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Thinopyrum* trigenetic hybrids**

Journal:	<i>Genome</i>
Manuscript ID	gen-2017-0025.R2
Manuscript Type:	Article
Date Submitted by the Author:	10-Apr-2017
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Is the invited manuscript for consideration in a Special Issue? :	This submission is not invited
Keyword:	triticale, <i>Th. elongatum</i> , GISH, chromosome-specific marker, chromosome identification

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Chromosome identification by new molecular markers and genomic *in situ* hybridization in the *Triticum-Secale-Thinopyrum* trigenic hybrids

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Abstract: It is very important to use chromosome-specific markers for identifying alien chromosomes in advanced generations of distant hybridization. The chromosome-specific markers of rye and *Thinopyrum elongatum* as well as genomic *in situ* hybridization were used to identify the alien chromosomes in eight lines which were derived from the crossing between *Triticum trititrigia* (AABBEE) and triticale (AABBRR). The results showed that four lines contained all rye chromosomes but no *Th. elongatum* chromosomes. The line RE36-1 contained all of the rye chromosomes except for chromosome 2R. The lines RE33-2 and RE62-contained all rye chromosomes and 1E and 5E translocated chromosome, respectively. The line RE24-4 contained 12 rye chromosomes plus a 7E chromosome or 12 rye chromosomes plus one R-E translocated chromosome. Chromosome identification in the above lines was consistent using chromosome-specific markers and genomic *in situ* hybridization. These chromosome-specific markers provide useful tools for detecting alien chromosomes in trigenetic hybrids and these lines could be utilized as valuable germplasm in wheat improvement.

Abbreviations: GISH, genomic in situ hybridization; FISH, fluorescence in situ hybridization; PLUG, PCR-based landmark unique gene; SSR, simple sequence repeat; SLAF-seq, specific-locus amplified fragment; CS, Chinese Spring; LD, Langdon.

Keywords: triticale; *Thinopyrum elongatum*; GISH, chromosome-specific marker; chromosome identification

Introduction

Rye (*Secale cereal* L., $2n = 2x = 14$, RR) is an important cereal crop in Eastern, Central and Northern latitude countries (Geiger and Miedaner 2009). It has some significant advantages over wheat and other cereals. Specifically, rye has the highest tolerance to drought, salt, and aluminum stress among all small-grain cereals, and it is also an important reservoir for resistance to biotic stresses (Geiger and Miedaner 2009). For example, the short arm of rye chromosome 1R carries the leaf rust resistant gene *Lr26*, stem rust resistant gene *Sr31*, stripe rust resistant gene *Yr9* and powdery mildew resistant genes *Pm8*, *Pm17* (Zhu et al. 2015). Therefore there has been strong interest in producing new wheat varieties that include the superior agronomic performance of rye. To this end, both hexaploid (AABBRR) and octoploid (AABBDDRR) triticale have been produced by crossing of various cultivars of wheat species, *Triticum turgidum* (AABB) or *Triticum aestivum* (AABBDD), with different rye cultivars (Mergoum et al. 2009). Among the various types of triticale, hexaploid triticale has been the most successful because of its superior vigor and reproductive stability (Randhawa et al. 2015). In general, triticale combines the high yield potential of wheat with the biotic and abiotic stress tolerance of rye, making it more suitable for production in marginal areas. Despite having many advantages over wheat, global triticale production is still very low because of the inherent problems of partial floret sterility, shriveled seed, long growing season, lack of end-use markets, and competition from wheat (Blum 2014). Therefore, there is a need to improve the genetic diversity of triticale by different ways in triticale breeding programs. More recently, allelic variation from the D genome contributed by *Triticum tauschii* has been incorporated in hexaploid triticale (Lukaszewski 2006). Additionally, some breeding programs have crossed other species containing genomes derived from wheat relatives with triticale. For example, the 3S^v chromosome derived from *Aegilops variabilis* Eig., conferring powdery mildew resistance, has been transferred into triticale (Kwiaterek et al. 2016). Diploid wheatgrass, *Thinopyrum elongatum* (Host) D.R. Dewey ($2n = 2x = 14$, EE), is another wild relative of wheat which carries many superior characters, such as high protein content, resistance to biotic stresses and tolerance to abiotic stress (Shukle et al. 1987; Sharma et al. 1989; Yang and Ren 2001; Shen et al. 2004; Linc et al. 2012; Chen et al. 2013). Thus wheat geneticists and breeders around the world would like to transfer these superior characters into triticale to obtain germplasm with increased genetic variation through the use of trigeneric hybrids. This example illustrates that transferring multi-alien genomes into triticale can

improve its poor agronomic characteristics, and that the recombination between the R genome and other species chromosomes, such as addition, substitutions and translocations, may create new genetic constitutions, which could enhance the good agronomic traits of triticale.

Owing to the important roles of alien chromosomes in new hybrids with triticale, there is a great need to develop methods for the rapid and reliable identification of alien chromosomes in triticale breeding. Genomic *in situ* hybridization (GISH) is widely used for determining the alien rye chromosomes and chromosome segments in hybrids, and fluorescent *in situ* hybridization (FISH) is a powerful technique for detecting specific nucleic acid sequences and localizing highly repetitive DNA sequences in specific regions of individual chromosomes (Schneider et al. 2016). However these cytogenetic techniques are costly and difficult to implement. With the increasing availability of molecular markers for many agronomically important genes, molecular marker-assisted selection is gaining more attention in wheat breeding. Reliable markers to identify the chromosomal sources of useful genetic variability in intergeneric hybrids involving triticale are required. Consequently, a number of chromosome-specific DNA-based markers have been reported. For example, PLUG (PCR-based landmark unique gene), SSR (simple sequence repeat), and SLAF-seq (specific-locus amplified fragment) markers have been used in rye genetics, especially SLAF-seq (Li et al. 2013; Qiu et al. 2016; Tomita et al. 2012). The latter is a recently developed method that combines high-throughput and reduced-representation library sequencing for efficient and high-resolution large-scale genotyping (Xia et al. 2015). This approach also has high efficiency, low cost, less sequencing demand and high capacity for developing specific molecular markers (Geng et al. 2016). So SLAF-seq has been used in the successful development of specific molecular markers for rye and *Th. elongatum* chromosomes in triticale breeding (Chen et al. 2013; Qiu et al. 2016). In this study, using the chromosome-specific molecular markers developed by SLAF-Seq combined with GISH together can accurately identify the individual chromosomes in each hybrid line.

The diploid wheatgrass *Thinopyrum elongatum* (Host) D.R. Dewey [= *Lophopyrum elongatum* (Host) Á. Löve; = *Elytrigia elongatum* (Host) Nevski; = *Agropyron elongatum* (Host) Beauv.] ($2n = 2x = 14$, EE) is a significant wild relative of wheat that has many desirable genes for wheat improvement. Its high cross-compatibility with wheat and its superior characters, such as high protein content, resistance to biotic stresses, and tolerance to abiotic stress, has attracted the attention of wheat geneticists and breeders (Shukle

et al. 1987; Sharma et al. 1989; Yang and Ren 2001; Shen et al. 2004; Linc et al. 2012; Chen et al. 2013). In this study, we obtained eight lines from hybrids derived from durum (AABB) wheat $\times$ *Th. elongatum* amphiploid forms of *Triticum trititrigia* (AABBEE) and hexaploid triticales T182 (AABBRR). The chromosome constitution of some progenies could be distinguished by GISH, as in previous studies, but we used the chromosome-specific molecular markers developed by SLAF-Seq and GISH together to accurately identify the individual chromosomes in each line of the progenies. The specific molecular markers for rye and *Th. elongatum* developed in this study not only identify the chromosomes or chromosomal fragments of rye and *Th. elongatum* in hybrids, but also can be used to locate the resistance genes in the progeny. These markers can also be located on the chromosomes in order to develop a physical map. The resistant translocation lines will be used as a bridge to transfer the resistant genes into a wheat background.

Materials and Methods

Plant materials

The materials employed in the current study included common wheat (Chinese Spring), durum wheat (Langdon), rye (Imperial), seven Chinese Spring–Imperial rye addition lines (DA1R–DA7R, $2n = 6x = 44$), seven Chinese Spring–*Th. elongatum* addition lines (DA1E–DA7E, $2n = 6x = 44$), *Th. elongatum* ($2n = 2x = 14$, EE), hexaploid triticales cv. T182 ($2n = 6x = 42$, AABBRR), hexaploid amphiploid *Triticum trititrigia* cv. 8801 ($2n = 6x = 42$, AABBEE) (Guo et al. 2015). These materials were stored at Ottawa Research and Development Centre.

For the 8801 $\times$ T182 cross (Fig. 1), F_1 plants were obtained by embryo rescue, and the progeny were advanced to the F_5 generation in a greenhouse. Eight lines (RE24-1, RE24-4, RE26-1, RE26-2, RE33-2, RE33-3, RE36-1, and RE62-1) were selected based on good fertility and stable phenotypes (Fig. 2). Seeds of the eight F_6 and F_7 lines were used for molecular marker analysis and cytological studies.

Molecular marker development

SLAF library construction for high-throughput sequencing

The SDS method was used to extract genomic DNA from young leaves of the listed materials. DNA quality

and concentration were measured using 1% agarose gel electrophoresis, and adjustments were made for a final DNA concentration of 100 ng/μL in Tris/ethylenediamine tetraacetic acid (TE0.1) buffer at pH 8.0 for PCR amplification.

The genomic DNA of the above materials was sequenced using the SLAF-seq method (Biomarker, Beijing, China). Then, the SLAF library was constructed based on the scheme of Chen et al. (2013) with small modifications. Purified DNA was digested using an appropriate restriction endonuclease *RsaI* (New England Biolabs), then a single-nucleotide A overhang was added to the digested fragments with Klenow Fragment (3' → 5' exo -) (NEB) and dATP at 37° C, and then the Duplex Tag-labeled Sequencing adapters (PAGE purified, Life Technologies) were ligated to the A-tailed DNA with T4 DNA ligase. The reaction products were purified using Agencourt AMPure XP beads (Beckman Coulter, High Wycombe, UK) and electrophoresed in a 2% agarose gel. Fragments of 400~450bp (with indexes and adaptors) were isolated using QIAquick Gel Extraction Kit (QIAGEN) and then subjected to PCR. The products were sequenced on the Illumina HiSeq 2500 system (Illumina, Inc; San Diego, CA, U.S.) at Biomarker Technologies Corporation in Beijing.

SLAF-seq data analysis

The SLAF sequences were identified and filtered to ensure that the original sequencing data were accurate, and the sequences were clustered based on similarity using BLAT with focused recognition and correction techniques (Chen et al. 2013). The paired-end reads obtained from the DA1R–DA7R addition lines were first compared with the sequences obtained from Chinese Spring wheat and then with sequences on the NCBI (www.ncbi.nlm.nih.gov) and Bristol Wheat Genomics cereals (www.cerealsdb.uk.net) databases. The paired-end reads with low identity (<50%) with wheat were retained, and of those, the reads with high identity (>80%) with the sequences obtained from Imperial rye were obtained. Finally, primers were designed according to the rye-specific reads using the software Primer 5. In addition, the 1E-7E chromosome-specific molecular markers of *Th. elongatum* were obtained by the same way as above in this study, and 4E chromosome-specific molecular marker of *Th. elongatum* were designed by Chen et al. (2015) (Table S1).

