# Supplementary information

**Table S1** Compositions for the different PCR reactions

| Components | Template [ng/µl] | Primer [µM] | Megaprimer[a] [ng/µl] | dNTP [mM] | $Mn_2Cl$ [mM] | Polymerase [U] |
|---|---|---|---|---|---|---|
| epPCR | 35 | 5 | | 10 | 0.2 | 2.5[b] |
| MEGAWHOP | 35 | | 300 | 10 | - | 2.5[c] |
| PLICing insert | 35 | 20 | | 10 | 0.2 | 2.5[b] |
| PLICing vector | 35 | 20 | | 10 | - | 2.5[c] |
| Two step PCR | 35 | 20 | | 10 | - | 2.5[c] |

[a]Amplified CelA2 insert by epPCR; [b]Taq Polymerase; [c]PfuS Polymerase

**Table S2** Error prone PCR (epPCR) program

| Step | Temperature [°C] | Time [sec] | Cycle [-] |
|---|---|---|---|
| Initial denaturation | 94 | 120 | 1x |
| Denaturation | 94 | 30 | |
| Annealing | 66.7 | 30 | 25x |
| Elongation | 72 | 120 | |
| Final elongation | 72 | 600 | 1x |

**Table S3** Primers for epPCR

| Primer name | Sequence 5'-3' |
|---|---|
| epPCR_Fw | TAC CAT GGG TAG CAG CCA TCA CCA CC |
| epPCR_Rev | GTTATTGCTCAGCGGTGGCAGCAGC |

**Table S4** MEGAWHOP PCR program

| Step | Temperature [°C] | Time [sec] | Cycle [-] |
|---|---|---|---|
| Exonuclease activity | 72 | 300 | 1x |
| Initial denaturation | 94 | 120 | 1x |
| Denaturation | 94 | 30 | |
| Annealing | 60 | 30 | 25x |
| Elongation | 72 | 210 | |
| Final elongation | 72 | 600 | 1x |

**Construction of CelA2 error-prone library with PLICing**

The modified nucleotides used for PLICing are listed in Table S7. PCR program for amplification of insert and vector is described in Table S5 and Table S6.

**Table S5** PLICing program for insert

| Step | Temperature [°C] | Time [sec] | Cycle [-] |
|---|---|---|---|
| Initial denaturation | 94 | 120 | 1x |
| Denaturation | 94 | 30 | |
| Annealing | 60 | 30 | 25x |
| Elongation | 68 | 120 | |
| Final elongation | 68 | 600 | 1x |

**Table S6** PLICing program for vector

| Step | Temperature [°C] | Time [sec] | Cycle [-] |
|---|---|---|---|
| Initial denaturation | 94 | 120 | 1x |
| Denaturation | 94 | 30 | |
| Annealing | 60 | 30 | 25x |
| Elongation | 68 | 270 | |
| Final elongation | 68 | 600 | 1x |

**Table S7** List of primers used for PLICing and cloning of cellulase (Asterisks mark the locations of phosphorothioate bonds)

| Primer name | Sequence 5'-3' |
|---|---|
| CelA2 Rev | **T*T*C*G*G*C*C*A*C*G*G*T*** ATTCAGGCTATAAACAT |
| CelA2 Fw | **A*G*C*A*G*C*G*G*T*G*A*A*** AATCTGTATTTTCAGG |
| Vector Rev | **T*T*C*A*C*C*G*C*T*G*C*T*** ATGATGATGGTGG |
| Vector Fw | **A*C*C*G*T*G*G*C*C*G*A*A*** TAATAAGAATTCGAGC |

**Table S8** Two step PCR program for site-saturation and site-directed mutagenesis

| Step 1 | Temperature [°C] | Time [sec] | Cycle [-] |
|---|---|---|---|
| Initial denaturation | 98 | 30 | 1x |
| Denaturation | 98 | 30 | |
| Annealing | 55 | 60 | 3x |
| Elongation | 72 | 300 | |

| Step 2 | Temperature [°C] | Time [sec] | Cycle [-] |
|---|---|---|---|
| Initial denaturation | 98 | 30 | 1x |
| Denaturation | 98 | 30 | |
| Annealing | 55 | 60 | 15x |
| Elongation | 72 | 300 | |
| Final elongation | 72 | 600 | 1x |

