Figure S1 A

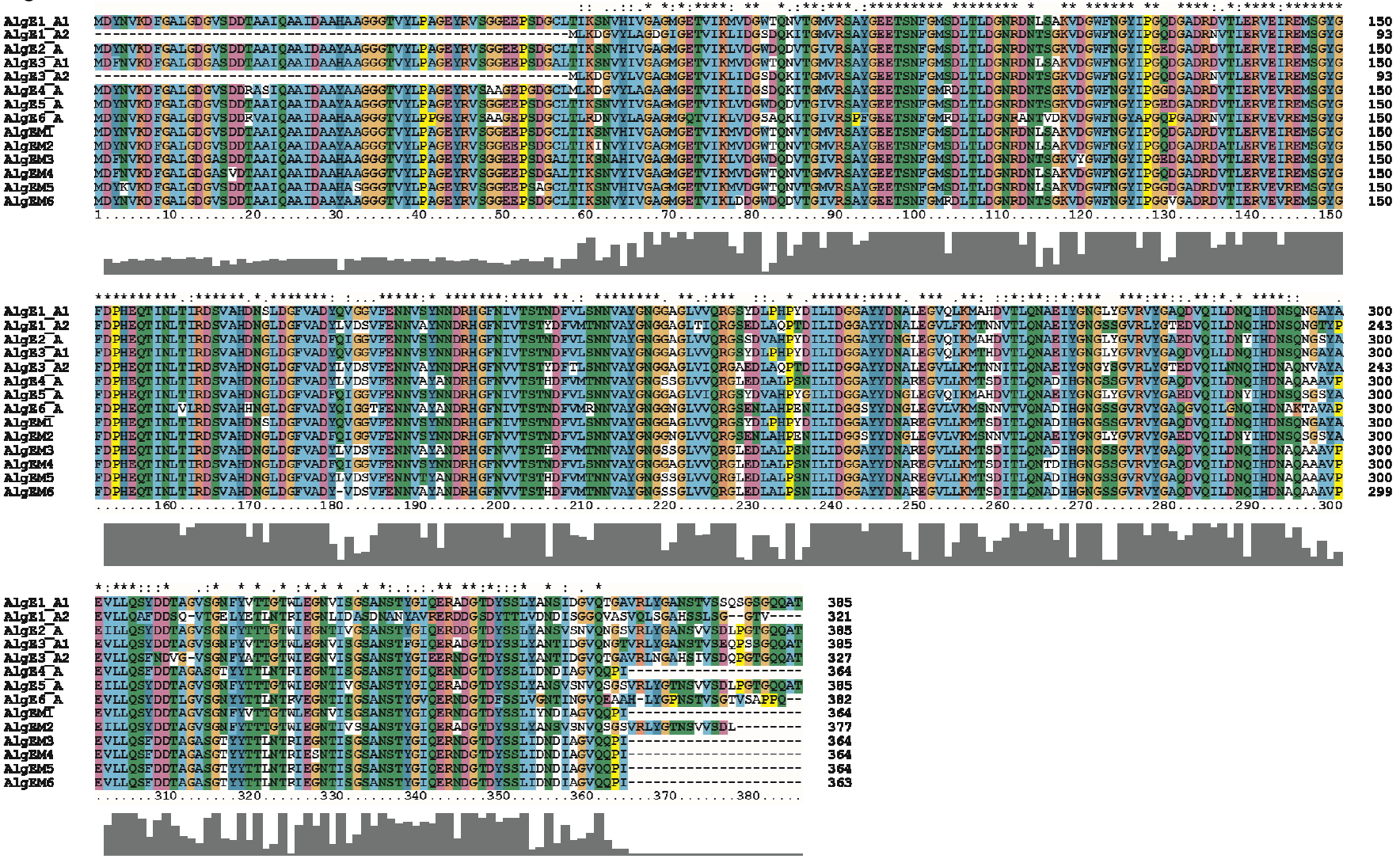
AlgE1_A1
AlgE1_A2
AlgE2_A
AlgE3_A1
AlgE3_A2
AlgE4_A
AlgE5_A
AlgE6_A
AlgEM1
AlgEM2
AlgEM3
AlgEM4
AlgEM5
AlgEM6