PCR analysis

PCR amplification was carried out in a 25 μ L reaction containing 1 μ L genomic DNA (100 ng/ μ L), 2.5 μ L PCR buffer (10 $\times$), 2 μ L dNTPs (2.5 mM), 1 μ L (each of primers 1 and 2) (10 mM), 0.2 μ L Taq (5 U/ μ L) and 18.3 μ L double-distilled water. The PCR procedure was as follows: 94°C for 5 min followed by 35 cycles of 94°C for 45 s, appropriate anneal temperature (50–65°C) for 45 s, 72°C for 40 s and a final extension for 10 min at 72°C. The amplified products were electrophoresed in a 1% agarose gel in 1 $\times$ TAE buffer. For each pair of primers, PCR reactions were repeated three times.

Molecular cytogenetic analysis

Chromosome preparations of root tip cells at mitotic metaphase followed the protocol of Chen et al. (1995). GISH followed the procedure described by Zhang et al. (2004). Total genomic DNA of Imperial rye and *Th. elongatum* was labeled with the Biotin-Nick Translation Mix (Roche, Germany) and used as probes for GISH. Hybridization signals were observed using a Zeiss Axioplan fluorescent microscope and images obtained with a CCD camera (Zeiss, Germany).

Results

Development of chromosome-specific markers for rye

Using the SLAF-seq approach, a total of 2,477,872 SLAF reads were obtained from the addition lines DA1R–DA7R (Table 1). The sequencing depth was >11.89 $\times$. After comparing with wheat and rye sequences on the GenBank and Bristol Wheat Genomics cereals, the reads that had <50% identity with Chinese Spring and other wheat species and >80% identity with Imperial rye were retained. Finally, 350 randomly selected rye-specific paired-end reads (50 pair-end reads per chromosome) were used to design primer sets. Of these primer pairs, 253 amplified specific bands from Imperial rye by PCR, but did not amplify sequences in Chinese Spring and Langdon wheat (Table S1, Fig. 3). Of these, 44 were genome-specific markers, amplifying rye-specific bands from all seven of the addition lines (Fig. 3h). Another 33 pairs of primers produced rye-specific bands for more than one R chromosome in the wheat–rye addition lines (Fig. 3i). The remaining 176 of the 253 pairs of primers produced only one specific band in one wheat–rye addition line. The results indicated that markers 22, 21, 28, 28, 24, 20 and 33 were located on chromosomes 1R, 2R, 3R, 4R, 5R, 6R, 7R, respectively (Fig. 3a–g).

Development of chromosome-specific markers for *Thinopyrum elongatum*

Using the SLAF-seq approach, the chromosome-specific markers for *Th. elongatum* were developed in this study, and the total numbers of SLAF reads are shown in Table 1. Based on 175 sequences randomly selected from the specific sequences of the 1E-7E chromosomes, 175 pairs of primers were designed for developing specific molecular markers (25 pairs of primers per chromosome). PCR products were amplified from DA lines (DA1E-7E), CS, *Th. elongatum* (2n=2X), and Langdon, respectively. Of these primer pairs, 108 amplified specific bands from *Th. elongatum* by PCR, but did not amplify sequences in Chinese Spring and Langdon wheat (Table S2, Fig. 4). In addition, of the 108 pairs of primers, 20 were genome-specific markers, amplifying *Th. elongatum* -specific bands from all seven of the addition lines (Fig. 4h). Another 31 pairs of primers produced *Th. elongatum* -specific bands for more than one E chromosome in the wheat–*Th. elongatum* addition lines (Fig. 4i-j). The remaining 57 of the 108 pairs of primers produced only one specific band in one wheat–*Th. elongatum* addition line. The results indicated that markers 10, 6, 11, 1, 13, 6 and 10 were located on chromosomes 1E, 2E, 3E, 4E, 5E, 6E, 7E, respectively (Fig. 4a–g).

Chromosome identification using chromosome-specific markers

The eight lines in the F₆ generation obtained from two original F₂ plants of the 8801×T182 combination were studied for chromosome composition by chromosome-specific markers. The eight lines were designated as RE24-1, RE24-4, RE26-1, RE26-2, RE33-2, RE33-3, RE36-1, and RE62-1. Line RE62-1 was derived from one F₂ plant and the other seven lines were derived from a different F₂ plant. These eight lines were screened with a total of 35 rye chromosome-specific markers and 21 *Th. elongatum* chromosome-specific markers. The PCR analysis showed that four lines (RE24-1, RE26-1, RE26-2 and RE33-3) contained all seven rye chromosome-specific markers but no *Th. elongatum* markers, indicating that these lines contained 14 A, 14 B and 14 R chromosomes, with a chromosome number of 2n = 42. However, the line RE36-1 appeared to contain 40 chromosomes, as the primers amplified bands from six pairs of rye chromosomes, but not chromosome 2R, and no bands from the E genome chromosomes; thus, this line contained 12 R, 14 A and 14 B chromosomes. For lines RE24-4, RE33-2 and RE62-1, the primers amplified the bands for all seven rye chromosomes, plus 7E-, 1E- and 5E-specific bands, respectively, suggesting the presence of R–E chromosome translocations in these three lines. The results of this analysis are shown in Fig. 5.

We also analyzed eight lines in F_7 generation, randomly selected from specific F_6 plants, using five markers for each rye chromosome and three markers for each *Th. elongatum* chromosome. The PCR results for these lines at the F_7 generation were mostly the same as for the F_6 generation (Table 2, Fig. 6). The genomic constitutions of lines RE24-1, RE26-1, RE26-2 and RE33-3 were $2n = 42$, consisting of 28 durum wheat chromosomes and 14 rye chromosomes. Five plants from line RE36-1 also showed the same constitution as in the F_6 generation, namely $2n = 40$ and lacking chromosome 2R. For lines RE33-2 and RE62-1, showed markers for all rye chromosomes plus markers for either 1E (RE33-2) or 5E (RE62-1). Interestingly, in the 14 progenies of line RE24-4, one plant showed markers for all rye chromosomes plus a marker for 7E, four plants showed markers for six rye chromosomes (lacking chromosome 7R), plus a marker for 7E, and nine plants had the constitution of $2n = 40$, with only six pairs of rye chromosomes, lacking chromosome 7R.

Chromosome identification by multicolor GISH

To confirm the accuracy of the PCR results, GISH was used to confirm the chromosome composition of the eight lines at the F_7 generation. The GISH results indicated that lines RE24-1, RE26-1, RE26-2 and RE33-3 carried 28 chromosomes of durum wheat and 14 chromosomes of rye but no *Th. elongatum* chromosomes. The chromosome composition therefore was the same as in the F_6 generation (Fig. 7a). The line RE36-1 in F_6 generation had 40 chromosomes, consisting of 28 durum wheat chromosomes plus 12 rye chromosomes (Fig. 7b). Line RE33-2 carried 28 durum wheat chromosomes, 13 rye chromosomes and one R–E translocated chromosome (Fig. 7c). The line RE62-1 carried 28 durum wheat chromosomes, 12 rye chromosomes and 2 R–E translocated chromosomes (Fig. 7d). The line RE24-4 carried 28 chromosomes of durum wheat (A and B genomes) and 12 rye chromosomes plus one *Th. elongatum* chromosome (Fig. 7e) or 12 rye chromosomes plus one R–E translocated chromosome (Fig. 7f).

Discussion

Triticale is an artificial species recently developed. Considering its narrow genetic diversity, it is necessary to utilize its varied relatives to enrich the genetic pool of triticale by interspecific (wheat $\times$ triticale) and intraspecific (winter triticale $\times$ spring triticale) crosses. Another recently utilized breeding strategy for triticale improvement has been to introduce chromosomes of other genomes into hexaploid triticale to produce the trigeneric hybrid. For example, Kwiatek et al. (2015; 2016) transferred the D genome

chromatin carrying leaf rust resistance gene *Lr33* from *Aegilops tauschii* to triticale (AABBRR, $2n = 6x = 42$), and they have developed addition and substitution lines by crossing triticale with *Ae. variabilis* $\times$ *S. cereale* ($2n=6x=42$, $U^vU^vS^vS^vRR$) amphiploid that carried powdery mildew resistance. Yang et al. (2015) transferred the E genome of *Th. elongatum* to hexaploid triticale (AABBRR) and found E and R genome chromosome substitutions and translocations in the progenies. However, the identities of the individual R and E chromosomes in the progenies were not reported. In this study, we not only obtained the lines with the E genome introgression, but also identified the individual R and E chromosomes by chromosome-specific markers and GISH.

In germplasm development involving crosses of divergent species, alien chromosome identification is essential for monitoring chromosome composition changes. C-banding staining allows the differentiation of all chromosomes and most chromosome arms between wheat and its relatives through distinct heterochromatin patterns (Sharma and Gill 1983; Sharma 1995). With the development of molecular biology, the identification of alien chromosomes has greatly improved. GISH is now widely used for determining the origin of genomes, chromosomes and chromosome segments in hybrids (Autrique et al. 1995). Furthermore, the application of repetitive DNA clones as a probe in FISH has made it possible to identify individual chromosomes within a species or to follow intergenomic chromosome rearrangements and chromosome polymorphisms in polyploids (Linc et al. 2012). The combination of the GISH and FISH techniques can identify individual chromosomes, but because these two techniques are time and labor intensive, other methods need to be applied to properly assess the nature of alien chromatin in progeny and eventually identify stable addition lines. So many PCR-based markers have been developed for utilization in wheat breeding programs, and some of these markers are specific to individual chromosomes. For example, rye-specific SSR, PLUG, and SLAF-seq markers had been used to identify individual chromosomes or chromosomal segments in a wheat background (Saal and Wricke 1999; Li et al. 2015). Therefore, using molecular cytogenetic techniques and chromosome-specific markers together could accelerate the identification of the alien chromosomes in wheat breeding. In our study, we developed 253 rye chromosome-specific markers and 108 *Th. elongatum* chromosome-specific markers by SLAF-seq technology and some of these chromosome-specific markers were chromosome specific markers. These markers have greatly enriched the available resource of rye and *Th. elongatum* chromosome-specific

markers that can be used to investigate the diversity of species in wheat backgrounds. These markers have great promise for practical application, because they are PCR and agarose gel electrophoresis-based. So we used chromosome-specific markers and GISH for understanding the chromosome composition for 8 lines with different chromosome rearrangements between E genome chromosomes and R genome chromosomes. And the results were entirely consistent between PCR and GISH.

