Intron gain by tandem genomic duplication: a novel case and a new version of the model

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Origin and subsequent accumulation of spliceosomal introns are prominent events in the evolution of eukaryotic gene structure. Recently gained introns would be especially useful for the study of the mechanisms of intron gain because randomly accumulated mutations might erase the evolutionary traces. The mechanisms of intron gain remain unclear due to the presence of very few solid cases. A widely cited model of intron gain is tandem genomic duplication, in which the duplication of an AGGT-containing exonic segment provides the GT and AG splicing sites for the new intron. We found that the second intron of the potato RNA-dependent RNA polymerase gene *PGSC0003DMG402000361* originated mainly from a direct duplication of the 3′ side of the upstream intron. The 5′ splicing site of this new intron was recruited from the upstream exonic sequence. In addition to the new intron, a downstream exonic segment of 178 bp also arose from duplication. Most of the splicing signals were inherited directly from the parental intron/exon structure, including a putative branch site, the polypyrimidine tract, the 3′ splicing site, two putative exonic splicing enhancers and the GC contents differentiated between the intron and exon. We propose a new version of the tandem genomic duplication model, termed as the partial duplication of the preexisting intron/exon structure. This new version and the widely cited version are not mutually exclusive.

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ABSTRACT

Origin and subsequent accumulation of spliceosomal introns are prominent events in the evolution of eukaryotic gene structure. Recently gained introns would be especially useful for the study of the mechanisms of intron gain because randomly accumulated mutations might erase the evolutionary traces. The mechanisms of intron gain remain unclear due to the presence of very few solid cases. A widely cited model of intron gain is tandem genomic duplication, in which the duplication of an AGGT-containing exonic segment provides the GT and AG splicing sites for the new intron. We found that the second intron of the potato RNA-dependent RNA polymerase gene *PGSC0003DMG402000361* originated mainly from a direct duplication of the 3′ side of the upstream intron. The 5′ splicing site of this new intron was recruited from the upstream exonic sequence. In addition to the new intron, a downstream exonic segment of 178 bp also arose from duplication. Most of the splicing signals were inherited directly from the parental intron/exon structure, including a putative branch site, the polypyrimidine tract, the 3′ splicing site, two putative exonic splicing enhancers and the GC contents differentiated between the intron and exon. We propose a new version of the tandem genomic duplication model, termed as the partial duplication of the preexisting intron/exon structure. This new version and the widely cited version are not mutually exclusive.

INTRODUCTION

Although, spliceosomal introns are the characteristic feature of eukaryotic nuclear genes, their origin and subsequent accumulation during evolution remain obscure. Several models of spliceosomal intron gain have been proposed, including intron transposition, transposon insertion, tandem genomic duplication, insertion of an exogenous sequence during double-strand-break repair, insertion of a group II intron, intron transfer and intronization (Yenerall & Zhou 2012). Comparative analyses of discordant intron positions among conserved homologous genes have been carried out in diverse eukaryotic lineages. Although intron gains are generally reported at a lower frequency than intron losses, the reported intron gains have been accumulated to a considerable number (Csuros et al. 2011; Fablet et al. 2009; Hooks et al. 2014; Irimia & Roy 2014; Li et al. 2009; Li et al. 2014; Roy & Gilbert 2005; Roy & Penny 2006; Torriani et al. 2011; van der Burgt et al. 2012; Verhelst et al. 2013; Yenerall et al. 2011; Yenerall & Zhou 2012; Zhu & Niu 2013a). Unfortunately, the source sequences of most of these reported intron gains have not been identified. As a consequence, these intron gains provide very limited supporting evidence for the intron gain models. Collemare et al. (2013) claimed that the abundance of introns in extant eukaryotic genomes could not be explained by traditional models of intron gain, but can be possible by a new model, the insertion of introner-like elements (van der Burgt et al. 2012). Among the traditional models, intron gain by tandem genomic duplication is not expected to occur rarely, because frequent internal gene duplications are observed (Gao & Lynch 2009). This model was originally put forward by Rogers (1989), suggests that tandem duplication of an exonic segment harboring the AGGT sequence generates two splice sites for the new intron: 5′-GT and 3′-AG. In this model, the new intron comes from the duplication of an exonic sequence and the translated peptide is not altered by the intron gain. An example strictly

