Synthetic Fungal Multifunctional Cellulases for Enhanced Biomass Conversion

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Substrate	Glucan (%)	Xylan (%)	Galactan (%)	Arabinan (%)	Lignin (%)	Ash (%)	Acetyl (%)	Total Mass Closure (%)
DDR CS	43.6	33.1	1.4	2.5	12.6	0.6	0.3	95.1

Table S1: DDR biomass composition

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† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

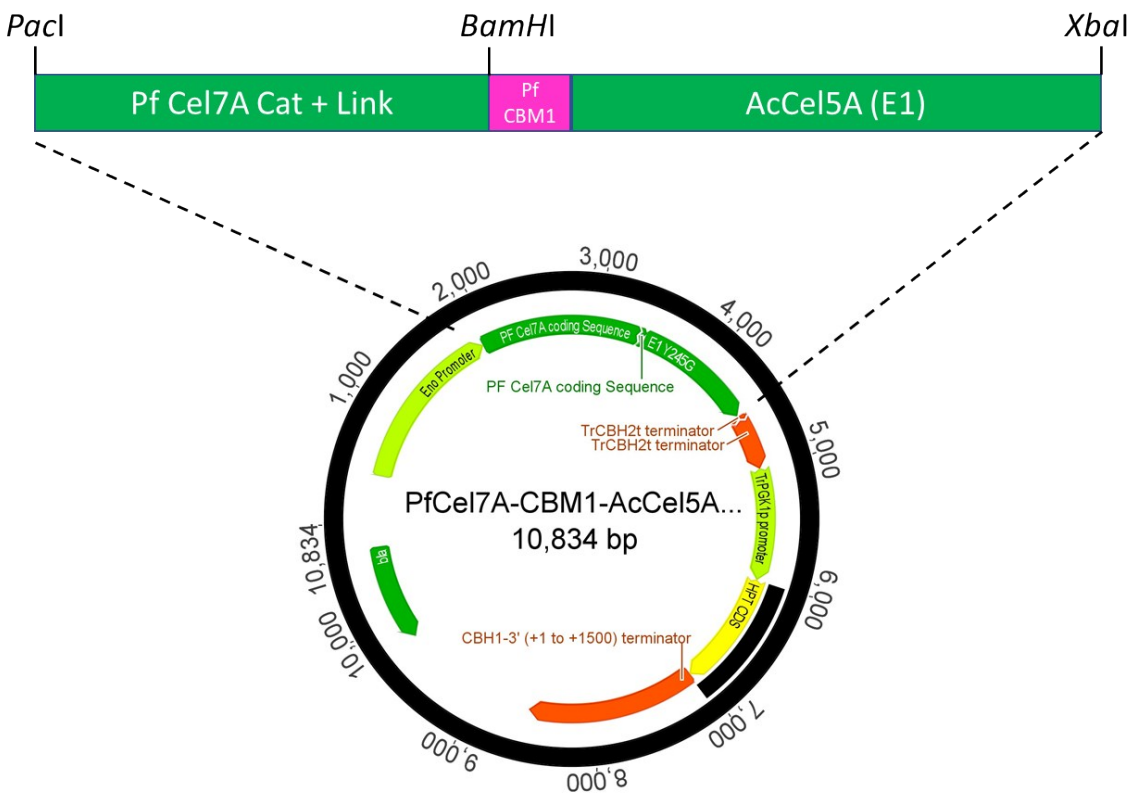


Figure S1. Plasmid map of PfCel7A-CBM1-AcCel5A construct in the vector pTreno-PF. The region from *Pac*I to *Xba*I containing the multifunctional cassette is shown above the vector map.