Both PCR and GISH indicated that lines RE24-1, RE26-1, RE26-2 and RE33-3 carried all of the rye chromosomes but no *Th. elongatum* chromosomes (Fig. 7a). Similarly, in line RE36-1, PCR detected six rye-chromosomes and all E chromosome but not 2R, and GISH also detected only 12 R chromosomes in this line (Fig. 7b). According to the PCR results, RE33-2 and RE62-1 had all of the R chromosomes, plus the 1E and 5E chromosomes, respectively, suggesting that introgression occurred from *Th. elongatum* and GISH confirmed that one R–E translocated chromosome was present in line RE33-2 and two R–E translocated chromosomes were present in line RE62-1 (Fig. 7c and 7d). This observation is consistent with the findings for other research, such as the translocation line T4DL.4Ai#2S, in which the 4Ai short arm of *Th. intermedium* replaces the 4DS arm of wheat (Qi et al. 2007), the 4V(4D) substitution and T4DL·4VS translocation (Zhao et al. 2013). This indicates that whether substitution or translocation, the chromosome recombination generally occurred in homoeologous groups. In our study, GISH detected the translocated chromosomes in the lines RE33-2 and RE62-1, and PCR identified the 1E and 5E chromosomes as being involved in the R–E translocation. However, we do not know which wheat chromosome arms were involved in the translocation. Additionally, line RE24-4 was still unstable in the F₇ generation based on the results of PCR and GISH. The PCR showed all rye chromosome-specific bands and a 7E chromosome-specific band in the F₆ generation, but in the F₇ generation, some progenies had six rye chromosome-specific bands (lacking chromosome 7R). The GISH showed 12 rye chromosomes and 1 *Th. elongatum* chromosome in the progenies (Fig. 7e), and some progenies still showed all rye chromosome-specific bands, plus the 7E chromosome-specific band. The GISH showed 12 rye chromosomes and 1 R–E translocated chromosome, suggesting that this might be a translocation line containing 12 rye chromosomes and 1 R–E translocated chromosome (Fig. 7f). We thus speculated that the chromosome constitution of this line was $2n = 42$, and that it contained 14 A and 14 B and 12 R chromosomes of rye, one *Th. elongatum* chromosome and one R-E translocated chromosome in F₆ generation. Overall, the GISH results agreed with

the PCR, indicating that it is possible to introgress the E genome into triticale, and that these individual chromosome-specific markers could be used to identify the rye and *Th. elongatum* chromosomes in triticale-improvement programs.

In recent years, crosses between wheat and its wild relatives, such as *Agropyron*, *Elymus*, and *Secale*, have attracted considerable attention as a way to extend the resources for genetic improvement of wheat (Sharma and Gill 1983). The most well-known and widely used rye chromosome for wheat improvement is chromosome 1R translocated as 1RS into the wheat genome, such as 1AL.1RS, 1BL.1RS and 1DL.1RS (Merker 1982; Gupta and Shepherd 1993;). For example, a new wheat-rye 1BL.1RS translocation line derived from the hexaploid triticale cultivar Zhongsi 828 ($2n=6x=42$, AABBRR) and wheat-*Psathyrostachys huashanica* amphiploid PHW-SA ($2n=8x=56$, AABBDDNsNs) has the characteristics of superior stripe rust resistance, high thousand-kernel weight and high grain number per spike (Qi et al. 2016). In relation to our present research, the eight lines, especially the translocation lines, obtained in this study could be used as a donor source for introducing and tracking novel genes from rye and *Th. elongatum* in wheat backgrounds. The two translocation lines carried the most promising characteristics, such as earlier maturity and nearly 90% spike fertility. However, the locations of the sequences conferring these enhanced traits in the translocated chromosomes needs to be verified before proceeding with the process of introgressing these traits into wheat. Thus, these lines carrying translocation chromosomes could be used for genetic improvement with the marker-assisted selection in further studies.

In conclusion, we developed rye and *Th. elongatum* molecular markers by the SLAF-seq method. These markers have greatly enriched the available rye and *Th. elongatum* molecular markers, which can be used to investigate the diversity of species in wheat-rye-*Th. elongatum* trigenic hybrids. And molecular analysis combined with molecular cytological techniques enhanced the ability to monitor chromosome composition changes in progeny of wide crosses in the triticale background.

Conflict of Interest: None declared

Violence upon Ethical Standards: None declared

Author Contribution Statement

YD, GF and J-MC participated in the study conception and design. H-PL, Y-MD, J-JT and YD contributed

to DNA extraction and molecular marker identification. YD, DC and W-GC performed hybridization. YD, H-PL and GY analyzed the data. YD, J-MC and GF wrote the manuscript. All authors approved the final version of the manuscript.

Acknowledgements

This work was supported by the MOE-AAFC PhD Research Program; the National Natural Science Foundation of China (No. 31071406); Genetically Modified Organisms Breeding Major Projects (2014ZX0800205B); the Fund of Jiangsu Provincial Key Lab for Agrobiolgy (2015K003); A project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions and Joint Laboratory in Agricultural Sciences between ORDC and YZU. We also thank another financial support for the Priority Academic Program Development of Jiangsu Higher Education Institutions and State Key Laboratory of Crop Biology.

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Figure Legends

Fig. 1 Procedure for synthesis of eight lines in progenies of crosses between 8801 and T182.

Fig. 2 The spikes of the eight lines derived from crosses between 8801 and T182.

Fig. 3 Examples of amplification of chromosome-specific sequences from individual wheat-rye addition lines.

a-j: Products amplified by chromosome-specific markers CMS1R-28, CMS2R-27, CMS3R-13, CMS4R-33, CMS5R-27, CMS6R-21, CMS7R-30, CMS5R-25, CMS6R-30 and CMS6R-33, respectively. M: DNA size markers; 1-7: DA1R-DA7R; 8: Imperial; 9: Chinese Spring; 10: Langdon

Fig. 4 Examples of amplification of chromosome-specific sequences from individual wheat-*Th. elongatum* addition lines.

a-j: Products amplified by chromosome-specific markers CMS1E-1, CMS2E-4, CMS3E-1, CMS4E-5, CMS5E-1, CMS6E-2, CMS7E-1, CMS1E-11, CMS2E-3 and CMS5R-5, respectively. M: DNA size markers; 1-7: DA1E-DA7E; 8: Chinese Spring; 9: Langdon; 10: *Th. elongatum* (2X)

Fig. 5 PCR amplification of rye chromosome-specific sequences of 1R to 7R and *Th. elongatum* chromosome-specific sequences from 1E to 7E in the F₆ generation of 8801 × T182.

M: DNA size markers; 1: RE24-1, 2: RE24-4, 3: RE26-1, 4: RE26-2, 5: RE33-2, 6: RE33-3, 7: RE36-1, 8: RE62-1; 9: Imperial; 10: T182; 11: 8801; 12: *Th. elongatum* (2X); 13: Chinese Spring; 14: Langdon.

The amplified bands of the 3R to 7R chromosome-specific markers were the same as 1R, and the amplified bands of 2E, 3E, 4E and 6E were the same as 1E.

Fig. 6 PCR amplification with 1R, 2R, 7R, 1E, 5E and 7E chromosome-specific markers in plants of the F₇ generation derived from 8801 × T182.

The amplified bands of 3R to 6R chromosome-specific markers were the same as 1R. All plants from eight lines without the amplified bands of 2E, 3E, 4E and 6E except for 1E, 5E and 7E.

Fig. 7 Multicolor GISH of eight lines using rye genomic DNA (green) and *Th. elongatum* (2X) genomic DNA (red) as probes. Chromosomes were counterstained with DAPI (blue). Bar = 10 μm.

a: Line RE24-1, with 14 A, 14 B and 14 R chromosomes of rye; b: Line RE36-1, with 14 A, 14 B and 12 R chromosomes; c: Line RE33-2, with 14 A, 14 B and 13 R chromosomes and 1 R-E translocated chromosomes; d: Line RE62-1, with 14 A, 14 B and 12 R chromosomes and 2 R-E translocated chromosomes; e: Line RE24-4, with 14 A, 14 B and 12 R chromosomes and 1 *Th. elongatum* chromosome; f: Line RE24-4, with 14 A, 14 B and 12 R chromosomes and 1 R-E translocated chromosome

Table 1 The numbers of SLAFs and chromosome-specific SLAFs obtained from Chinese Spring, Imperial rye, *Thinopyrum elongatum*, wheat-rye addition lines and wheat- *Th. elongatum* addition lines.

Sample	SLAF number	Chromosome-specific SLAF number
CS	286,282	
Imperial rye	298,964	
<i>Thinopyrum elongatum</i>	282,962	-
DA1R	347,048	3,220
DA2R	286,417	2,120
DA3R	334,109	4,012
DA4R	413,878	6,128
DA5R	317,443	1,101
DA6R	350,338	4,935
DA7R	351,559	4,545
DA1E	322,508	664
DA2E	313,687	1,501
DA3E	301,667	1,142
DA4E	322,102	540
DA5E	338,323	463
DA6E	322,503	1,258
DA7E	280,837	1,255

Table 2 The chromosome numbers and PCR amplifying bands of rye, *Th. elongatum* chromosome in the eight lines derived from crosses between 8801 and T182.

lines	Chromosome number	Rye chromosome bands	Size of amplified bands	<i>Th. elongatum</i> chromosome bands	Size of amplified bands
RE24-1	42	1R-7R	440	-	
RE24-4	40-41	1R-7R or 1R-6R	440	7E	400
RE26-1	42	1R-7R	430	-	
RE26-2	42	1R-7R	430	-	
RE33-2	42	1R-7R	440	1E	430
RE33-3	42	1R-7R	440	-	
RE36-1	40	1R, 3R-7R	440	-	
RE62-1	42	1R-7R	440	5E	420

Fig. 1

123x142mm (300 x 300 DPI)

Fig. 2

197x74mm (300 x 300 DPI)

Draft

Fig.3

191x107mm (300 x 300 DPI)

Fig.4

197x109mm (300 x 300 DPI)

Fig.5

199x117mm (300 x 300 DPI)

Fig. 6

193x96mm (300 x 300 DPI)

Fig. 7

163x107mm (300 x 300 DPI)

Supplementary Table 1 The chromosome-specific molecular markers and PCR primers of Imperial rye

