRdelta3_MDH    1  ---------MITIIGS-GKVGGAAALFTALKKLDEQILLDVVKGLPQGEAM--DLNHALSEQG--VDVEIIGSNDYSDM
NPMRdelta2_MDH    1  ---------MITIIGS-GKVGGEAALFTALKKLDDQILLDIVKGLPQGEAM--DLNHSLSELG--IDVEIIGSNDYSDM
NPMRiota1_MDH     1  ---------MITIIGA-GKVGAAAISIALRNLSDEILLDVIKGLPEGEAM--DINHMLSEKG--INVDVRGSNDYSDI
YK1309iota_MDH    1  ---------MITIIGA-GKVGAAAISIALRNLSDEILLDVIKGLPEGEAM--DINHMLSEKG--INVDVRGSNDYSDI
Nuzo_MDH          1  --------MLTIIGS-GKVGGDAALFSALRKVDKDILLDVVNGLPQGEAM--DINHMLSEQG--VDVHIRGSNDYADM
Mjan_MDH          1  --------MKVTIIGASCRVGSATALLLAKEPFMKDLVIGREHSINKLEGLREDIYDALAGTRSDANIYVESDENLRII
Iisl_MDH          1  MARI---PYKVAVIGT-CRVGATFAYTMAVVPGIARMTLVDVVPGLAKGVME--DIKHAAAVFR--RSITVEAFEDVSKV
Msed_MDH          1  M-------AKVGFIGA-CKIGQTIAYSALVSGAVDEAVLYDIIPELPDKFEH--ELRHAFATKG--IKANVLGTNSLDDV

                         102
Gster_LDH        73  RDADLVVICAGANQKPGETRLDLVDKNIAIFRSIVESVMASGFQGLFLVATNPVDILTYATWKFSGLPHERVIGSCTILD
Tth_LDH          66  EGARAVVLAAGVAQRPGETRLQLLDRNAQVFAQVVPRVLEAAPEAVLLVATNPVDVMTQVAYRLSGLPPGRVVGSCTILD
Blon_LDH         75  RDADMVVITAGPRQKPGQSRLELVGATVNILKAIMPNVVKVAPNAIYMLITNPVDIATHVAQKLTGLPENQIFGSCTNLD
Ctep_MDH         68  ANSDIVVITAGLPRKPGMTREDLLSMNAGIVREVTGRVMEHSKNPIIVVSNPLDIMTHVAWQKSGLPKERVIGMAGVLD
Nvie_MDH         67  RGSDYVVLVAGVGRKPGMTRMDLLKINAGIVKDVASKIATYAKDATVIVVTNPLDPMTYLALKTIGAQKSKVMGMGGMLD
Nmar_MDH         67  KGSNIVVVAGSGRKPGMTRMDLLKINASIVKSVVENVKKYADDSMIIPVTNPLDPMAYITYKVSGFDRSRVFGMGGMLD
Nkor_MDH         67  KGSKIVVVAGSGRKPGMTRMDLLKINATIVKSVVENVKKYANDSMIIPVTNPLDPMAYITYKVSGFDRSRVFGMGGMLD
NPMRtheta1_MDH   67  KGSNIVVVAGLGRKPGMTRMDLLQKNAGIVKSVVENVKKYADNSMIIPVTNPLDPMTYICYKVSGFERNRVFGMGGMLD
NPMRtheta5_MDH   67  KGSNIVVVAGLGRKPGMTRMDLLQKNAGIVKSVVENVKKYADDSIIIPVTNPLDPMTYICYKVSGFERNRVFGMGGMLD
NPMRtheta2_MDH   67  KGSNIVVVAGLGRKPGMTRMDLLQKNAGIVKSVVENVKKYADDSIIIPVTNPLDPMTYICYKVSGFERNRVFGMGGMLD
YK1312theta_MDH  67  KGSDIVVTVAGSGRKPGMTRMDLLKINAGIVKSVVENVKKYANDAMIIPVTNPLDPMAYICYKVSGFEKNRIFGMGGMLD
NPMRtheta4_MDH   67  KGSDIVVVAGLGRKPGMTRMDLLQKNAGIVKSVVENVKKYANDSMIIPVTNPLDPMAHICYKVSGFEKNRVIGMGGMLD
Nbrev_MDH        67  KGSKIVVVAGAGRKPGMTRMDLLKINAGIVKDVVGNVKKFADDAMIIPVTNPLDPMAQIAYKTSGFDRSRVFGMGGMLD