Figure S1 B

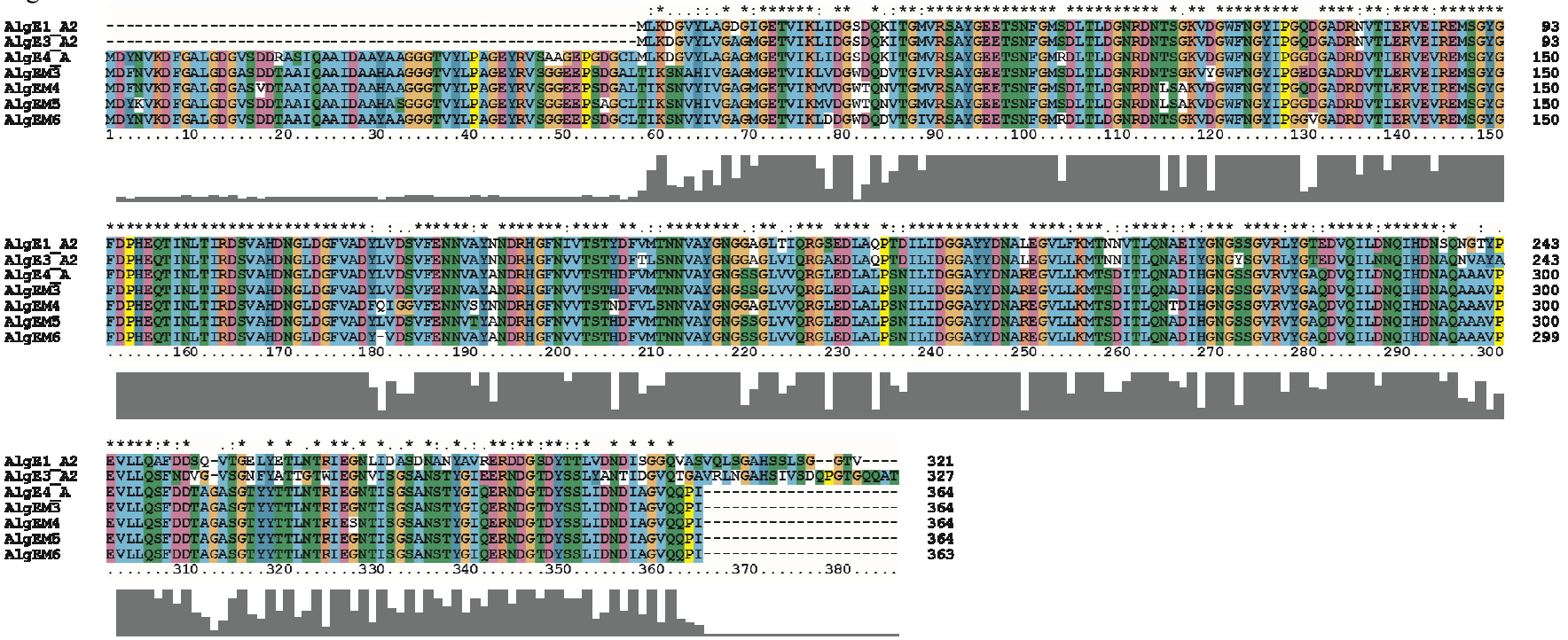

Figure S1 C

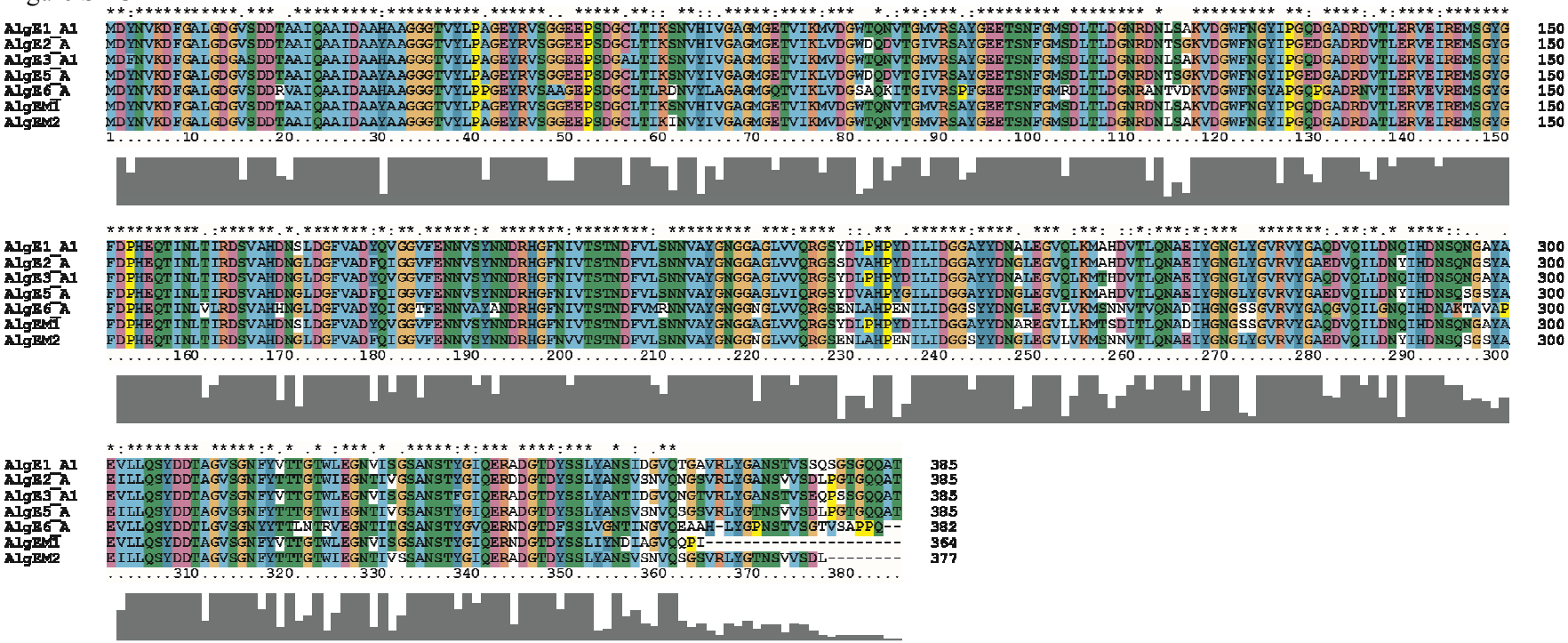

Figure S1: Multiple sequence alignment of epimerase A-module from *Azotobacter vinelandii* and the mutant A-modules for AlgEM1-6. Standard colour code for the amino acid types in ClustalX2 software is used. *indicates residues identity, : means conserved amino acids, · shows semi-conserved amino and the height of the bar indicate the degree of conservation (ClustalX2 definitions). (A) Multiple sequence alignment of epimerase from AlgE1-AlgE6 from *A. vinelandii* and the mutant A-modules for AlgEM1-6. (B) Multiple sequence alignment of MG –block forming epimerase from AlgE1,AlgE3 and AlgE4 from *A. vinelandii* and the mutant A-modules for AlgEM3-6. (C) Multiple sequence alignment of GG –block forming epimerase from AlgE1-AlgE3 and AlgE5-AlgE6 from *A. vinelandii* and the mutant A-modules for AlgEM1-2.