Specific Markers	Specific Primers	Sequences of the Special Primers (5'-3')		Annealing temperatures	Amplified Chromosomes
		Forward	Reverse		
CMS1R-1	SLAF1R-1	CTGGGGAGGTGGTTGATT	TCGGCGTTATCGCTCTAT	62	1R
CMS1R-2	SLAF1R-3	TGCTGACGTGGGATGTTTT	CTTTTGCTGATGGTTGGTAG	62	1R
CMS1R-3	SLAF1R-4	TTATGAAGGCTTGAAGGTG	ATCGTTTATGTAGTGTTCG	60	1R
CMS1R-4	SLAF1R-5	AGTAAGGGATCAACTGCTGC	ACGATTTGCTCAAGGACATT	61	1R
CMS1R-5	SLAF1R-6	TATGTCGGTAGCATCAATCA	AATTCTAGACGTGACCCAAG	59	1R
CMS1R-6	SLAF1R-7	CCTCTATCTCTTAGTTGCCA	TAAAACTCATACCTCAGCAG	55	1R-7R
CMS1R-7	SLAF1R-9	CCACCACATTCATTCCACCA	GCAGGCAGGGCTAGAAGTTC	64	1R
CMS1R-8	SLAF1R-10	TACAACAACACAAATATTACCCCC	GGACTCGGAACAGGAGCAGA	64	1R
CMS1R-9	SLAF1R-11	CACCACCACATTCATTCCAC	TCGGGGAGCCAGAAATTC	64	1R
CMS1R-10	SLAF1R-12	ATACAACAACACAAATATTACCC	TTCCACGACACCGACAAT	59	1R
CMS1R-11	SLAF1R-14	TGCAAAAGCCTCGGATCT	GCCCATAAACAGAAAACAAA	62	1R
CMS1R-12	SLAF1R-16	ATCCATCTCCCATCCTTC	CAGCTAGCCATTCATTTT	58	1R
CMS1R-13	SLAF1R-17	ATCCATCTCCCATCCTTC	CAGCTAGCCATTCATTTT	58	1R
CMS1R-14	SLAF1R-18	ATCCATCTCCCATCCTTC	CAGCTAGCCATTCATTTT	58	1R
CMS1R-15	SLAF1R-19	TATCTATCTCCCATCCTTC	GCCATTTATTTTACTTGTCA	57	1R
CMS1R-16	SLAF1R-20	TTGGTAAAGGGGCTTCAGG	GCATAACATCGCTCGGGC	62	1R-7R
CMS1R-17	SLAF1R-27	AAAAACCCTACCCTCCTGA	AGCCTCTTACCGTTGAACA	58	1R-7R
CMS1R-18	SLAF1R-29	TCCATCTTGCTAAGCGCC	CTCCTGTCTTCATACGTTTGC	58	1R
CMS1R-19	SLAF1R-30	TCAGTAGGTTGACGAAAGC	TAGAGTGTATGGAGGGTGC	58	1R
CMS1R-20	SLAF1R-31	GTCATAGAAAGTGAGGAAAAA	AGCAATAACCATCAGCAAG	53	1R
CMS1R-21	SLAF1R-32	TTGGTGTTCGTCTTGTTGA	TGTATTTTGCTTGGAACCTCT	53	1R
CMS1R-22	SLAF1R-34	TAGACTTGACCGTGGACAG	CCACGCAGGATAGGAGTTA	58	1R-7R
CMS1R-23	SLAF1R-35	AATTGGATTTTATTTTGTTAC	GATTAGGAGGTCCCGTTTT	55	1R
CMS1R-24	SLAF1R-38	CCGCCCTGACATCCACTGC	CAGCAGCCCCATTTTCATCCTT	61	1R
CMS1R-25	SLAF1R-42	CGCCCATCAGGCAGAAAGA	GGACCCTGACCCAGCGG	60	1R-7R
CMS1R-26	SLAF1R-43	GCATCCATGCACAAGGTAAAGT	TCTACCCCATTCTGTCACTCCA	60	1R-7R
CMS1R-27	SLAF1R-45	TGAGTTTCAGAGAGGGGTTAG	CTTCGTCAGTCTTGGGTTAGGT	58	1R
CMS1R-28	SLAF1R-48	GAGCACAATTTTCCGTCCCA	TCTTGCTAAGCGCCTGAGTT	60	1R

CMS1R-29	SLAF1R-50	TAGTCATCCACTCCTTGAATCA	ATAGACCCTAAACTTGGTAACG	55	1R-7R
CMS1R-30	SLAF1R-2	GGTAAGTCCTGGCTGTAATA	CTCCACCACGCTTTCATA	58	1R, 4R
CMS1R-31	SLAF1R-23	TAAAAAACCTACCCTCC	GCCTCTTACC GTTGAACA	55	1R, 4R, 6R, 7R
CMS1R-32	SLAF1R-24	AAAAACCTACCCTCCTG	TGTCTGATCCCCCTTGAA	56	2R, 4R, 6R, 7R
CMS1R-33	SLAF1R-26	CCCTCTTGAGTCAGTTCCGC	CCTCGAGCCTCTTACCGTTG	62	1R, 2R, 4R, 6R, 7R
CMS1R-34	SLAF1R-31	GTCATAGAAAGTGAGGAAAAA	AGCAATAACCATCAGCAAG	53	1R, 5R
CMS1R-35	SLAF1R-39	ATGAGTCCCTGGACAAAAA	ATACGGAGCTTCACCCAAA	55	1R-4R, 6R, 7R
CMS1R-36	SLAF1R-41	TCAAACTTATCTCCGAACGAAT	GATAGTGCTCTTAAGGGAATGG	55	1R, 3R-7R
CMS2R-1	SLAF2R-1	CCTTAGATTCTCTTTTTT	TTTCTGT TACTATCCCCTC	52	2R
CMS2R-2	SLAF2R-2	CCATGCAACGTTCCCCA	TCTCCAAAGTTTTCCCCC	65	2R
CMS2R-3	SLAF2R-3	CATATGGCCTCCACCTCCT	CAGTTTAGCTACCCGGAGAT	62	1R-7R
CMS2R-4	SLAF2R-4	TACCAGCCGCCTCGTCTTG	AATCAGTGGGGGCACGCTC	67	1R-7R
CMS2R-5	SLAF2R-5	GGGAACTGGAGGGAACCG	GCTGAGAAGACGATGGAGGG	65	2R
CMS2R-6	SLAF2R-8	GAAGTGGCAGTCTCAGATTG	AAACTCCAATCAGTATTATCGT	58	2R
CMS2R-7	SLAF2R-10	CTAATGATCTCCGCCCCG	ATAGTGACACAAATTTTATGCAA	58	2R
CMS2R-8	SLAF2R-11	CTTCAAGCAAAGCACAGAC	GGAGATAACACAAGGCAAC	59	2R
CMS2R-9	SLAF2R-12	CTATACGTTTTTCTACCCCA	TCCGCACACTTGTTTCTTT	59	2R
CMS2R-10	SLAF2R-14	CCCTCATCTTCGTCCAGTG	CCGAAGAGTATCGCCAAAT	61	2R
CMS2R-11	SLAF2R-15	AGCGTCACAATGGATCTCA	ATCGGAACCACGACAAAAA	62	2R
CMS2R-12	SLAF2R-19	ACTCGCACATCAGGGAGAAAT	CAAAGCCCCAAACATCAAA	63	2R
CMS2R-13	SLAF2R-21	GGCTGCCCAATGAATGA	CATCACAATTGGCAAAGGG	55	2R
CMS2R-14	SLAF2R-22	AGCACCTGTGTGACCGTAT	CAAGCGACTTCAAGGCATC	58	2R
CMS2R-15	SLAF2R-23	ATCTAGGCATGATTATTTG	ATACATACCAATGCACCAC	55	1R-7R
CMS2R-16	SLAF2R-24	TATCGGATGTGTGATTGGA	AAAGTTGGGTCTTGCTGGA	55	2R
CMS2R-17	SLAF2R-27	GTTTTCTCTTAAATTCGCAA	GTGTGTGCCCTATCAAGA	55	2R
CMS2R-18	SLAF2R-29	CTGCTAGCTGATCGTGGC	CGTGGGTTTAATCTGGTTT	55	2R
CMS2R-19	SLAF2R-31	TCCTTCTACATACTTGATT	ATAGTTACATTCTTTCTT	50	2R
CMS2R-20	SLAF2R-34	CAGCACCCCTGAGTGTAGC	CGGCGGAAGAAGATAGAG	62	1R-7R
CMS2R-21	SLAF2R-37	CACAAAGGTCTAATTTTAAAAG	GAATGGTTATGAAGGGGTT	53	1R-7R
CMS2R-22	SLAF2R-38	ATGACCTACCGTGACAAAC	GGATGAGGAGACAACTAAAA	58	2R
CMS2R-23	SLAF2R-40	CCCAACTGTGTTAGCCAAA	CATCGCTTCGGTGTTATTA	55	2R
CMS2R-24	SLAF2R-41	TTCCCAACTGTAAATGCTCCCA	CCCCCAGATCCCAGAAGGC	58	1R-7R
CMS2R-25	SLAF2R-47	TCTCTCTTTTCAGTGGTTCCG	TCCTGGTTCATGCTCTTGGC	62	2R

CMS2R-26	SLAF2R-48	TACGGGTGAAGAACCAAAAG	AATAGGGATTCGGGAGTTGA	55	1R-7R
CMS2R-27	SLAF2R-49	GCACGATAACACACTGGACAAC	GTCTCTCTTATTTCCCTTCCGA	55	2R
CMS2R-28	SLAF2R-50	CAAAAGGTAAATAAAAACAACAAG	CTATGTGCACTATCCCGATGA	55	1R-7R
CMS2R-29	SLAF2R-32	TATATTAAAAGATGAGAACCA	TAAACAATTAGAGTAACAGGA	50	2R, 3R
CMS2R-30	SLAF2R-33	CGAGATAAGAATGGGGAGG	AGAGGGGTCGTGAGGTGTA	60	2R, 4R, 5R
CMS2R-31	SLAF2R-39	CTCCATAAACCGAAAGGCTT	CTGGGTGAAGCCGAAAGC	58	1R-3R, 6R
CMS2R-32	SLAF2R-42	GGTTTGCTGCTTGGTTTTG	CACCGGATCCAACCTGCTTA	58	2R-7R
CMS2R-33	SLAF2R-45	CACACACATCCGTATGATTTTG	AGAAGTGGTGCGTGGGTAAG	58	1R-4R, 6R-7R
CMS3R-1	SLAF3R-3	CCCAGAGCCCTCATGCA	ATTTCATCTAGTCACCTTCATCAT	63	3R
CMS3R-2	SLAF3R-5	CACATTAATAAAAGTTCCAAA	AGCTATTATTCTTTCTTCTTGA	53	3R
CMS3R-3	SLAF3R-6	TTTGAATATATGTCATGAGTT	AGGACTAGAACACACGACT	55	3R
CMS3R-4	SLAF3R-7	CCAGCTATCTTCCCCCACG	TACATATGGCACGAGAGGACC	60	3R
CMS3R-5	SLAF3R-9	GTAGCTTCTGTTCCCGTGG	TTGTAAGCCAACCTATTTTTTCAG	62	3R
CMS3R-6	SLAF3R-11	AGTGAGAGGCGACGGAAGG	CCCAATTTAACGCAAAACCTC	65	3R
CMS3R-7	SLAF3R-12	TTAGGAGATTGACTAGAGTTGCT	TGTCTTGAAGTATGCGCC	60	3R
CMS3R-8	SLAF3R-14	TTGAGGCAAGGAATGCGAAAGAG	GTGTGACGCCCCGAGACCGA	68	1R-7R
CMS3R-9	SLAF3R-16	AACTTTGGTAACACATCCTCGTC	GTTATCAAACCTGAACCTCTCTCG	63	3R
CMS3R-10	SLAF3R-17	ATTTTGCAATGTGATTTCTCTGT	TAGTGAAAGTCCCTCTTCTGGTGG	62	3R
CMS3R-11	SLAF3R-18	CACCCAGTCTGACATGTTCC	GGCCCTGAAATGCTTGTGTT	65	3R
CMS3R-12	SLAF3R-22	AGCTACAGGTTGGGGAGGA	AGTTGCTTAGAATTTTCGAGGA	58	3R
CMS3R-13	SLAF3R-24	ACCTGTGGCTTTTATTTATTTG	GTGATTTTCATTGGTGTCTTCT	55	3R
CMS3R-14	SLAF3R-25	GGTAAAGAACCACACATGAACG	TATGTCAGAATGTTGCTGGAGG	58	3R
CMS3R-15	SLAF3R-26	ATACTTCGGTGCTCCCAAT	CACTCTCCCCTCTCATTCC	58	3R
CMS3R-16	SLAF3R-27	TACGAGGGTTTACCTGTCGG	ATTCCTCTTATGAAGAGTCAACTAC	58	3R
CMS3R-17	SLAF3R-28	TTTGTTCCACTTTGTTTCTATC	TTTCTAGGCTAAGTATTTTGCT	53	3R
CMS3R-18	SLAF3R-29	CCTTGGGGTAAGCGTTATATC	CCAAAAATGATGGGTCAATACT	55	1R-7R
CMS3R-19	SLAF3R-30	TTGTTGTATGTAGGACGCCGTG	GCAATCTTGCTAGCGAGTGGA	58	1R-7R
CMS3R-20	SLAF3R-31	GCCACCTCATAGCGAGACCC	GGCTTCGGATTATTGCACCT	63	3R
CMS3R-21	SLAF3R-33	CTCGCTGCTGAACGACTTAT	TTGGGCTCATCGATTTTATT	58	3R
CMS3R-22	SLAF3R-34	TTATGGAGTGCTCACCGTCA	AATATGTGCATTTAATCTGGATCT	53	3R
CMS3R-23	SLAF3R-35	GCTTTTTTCTGCTGCTATTG	TGTGGTTTATTTATCTAAGTTTGG	55	3R
CMS3R-24	SLAF3R-36	ATCACTTATCAATACCTTCTGC	CATATATAATTCTTTACCTCCG	53	3R
CMS3R-25	SLAF3R-37	GCGGAGAGGTCGAGACGTG	TGTTTCTTTCAATTTTTGTGCTCT	60	1R-7R