**Table S9** Primers for site-saturation and site-directed mutagenesis library (Underlined indicates randomized codons)

| **Primer name** | **Sequence 5'-3'** |
|---|---|
| SSM_21_Fw | GTATGAAGATGATACC**NNK**GCACGCTTTTGTTTTAATCTGG |
| SSM_21_Rev | CCAGATTAAAACAAAAGCGTGC**MNN**GGTATCATCTTCATAC |
| SSM_184_Fw | GAATATGTTGGTGAA**NNK**TTTGCACGTGAAGCAGGTC |
| SSM_184_Rev | GACCTGCTTCACGTGCAAA**MNN**TTCACCAACATATTC |
| SSM_288_Fw | CATAAAGTTACCGCAAAAGAT**NNK**GCACCGATGACCATTCTG |
| SSM_288_Rev | CAGAATGGTCATCGGTGC**MNN**ATCTTTTGCGGTAACTTTATG |
| SSM_299_Fw | CTGCCGCATGAAGAT**NNK**AGTCCGCTGTATCTGAGTCC |
| SSM_299_Rev | GGACTCAGATACAGCGGACT**MNN**ATCTTCATGCGGCAG |
| SSM_330_Fw | GTATAAAGATATT**NNK**GAAGAATTTGCCAATCAGTGTCTGGATGC |
| SSM_330_Rev | GCATCCAGACACTGATTGGCAAATTCTTC**MNN**AATATCTTTATAC |
| SSM_442_Fw | CCAAAAATGCCTTTATT**NNK**AATGCCCAGAGC |
| SSM_442_Rev | GCTCTGGGCATT**MNN**AATAAAGGCATTTTTGG |
| SDM_580_Fw | GCTATGCCACCAAT**CAG**ATTTGCATTTATTGGAATAGTCCG |
| SDM_580_Rev | CGGACTATTCCAATAAATGCAAAT**CTG**ATTGGTGGCATAGC |

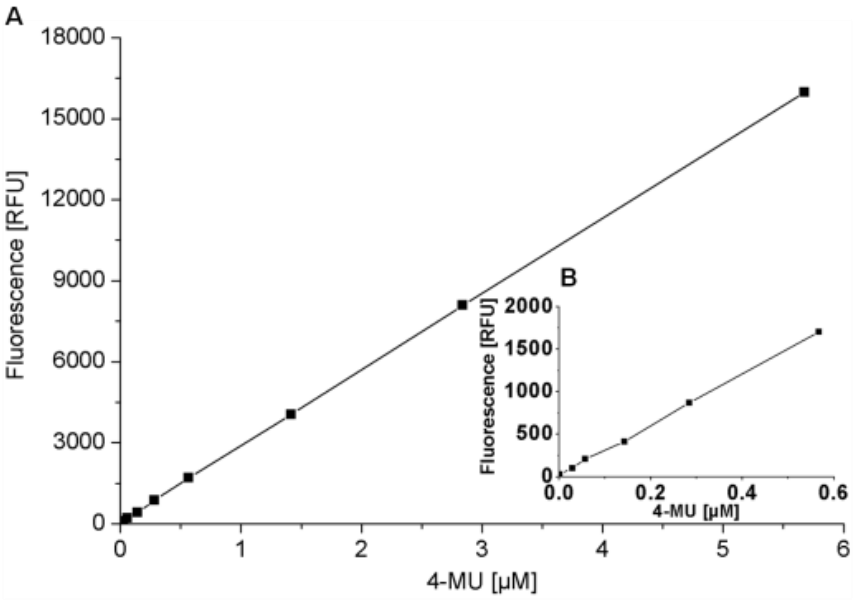


**Fig. S1** **A)** Correlation of fluorescence intensity (Y-axis) to 4-Methylumbelliferone concentration (0.02-6 µM 4-MU; $R^2$ value= 0.9999) in potassium phosphate buffer (0.2 M, pH 7.2). **B)** Enlargement of **A)** to indicate the linearity of the 4-MUC fluorescence detection system at low 4-Methylumbelliferone concentrations (0.02-0.06 µM 4-MU; $R^2$ value= 0.9994). The reported values are the average of three measurements and deviations are calculated from the corresponding mean values. The error values do not exceed the marker symbols.