consistent with this model is in the vertebrate gene *ATP2A1* (Hellsten et al. 2011). The duplicated region of *ATP2A1* not only has the AGGT signal, but also happen to include a polypyrimidine tract and a branch point. In addition to it, the birth of the intron has been successfully recapitulated in a conserved paralogous gene, *ATP2A2*, by Hellsten et al. (2011). In fission yeasts, multiple tandem duplication of a 24 bp exonic segment containing AGGT occurred in genes *SPOG_01682* and *SOCG_00815*. Comparison of these two genes with their expressed sequence tags indicates an intron across four duplicates in the gene *SPOG_01682* and an intron across two duplicates in the gene *SOCG_00815* (Zhu & Niu 2013b). In these two cases, intronization of the duplicated region possibly alleviated the potential negative effects of the duplications on the translated proteins. In the present study, we found a new intron gained by duplicating a gene segment across an intron-exon boundary in a potato RNA-dependent RNA polymerase (*RdRp*) gene. The *RdRp* genes encode those enzymes which catalyze the replication of RNA from an RNA template. They have been identified in all the major eukaryotic groups and play crucial roles in the regulation of development, maintenance of genome integrity, and defense against the foreign nucleic acids (Willmann et al. 2011; Zong et al. 2009).

MATERIALS AND METHODS

The genome sequences and annotation files of domesticated potato (*Solanum tuberosum*, PGSC_DM_v3), domesticated tomato (*Solanum lycopersicum*, ITAG2.3), wild tobacco (*Nicotiana benthamiana*, version 0.4.4), and wild tomato (*Solanum pennellii*, spenn_v2.0) were downloaded from Sol Genomics Network (Bombarely et al. 2011), and those of pepper (*Capsicum annuum* L., Zunla-1) were downloaded from the Pepper Genome Database (Qin et al. 2014). The scaffold sequences of Commerson's wild potato (*Solanum commersonii*,

JXZD00000000.1), wild tomato (*Solanum habrochaites*, CBYS000000000.1), and eggplant (*Solanum melongena*, SME_r2.5.1) were downloaded from the NCBI Genome database (http://www.ncbi.nlm.nih.gov/genome/). The SAR files of the whole-genome shotgun (WGS) reads (SRP007439) and the leaf, tuber, and mixed-tissue transcriptomes (SRP022916, SRP005965, SRP040682, and ERP003480) of *S. tuberosum* were retrieved from the Sequence Read Archive of NCBI (http://www.ncbi.nlm.nih.gov/sra/). We mapped the RNA-Seq reads to the genomes using TopHat version 2.0.8 (Kim et al. 2013), while BWA (alignment via Burrows-Wheeler transformation, version 0.5.7) (Li & Durbin 2009) was used for the WGS reads. We used default parameters for both programs except that the minimum intron length was adjusted to 20 bp for TopHat. The orthologous genes of the *S. tuberosum RdRp* gene *PGSC0003DMG402000361* were identified by using the best reciprocal BLAST hits with a threshold E value of $< 10^{-10}$. In addition, the orthologous relationship between the gene *PGSC0003DMG402000361* and its ortholog in *S. lycopersicum* was confirmed by their synteny using the SynMap (http://genomevolution.org/CoGe/SynMap.pl). The orthologous sequences of the gene *PGSC0003DMG402000361* in *S. commersonii*, *S. habrochaites*, *S. melongena* were manually annotated with references to the annotations in *S. tuberosum*, *S. lycopersicum*, *C. annuum*, and *N. benthamiana*.

We found that the intron gain was involved in a duplication using BLAT search (Kent 2002) and then identified the exact duplicated sequences using the programs REPuter (Kurtz et al. 2001) and Tandem Repeats Finder (Benson 1999).

By aligning 9,883 groups of orthologous mRNAs among *S. tuberosum*, *S. lycopersicum*, and *C. annuum*, we found all the introns conserved among these three species. After filtering them with a length of > 60 bp in *S. tuberosum*, 34,364 groups of conserved introns were retained.