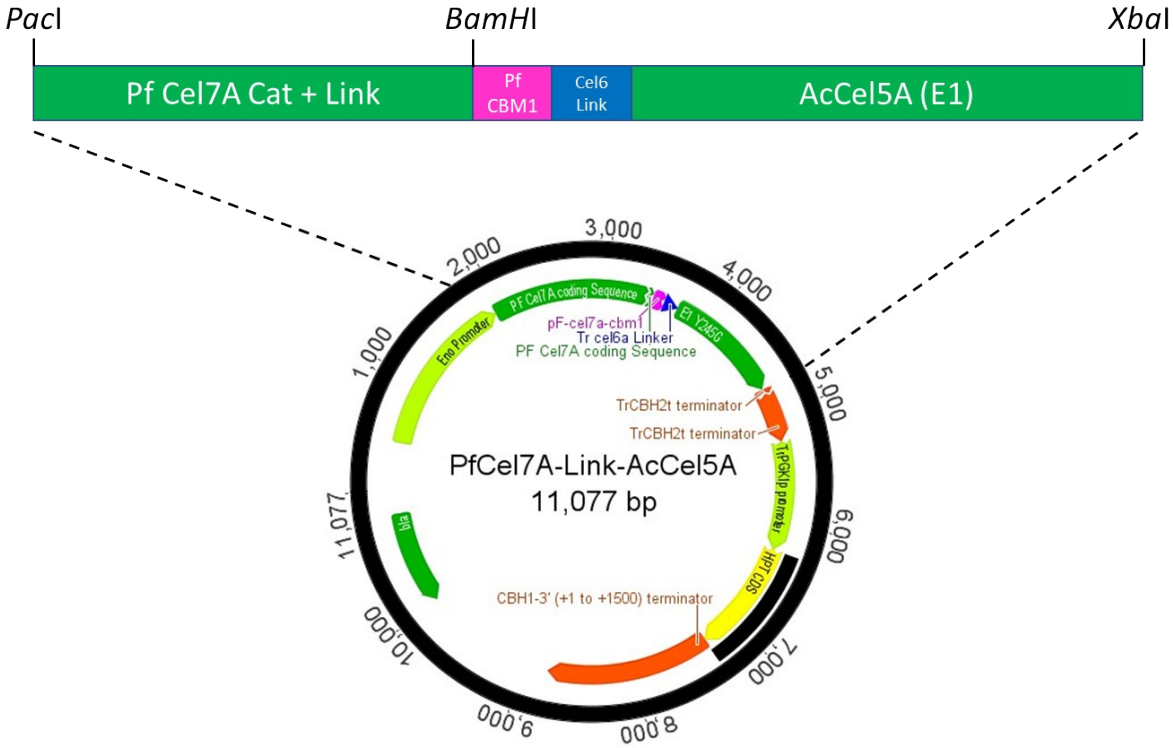


Figure S2. Plasmid map of PfCel7A-Link-AcCel5A construct in the vector pTreno-PF. The region from *Pac*I to *Xba*I containing the multifunctional cassette is shown above the vector map.