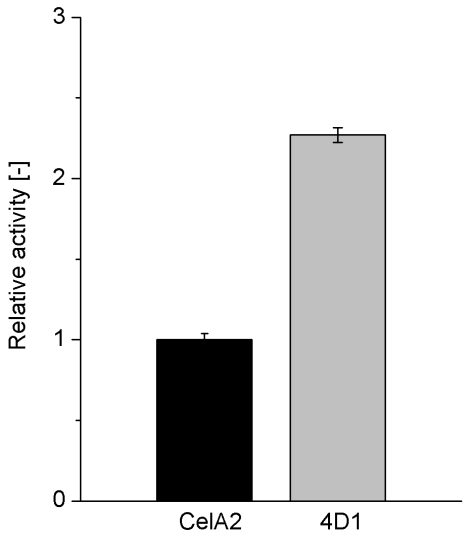


**Fig. S2** Relative cellulolytic activities in potassium phosphate buffer (0.2 M, pH 7.2) containing 5% (v/v) ChCl:Gly of 4D1 and CelA2 wild type. Relative activity is calculated as the ratio between product formation rate (µM/sec) of variant 4D1 and product formation rate (µM/sec) of CelA2 wild type. The reported values are the average of three measurements and average deviations from the mean values are shown.

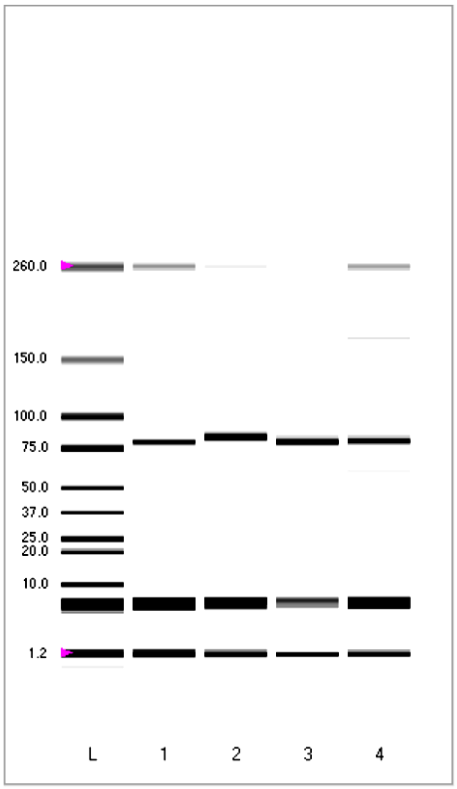


**Fig. S3** BioRad chip analysis of the purified CelA2 wild type, 4D1, M1, and M2 variant. The samples were produced as described in the manufacturer`s protocol and analyzed in duplicate. **(L)** Ladder; **(1)** CelA2 wild type, dilution 1:4; **(2)** 4D1, dilution 1:10; **(3)** M1, dilution 1:4; **(4)** M2, dilution 1:4.

**Table S10** Specific activity of CelA2 wild type, 4D1, M1, and M2 in the presence of ChCl:Gly, seawater and 3-fold concentrated seawater

| | Specific activity [U/mg] | | | | |
|---|---|---|---|---|---|
| **Variant** | **Buffer** | **5% (v/v) ChCl:Gly** | **30% (v/v) ChCl:Gly** | **Seawater** | **3x Seawater** |
| **CelA2** | 15.1 ± 0.4 | 3.7 ± 0.2 | 0.4 ± 0.1 | 5.5 ± 0.8 | 6.9 ± 0.1 |
| **4D1** | 10.8 ± 0.5 | 8.2 ± 0.2 | 3.0 ± 0.1 | 9.3 ± 0.3 | 11.3 ± 0.6 |
| **M1** | 10.2 ± 0.3 | 7.6 ± 0.1 | 3.0 ± 0.2 | 11.9 ± 1.3 | 15.0 ± 0.1 |
| **M2** | 131.2 ± 2.4 | 36.5 ± 1.2 | 3.4 ± 0.4 | 50.8 ± 5.7 | 25.5 ± 2.1 |