CMS3R-26	SLAF3R-38	TAAGAGCAGTTTTGATTTTGTC	ACGTGGTTTAATTACTTTTGAG	55	3R
CMS3R-27	SLAF3R-39	AGGAACGCAAAATCAAGGAA	CGACGACTCGACAGGAAAGA	58	1R-7R
CMS3R-28	SLAF3R-40	GAACTTTGAAATAAAATTTCTGGC	GCTCTTTTGTATGCTATGATGAT	55	1R-7R
CMS3R-29	SLAF3R-41	CATCAATGCTCGCTTCGTAA	CTCCATCAACCGCGTTCTAG	60	3R
CMS3R-30	SLAF3R-44	GAACATGGTGTGATAGATAGATTTA	TTTGAGACGCGCTACTGGT	55	3R
CMS3R-31	SLAF3R-46	ACTGTTTCGGTGTTGGTGCTTT	ATGTGTTGTGCCGGTGGATG	58	3R
CMS3R-32	SLAF3R-47	GCCCGATAGTGGTGATGTTTAC	AAGATTCATGGCTAGGGTGC	60	3R
CMS3R-33	SLAF3R-48	CCCCACCCCTGAAAATCG	AACGCTATGAAGTAGACGCAA	58	3R
CMS3R-34	SLAF3R-50	TCACAAACGTGGGATTCACC	TGGGAAAAGGTTTTGGATCAAGG	60	3R
CMS3R-35	SLAF3R-15	GAGACCCGAAGTCCAAGCG	TGAGTTCGTGCGGCAGATG	68	1R-4R, 6R, 7R
CMS3R-36	SLAF3R-45	TACATACCAGTTGGACACATTG	GTTTGTTTCAACCATACTTT	55	1R, 3R-7R
CMS3R-37	SLAF3R-49	AATTAGTGTCAATGAACCAGCC	AAATATTCGTTAGGATTGCAGG	55	3R, 5R-7R
CMS4R-1	SLAF4R-1	ATGAAAGGTAAAGAGTTCGTGC	GTGTTTGATGCCGAGAAGTG	62	1R-7R
CMS4R-2	SLAF4R-2	GAGGGATGTCGGGGGAGCAA	GGAACCCAAAAAGAACGAAGCA	67	4R
CMS4R-3	SLAF4R-3	GTTTCTACTAGGGGCGATTC	ATCTACACCAAAACACACGG	62	4R
CMS4R-4	SLAF4R-4	AGCCACCATAATTGATGCAGG	AGGCCAATCCACAACAAAGC	65	4R
CMS4R-5	SLAF4R-5	CTCGGTGATGATGTTTCGTCC	CTGCTTGCCTATAGCCCATG	65	4R
CMS4R-6	SLAF4R-6	ATGGACATAATTGAAAAGGAAA	ACAAGGGAATGGTAAAAAAGTT	60	4R
CMS4R-7	SLAF4R-7	GCATTCATCTTCCCCCAGTG	TAGGCGTGTTTGTTGATCTTCC	65	1R-7R
CMS4R-8	SLAF4R-8	CTCACAGTCTTTGATGCCTTCT	CTCGTTGCTATGCTTCTCCT	62	4R
CMS4R-9	SLAF4R-9	TTTTGTAGTGGAGGAGAGTTTG	GATCATTTTGACATATCATTTG	58	4R
CMS4R-10	SLAF4R-11	GAATGTGTTTGGCCCTTTCGG	CAGTGCAAATCCCAATTCAGGG	66	4R
CMS4R-11	SLAF4R-12	CCGTCGTGTCTGTGTCTCCT	TGCCCCCTTCTTTACCTCGT	65	4R
CMS4R-12	SLAF4R-13	ATATTGCTGTGGCTCTCCCG	CAGAAGATCAAGGAATTGGGGC	67	4R
CMS4R-13	SLAF4R-14	TTTAGTTAGCAGGGGTAGGTGG	TAGACAGAGTGATTGTTTCGGGG	63	4R
CMS4R-14	SLAF4R-15	CGCTTGAGCTTGTTCTGGTA	AAAGTCTCTGGCGCTATTGA	62	4R
CMS4R-15	SLAF4R-16	TAGCAAAAATGAAATATGAGAT	TGAACATAAAAAGGTGATGAAAG	58	4R
CMS4R-16	SLAF4R-17	ATGGCTTAGGCATGTGTATTTG	GCGATACTATGTGTCTTACCCAG	62	4R
CMS4R-17	SLAF4R-19	CACACTCTCCCTCTCGTTGAT	GGTGGCGGTCTCTTCTCTGA	65	4R
CMS4R-18	SLAF4R-21	AGTCGTTAATTTGACCAACCCA	GACGGATGCGGGAGGAAAT	58	4R
CMS4R-19	SLAF4R-22	GAATTGGAGGCATCCGGAA	GCTTATTTGCGGGAGCAGAC	60	4R
CMS4R-20	SLAF4R-23	TTGGGAAGTTCTCTTGTGTTGG	GGATTGGGCTATTTTGGTGC	58	4R
CMS4R-21	SLAF4R-24	AGGGCCGAAGTTCGATCTTT	GCTGCACCTTCTCTTTAGCTG	62	4R

CMS4R-22	SLAF4R-25	ACTCACTGCAGCATCCTCTAC	AGTATGTGGAGCCCAGTCTA	60	4R
CMS4R-23	SLAF4R-26	GCACCACCTATCGGAACCAC	GATCGACCACATCAAACACA	62	1R-7R
CMS4R-24	SLAF4R-27	ACAATGAAACAGGCAAGTGA	TCCATAATGAGGTATTCCGA	58	1R-7R
CMS4R-25	SLAF4R-28	AACACGATGTAGTTGCCCCCT	GCAGATAGAAGAAACCTCCC	58	4R
CMS4R-26	SLAF4R-33	CAGCGTTGATCGTTGTGAGG	TGCCAAGAGGCTACAAAAGGA	58	4R
CMS4R-27	SLAF4R-36	CACAAGTCAATCCTTCGGGAC	ACAACCTTTGTAGTTATCCAGTCTT	58	1R-7R
CMS4R-28	SLAF4R-38	ATTTTGGGGGCTTCACACAA	GCCGCTTGCTACTTTGAGTC	60	4R
CMS4R-29	SLAF4R-43	ACGAACACAACCTTCGGGCT	ACCGCTTTTCCCTCCAAAGA	60	4R
CMS4R-30	SLAF4R-44	ATTAGCCCCCAAGTGTCGAG	ACAAGTGATGAGTTAAGCCG	60	1R-7R
CMS4R-31	SLAF4R-45	TTATTTGTTGACTTATCATGGA	ATCAGCTCTGGACAGTATTTAT	52	4R
CMS4R-32	SLAF4R-46	TAGGAAAAGGGAATAAAAGC	TATGTAGAGATACACGGGGG	57	4R
CMS4R-33	SLAF4R-47	ACCACGAGGAGAAGTTTGCT	AGTAATCAATTGTTGGCCCTT	55	4R
CMS4R-34	SLAF4R-50	ATGCTTGATGTTTAGCCCGTG	TGGAGATGAGTGTGTGGCTCT	58	4R
CMS4R-35	SLAF4R-20	ACAACATCAAACGTCCGACC	TGCGAGAAAAACAAAGCATCAT	65	1R-5R, 7R
CMS4R-36	SLAF4R-30	CCTGGTATTGGTGATCCTGAA	GACAACGGCAACCCATTATTT	55	4R-7R
CMS4R-37	SLAF4R-31	GAATAAGCAGCCTCCACTAAG	AGCTCATACAGAAGACGAACC	58	4R, 6R
CMS4R-38	SLAF4R-34	AAAAAACCCCTACCCTCCTGAGTC	TCCCCCTTGCTGATCCCCCT	62	1R, 2R, 4R, 6R, 7R
CMS4R-39	SLAF4R-39	GGCTTGACGATGACCTCCTT	CCCATTACGTGGCTCCTACA	62	4R, 6R
CMS4R-40	SLAF4R-41	TGATGTTATTGCTGTTTTAGGA	TTGGACATATTGAACTTTGAAA	53	1R-6R
CMS5R-1	SLAF5R-2	ATGTTGAGCAAGTAAAGAGTCG	GGCAAAAGGTATCAAGGTATTC	60	5R
CMS5R-2	SLAF5R-3	AATGGAAAGCAAAACACGGG	GGATATGCCTGGTTGGATGA	65	5R
CMS5R-3	SLAF5R-4	TGGTCCCTTCGGCGATGG	TCCCGGAGGCAGCAACAA	68	5R
CMS5R-4	SLAF5R-5	TCATCCGAGTTACCGATTATT	CAGCACTTTCTGGGCTTCTT	62	5R
CMS5R-5	SLAF5R-6	GTCTGCTCCAGGACCCTTG	TCGTCATACCCAAGAAAGGC	65	5R
CMS5R-6	SLAF5R-7	AACAACGCGCGGGGTATCAT	CGTCTCCCTCACCGAAGCAA	68	5R
CMS5R-7	SLAF5R-8	AAGATACCAACTGAAACATAAGGA	CGATACAAGTAGGATCTAAAGGC	63	5R
CMS5R-8	SLAF5R-12	GGACGCCTTCCCCAACAT	AGCCGCTCCCCCTACAC	66	1R-7R
CMS5R-9	SLAF5R-15	CAAGAAAGGCAGAAAAGAGG	CCAGAGCTGTGATAAGGGTT	61	5R
CMS5R-10	SLAF5R-21	CTTCTTCGTCAATCATACCA	CCAAAACACCGACACTATTC	58	5R
CMS5R-11	SLAF5R-24	CAATGAAAAATGCCGCCTTAT	CCCATCGCCGTCTATACCC	60	5R
CMS5R-12	SLAF5R-25	TCTCTGACACCCGACGCACC	CGTCCCACCACACAGTTGA	65	1R-7R
CMS5R-13	SLAF5R-28	AAACTACATCTTTTTTGCCT	TAGCCTGGTTTTGACGATA	55	5R
CMS5R-14	SLAF5R-29	GTCCACTTGTGGTTATTTCTCA	GGAGGGTCTTCCGCTTCTAT	58	5R