Among these conserved introns, we searched the consensus sequences of the 5′ splicing sites, the branch sites, the polypyrimidine tracts, and the 3′ splicing sites according to Irimia and Roy (2008) and Schwartz, et al. (2008). Sequence logos were generated using the WebLogo 3.4 online (http://weblogo.threeplusone.com/create.cgi) (Crooks et al. 2004) from multiple alignments of the 34,364 conserved introns in potatoes. The exonic splicing enhancers (ESEs) of *Arabidopsis thaliana* were identified by Pertea et al. (2007). We used them as query and searched 50 bp exonic sequences upstream and downstream of the target intron.

The phylogenetic tree of the gene *PGSC0003DMG402000361* and its orthologs was constructed using MEGA 6.0 by employing the Neighbor-Joining method (Tamura et al. 2013). The tree topology is consistent with the species tree constructed by Särkinen et al. (2013). The schematic diagram of gene structures was drawn using the program GSDraw (Wang et al. 2013).

RESULTS AND DISCUSSION

By comparing the orthologous genes of *S. lycopersicum*, *S. tuberosum*, and other Solanaceae plants, we found 11 cases of precise intron loss and six cases of imprecise intron loss (Ma et al. 2015). At the same time, we found the sign of an intron gain in the *S. tuberosum* gene, *PGSC0003DMG402000361* (Fig. 1). According to the potato genome version PGSC_DM_v3, this gene has eight introns and nine exons. By comparing the annotations of other Solanaceae genomes, we manually annotated 16 exons in the orthologous gene in *S. commersonii* (Fig. 2). The orthologous genes in *S. lycopersicum*, *S. habrochaites*, *S. pennellii*, *S. melongena*, *C. annuum*, and *N. benthamiana* have 15, 15, 16, 15, 18, and 17 exons, respectively. The second introns of *S. tuberosum* and *S. commersonii* are absent from other Solanaceae genomes. Meanwhile, the third exons of these two species have sequences similar to the upstream ones as

well as the second exons of other Solanaceae species (Fig. 2). By analyzing the transcriptomic data of *S. tuberosum*, we found 106 RNA-Seq reads that are exclusively mapped to the annotated exon-exon boundary (Supplemental Information 1: Table S1; Supplemental Information 2: Fig. S1), which confirmed the annotation of this intron.

Based on the phylogenetic tree constructed using the gene *PGSC0003DMG402000361* and its orthologs (Fig. 2), there were two possible explanations for the presence/absence of the intron. The first was the gain of a new intron in the common ancestor of *S. tuberosum* and *S. commersonii*, and the second was four intron loss events independently occurred in the other four evolutionary branches: tomatoes (including *S. lycopersicum*, *S. pennellii*, and *S. habrochaites*), *S. melongena*, *C. annuum*, and *N. benthamiana*. According to the principle of parsimony, we concluded that the second intron of the gene *PGSC0003DMG402000361* was gained after the divergence of potatoes (*S. tuberosum* and *S. commersonii*) from other *Solanum* plants, but prior to the divergence between *S. tuberosum* and *S. commersonii*.

The new intron and the inserted exonic sequence (Fig. 1) was used as a query sequence against the whole genome of *S. tuberosum*. We found that this insertion is a tandem genomic duplication (Fig. 3A). The major part of the new intron and inserted exon region is a direct duplicate of the upstream intron-exon structure (Fig. 3B). Meanwhile, 10 nucleotides at the 5′ end of the new intron was recruited from the upstream exon (Fig. 3A). We were aware of the fact that two nearly identical regions in a reference genome might either be a true duplication or a false due to an error in genome assembly. To verify the duplication, we found three sources of evidence in *S. tuberosum*. Firstly, 53 WGS reads were exclusively mapped crossing the three boundaries of two duplicates (Supplemental Information 1: Table S2; Supplemental Information 2: Fig. S2-S4). Secondly, 106 RNA-Seq reads were exclusively mapped crossing the exon

147 boundary of the mature mRNA (Supplemental Information 1: Table S1; Supplemental
148 Information 2: Fig. S1). The exon boundary sequence would not exist in mature mRNA if the
149 duplication did not happen. Thirdly, there are ten nucleotides different between the duplicates
150 (Fig. 3B).