NPMRdelta3_MDH   67  KDSKIVVIVAGSGRKPGMTRMDLLKINAGIVKDVVENVKKYSNDAILVPVTNPLDPMAHIAYKTSGFERNRILGMCNMLD
NPMRdelta2_MDH   67  KNSKIVVVAGSGRKPGMTRMDLLKINAGIVKDVVENVKKYSKDSFIIPVTNPLDPMAHIAYKVSGFSRNRIFGMCNMLD
NPMRiota1_MDH    67  KGSEIVVVAGSGRKPGMTRMDLLKVNASIVKDVTENVKKYANNSIIVPVTNPLDPMAYVAFKVSGFSKNRIVGMCNMLD
YK1309iota_MDH   67  KGSEIVVVAGSGRKPGMTRMDLLKVNASIVKDVTENVKKYANNSIIVPVTNPLDPMAYVAFKVSGFSKNRIVGMCNMLD
Nuzo_MDH         67  KGSDIVVVAGSGRKPGMTRMDLLKINAGIVKDVVENVKKHASNAIIVPVTNPLDPMVHITYKTSGFEPSRIVGMCNMLD
Mjan_MDH         73  DESDVVIITSGVPRKEGMSRMDLAKTNAKIVGKYAKKIAEICDTKIF-VITNPVDVMTYKALVDSKFERNQVFGLCTHLD
Iisl_MDH         73  ENADAIVITAGKPRKADMSRRDLANVNAQIIRDIGDKIRDRNPGALYVVVTNPVDVMTMVLDDVIG-SKGTVIGTCTSLD
Msed_MDH         69  SGMDIVVISAGKPRKPGMSRRDLFVDNAKIMIDLAQKIPSKNPGAIYLMVANPVDMMASVFMKY---SKQFTISACDQVE

                         171          199
Gster_LDH       153  TARFRFLLGEYFSVAPQNVHAYIIGEHGDTELPVWSQAYIGVMPIRKLVESKGEE--AQKDLERIFVNVRDAAYQIIE--
Tth_LDH         146  TARFRALLAEYLRVAPQSVHAYVLGEHGDSEVLVWSSAQVGGVPLLEFAEARGRA-LSPEDRARIDEGVRRAAYRIIE--
Blon_LDH        155  SARLRFLIAQQTGVNVKNVHAYIAGEHGDSEVPLWESATIGGVPMCDWTPLPGHDPLDADKREEIHQEVKNAAYKIIN--
Ctep_MDH        148  SARFRSFIAMELGVSMQDVTACVLGGHGDAMVPVVKYTTVAGIPVADL--------ISAERIAEIVERTRTGGAEIVNHL
Nvie_MDH        147  LSRFKSYIQEATGVSRDSIQAMVISEHGNMLPLTRFSSLGGIPIHDF--------ITKEQATDIFEKTKKVAAEVIA--
Nmar_MDH        147  LSRFRQFIHEATGHSRDSIRALVIGEHGNMLPLPRFSSVGGIPLPSL--------LPKEKLEEIVQNTKQVAAKVIE--
Nkor_MDH        147  LSRFRQFIHEATGHSRDSIRALVIGEHGNMLPLPRFSSVSGIPLSSF--------LPKQKLDEIVQNTKQVAAKVIE--
NPMRtheta1_MDH  147  ISRFKQFIHEATGHSRHSIRALVIGEHGNMLPLPRFSSVSGIPLTSL--------LSKEKLDEIVQNTRNVAAKVIE--
NPMRtheta5_MDH  147  ISRFKQFIHEATGHSRHSIRALVIGEHGNMLPLPRFSSVSGIPLTSL--------LPKEKLDEIVQNTRQVAAKVIE--
NPMRtheta2_MDH  147  LSRFKQFIHEATGHSRHSIRALVIGEHGNMLPLPRFSSVSGIPLTSL--------LSKEKIDEIVQNTRQVAAKVIE--
YK1312theta_MDH 147  ISRFIQFIHEATGHSRDSIRALVIGEHGNMLPLPRFSSVSGIPLTSL--------LSKEKLDEIVQNTKQVAAKVIE--
NPMRtheta4_MDH  147  LSRFKQFIHEATGYSRDSIRALVIGEHGNMLPLPRFSSVAGIPVSL--------LSKEKLDEIQNTRQIAAKVIE--
Nbrev_MDH       147  LSRFKQFIHEATGYSRESTKALVIGEHGNMLPLTRFATVSGIPLPTL--------LPKEKLDEIFTATKGVAAEVIK--