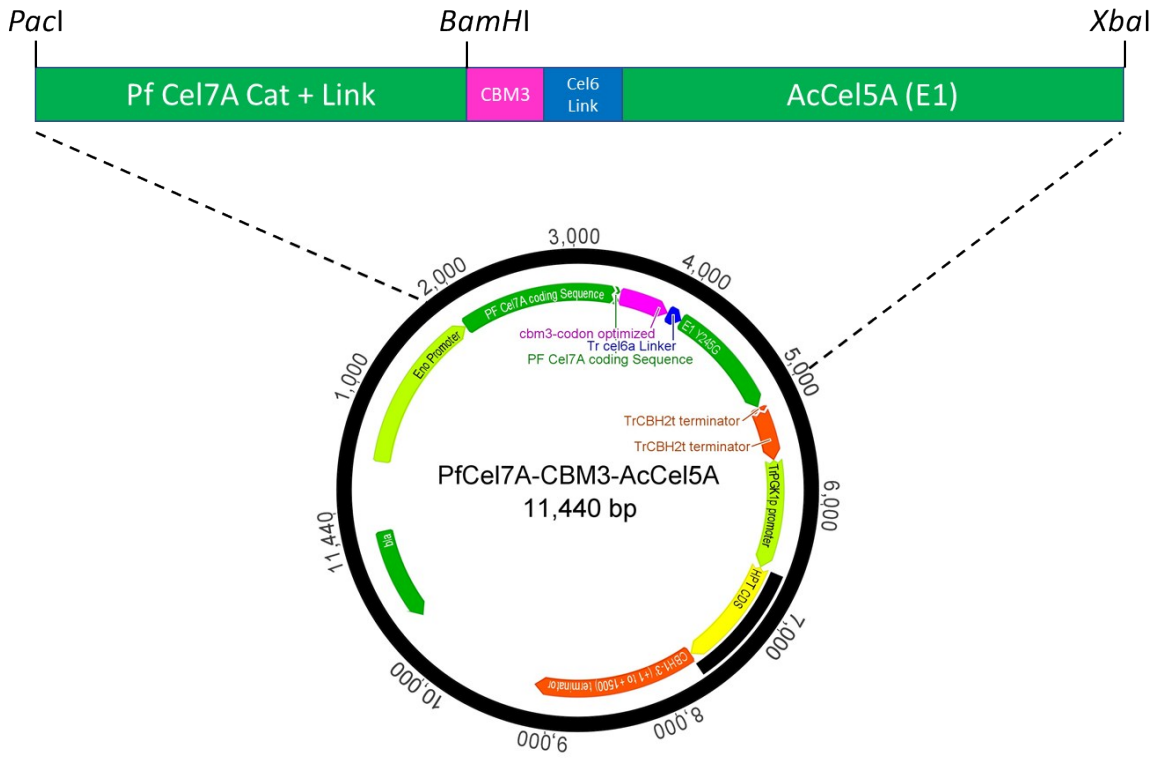


Figure S3. Plasmid map of PfCel7A-CBM3b-AcCel5A construct in the vector pTreno-PF. The region from *Pac*I to *Xba*I containing the multifunctional cassette is shown above the vector map.

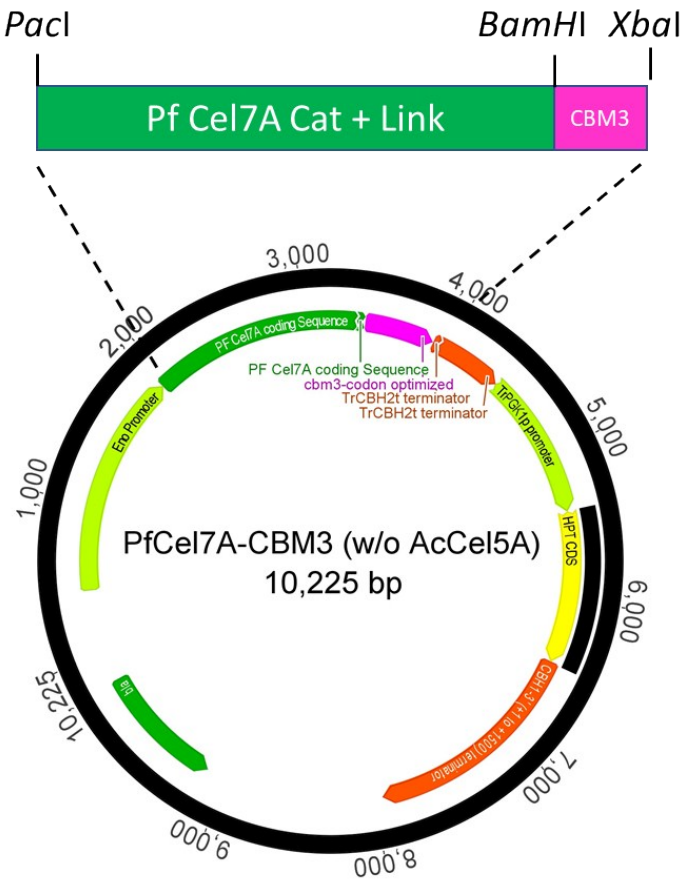


Figure S4. Plasmid map of PfCel7A-CBM3b construct in the vector pTreno-PF. The region from *Pac*I to *Xba*I containing the multifunctional cassette is shown above the vector map.