151 Close examination of the coding region confirmed that the duplication did not cause any
152 frame-shifts. Furthermore, using the phylogenetic tree of *PGSC0003DMG402000361* and its
153 orthologous genes in tomato, pepper, and tobacco, we performed a likelihood-ratio test (LRT) to
154 compare two hypotheses. The null hypothesis is that the gene is actually a pseudogene and so
155 was undergoing neutral evolution, in which case the d_N/d_S value of *PGSC0003DMG402000361*
156 would be equal to one. In the alternative hypothesis, the gene is still functional and under
157 purifying selection, in which the estimated value of d_N/d_S would be < 1 (Yang 2007). The d_N/d_S
158 that we observed was 0.3101; the LRT statistic, $2\Delta\ell$ (twice the log likelihood difference between
159 the two compared models), was 74.7; and the χ^2 test supported the second model ($P < 10^{-16}$).
160 Although this result indicates that this protein-coding gene is still functional after the duplication,
161 we do not think that producing functional proteins is a prerequisite in the identification of a
162 sequence as a new intron. An intron is defined by its being spliced out during the maturation of
163 any RNA molecules, including both protein-coding mRNAs and noncoding RNAs. In recent
164 years, numerous sequences have been found to be spliced out of long noncoding RNAs, and been
165 described as introns without any debate (Derrien et al. 2012; Guttman et al. 2009; Jayakodi et al.
166 2015; Kapusta & Feschotte 2014).

167 According to Logsdon et al. (1998), strong evidence of intron gain must satisfy the two
168 conditions. The first one is a clear phylogeny to provide support for the intron gain, while the
169 second is an identified source element of the gained intron. Given the clear phylogeny and the

identity of the source sequence, we consider the second intron of the potato gene *PGSC0003DMG402000361* to be a well-supported case of a newly gained intron.

The present case of intron gain is somewhat different from the tandem genomic duplication model of intron gain that was originally put forward by Rogers (1989). In that model, tandem duplication of an exonic segment harboring the AGGT sequence generates two splice sites for the new intron: 5′-GT and 3′-AG, and the new intron comes from the duplication of exonic sequence. It is now well known that the two splice sites do not contain sufficient information to unequivocally determine the exon-intron boundaries (Lim & Burge 2001). Accurate recognition and efficient splicing of an intron also requires a polypyrimidine tract, an adenine nucleotide at the branch site, and many other *cis*-acting regulatory motifs (Schwartz et al. 2009; Spies et al. 2009; Wang & Burge 2008; Wang et al. 2004). In addition, introns are often remarkably richer in AU than exons (Amit et al. 2012), and this difference has been demonstrated to be a requirement for efficient splicing (Carle-Urioste et al. 1997; Luehrsen & Walbot 1994). At the first glance, it seems unlikely for a coding segment to have a full set of the splicing signals. Contrary to this expectation, intronization of coding regions has been observed in several different organisms including both animals and plants (Irimia et al. 2008; Kang et al. 2012; Szczesniak et al. 2011; Zhan et al. 2014; Zhu et al. 2009). These observations indicate that it is possible for coding sequences to contain cryptic splice signals. Furthermore, an experimentally duplicated coding segment of the vertebrate gene, *ATP2A2*, has been shown to be successfully spliced out of the mature mRNA (Hellsten et al. 2011). Therefore, a full set of the splicing signals require for active splicing is present in the coding sequence of the gene *ATP2A2*. Although a full set of the splicing signals could preexist in coding sequences, we believe that utilization of the active splicing signals of the parental intron/exon structure is a more efficient method of intron gain. In

