

Supplementary figure 1: Temperature stability of FaGH16A, FaGH17A and FbGH30 measured by dynamic light scattering. The melting curves showed the mesophilic character of all three enzymes. A: FaGH17A started to denature at ~40°C. B: FbGH30 started to denature at ~42°C. C: FaGH16A started to denature at ~55°C. Each measurement comprised ten times 5 s acquisitions. The temperature was increased at a rate of 0.25°C per min, from 25 to 80°C.



Supplementary figure 2: Fluorophore assisted carbohydrate electrophoresis (FACE) of hydrolysis products revealed the mode of action of FaGH16A, FaGH17A and FbGH30. Hydrolysis of laminarin was monitored over 60 min (T0-T60). Untreated Glucose (Glc), Laminaribiose (L2), Laminaritriose (L3) and Laminaritetraose (L4) were added as a reference. **A:** On a 4-20% acrylamide gel FaGH16A and FaGH17A exhibited an endo-acting activity by creating a ladder type profile. **B:** On a 36% acrylamide gel FbGH30 produced only glucose, even during initial phases of the kinetic, which is typical for an exo-acting enzyme 0.1% (w/v) laminarin was hydrolyzed by 100 nM (~5 μg ml⁻¹) purified enzyme at 37°C for 30 min in PBS buffer at pH 7.5. The reaction was stopped by heating for 10 min at 100°C. Derivatization and electrophoresis were performed as previously described (Jackson, Biochem J 270:705–713, 1990, DOI: 10.1042/bj2700705)



Supplementary figure 3: Enzyme specificity tested with defined oligosaccharides substrates using thin layer chromatography. FaGH16A released laminaribiose and glucose from laminarin, laminaritriose and -tetraose. This enzyme also hydrolyzed mixed linked cellotetraose and mixed linked β -glucan from barley suggesting it can hydrolyze β -1,3-linkages adjacent to β -1,4-linkages. FaGH17A showed a similar product profile of laminaribiose and glucose released from laminarin, laminaritriose and laminaritetraose. However, this enzyme did not cleave the mixed linked cellotetraose. FbGH30 released glucose from laminarin and hydrolyzed gentiobiose, a β -1,6 linked glucose disaccharide into glucose. In these experiments 0.1% (w/v) of the substrates were hydrolyzed by 100 nM (~5 µg ml⁻¹) purified enzyme at 37°C for 30 min in PBS buffer at pH 7.5.



Supplementary figure 4: Yield of laminarin enzyme hydrolysis (~51%) compared to total acid hydrolysis (100%). 0.1% (w/v) of laminarin was hydrolyzed either by 100 nM (~5 μ g ml⁻¹) purified enzyme at 37°C for 30 min in MOPS buffer at pH 7.5. Acid hydrolysis was conducted with 1 M HCl for 24 h at 100°C.



Supplementary figure 5: PAHBAH calibration curves of laminarin hydrolysis. Comparison of acid hydrolysis (A) and enzyme hydrolysis using FaGH17A and FbGH30 (B).