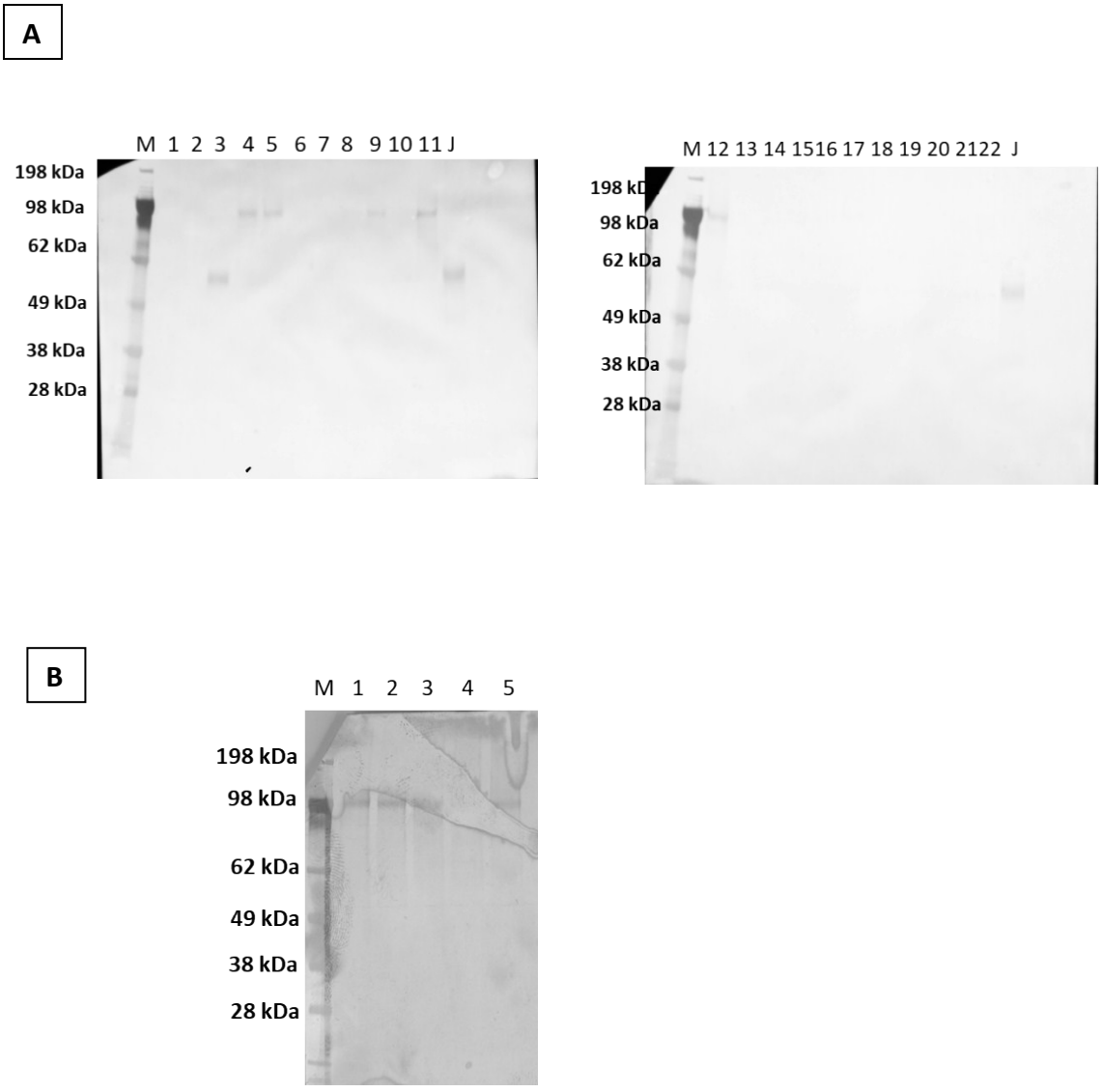


Figure S5. Transformant screening for PfCel7A$_{cat}$-PfCel7A$_{linker}$-PfCel7A$_{CBM1}$-TrCel6A$_{linker}$-AcCel5A$_{cat}$. An AST1116 strain was transformed with this construct and plated on potato dextrose agar containing hygromycin and triton X-100. Cell-free broth from 22 colonies were screened by western blotting using anti-Cel7A antibody. Transformant #13 showed the correct size protein product (~ 97.15 kDa) B. Colony #11 was subjected to clonal isolation procedure followed by screening 5 independent isolates for expression of this construct. Clonal isolate #2 was selected for large scale fermentation. M, molecular weight marker; 1-24, transformant colonies; J, JLT102A (Cel7A overexpressing strain).