Table S1. Plasmids used in this study

Plasmid	Description	Reference or source
pLITMUS28	Cloning vector, Ap^r	New England BioLabs
pUC128	Cloning vector, Ap^r	[1]
pTrc99A	Expression vector, Ap^r	[2]
pBL5	pTrc99A encoding AlgE4	[3]
pBL6	pTrc99A encoding AlgE2	[3]
pBG29	pTrc99A encoding AlgE6	[4]
pHE37		[5]
pHE133		
pHH5	pTrc99A encoding AlgE5	[6]
pHE88	Expression plasmid encoding the A2-module of AlgE3	[7]
pHE58	Expression plasmid encoding the A2-module of AlgE1	[5]
pBS5	pLITMUS28 in which a 0.3 kb EcoRI-EcoRV fragment from pBG29 was inserted	This study
pBS7	pLITMUS28 in which a 1.6 kb NcoI-KpnI fragment from pHE133 was inserted.	This study
pBS8	pTrc99A encoding the A- module of AlgE5. *algE5A* was PCR-amplified as a 1.13 kb NcoI-XmaI fragment using pHH5 as template and M13/pUC rev and EU5-E5 as primers and inserted into the same sites of pTrc99A.	This study
pBS9	pTrc99A encoding the A1-module of AlgE1. *algE1A1* was PCR-amplified as a 1.085 kb NcoI-XmaI fragment using pHE37 as template and M13/pUC rev and EU12-E1.1 as primers and inserted into the same sites in pTrc99A.	This study
pBS10	pTrc99A encoding the A-module of AlgE4. A 1.13 kb NcoI-KpnI fragment from pBL5 containing *algE4A* was inserted into the same sites in pTrc99A.	This study
pBS11	pTrc99A encoding the A-module of AlgE2. A 1.13 kb NcoI-KpnI fragment from pBL6 containing *algE2A* was inserted into the same sites in pTrc99A.	This study
pBS12	pTrc99A encoding the A1-module of AlgE3. *algE3A1* was PCR-amplified as a 1.085 kb NcoI-XmaI fragment using pBS7 as template and M13/pUC rev and EU6-E3.1 as primers and inserted into the same sites in pTrc99A.	This study
pBS13	A 1.127 kb PCR-amplified NcoI-XmaI fragment containing *algE3A2* was cut with NcoI and EcoRV and the resulting 0.704 kb fragment was inserted into the same sites of pLITMUS28. pHE88 was used as template and EU10-E3.5 and EU11-E3.6 as primers.	This study
pBS14	A 1.127 kb PCR-amplified NcoI-XmaI fragment	This study

