Information notices issued by ACRE – PBM/26/FRAN/008

1. Information notice issued 16th February 2026

ACRE:

Please may the applicant provide sufficient gene/genomic information that would allow unambiguous identification for each of the target genes using publicly accessible data, e.g. unique gene identifiers (e.g. Entrez Gene ID/Ensembl ID) for each of the Tfl1 genes targeted or their homologues/homeologues (where necessary).

Answered on 18th February 2026:

The edited target in all S4A lines is the strawberry *Tfl1* gene with the GenBank Accession number JN172097.1. All alleles in the parent strawberry align to accession number JN172097.1. This was the sequence used to build the guide RNA for these edits.

2. Information notice issued 12th March 2026

ACRE:

Please may the notifier provide independent identifiers for each of the edited *Tfl1* alleles using relevant gene model identifiers from the octaploid strawberry (*Fragaria ananassa*) as opposed to that previously supplied for the diploid strawberry (*Fragaria vesca*). Please may the notifier also provide information on which *Tfl1* alleles have been edited in each of the eight S4A strawberry lines described within the marketing notice.

Answered on 17th March 2026:

Strawberry (*Fragaria x ananassa*) is an octoploid species (8x - allopolyploid) composed of four diploid sub genomes, designated A, B, C, and D (Folta and Barbey, 2019). The *Tfl1* gene is present in all sub genomes with sub genome D containing only one copy of *Tfl1* (present in D1 and absent on D2), resulting in seven copies of *Tfl1* in the strawberry genome (Table 1).

Table1: Identifiers from *Fragaria* x *ananassa* for Fa*Tfl1*

Allele	Accession	Description
A1	AB822995.1	*Fragaria x ananassa* FaTFL1-1 mRNA for terminal flower 1, complete cds
A2	AB822995.1	*Fragaria x ananassa* FaTFL1-1 mRNA for terminal flower 1, complete cds
B1	JN788264.1	*Fragaria x ananassa* TFL1-like protein (TFL1) mRNA, complete cds
B2	JN788264.1	*Fragaria x ananassa* TFL1-like protein (TFL1) mRNA, complete cds
C1	JN788264.1	*Fragaria x ananassa* TFL1-like protein (TFL1) mRNA, complete cds
C2	JN788264.1	*Fragaria x ananassa* TFL1-like protein (TFL1) mRNA, complete cds
D1	KR139757.1	*Fragaria x ananassa* TFL1 protein (TFL1) mRNA, complete cds

Table 2: *Tfl1* Alleles Edited in Eight S4A Lines

Line	DEFRA PBO Reference	Edited *Tfl1* Alleles
S4A-210 (Primary line)	PBM/26/FRAN/008-1	A1, A2, B1, B2, C1, C2, D1
S4A-115	PBM/26/FRAN/008-2	A1, A2, B1, C1, C2, D1
S4A-293	PBM/26/FRAN/008-3	A1, A2, B1
S4A-334	PBM/26/FRAN/008-4	A1, B1, B2, C2, D1
S4A-336	PBM/26/FRAN/008-5	A1, A2, B1, B2, C1, D1
S4A-444	PBM/26/FRAN/008-6	A1, A2, B1, B2, C1, C2, D1
S4A-448	PBM/26/FRAN/008-7	A1, A2, B1, B2, C1, C2, D1
S4A-480	PBM/26/FRAN/008-8	A1, A2, C1, C2, D1

3. Information notice issued 1st April 2026

ACRE: The notifier needs to provide allele specific identifying information (e.g. gene models) for each of the allelic variants of *Tfl1* targeted.

If this is not possible, the committee requires that the notifier provide scientific justification as to why that is the case and an explanation therefore as to how the notifier has been able to distinguish which of the seven *Tfl1* alleles have been edited in each of the eight S4A lines contained within the marketing notice (*Reference to Table 2 in notifiers response to the previously issued information notice*).

Answered 7th April 2026:

The *Tfl1* gene was sequenced in the S4A parental genome, and inherent, allele-specific SNPs flanking the gene in the octoploid *Fragaria* × *ananassa* genome were used to identify and distinguish seven distinct *Tfl1* alleles. Transcript sequences derived from the parental variety were aligned to these alleles to confirm sequence variation and exon–intron organisation, thereby defining allele-specific gene models (Table 1). Chromosome assignment and gene identity were further confirmed by homology to the *Tfl1* locus in the *Fragaria vesca* reference genome. Following genome editing, long-read sequencing spanning the target site and flanking SNPs enabled unambiguous assignment of edits to individual *Tfl1* alleles in each S4A line.

Each *Tfl1* allele is therefore uniquely identified by a combination of its allele-specific SNP profile and its location on a distinct chromosome.

All seven *Tfl1* alleles targeted in the S4A parental *Fragaria* × *ananassa* genome correspond to the strawberry *Tfl1* gene represented in GenBank under accession JN172097.1. In addition, publicly available *Tfl1* sequences with high sequence homology to the edited alleles were provided previously (Table 1, Response to 2nd Questions, dated 17th March 2026) to support gene identity and confirm that the targeted locus corresponds to the *Tfl1* gene.

At present there are no publicly accessible sequence accessions that uniquely distinguish each individual octoploid *Tfl1* allele (A1–D1). The available public sequences represent conserved *Tfl1* coding regions and do not capture allele-specific sequence variation present among homoeologous copies in the octoploid genome. As a result, multiple alleles (e.g. B1, B2, C1, C2) share identical or near-identical public reference sequences and cannot be distinguished using public data alone.

Allele-level discrimination was therefore achieved using allele-specific SNPs located in upstream and downstream flanking regions of the *Tfl1* coding sequence, identified from the S4A parental genome. These allele-specific sequence features, together with long-read sequencing spanning the edited region and adjacent polymorphisms, enabled unambiguous identification of individual *Tfl1* alleles and assignment of edits in each S4A line.

Multiple alleles map to the same public accession due to sequence homology, as these alleles share highly conserved coding sequence. As such, the allele designations (A1-D1) and corresponding gene-models do not have unique allele-specific accession numbers. We are not aware of any allele-specific public accessions that exist for individual octoploid *Tfl1* alleles.

Although allele-specific public accessions are not available for each *Tfl1* copy, this does not prevent unambiguous verification of the edited alleles reported in the marketing notice. Allele identity and edits were determined using allele-specific SNPs present in the parental genome, together with long-read sequencing spanning the edited region and flanking polymorphisms. The allele-specific edit assignments reported in Table 2 (Response to 2nd Questions, dated 17 March 2026) are directly derived from this approach. We therefore confirm that, while public databases currently allow verification of the targeted *Tfl1* locus, allele-level resolution relies on internal genomic information rather than distinct public accessions.