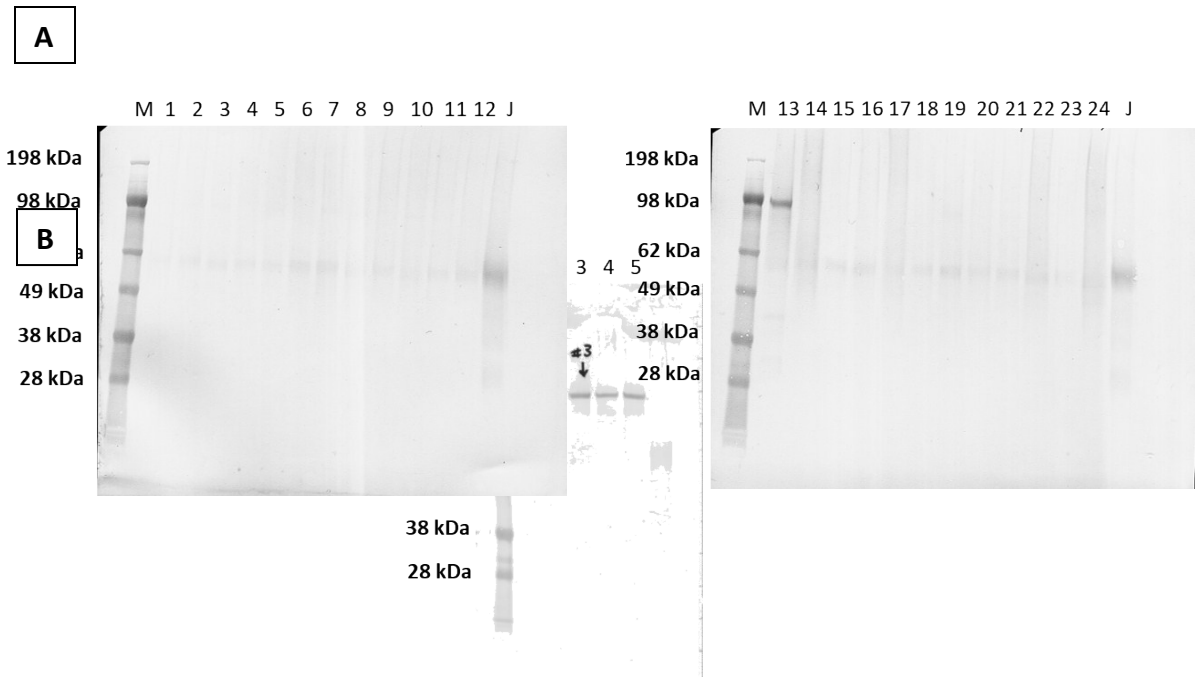
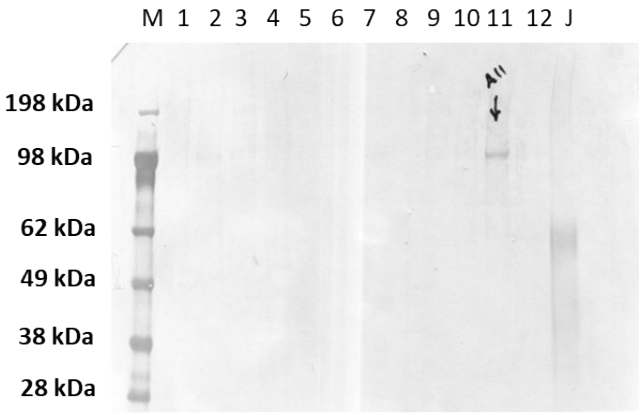


Figure S6. Transformant screening for PfCel7A$_{cat}$-PfCel7A$_{linker}$-TrCel6A$_{linker}$-AcCel5A$_{cat}$. A. QM9414 strain was transformed with the C2 construct and plated on potato dextrose agar containing hygromycin and triton X-100. Cell-free broth from 24 colonies were screened by western blotting using anti-Cel7A antibody. Transformant #13 showed the correct size protein product (~ 93.44 kDa) B. Colony #13 was subjected to clonal isolation procedure followed by screening 5 independent isolates for expression of this construct. Clonal isolate #3 was selected for large scale fermentation. M, molecular weight marker; 1-24, transformant colonies; J, JLT102A (Cel7A overexpressing strain).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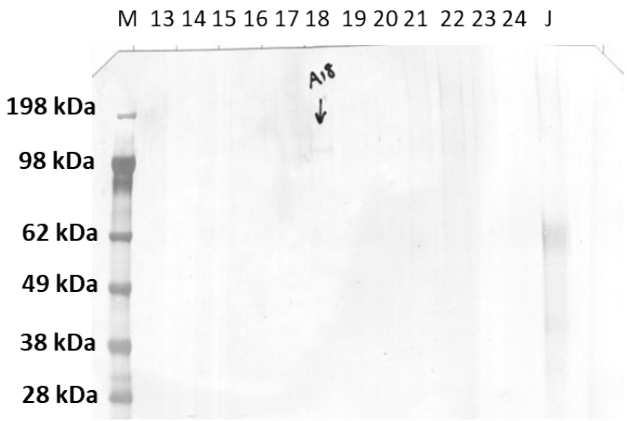
98 kDa
62 kDa
49 kDa
38 kDa
28 kDa
A11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A18