CMS5R-15	SLAF5R-30	AGTTCACACCCCTATCCCCT	CGTTACATGATATACGCTGCTT	60	5R
CMS5R-16	SLAF5R-32	GAGCACTGGAGGAGGCGGTA	TTTGC GTTCTCGTCACATTCC	62	1R-7R
CMS5R-17	SLAF5R-34	TGTTATGCTCAAGTTTGGGTCT	ACCGATGTTCAATTTAGTGCA	53	5R
CMS5R-18	SLAF5R-35	AATGGTGCCATGTCATCATCTTA	AGCCTGGCCCCACCAAAAC	56	5R
CMS5R-19	SLAF5R-36	GTCGTTCTACGGATACACCTCA	ACCACGGGAAAAATCATTGC	58	5R
CMS5R-20	SLAF5R-37	TACAGTCCACTTGTGGTTATTTT	GGTAGGGTCTTCCGCTTCT	55	5R
CMS5R-21	SLAF5R-38	CATGCACATTAGAGTTGTTACCA	GCTTCAATCTTGTTCCTGTG	58	5R
CMS5R-22	SLAF5R-40	TCCCATTTCCCGGATATCAAA	TTCGGCGCTGGACTCATC	58	5R
CMS5R-23	SLAF5R-41	AGCTTAAAACCCCCAGCAAAT	AAAGCGTCAAATAGGGGCACA	55	5R
CMS5R-24	SLAF5R-42	GAAC TTGGCAGGAAATCCCC	AGTCTTGAAAACATGCCGAC	60	5R
CMS5R-25	SLAF5R-45	CACTTTGGTTATGTCCTCACG	CGATGTTTATCACAACCCGAC	55	1R-7R
CMS5R-26	SLAF5R-47	TACTCCAAGTTAGGACCCGTC	CGTTGCAGATGCCCTTAGAAT	58	5R
CMS5R-27	SLAF5R-49	TCCGCTGACTATGTGTGTTAC	CTTA ACTCGTTACTTGTCTCA	58	5R
CMS5R-28	SLAF5R-50	GGAAGGATTTGGAGCAAGGAGT	CGACGAGCAACAACAGAATGG	58	5R
CMS5R-29	SLAF5R-9	CGTAGGAGAGGAACCAGTCAGG	CACGAAGAAGGACGGCTGAA	66	1R, 3R-7R
CMS5R-30	SLAF5R-13	GAGCAAACCTATCAACAAG	GCATGAGTCTCAAAAATCCT	61	3R-6R
CMS5R-31	SLAF5R-16	GAATTAGTGTCAATGAACCAGC	AAATATTCGTTAGGATTGCAGG	61	3R, 5R-7R
CMS5R-32	SLAF5R-18	CCTGGCTCCTGTGACCGT	TCCTTCCATGCAAATATGTAGC	64	2R-7R
CMS5R-33	SLAF5R-20	CCTCCCTACTTTCTACCCCT	AGACTCAGGAAGGACGAAGC	61	1R, 3R-7R
CMS5R-34	SLAF5R-31	GGCTGTGTCTCCTTCGTCTT	TCCTTCGGAACCATCCCTAG	62	1R, 3R, 6R, 7R
CMS5R-35	SLAF5R-46	ACTCTAGTGCAAACCTCAATTGTTG	TCATGACACTTACACCTACAC	55	1R, 3R, 5R
CMS6R-1	SLAF6R-5	TTCGCAGCTAAACCTTCTTGG	CCTCCGGTGGTTCGTTAGTCCT	65	6R
CMS6R-2	SLAF6R-8	ACTCTGTGTTTCATCCCATACT	CAAATAATCAAGGGAGTCATC	58	1R-7R
CMS6R-3	SLAF6R-9	GTGGGCGATACTACGTGTCTT	TGTGTTTCCAGTGTCCCTTCT	63	6R
CMS6R-4	SLAF6R-10	CACTATTTGCCAGATGTTGTC	TGATCTTTTTACAGCAGCTCC	60	1R-7R
CMS6R-5	SLAF6R-11	TATGGAAATCCTGCACACTAG	TTGTGATTAAATATGATGTTGTT	58	6R
CMS6R-6	SLAF6R-15	CCAGGGTGGTGGAGTGTCT	TATAAACCTTGTGACACCTGCCT	65	1R-7R
CMS6R-7	SLAF6R-16	GGTAGACTGCAACTCTTGGTGT	AGTTATTTGGTAATCACGAAGC	60	6R
CMS6R-8	SLAF6R-17	AAGGAATCTATTCTATCCAGGC	GAGTTGTCAGCAGCTGGTAGT	60	6R
CMS6R-9	SLAF6R-18	TTGACACAGAGAAAGGGAAAG	CGTGTGCCATCTTAAAAAATC	61	6R
CMS6R-10	SLAF6R-20	AGTTACTGAGGGAAGCTGGAAT	AAATAGCGGTCACCTAATGGGA	63	6R
CMS6R-11	SLAF6R-22	TTCGTCTTCATCCATCGGTAA	TGCCCCAAACAAGTAGATCCAA	55	6R
CMS6R-12	SLAF6R-23	TTGAAGCAGTAAACAACAGAAA	ATATGAGCGCCCCAAGAAA	52	1R-7R

CMS6R-13	SLAF6R-24	GGACATTATGGAGTCGGAGCC	CGAACGTCCAAATAGTGCTGC	57	6R
CMS6R-14	SLAF6R-25	TCGACTCATGAGAAGACCCA	TTCCTAGGATTTACCATGACTT	58	6R
CMS6R-15	SLAF6R-26	TAGGAGCAGTCAACACAGGCAA	CGGCTACAAAAGTCGAAAGGAA	58	6R
CMS6R-16	SLAF6R-27	GCTTCTTCCTCGGTATCTCGTA	AATCTCCATCTATGCCGCAGTT	58	6R
CMS6R-17	SLAF6R-28	GCAGGAGGGGATGTTATGG	TATGTTGCACCTGTATTCTCA	58	6R
CMS6R-18	SLAF6R-29	ATAGGTAAACCAACGTTGAGC	CGATCACATGCGAATGACCC	58	6R
CMS6R-19	SLAF6R-30	AAGGAGATAAAACAGGGAAGGC	CCTATATCCTGAACCGGAATTG	58	1R-7R
CMS6R-20	SLAF6R-31	ATTCTTTGGATACCATTACAGG	TGAAAACAAAAGGACTACAGTG	55	6R
CMS6R-21	SLAF6R-33	GGTGCCAGCGATAACCCAAA	GCCGTATGCTTGTGACGTTT	62	6R
CMS6R-22	SLAF6R-35	TCTCGTGTTTTTCTTGTGCC	CTCGTGGTGACGCTGTTGTG	58	6R
CMS6R-23	SLAF6R-42	ACATCTTCCAACCCGATCTT	CACGCATAGCTTCACTCTCTT	58	1R-7R
CMS6R-24	SLAF6R-48	TCTACAAGGCCCGCGTTTC	CGTGGTGGTGATGAAGAATTTACC	60	6R
CMS6R-25	SLAF6R-49	TTAGTATGGCTTGTAATCTTGGA	CCTCTTGCACTCTGTTTTCTC	55	6R
CMS6R-26	SLAF6R-50	CCAGGGAATAACAGGGCGAT	AGCCCAAGCGAACACGAAA	58	6R
CMS6R-27	SLAF6R-13	TGGGCAACGATGAAAAAATA	CGAAGATCGAGGCAACAGTAT	65	1R-3R, 5R-7R
CMS6R-28	SLAF6R-34	AAAAACCCTACCCTCCTGAGTC	TCCCCTTGTCTGATCCCCCT	60	1R-4R, 6R, 7R
CMS6R-29	SLAF6R-37	TAAAGCGCCGACAAAGTTG	ATCTGAGGAGGGGGCATGG	60	1R, 2R, 4R, 6R, 7R
CMS6R-30	SLAF6R-38	AGGTGAGCCTGTTGCCTAAT	AAACCATCATAATACCAACCAGA	58	6R, 7R
CMS6R-31	SLAF6R-43	AACCTTGTTAGTGATGTCCTC	ATGTTATATGTCGTCAATGCA	55	5R, 6R
CMS6R-32	SLAF6R-45	CACCCTTGAGAGTCACGG	TTGGGGCGAAGCCTTAGT	60	1R, 3R-7R
CMS6R-33	SLAF6R-44	GCGGCAAAAAGAGTGGGATAA	AGCTGCAGTGAAAGAGAAGAGGAT	58	-
CMS7R-1	SLAF7R-1	GGTGCTCATATGCATGGCTC	GAGCGCTGACAAGCGAATAAC	65	7R
CMS7R-2	SLAF7R-2	CTCGAGCATCCCTGTGAGAT	CTCGAGCATCCCTGTGAGAT	65	7R
CMS7R-3	SLAF7R-3	TCTGCAATTCCAAATAGCGCA	TCTGGTTGTAACCTTCCCTGG	65	7R
CMS7R-4	SLAF7R-4	GCCGCAATAGTGCAGTTCTC	GCAGTGCAGTTGTGTTCTGTC	65	7R
CMS7R-5	SLAF7R-6	GCGTCAAGTGGGTGACAAAG	GGGCGAAGCCATTAACAATCG	67	1R-7R
CMS7R-6	SLAF7R-7	CAATTTTGCGGGCCGTTTTT	TAGAAGGAGGAGGCTCTTCGG	67	7R
CMS7R-7	SLAF7R-8	CTGGTTGATGACGCGTAGGTA	CAGCACGTGGCTCCATCAAT	67	7R
CMS7R-8	SLAF7R-11	TATCCCGACTTGTGCACGGA	GGAGCGCAGGCATACAGT	66	1R-7R
CMS7R-9	SLAF7R-12	AACCTCTATGCCCAGAGCAAC	TGTGGTCGCAGAGTGAAGAA	65	7R
CMS7R-10	SLAF7R-13	AGGAAAATCTACACCCGAGCC	TATGCACGATCTCGGTGGGA	66	7R
CMS7R-11	SLAF7R-14	TCATCAACGAGAGCCAGCTTA	AGGTGTTTGGTTGCATGTGTC	65	7R
CMS7R-12	SLAF7R-15	TGGGCAACACAAAGGACACA	TAGGCACTTCAACATCGGGG	67	7R