One Unit was defined as the amount of cellulase that catalyzes the conversion of 1 µmol of 4-MUC per minute. All values reported the average of three measurements; average deviations from the mean values are shown.

**Table S11** Residual activity of CelA2 wild type, 4D1, M1, and M2 in the presence of ChCl:Gly, seawater and 3-fold concentrated seawater

| | Residual activity [%] | | | | |
|---|---|---|---|---|---|
| **Variant** | **Buffer** | **5% (v/v) ChCl:Gly** | **30% (v/v) ChCl:Gly** | **Seawater** | **3x Seawater** |
| **CelA2** | 100 ± 2.0 | 24.3 ± 3.9 | 2.6 ± 9.0 | 36.7 ± 11.3 | 45.6 ± 1.6 |
| **4D1** | 100 ± 3.9 | 75.8 ± 1.6 | 27.9 ± 1.3 | 85.6 ± 2.7 | 104.1 ± 3.9 |
| **M1** | 100 ± 2,1 | 74.8 ± 0.7 | 29.3 ± 7.1 | 116.2 ± 8.3 | 147.1 ± 4.6 |
| **M2** | 100 ± 1,4 | 27.8 ± 2.6 | 2.6 ± 9.7 | 38.7 ± 8.5 | 19.4 ± 6.2 |

One Unit was defined as the amount of cellulase that catalyzes the conversion of 1 µmol of 4-MUC per minute. All values reported the average of three measurements; average deviations from the mean values are shown. Residual activity (%): activity of variant with addition of co-solvent divided by the activity of the same variant in absence of co-solvent (0.2 M potassium phosphate buffer, pH 7.2).

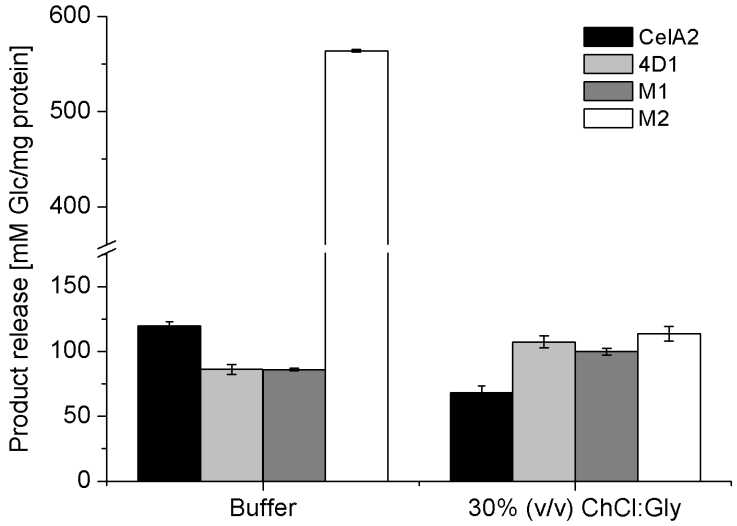


**Fig. S4 Measured activity of CelA2 and variants using the complex substrate carboxymethyl cellulose (CMC) A)** CMC conversion with the purified CelA2 wild type and the variants 4D1, M1 and M2. **B)** Enlargement of **A)** to indicate the performance of the variants in the lower release range. The reaction was Incubated for 20 min at 37 °C with 1% (w/w) CMC in potassium phosphate buffer (0.2 M, pH 7.2) or 30% (v/v) ChCl:Gly. Product release (glucose) was defined as mM glucose per mg protein after 20 min reaction. Glucose concentration was measured using the DNSA assay.