B

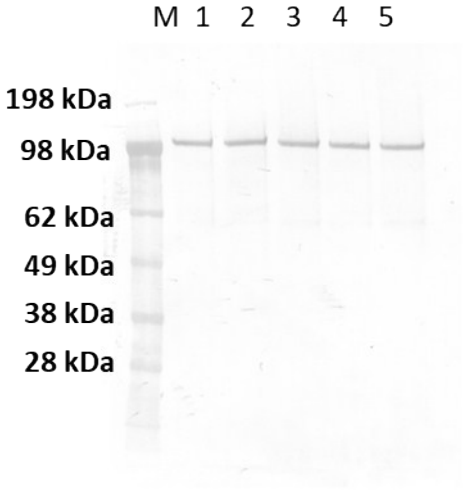
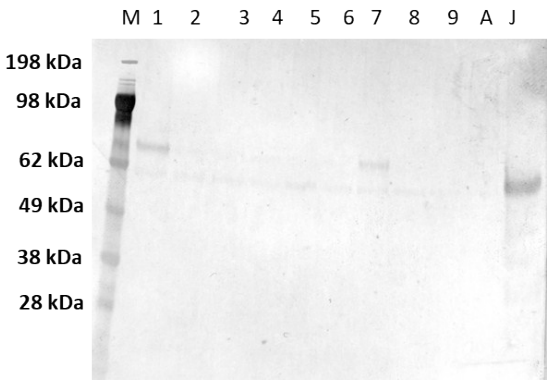
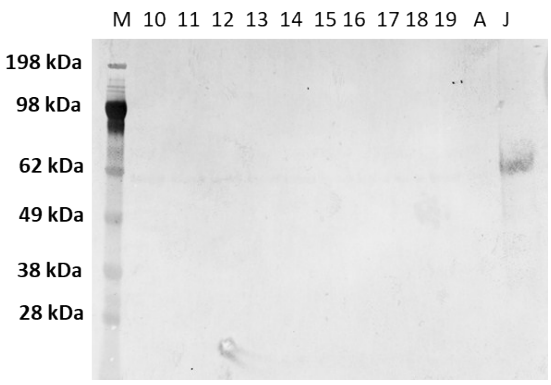


Figure S7. Transformant screening for PfCel7A$_{cat}$-PfCel7A$_{linker}$-CBM3b-TrCel6A$_{linker}$-AcCel5A$_{cat}$. A. AST1116 strain was transformed with this construct and plated on potato dextrose agar containing hygromycin and triton X-100. Cell-free broth from 24 colonies were screened by western blotting using anti-Cel7A antibody. Transformants #11 and #18 showed the correct size protein product (~ 110.65 kDa) B. Colony #11 was subjected to clonal isolation procedure followed by screening 5 independent isolates for expression of this construct. Clonal isolate #5 was selected for large scale fermentation. M, molecular weight marker; 1-24, transformant colonies; J, JLT102A (Cel7A overexpressing strain).

A





B

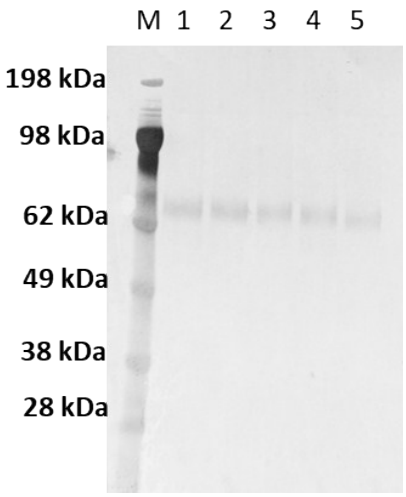


Figure S8. Transformant screening for PfCel7A$_{cat}$-PfCel7A$_{linker}$-CBM3b. A. AST1116 strain was transformed with the C3 w/o E1 construct and plated on potato dextrose agar containing hygromycin and triton X-100. Cell-free broth from 19 colonies were screened by western blotting using anti-Cel7A antibody. Transformants #1 and # 7 showed the correct size protein product (~ 66.08 kDa) B. Colony #1 was subjected to clonal isolation procedure followed by screening 5 independent isolates for expression of this construct. Clonal isolate #2 was selected for large scale fermentation. M, molecular weight marker; 1-19, transformant colonies; J, JLT102A (Cel7A overexpressing strain); A, AST1116 (Cel7A deleted strain of QM6A).