193 the potato gene *PGSC0003DMG402000361*, the duplication includes the 3′ side sequence of an
194 intron and the 5′ side of the downstream exon (Fig. 3A). The 3′ splicing site signal (CAG), the
195 polypyrimidine tract (TCTTCCAATGCCT), and the putative branch site (TTTAC) of this novel
196 intron was inherited from the parental intron (Fig. 3B, 3C). Moreover, the two overlapped
197 putative ESEs of the 3′ flanking exon, TCAGCT and CAGCTC, and the GC contents
198 differentiated between the intron and exon (36% *vs.* 46%) were also inherited from the parental
199 copy. The 5′ splicing signal of the novel intron, GTAAG, was activated from a cryptic splice site
200 which was recruited from the upstream exon. One putative 5′ ESE, GAGGAA, has been
201 identified in the 5′ flanking exon of this new intron. Before the duplication event, the signal
202 GAGGAA was 73 bp far from its downstream intron. It was more likely a cryptic ESE than an
203 active one. The duplication event made it close to an intron and so ready to act as an ESE.
204 Therefore, we propose a new version of the tandem genomic duplication model, termed as partial
205 duplication of a preexisting intron/exon structure. Apparently, the traditional version of the
206 tandem genomic duplication model and this new version is not mutually exclusive. Each of them
207 might account for some cases of intron gain in evolution. Segmental duplication containing
208 entire introns would be more likely to increase the intron number of genes and also has been
209 observed previously (Gao & Lynch 2009). In the present paper, we confine our discussion to the
210 creation of new introns rather than the propagation of preexisting introns.
211 The new version of the tandem genomic duplication model also highlights the co-occurring
212 insertion of coding sequence with an intron gain. Generally, the researchers seek intron gains in
213 highly conserved orthologous genes. Thus, only introns flanking conserved exonic sequences are
214 likely to be identified as a new one. Due to this methodology, the frequency of intron gain by
215 segmental duplication might have been underestimated previously. To be consistent with this

idea, a study that specifically explored intron gains by segmental duplications revealed tens of new introns in humans, mice, and *A. thaliana* (Gao & Lynch 2009). This result is in stark contrast to the comparative studies of their highly conserved orthologous genes, which found very few or no intron gains at all (Coulombe-Huntington & Majewski 2007; Fawcett et al. 2012; Roy et al. 2003; Yang et al. 2013). Considering the high frequency of internal gene duplications, which is 0.001–0.013 duplications/gene per million years (Gao & Lynch 2009), it can be stated that intron gain by segmental duplication may be an important force shaping the eukaryotic gene structure. With the increasing number of very closely related genomes (*i.e.*, diverged within ten million years) to be sequenced, we expect to find more intron gains by segmental duplication in the near future.

CONCLUSIONS

In the gene *PGSC0003DMG402000361* of last common ancestor of domesticated potato *S. tuberosum* and wild potato *S. commersonii*, a tandem duplication event created a novel intron. The duplicate includes the 3′ side sequence of an intron and the 5′ side of the downstream exon. Most splicing signals which include, a putative branch site, the polypyrimidine tract, the 3′ splicing site, two putative ESEs and the GC contents differentiated between the intron and exon were inherited from the parental intron/exon structure. By contrast, the widely cited model of intron gain is tandem duplication of an exonic segment containing AGGT, which would create the GT and AG splicing sites. The case of intron gain which we observed, requires a new version of the tandem genomic duplication model: partial duplication of the preexisting intron/exon structure. This version is a supplement to the widely cited version of the tandem genomic duplication model (Rogers 1989; Yenerall & Zhou 2012).

239

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243

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Figures

Figure 1. Alignments indicating an intron gain and a flanking insertion of coding sequence in the potato gene *PGSC0003DMG402000361*.

The orthologous genes used as references are *Solyc12g008410.1* in *S. lycopersicum*, *Capana09g000243* in *C. annuum*, and *NbS00003153g0003* in *N. benthamiana*. The orthologous region in eggplants was manually identified by the best reciprocal program, BLAST, and manually annotated. Only aligned sequences close to the intron variation are shown here. Abbreviations: Stub: *S. tuberosum*; Slyc: *S. lycopersicum*; Smel: *S. melongena*; Cann: *C. annuum*; Nben: *Nicotiana benthamiana*.

Figure 2. Identification of the intron gain in potatoes.

The phylogenetic tree was constructed using the coding sequences of the gene *PGSC0003DMG402000361* and its orthologs: *Solyc12g008410.1* in *S. lycopersicum*, *Sopen12g003370* in *S. pennellii*, *Capana09g000243* in *C. annuum*, and *NbS00003153g0003* in *N. benthamiana*, and the orthologous regions manually annotated in *S. commersonii*, *S. habrochaites*, and *S. melongena*. The tree is not scaled according to substitution rates. As the untranslated regions have not been annotated in *S. commersonii*, *S. lycopersicum*, *S. habrochaites*, or *C. annuum*, the presented sequences start from the initiation codon ATG. In the schematic diagram of gene structures, boxes represent exons and horizontal lines represent introns. Due to the limited space, two extraordinarily long introns are not scaled according to their lengths. They are represented by broken lines. The new intron/exon structure is marked in red color. Abbreviations: Stub: *S. tuberosum*; Scom: *S. commersonii*; Slyc: *S. lycopersicum*; Shab: *S. habrochaites*; Spen: *S. pennellii*; Smel: *S. melongena*; Cann: *C. annuum*; Nben: *Nicotiana benthamiana*.