NPMRdelta3_MDH  147  LSRFSQFIHEATGQSRESIRALVIGEHGNMLPLRFSSVSGIPLTSL--------LAKDKLEEIEKNTRQVAAKVIE--
NPMRdelta2_MDH  147  ISRFTQFIHEATGYSRQSIRALVIGEHGNMLPLMRFSSVSGIPLTSL--------LSKEKLDEIEKNTRQVAAKVIE--
NPMRiota1_MDH   147  LSRFKQFIHESTGFSRDSIRALVIGEHGNMVPLPRFSSVAGIPLMSL--------LSKEKIDEIVVSTREVAAKVIE--
YK1309iota_MDH  147  LSRFKQFIHESTGFSRDSIRALVIGEHGNMVPLPRFSSVAGIPLMSL--------LPKEKIDEIVISTREVAAKVIE--
Nuzo_MDH        147  LSRFIQFIHESTGHSRDSIRALVIGEHGNMLPLPRFSTVSGIPLASI--------LPKEKLDQIVKDTRGVAAKVIE--
Mjan_MDH        152  SLRFKVAIAKFFGVHIDEVRTRIIGEHGDSMVPLLSATSLGGIPIQKFER------FKELPIDEIEDVKTKGEQIIR--
Iisl_MDH        152  TFRFRAAVSELLNVPIVAVDGYVVGEHGEEAFVAWSTVTKGIHIDQYIKERNIN-ISR---EQIEKYVKDVAASIIA--
Msed_MDH        146  TMRMRSFIAKKLKIPVTSVDGFVGGEHGEDAVVLWSTVKLKGKPVDEFN-------INK---DEVSDYVKKIPGEIIR--
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                      246
Gster_LDH     229 KKGATYYCIAMGLARVTRAILHNENALLTVSAYLDGLY-GERDVYIGVPAVLNRNGIREVIEIELNDDEKNRFHHSAATL
Tth_LDH       223 GKGATYYGIGAGLARLVRAILTDEKGVYTVSAFTPEVE-GVLEVSLSLPRILGAGGVEGTVYPSLSPEEREALRRSAEIL
Blon_LDH      233 GKGATNYAIGMSGVDLIEAVLHDTNRLPVSSMLKDFH-GISDICMSVPTLNRQGVNNTINTPVSDKELAALKRSAETL
Ctep_MDH      220 KQGSAFYAPATSVVEVVESIVLDRKRVLTCAVSLDGQY-GIDGTFVGVPVKLGKNGVEHIYEIKLDQSDLDLLQKSAKIV
Nvie_MDH      217 LKGATVYAPGNAVATVIESMAKDKKMVIPVSAYLDGQY-GVSDLCIGVPAVLGAGGVEKIVELKLDSFEQGVFDKGVASV
Nmar_MDH      217 LKGATVHAPGNAISAVVEAVVRDRKQVIPVATYLDGEY-DHSDVTIGVPAVLGKNGVEKIIELDLNDEEKQVFNKAVENV
Nkor_MDH      217 LKGATVHAPGNAISAVIEAVVRDRKQVIPVATYLDGEY-GHSDVTIGVPAVLGKKGVEKIIELDLNAEEKQLFDAGIQNV
NPMRtheta1_MDH 217 LKGATVHAPGNGISALIDSVVNDKKQVLPIATYLDGEY-GYSDVTIGVPAVLGKNGVEKIIELDLNDEEKEMFNKGIESV
NPMRtheta5_MDH 217 LKGATIHAPGNGISALIDSVVNDKKQVLPIATYLDGEY-GYSDVTIGVPAVLGKNGVEKIIELDLNDEEKEMFNKGIESV
NPMRtheta2_MDH 217 LKGATIHAPGNGISTLIDSVVNGKMQVLPIATYLDGEY-GYSDVTIGVPAVLGKNGVEKIIELDLNDEEKEIFNKGIESV
YK1312theta_MDH 217 LKGATIHAPGNGISSLIDAVVNNKRRVIPVATYLDGEY-GYSDVTIGVPAVLGRNGVEKIVELDLNDEEKSMFQKGIESI
NPMRtheta4_MDH 217 LKGATIHAPGNAISSLIDSIVNNRKQVLPVATCLDGEY-GQSDITIGVPAVLGKKGIEKIIELDLNEEEKDWFNKGIVSV
Nbrev_MDH     217 LKGATVHAPGNAISAVIESVVQDKKQVIPVSTNLDGEY-GQKDVSIGVPAVLGKKGVEKILELELNDEEKEWFNKGVDSV