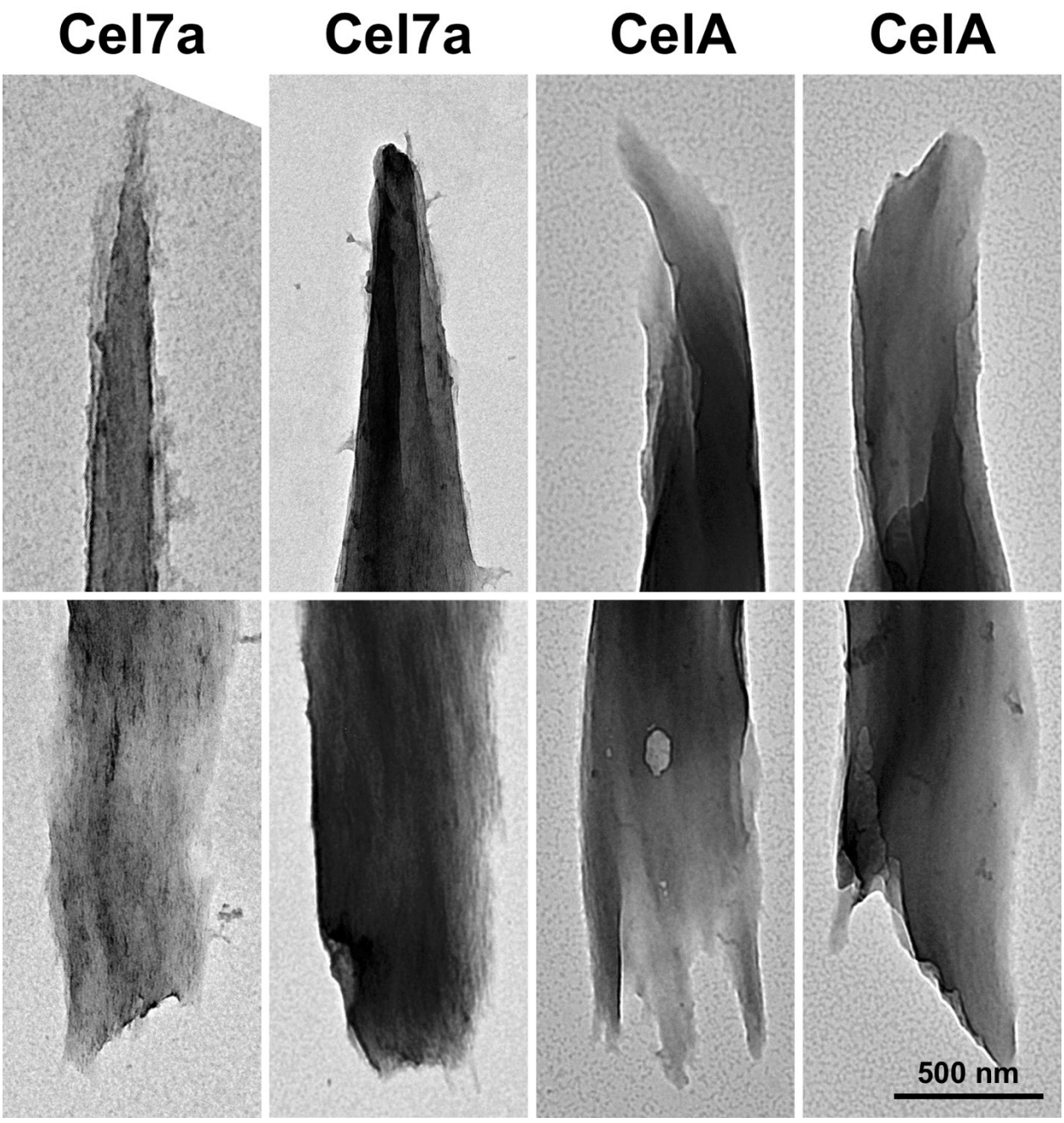


Figure S9. TEM micrographs of Avicel particles digested with Cel7a (left panels) or CelA (right panels). The Cel7a digested particles display a tapered end morphology on one end of the particle and a blunt or angled broad end on the opposite end. The CelA digested particles display irregular, scalloped ends and cavities along the particle surface.