Figure 3. An intron gained by tandem genomic duplication within the potato gene *PGSC0003DMG402000361*.

(A) A schematic diagram showing the creation of a new intron by partial duplication of the parental intron (marked in blue line) and recruitment of a 10 bp exonic segment (marked in red line). **(B)** Alignment of the two copies of the duplication. The splicing sites, the putative branch site, the polypyrimidine tract, and putative exonic splicing enhancers (TCAGCT, CAGCTC and

GAGGAA) are underlined. A cryptic 5′ exonic splicing enhancer, GAGGAA, and a cryptic 5′ splicing signal, GTAAG, was activated by the duplication event. This duplication was also found in the orthologous region of the wild potato *S. commersonii*. Besides this duplication, we also detected another 83 bp tandem genomic duplication within the first intron of the gene *PGSC0003DMG402000361*, but not in the orthologous region of *S. commersonii*. The second duplication did not change the intron/exon structure of the gene *PGSC0003DMG402000361*. So it is not described here in detail. Sites differing between the two copies are indicated with green letters. **(C)** The consensus sequences of the introns conserved among potatoes, tomatoes and peppers. These sequences were used to recognize the splicing signals for the new intron.

Figure 1(on next page)

Alignments indicating an intron gain and a flanking insertion of coding sequence in the potato gene *PGSC0003DMG402000361.*

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```
Stub   GAGACAGGGCCAGCACACGAGGAATAAGTGAACAGTTGCTGGCACTCAgtaagcttgaat
Slyc   GAGACAGGGCCAGCACACGAGGAATAAGTGAACAGTTGCTGGCACTCA------------
Smel   GAGACAGGGCCAGCACACGAGGAATAAGTGAACAGTTGCTGGCACTCA------------
Cann   GAAACAGTGCCAGCACACGAGGAATAAGTGAGCAGTTACTTGCACTCA------------
Nben   GAAACAGGGCTGGCATACAAGGAATAAGCGAACAGTTGCTGGCACTCA------------

Stub   tagtttaggcttaatgaagaacttgttcaatttttttattggttgcgatctcttcttcct
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   ttttttgcatatttacaactctacatgtaaactatgttgctcggactctcaaaaactgtt
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   gaacccgtgttggattctccaaaatgcactacttttggagtattcgatacacacttttga
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   agagtccgaacaacacaacatgtaatgtactcagacctttcaagaattctagtttaccaa
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   tgatggtcttccaatgcctgcagACATAGTAGGTGATGCATCTGATTCTCCTACATCAGC
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   TCCACGAATACCATCACCTCCAATGAGTCCAGTGACAACTAGCTTTCAAAGAGATCATTA
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   CGATCCTAGGCCATCTACATTCAGAGACAGGGCCAGCACACGAGGAATAAGTGAGCAGTT
Slyc   ------------------------------------------------------------
Smel   ------------------------------------------------------------
Cann   ------------------------------------------------------------
Nben   ------------------------------------------------------------