	containing *AlgE3A2* was cut with EcoRV-XmaI and the resulting 0.437 kb fragment was inserted into the same sites of pUC128. pHE88 was used as template and EU10-E3.5 and EU11-E3.6 as primers.	
pBS15	Derivative of pBS5 where an internal XmaI site was removed by site-directed mutagenesis. Primers used were EU2-E6.1 and EU3-E6.2.	This study
pBS17	Derivative of pBS13where an internal XmaI site was removed by site-directed mutagenesis. Primers used were EU8-E3.3 and EU9-E3.4.	This study
pBS18	Derivative of pBS29 in which a 0.218 kb PshAI-EcoRI fragment from pBS15 was inserted.	This study
pBS20	Derivative of pBS17 in which a 0.473 kb EcoRV-EcoRI fragment from pBS14 was inserted.	This study
pBS21	pTrc99A in which a 1.120 kb PCR-amplified NcoI-XmaI fragment containing *algE6A* was inserted. pBS18 was used as template and M13/pUC rev and EU4-E6.3 as primers.	This study
pBS22	pTrc99A encoding the A2-module of AlgE3. A 1.127 kb NcoI-XmaI fragment from pBS20 was inserted into the same sites of pTrc99A.	This study
pBS23	pTrc99A in which a 1.084 kb PCR-amplified NcoI-XmaI fragment containing *algE1A2* was inserted. pHE58 was used as template and EU14 and EU13 as primers.	This study
pBS29	pTrc99A encoding the A2-module of AlgE1. A nucleotide was inserted into pBS23 by site-directed mutagenesis. Primers used were EU23 and EU24.	This study
pBS30	pTrc99A encoding the A-module from AlgE6. A nucleotide was inserted into pBS21 by site-directed mutagenesis. Primers used were EU25 and EU26.	This study
pAT111	pTrc99A encoding AlgE4 with D119Y substitution. A synthetic[1] algE4 A-module with mutations leading to D119Y was inserted into pBL5 as a NcoI-SexAI fragment.	This study
pTB68	pTrc99A encoding AlgE4 with D119F substitution	This study
pTB77	pTrc99A encoding AlgE4 with D119A substitution	This study
pAT112	pTrc99A encoding AlgEM3 with Y119D substitution. A synthetic[1] *algEM3* A-module with mutations leading to D119Y was inserted into pBL5 as a NcoI-SexAI fragment.	This study
pAT113	pTrc99A encoding AlgEM3 with Y119R substitution. A synthetic[1] gene fragment encoding the *algEM3* A-module with mutations leading to Y119R was inserted into pBL5 as a NcoI-SexAI fragment.	This study
pAT114	pTrc99A encoding the A-module of AlgEM1 combined with the R-modules of AlgE6. A synthetic[1] gene fragment encoding the R-modules of AlgE6 was inserted as a XmaI-EcoRI fragment into the vector encoding AlgEM1 isolated from the mutant library	This study

pAT115	pTrc99A encoding the A-module of AlgEM2 combined with the R-modules of AlgE6. A synthetic[1] gene fragment encoding the R-modules of AlgE6 was inserted as a BglII-EcoRI fragment into the vector encoding AlgEM2 isolated from the mutant library	This study

1. Synthetic gene fragments were obtained from Genescript.

1. Keen, N. T., Tamaki, S., Kobayashi, D., and Trollinger, D. (1988) *Gene* **70**, 191-197
2. Amann, E., Ochs, B., and Abel, K. J. (1988) *Gene* **69**, 301-315
3. Bjerkan, T. M., Lillehov, B. E., Strand, W. I., Skjåk-Bræk, G., Valla, S., and Ertesvåg, H. (2004) *Biochem J* **381**, 813-821
4. Svanem, B. I. G., Skjåk-Bræk, G., Ertesvåg, H., and Valla, S. (1999) *J. Bacteriol.* **181**, 68-77
5. Ertesvåg, H., and Valla, S. (1999) *J. Bacteriol.* **181**, 3033-3038
6. Ramstad, M. V., Ellingsen, T. E., Josefsen, K. D., Høidal, H. K., Valla, S., Skjåk-Bræk, G., and Levine, D. W. (1999) *Enzyme Microb. Technol.* **24**, 636-646
7. Ertesvåg, H., Høidal, H. K., Schjerven, H., Svanem, B. I., and Valla, S. (1999) *Metab Eng* **1**, 262-269

Table S2. Primers used for the staggered extension process, error prone PCR and for construction of StEP templates