CMS7R-13	SLAF7R-16	AGGTGCCTACATTGTATCTTGTGTTG	TGCTATTTTCCTTCACTCTGCT	63	7R
CMS7R-14	SLAF7R-17	AGGTGCCTACATTGTATCTTGT	TGCTAGACTACATGACTTGGTCC	60	7R
CMS7R-15	SLAF7R-18	GTTTCATCAGATACAGAAAATGGTCA	TCGATCGACCGCTAACTGC	64	7R
CMS7R-16	SLAF7R-20	TAGGCACTTCAACATCGGGG	TGGGCAACACAAAGGACACA	67	7R
CMS7R-17	SLAF7R-22	CCTCACCACGCGAACAAGAA	AGGCATTCCACGATTTGCGA	60	7R
CMS7R-18	SLAF7R-25	TCTTTGATTCTTGGCGGGA	TCTCCAAGATAAGGCATCGGC	60	7R
CMS7R-19	SLAF7R-26	TCTTGTAGGAGAGACCCCG	GCTCTTACCATAACCGTTGCTC	60	7R
CMS7R-20	SLAF7R-27	CACAGATGGTGGTTGCGTTG	CACTGCTTGCTCGCTCAAAT	60	7R
CMS7R-21	SLAF7R-28	GGCGTTGCACCTTATATGCC	GGGGGCAAGTTACTCGTCTTT	60	7R
CMS7R-22	SLAF7R-29	CATATCCCGGAGCTAACGACC	GCGGCTCGGGTGTTATACAT	60	1R-7R
CMS7R-23	SLAF7R-30	TATCCCACACCATCTCATAAGCA	TCCGACCCCTTCAGATAGCC	60	7R
CMS7R-24	SLAF7R-31	TCGCCCTCCATGTGGTGA	GGGCTTCACAGACTTAGGAACA	60	1R-7R
CMS7R-25	SLAF7R-33	CGCTTCCGCTGACTATGTGT	TACCCCTGTTTTGTGCCTCTG	60	7R
CMS7R-26	SLAF7R-34	CCCCAAAACCATCAGCAACAG	CGCCCTTAGCATTGCATTAC	60	7R
CMS7R-27	SLAF7R-36	TCTGTTTCAGCCCAAATGCG	CTGGGGGCGACAAATACTCC	58	1R-7R
CMS7R-28	SLAF7R-37	CCTCCAAGAACTACGGCTCC	GGGGGCGACTTGATGTAGTG	63	7R
CMS7R-29	SLAF7R-38	CTTCTCGGTCTCCAGGTCGG	GTAGCCCCTGATGCTTTGTTTG	63	1R-7R
CMS7R-30	SLAF7R-39	GCTGCACTTGTCTTAATGGACG	GATTGGGTTTCCATGCCTCG	60	7R
CMS7R-31	SLAF7R-40	GCAGGAGTAGGGTTTTACGC	TTCTTGCGCTCCATGACACC	62	7R
CMS7R-32	SLAF7R-41	CACCACACTGGATTTGGGTATTA	TTGCTCGACTGAAGTTGCATTAA	58	7R
CMS7R-33	SLAF7R-42	TCGTCAGTTCAAGTTTGGTTA	ACAGAAGTGCGGTTATTTTTT	53	7R
CMS7R-34	SLAF7R-43	TTGCACCAGAATGGAAACCTT	AAGAAGGGGAGGATGATGAGG	58	7R
CMS7R-35	SLAF7R-44	TCCTCTGGTTGTATTCTGGTC	AGGTGAAACGATGAACTCTTAT	55	7R
CMS7R-36	SLAF7R-46	ATCATTTGCGCATTCACACCTC	TAGCACATCATCGACACGGA	53	7R
CMS7R-37	SLAF7R-47	AGGAATAACTTGGACTTGGCTAG	ACACAAAAGAGAGGATGGGGT	56	7R
CMS7R-38	SLAF7R-48	GAGCCAGACATGATTGTATTAGC	CAGACTCTTCTCTACCTCGACCT	60	7R
CMS7R-39	SLAF7R-49	AGCATCCGCAGATTTAGCC	CACCACCCGTTTTCTTTTA	58	7R
CMS7R-40	SLAF7R-50	CAAGCCGTCTGGTAAAGATAGT	TAATGCTTCCTTCAGCGTGTC	56	7R

Supplementary Table 2 The chromosome-specific molecular markers and PCR primers of *Th. elongatum*

Specific Markers	Specific Primers	Sequences of the Special Primers (5'–3')		Annealing temperatures	Amplified Chromosomes
		Forward	Reverse		
CMS1E-1	SLAF1E-1	CGGCTTGGTCGGGTTCTT	AACCCAAGATACTCAAACACCT	64	1E
CMS1E-2	SLAF1E-2	GCAAGCATAGTAAGGAGCAA	TAAGGTCGTCCCAACAGG	60	1E
CMS1E-3	SLAF1E-3	TAGCAAGCATAGTAAGGAGCAA	GAAGAACAGAGTCGCAAAA	59	1E
CMS1E-4	SLAF1E-4	CCTTCTGCCAATACAATACTCC	CTGGAACAGGCTCCTATACTTT	61	1E
CMS1E-5	SLAF1E-6	CTTGGCCCAGTTAAGGTGTT	TACCATGTAAGTTCCTCTAAGC	58	1E, 4E
CMS1E-6	SLAF1E-9	ATGGTGATGTCAGTATCCTTCTC	GATGGGGACACCCTTTGCT	64	1E
CMS1E-7	SLAF1E-10	GAGGCCCTGGCTTGTAAT	TTGCTCGATATGTTGCTGAATGTG	65	1E, 5E
CMS1E-8	SLAF1E-11	TGGTGGCTGCTACTTCTT	GCTATGCTTTACCCTCAA	54	1E
CMS1E-9	SLAF1E-12	GCTTCAAGACACTGACAGATGG	TTTTTGCGGGGAAGCTGGT	68	1E
CMS1E-10	SLAF1E-13	TTCTCCTCATCCATGCCAACC	TGCTGTGCGGGTGGAGT	68	1E
CMS1E-11	SLAF1E-16	CAGGGTTTTGGCAAAGTGAT	GCAAGCGATATGAGTGAAT	63	1E-7E
CMS1E-12	SLAF1E-17	TCTCCTCTGTTTCGCTGTCG	CCCGACGAAGTTCAAGGAC	64	1E, 3E
CMS1E-13	SLAF1E-18	TGAACAAGATGCCAGATAGG	TCGCAGCTGGTAATAGTCAT	60	1E
CMS1E-14	SLAF1E-19	TATCCACATGGCAAAACCT	CAAAAGACCAAGATATCCAGGC	63	1E
CMS2E-1	SLAF2E-1	TGCCTTCTTGCTGATTTGAT	TCTTGCGAGATTTGTGTGCT	63	1E-7E
CMS2E-2	SLAF2E-2	ACATACAGCCCACTACTACGC	CCAGGGACCCGATAGTAAAT	61	4E, 7E
CMS2E-3	SLAF2E-3	GAGTCTGCGTCAAGAAGTGC	GCTTCAATGGGTTTCATAGGTG	63	2E, 6E
CMS2E-4	SLAF2E-4	AGAATCAGCGGGTTCATCT	GTAGCGAGTAGAAACAGGGAT	60	2E
CMS2E-5	SLAF2E-5	GCCTCTTTGTTAGATCATCAG	CTCTGCGTTTCCACCAC	59	2E, 6E
CMS2E-6	SLAF2E-6	CTGCCTTGTTCTTTACTCGC	CTCGTCCTGTCCGTCAT	61	2E, 6E
CMS2E-7	SLAF2E-7	GCCTTGTTCTTTACTCGC	CTCGTCCTGTCCGTCAT	59	2E, 6E
CMS2E-8	SLAF2E-9	TGGTCTTCCCTTGCTCTGT	GCCCTCTTCCACCATTACTT	62	1E-7E
CMS2E-9	SLAF2E-14	CCAAACCCTAACAAATCG	CATGGCTAGTGTAGTTTATCTTCC	59	2E
CMS2E-10	SLAF2E-17	AACACTGGCAGTAGTTCATG	CAGCCACTCCGACAAAC	59	1E-7E
CMS2E-11	SLAF2E-18	CGGAGACCCTGTTGAAGTA	TACCAACTATACCTTGCTCCA	60	2E, 6E
CMS2E-12	SLAF2E-19	ACAAGGCTTACATGCTCG	TATGCTCCAAAGATACACC	57	1E-7E
CMS2E-13	SLAF2E-20	GATGTGATGTGCGGTGCTG	GCGTATTTATTTGTTTCTTCTCCC	65	1E-7E
CMS2E-14	SLAF2E-22	TTTGATGGCGACTTTAGG	CTTGGTTCTGACCCTTT	60	2E
CMS2E-15	SLAF2E-23	TCAGGAAGGAGAAGGAGCAG	CCACGAGGACGACAAAGGT	64	2E