NPMRdelta3_MDH 217 LKGATIHAPGNAISSLIESVVRDKKRVMPVSTPLNGEY-GYSDVTVGVPAVLGKNGVEKIIELELNDEEKDWFRKSVDSV
NPMRdelta2_MDH 217 LKGATVHAPANAISSLIESVVRNQRRVIPVATPLDGEY-GYSDVTIGVPAVLGKNGVEKIIQLDLDEKEKEWIKKSVESV
NPMRiota1_MDH  217 LKGATVHAPANGISAVVESIVRDTKQVIPVATYLDGEY-GFSDVTIGVPAVLGKNGIEKIIELDLDSSEKE---------
YK1309iota_MDH 217 LKGATVHAPANGISVVVESIVRDTKQVIPVATYLDGEY-GFSDVTIGVPAVLGKNGIEKIIELDLDSSEKEWFKKGVDSV
Nuzo_MDH      217 LKGATVHAPGNAISAVVEAIIKDRKKVIPVATPLTGQY-GHSDVSIGVPAVLGRKGVEKIIELDLNSEEKEWFEKGVNSV
Mjan_MDH      224 LKGGSEFGPAAAILNVVRCIVNNEKRLLTLSAYVDGEFDGIRDVCIGVPVKLGRDGIEEVVSIELDKDEIIAFRKSAEII
Iisl_MDH      226 SQGATIWGPAATFQELVVSHLANESKLIPISLPQNIE--GVGRVAVSVPTILSGR-LKPLVQL-LNEEEQERLKRAAKAI
Msed_MDH      214 VIGGTTWGPGTIIADLIKSIAFSENRVMSIATP--KEY-EKEIIHVSAPTVVGSS-IGPSLESLLDEKDRWHLNSAMKDF
```
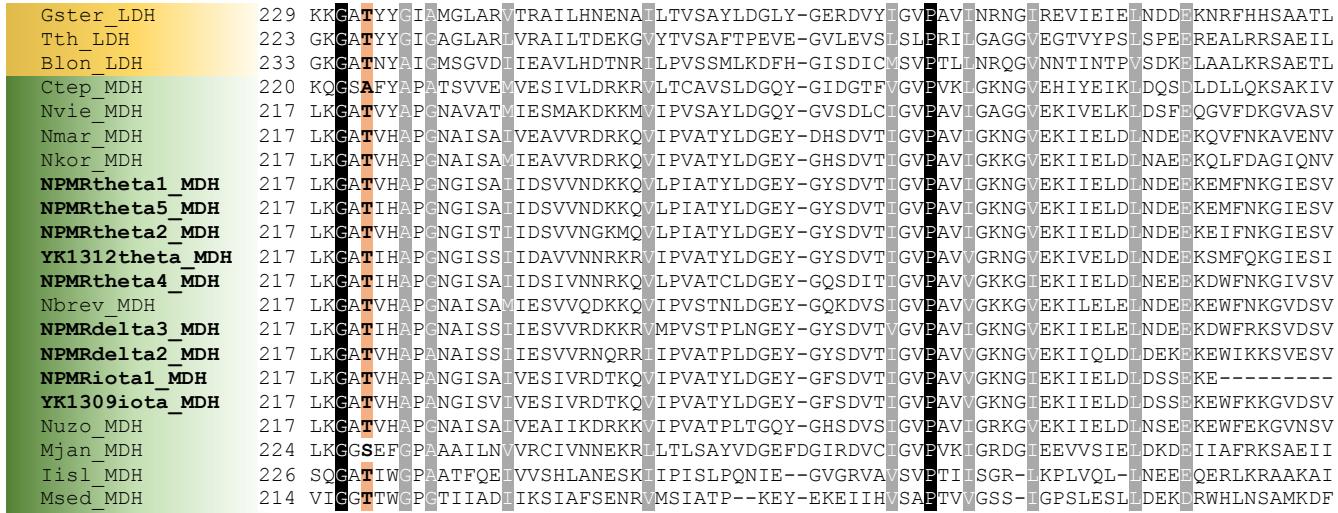
Fig. S5. Sequence alignment of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) homologs, shaded in yellow and green, respectively. Conserved residues are shaded in black and grey. The universally conserved in the LDH/MDH superfamily substrate binding Arg171 is indicated in red. Residues important for substrate discrimination and active site architecture are shaded in orange and discussed in the text. The residue determining the cofactor specificity is shaded in green. Residue numbering refers to the LDH numbering as in Roche *et al.* 2019.

Primary sequences from the following organisms were used to generate the alignment (Uniprot accession numbers in parentheses): Gster, *Geobacillus stearothermophilus* (P00344); Tth, *Thermus thermophilus* (Q5SJA1); Blon, *Bifidobacterium longum* (E8ME30); Ctep, *Chlorobaculum tepidum* (P80039); Nvie, *Nitrososphaera viennensis* (A0A060HG74); Nmar, *Nitrosopumilus maritimus* (A9A450); Nkor, *Nitrosarchaeum koreense* (F9CUM5); Nbrev, *Ca.* Nitrosopelagicus brevis (A0A0A7V4F4); Nuzo, *Ca.* Nitrosotenuis uzonensis (V6AR53); Mjan, *Methanocaldococcus jannaschii* (Q60176); Iisl, *Ignicoccus islandicus* (A0A0U3FQH7); Msed, *Metallosphaera sedula* (A4YDY0). Sequences from the marine sediment AOA MAGs reported in this study are in bold and their locus tags can be found in Table S3.