Stub   ACTGGCACTCAGTAAGCTTGAATTCAGGAAATTCTTTTTGATTCTAAACTACATTGGGAG
Slyc   ------------ATAAGCTTGAATTCAGGAAATTCTTTTTGATTCTAAACTACATTGGGAG
Smel   ------------GTACGCTTGAATTCAGGAAATTCTTTTTGATTCTAAACTACATTGGGAG
Cann   ------------ATAAGCTTGAATTCAGGAAATTCTTTCTGATTCTGAATTACATAGGGAG
Nben   ------------GTGATGTTGAGTTCAGGAAATTATTTTTGATTCTACACTACATTGGAAG
```

Figure 2(on next page)

Identification of the intron gain in potatoes.

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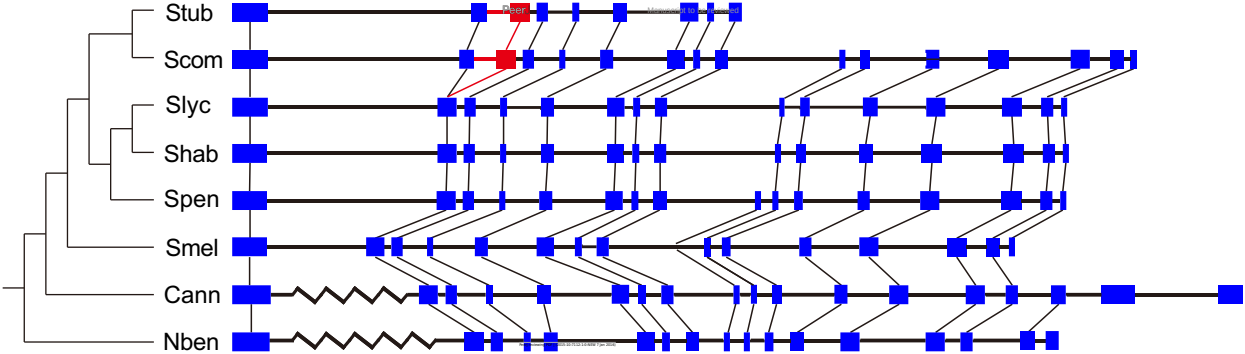
Stub
Scom
Slyc
Shab
Spen
Smel
Cann
Nben

Figure 3(on next page)

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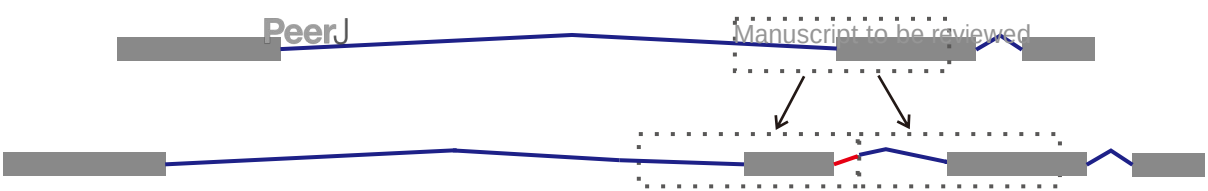
A



B

```
Downstream attagtttaggcttaatgaagaacttgttcaattttttattggttgcgatctcttcttc
Upstream   attagtttaggcttaatgaagaacttgttcaaattttttattggttgcgatctcttcttc

Downstream c-ttttttgcatatttacaactctacatgtaaactatgttgctcggactctcaaaaact
Upstream   cttttttttgcatatttacaactctacatgtaaactatgttgctcggactctcaaaaact

Downstream gttgaacccgtgttggattctccaaaatgcactacttttggagtattcgatacacacttt
Upstream   gttgaacccgtgttggattctccaaaatgcactacttttggagtattcgatacacacttt

Downstream tgaagagtccgaacaacacaacatgt-aatgtactcagacctttcaagaattctagtttta
Upstream   tgaagagtctgaacaacacaacatgtaaacatactcagacctttcaaaaattctagtttta

Downstream ccaatgatggtcttccaatgcctgcagACATAGTAGGTGATGCATCTGATTCTCCTACAT
Upstream   ccaatgatggtcttccaatgcctacagACATAGTAGGTGATGCATCTGATTCTCCTACAT

Downstream CAGCTCCACGAATACCATCACCTCCAATGAGTCCAGTGACAACTAGCTTTCAAAGAGATC
Upstream   CAGCTCCACGAATACCATCACCTCCAATGAGTCCAGTGACAACTAGCTTTCAAAGAGATC

Downstream ATTACGATCCTAGGCCATCTACATTCAGAGACAGGGCCAGCACACGAGGAATAAGTGAGC
Upstream   ATTACGATCCTAGGCCATCTACATTCAGAGACAGGGCCAGCACACGAGGAATAAGTGAAC

Downstream AGTTACTGGCACTCAGTAAGCTTGA
Upstream   AGTTGCTGGCACTCAgtaagcttga
```

C