

APPENDIX S10. SSR validation using PCR. We designed primers based on the de novo assemblies of the three *Brachypodium sylvaticum* genotypes and *B. distachyon* genome sequence v1.2. From our sequenced transcriptome assemblies, we identified a putative SSR to be a CTG repeat in contigs Brasy-CorV1T113346, Brasy-EspV1T98857, Brasy-GreV1T116317, and the Bradi5g11660.1 gene locus. We then tested these primers and demonstrated the potential of SSR sequences for use as genetic markers in various *B. sylvaticum* genotypes. Forward: GGAGAAACCAGCACATGAAGTCG and Reverse: GCCGTGCGGAAGTGAAAGGGT primers were used for PCR validation.

50 bp Ladder
Neg Control
Spain
Greece 1
Greece 2
Greece 3
England 1
England 2
England 3
Oregon 1
Oregon 2
Oregon 3
Oregon 4
Oregon 5
Oregon 6
Oregon 7
Oregon 8
Oregon 9
Norway 1
Norway 2
Norway 3
Norway 4
Macedonia 1
Macedonia 2
Macedonia 3
Macedonia 4
B. Distachyon
50 bp Ladder