Primer name	Restriction site in primer sequence	Sequence (5'-3')[a, b]
M13/pUC rev		**AGCGGATAACAATTTCACACAGGA**
EU12-E1.1	XmaI	CGCCCCGG**G**CTGCACACC
EU14-E1.3	NcoI	CGCAAGACCATG**G**TCAATGCC
EU13-E1.2	XmaI	AGCTGC**CCCG**GGGCGACC
EU23		CCAGGTCG**G**CCCCGGGGATCC
EU24		GGATCCCCGGGG**C**CGACCTGG
EU6-E3.1	XmaI	GTACCGTCCCG**GG**CTGCAC
EU10-E3.5	NcoI	CAGACCATG**G**TCAATGCCAAGG
EU11-E3.6	XmaI	CTGGCCGGT**C**CCGGGCTG
EU8-E3.3		CACCGGGGA**T**CC**T**GGCGACGGCT
EU9-E3.4		AGCCGTCGCC**A**GGA**T**CCCCGGTG
EU5-E5	XmaI	TGGCCGGT**C**CCGGGCAGGTC
EU2-E6.1		CTGAACAC**A**CGGGTCGAGGGCAAC
EU3-E6.2		GTTGCCCTCGACCCG**T**GTGTTCAG
EU4-E6.3	XmaI	ACAGTGCC**C**G**G**GACCGTCG
EU25		CGAATTCGACGGT**A**CCCGGGGATC
EU26		GATCCCCGGG**T**ACCGTCGAATTCG
EU20		GAGCTGGTCGTCCGTGTCGC
Britt 35		AGCTTATCATCGACTGCACGGTG
Britt 28		CACACTACCATCGGCGCTACG

[a] Restriction sites are underlined

[b] Nucleotides shown in bold are not part of *A. vinelandii* wild type sequence

Table S3. NMR analysis of polyM epimerised with AlgE6 and mutant epimerases AlgEM1-6. Epimerisation was performed with polyM (10 mg) and enzyme (0.2 mg) in MOPS, pH 6.9 with 100 mM NaCl and 1.5 mM $CaCl_2$

Enzyme	F_G	F_M	F_{GG}	F_{MG}/F_{GM}	F_{MM}	F_{MGM}
AlgE6	0.77	0.23	0.69	0.074	0.16	0.048
AlgEM1	0.85	0.15	0.78	0.061	0.094	0.030
AlgEM2	0.83	0.17	0.74	0.088	0.085	0.027
AlgEM3	0.54	0.46	0.22	0.33	0.13	0.25
AlgEM4	0.67	0.33	0.44	0.23	0.10	0.15
AlgEM5	0.59	0.41	0.31	0.28	0.13	0.21
AlgEM6	0.40	0.60	0.039	0.36	0.23	0.33

Table S4. Sequence properties of the A-modules from epimerase mutants AlgEM1-6

Enzyme	% identity to wild type A-modules[1)]								New amino acid residues introduced by mutagenesis[2)]
	E1A1	E1A2	E2	E3A1	E3A2	E4	E5	E6	
AlgEM1	93	74	87	92	80	84	86	76	D/A/G354Y
AlgEM2	89	73	92	88	78	76	93	77	D/S61I, V136A, G/A332S
AlgEM3	81	75	81	82	78	92	80	77	V63A, D119Y
AlgEM4	85	74	82	86	78	88	82	77	D17V, A265T, G327S
AlgEM5	83	74	79	82	76	92	79	77	N4K, A32S, D54A, S/A190T
AlgEM6	81	76	81	80	78	95	81	79	V/I78D, P/D130V

1) Given as % amino acid identity over 376 N-terminal residues constituting the A-module.
2) Residues before the position number indicates amino acids present in the wild type A-modules.

Table S5. NMR analysis of polyM epimerised with AlgE4 and AlgEM3 and derivatives of these enzymes with substitutions in residue 119. Epimerisation was performed with polyM (10 mg) and enzyme (0.2 mg) in MOPS, pH 6.9 with 100 mM NaCl and 1.5 mM $CaCl_2$.

Epimerase	F_G*	F_{GG}*	F_{MGM}*
AlgE4 (D119)	0.47	0	0.47
AlgE4 D119Y	0.50/0.50	0.083/0.085	0.41/0.40
AlgE4 D119F	0.50	0.089	0.41
AlgE4 D119A	0.48	0.044	0.44
AlgEM3 (Y119)	0.64/0.64	0.39/0.38	0.17/0.18
AlgEM3 Y119D	0.57/0.58	0.27/0.27	0.23/0.23
AlgEM3 Y119R	0.36/0.37	0.024/0.032	0.35/0.33

*Were two values are shown, the results are obtained from two independent epimerization experiments.