CMS2E-16	SLAF2E-24	GCACCCTTGTAATCGAGTTC	GCAACGGCAATGACAGAAT	62	2E
CMS2E-17	SLAF2E-25	AGTCTCGCTATTTTCGCCTT	TGAACACTGAATCCTGCTGC	63	2E
CMS3E-1	SLAF3E-1	TGCGCTCGGAGATTTTAGTC	CCGTTCCGGTGCAACATACAT	65	3E
CMS3E-2	SLAF3E-2	CTGACTTCTCGCACGGATTG	CCACTCAGCCAACCAGAT	64	1E-7E
CMS3E-3	SLAF3E-3	TGGTCTTCCCTTGCTCTGT	GCCCTCTTCCACCATTACTT	62	1E-7E
CMS3E-4	SLAF3E-5	CTCCGAGAAAAGAAACGC	TGTCGGAGTTCGTTTGC	60	1E, 3E
CMS3E-5	SLAF3E-6	GTCTACTTTAGCACCTCACTCA	AAATTCGTCCGGTTGTT	58	3E
CMS3E-6	SLAF3E-7	CTACTTTAGCACCTCACTCAT	AAATTCGTCCGGTTGTT	57	3E
CMS3E-7	SLAF3E-12	GTTGGGGGAATCATGGTTTGT	ACATCTCCAACACTCTCCAGGT	66	3E
CMS3E-8	SLAF3E-13	GACTCTTGGGCTGGTAAATAT	CATATTCTTGACAGTTAGACCAT	58	3E
CMS3E-9	SLAF3E-15	GCTTCCCGATTTTACGCTTT	GTGGCAATCTGGAGAAAGCAA	65	3E
CMS3E-10	SLAF3E-16	GACACCACAGGGTCCTAAAGT	CCTTACCTGCCTGAGTTTCC	62	3E
CMS3E-11	SLAF3E-17	CTTTTGGGATGCTTGACCG	CCTTACCTGCCTGAGTTTCC	65	3E
CMS3E-12	SLAF3E-18	GAAGGTTTCGCCAACTGTGCT	CTATTCACCCAAGCAGGAGT	64	3E
CMS3E-13	SLAF3E-19	TCTCAATACCGAGGAGGGAT	CGAGGTCATCTATGGCTTCAAT	63	3E
CMS3E-14	SLAF3E-20	CAACGGAAGGAGGGAAGCAT	GATGCTCGTATGGTTGGCTT	66	1E-7E
CMS3E-15	SLAF3E-21	AAACAGCATTCAGAACCCAC	TGTCAGTCCAAGGAAACCC	62	1E, 2E, 3E, 6E
CMS3E-16	SLAF3E-22	ATAGGAAATGGAGGGGAGAT	CAACAGCAATACCAAACAGG	60	1E-7E
CMS3E-17	SLAF3E-23	GGATTACAGGCCAGCATAT	CTTCGGTGATAACCTTTGCT	62	3E
CMS3E-18	SLAF3E-25	GAATGTGGAATGTCCCGAGT	GCATCAGGATGAACTGCTCTAG	63	1E-7E
CMS4E-1	SLAF4E-1	GATTTCTGGGTATCGGCTCT	AGTCATTTCCGACATCCACC	63	1E-7E
CMS4E-2	SLAF4E-3	CAAAAGCACCTAACTCACCG	ACCAGGCTCTGTCCACTTAT	61	4E, 7E
CMS4E-3	SLAF4E-4	AAGTATGGAGACGGGAGACAGA	GGTAGCCCAGCAGGGTTTAT	64	7E
CMS4E-4	SLAF4E-5	CAACGGAAGGAGGGAAGCAT	ATGGATGCTCGTATGGTTGG	66	1E-7E
CMS4E-5	SLAF 4E-13	GACAAGAAGACTGAAGATCGACA	TGACAGATTAGGACGAACGC	62	4E
CMS4E-6	SLAF4E-14	GCCGTCATGTCTGCGTAGGT	AAGCAACACCTCGTTAGCAG	64	4E, 7E
CMS4E-7	SLAF4E-15	CGCCACCTGACCAAGTTTCT	CAAAGTCATTCCAACAACCC	64	4E, 7E
CMS4E-8	N ₂₈ -4E ₆₀₃	ATTCTCGCTCGCCACTAC	TGTATGCC ATCGAGCTATGTATT	60	4E
CMS5E-1	SLAF5E-1	CCTCCTGCCCTCTTATGTCC	TCTACATGCCGAGGAGCCTG	66.5	5E
CMS5E-2	SLAF5E-5	GTCCCCAGGGTGCATAACT	CGAAAAGATACAATGCGAGT	61.5	1E, 4E, 5E, 6E
CMS5E-3	SLAF5E-6	TGATTTCGTAGGAGGAAGCAAG	TTGTTGAGTGCTACTTCCGTC	62.5	5E
CMS5E-4	SLAF5E-7	GCAAGCCTTCACCCACACAT	TGGCATGTTTTTTGGCACTT	66	5E
CMS5E-5	SLAF5E-8	CTTGAGCCATAAAGCAGGAC	TTCGGTGAAGCTCTGAACTCG	64	2E, 5E, 6E

CMS5E-6	SLAF5E-10	AATTCCCACAAAGGTTCCAC	TATTGTAGGTCGCTCGCTTG	62.5	5E
CMS5E-7	SLAF5E-11	GTGGCGACAGCACCTTCTT	CCAAAGGCAGGAGTAGGGTT	65	5E
CMS5E-8	SLAF5E-12	CATCCACCACAAATAGGAGAAC	TGGCATGTTCCGCCAAGTGT	66.5	5E
CMS5E-9	SLAF5E-13	ATGACGACGATGACACCGAC	CATTTACTCACACGCCAACACT	65	5E
CMS5E-10	SLAF5E-14	TTAGCAGAGAGGTCAACACG	AGGGCACCCGAGAGAGATAT	62	1E-7E
CMS5E-11	SLAF5E-15	GATCCTCACCTCCTCAATTTT	TATCTTCTGGACCTTGTTTGC	60.5	5E
CMS5E-12	SLAF5E-16	CAGGCGAGGGAGCACTTGAT	GAATGTTCTGCCTCGTATG	65.5	5E
CMS5E-13	SLAF5E-19	CCACCACAAATAGGAGAACT	ATGTTCCGCCCAAGTGTCTT	60.5	5E
CMS5E-14	SLAF5E-21	TACACAGTGAGTTGGCAGCAT	TGAGGAAGCTGGCTTTGATAA	63.5	1E-7E
CMS5E-15	SLAF5E-22	CTCCCAGTATGGAAGATGCC	GGACTCCGTTTGGTATTGAT	62	5E
CMS5E-16	SLAF5E-23	AGAGCAAATTCCTGCACAAT	CCACCAAATCTGCCCATTAT	65.5	5E
CMS5E-17	SLAF5E-25	TCAAATTCAACGGTGACAGG	TGCCGTCTCTACAACCCT	64	5E
CMS6E-1	SLAF6E-1	GAGTCTGCGTCAAGAAGTGC	TCAATGGGTTTCATAGGTGTGTC	63	2E, 6E
CMS6E-2	SLAF6E-2	GGGTCAGCCAACAACACAAG	ATCCACCTTTTTCGCTTCTG	65	6E
CMS6E-3	SLAF6E-3	ACTATTTCCGTTGCCCTAT	GTGCAGCAGTGGTGATATTTT	61	2E, 6E
CMS6E-4	SLAF6E-4	GATTTCTGGGTATCGGCTCT	AGTCATTTCCGACATCCACC	63	1E-7E
CMS6E-5	SLAF6E-6	GCCTCTTTGTTAGATCATCAG	CTCTGCGTTTCCACCAC	59	2E, 6E
CMS6E-6	SLAF6E-7	GCTGCCTTGTTCTTTACTCG	TCTCTCGTCTGTCCGTCAT	63	2E, 6E
CMS6E-7	SLAF6E-8	CTGGTCTTCCCTTGCTCTGT	GCCCTCTTCCACCATTACTT	63	1E-7E
CMS6E-8	SLAF6E-9	TGGAAGTCTATGCGGTCAAT	GTCGAGAACCCAAGTGGAAT	62	6E
CMS6E-9	SLAF6E-10	ATGGGGGGCGTAAAAAGAC	GCATACAGACAAGATGTGGAGT	63	2E, 5E, 6E
CMS6E-10	SLAF6E-11	ATGTTGGGGAGGAAGGAGAT	TGTGAGAAACGTGAAACCT	64	2E, 6E
CMS6E-11	SLAF6E-12	ACCAACATGACGCCAGTGAT	TGCCACCTAGCTACACCTTG	64	2E, 6E
CMS6E-12	SLAF6E-15	AGTAATTGGAGACCCAGGCG	CAAATCAAACGACCATCACA	64	6E
CMS6E-13	SLAF6E-16	CGGAGACCCTGTTGAAGTAG	TGTGATTTAGGAGCTGGTGT	61	2E, 6E
CMS6E-14	SLAF6E-17	AAAGGGGATGTGATGTGCGG	TAGGGCACTCCTCGAACAAC	67	2E, 6E
CMS6E-15	SLAF6E-18	CAACACCACATGAAGCCACC	GCAAAAATGTGAAGCCGAGG	67	2E, 6E
CMS6E-16	SLAF6E-19	AATTGCGGTTAGGGTTCT	GGTCCAGCTTCCCATTATAT	59	6E
CMS6E-17	SLAF6E-20	TGATCGGCTTCCTTCCTATT	TGATTGCATCTTCCCGTTCT	63	2E, 6E
CMS6E-18	SLAF6E-21	TTTTGACATTTAGGCTTCGC	TGATGTTGCCCGAGTAAGAG	62	6E
CMS6E-19	SLAF6E-22	AGGTCTCGGCAAGGCACAAC	GTCTTCTCCTCTTCCTCGCC	66	2E, 6E
CMS6E-20	SLAF6E-23	ATCAGGCTAAGAATCCAGTC	TGACTTGATGGTTGGGACT	59	2E, 6E
CMS6E-21	SLAF6E-24	ACAGTAGCTTGTTTGGGTCAG	CCCTCTTAGACTCTGTCATTCTG	61	6E

CMS7E-1	SLAF7E-1	CATCATCCCTCAATACCGC	GGAGACCGCAATACCAAAT	62	7E
CMS7E-2	SLAF7E-3	GATTTCTGGGTATCGGCTCT	AGTCATTTCCGACATCCACC	63	1E-7E
CMS7E-3	SLAF7E-4	TGGTCTTCCCTTGCTCTGT	GCCCTCTTCCACCATTACTT	62	1E-7E
CMS7E-4	SLAF7E-6	TTATGCTCATGGTTAACTGG	ACTCCGACAAACTCCCCTT	62	1E-7E
CMS7E-5	SLAF7E-7	GCCTCATTACATCCAGTCCC	GGTCTGCGGCTAGAATCAAG	64	7E
CMS7E-6	SLAF7E-8	GCCTCATTACATCCAGTCCC	CAATCGGCTTGCGGTCT	64	7E
CMS7E-7	SLAF7E-9	GGGCAGGAGTGAATCAGAAT	CTTGCTCCACAAAAATCCGC	65	7E
CMS7E-8	SLAF7E-11	GAAATTGCTGACCCTGGACAT	GCAACAACACTCGCAATGGAAC	67	7E
CMS7E-9	SLAF7E-13	ACGCCCACATGCACATACCT	TTATGAACGCTTCTCGCCCT	66	7E
CMS7E-10	SLAF7E-14	ATACCATCACGGTCCAACAT	TCCTCTATCAGCTAGTCGCC	61	7E
CMS7E-11	SLAF7E-15	CCATCATCTGTTGCCCTTCA	TTTTGTGGGAATATCTAGCG	62.5	7E
CMS7E-12	SLAF7E-16	TAGGCAAGCCATTCTCGGT	TGACGCCTTCTCAGCCACAT	66	7E
CMS7E-13	SLAF7E-17	TCTTCTCCCTCCTTGTTGCT	CATTGGCAAGGCACATCAGT	64	2E, 7E
CMS7E-14	SLAF7E-18	TATCTTGGGCTCCCTACTCC	CTGGTTGAACAGCCGAAGGT	64	7E