Thenell & Associates LLC
Agricultural Biotechnology Regulatory Affairs Consulting

VIA EXPRESS COURIER

September 9, 2011

Michael C. Gregoire
Deputy Administrator
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
4700 River Road, Unit 98
Riverdale, Maryland 20737-1228

Dear Mr. Gregoire,

Cellectis S. A., a French biotechnology company, and its U.S. subsidiary Cellectis Plant Sciences focused on applications in plants (hereinafter collectively referred to as "Cellectis"), have developed a platform for making targeted modifications to plant genomes. The platform uses proteins called meganucleases – naturally occurring enzymes that recognize and cleave large DNA sequence targets. By altering specific amino acids that contact DNA, Cellectis can create meganuclease variants thereby making it possible to create targeted chromosome breaks at any locus of interest in the plant genome. Repair of the chromosome breaks enables a variety of precise genome modifications ranging from functional gene deletion (targeted gene knockout) to targeted insertion of transgenes.

Cellectis seeks confirmation from USDA-APHIS that plants modified by its meganuclease technology without the use of plant pest components are not regulated articles within the meaning of current regulations. A description of Cellectis' meganuclease technology follows below.

Cellectis' technology is based on a naturally occurring I-CreI meganuclease produced by the chloroplasts of the green algae *Chlamydomonas reinhardii*. Cellectis can create I-CreI variants with DNA sequence specificities by altering specific amino acids in the protein that contact DNA. Such modified meganucleases make it possible to create targeted chromosome breaks at any locus of interest in a plant genome. Repair of the chromosome break enables a variety of precise genome modifications. For example, if the chromosome is broken and rejoined by the cell's DNA repair machinery, small deletions are introduced at the break site that result in loss of gene function (targeted gene knockout). Alternatively, the chromosome break can be repaired through homologous recombination in which a DNA repair template is provided and sequence information on the repair template is copied into the genome at the break site. Repair of the chromosome break by homologous recombination allows a variety of DNA sequence modifications to be generated, ranging from single base pair substitutions to the targeted insertion of transgenes.

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Targeted gene knockouts. In many instances, loss of gene function can confer a trait of value. For example, a rice gene (Pi21) was recently described, which when knocked out, confers resistance to rice pathogens (Science 2009, 325:925). Traditionally, gene knockouts have been attained by chemical mutagenesis or by treating plants with ionizing radiation. Cellectis has the capacity to modify I-CreI meganucleases to create targeted gene knockouts. For convenience, we refer to targeted gene knockouts by such meganuclease technology as MGN1 events.

Protein and mRNA delivery of the meganuclease. One advantage of meganucleases over other targeted mutagens is that the meganuclease protein is very stable. This makes it possible to deliver purified meganuclease protein directly to plant cells by biolistics or electroporation. Alternatively, mRNA encoding the engineered meganuclease can be delivered to the plant cell, which is then translated by the plant into the protein mutagen. The meganuclease acts on the plant cell's DNA to create a double-stranded break, which is repaired by a non-homologous end-joining repair mechanism, creating small DNA deletions at the break site and loss of gene function. The mutagenized cells are regenerated into whole plants, which are then screened for the desired phenotype and genotype. For both protein and mRNA delivery, no DNA is introduced to the plant cell, and so this approach uses a mutagen that cannot integrate into the cellular genetic material similar to traditional, chemical mutagenesis. An added advantage over traditional chemical mutagenesis is this mutagen targets one specific site of interest.

Cellectis believes that a plant modified by MGN1 as described above, wherein neither the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, nor is an unclassified organism or organism whose classification is unknown, is not a regulated article under 7 CFR 340 because it does not satisfy any of the regulatory criteria that would subject it to APHIS oversight.

Cellectis requests that USDA-APHIS confirm that a plant modified by MGN1 without plant pest components as described above is not a regulated article within the meaning of the current regulations.

Targeted mutagenesis through homologous recombination. One of the most powerful applications of meganuclease for genome modification is its ability to make precise alterations to plant genes through homologous recombination. For example, a single nucleotide sequence change introduced into a plant gene such as acetolactate synthase confers resistance to certain herbicides (Nature 2009, 459:442). This approach for creating modified plant germplasm differs from transgenic approaches to herbicide resistance in that only a single or few nucleotides distinguish the herbicide resistant plant from the non-resistant progenitor. No foreign transgene is incorporated into the plant's genome. For convenience, we refer to targeted mutagenesis with genomic repair by homologous recombination as MGN2 events.

Protein or mRNA delivery of the meganuclease and use of oligonucleotides to mediate DNA repair. Targeted genome modification by homologous recombination requires the introduction of a chromosome break, which as described above can be accomplished by delivering the meganuclease to the plant cell as either protein or mRNA. Repair of the chromosome break by homologous recombination and incorporation of specific sequence modifications requires the use of a DNA template that is co-introduced with the meganuclease into the plant cell. Short, single-stranded DNA molecules (oligonucleotides) can be used for this purpose. It is our understanding that USDA-APHIS already considers plants created through the use of oligonucleotide-mediated mutagenesis to not meet the definition of a *regulated article*.

Protein or mRNA delivery of the meganuclease and use of double-stranded DNA to mediate repair. Chromosome breaks created by meganucleases delivered as mRNA or protein can also be repaired by double-stranded DNA, allowing for a variety of targeted genome modifications that range from single base pair substitutions to insertions of short DNA sequences originating from the same plant species. As described above, this approach could be used to create herbicide resistance by making single base pair substitutions in native plant genes. In another example, an enhancer or a promoter from the same species is inserted in the endogenous promoter to boost expression of the endogenous herbicide tolerant gene – also obtained through targeted mutagenesis. In this application of the technology, the repair DNA template and meganuclease mRNA or protein are delivered using biolistics or electroporation. Modified plants are characterized to ensure that the repair template has not integrated into the genome at a non-targeted site and that only the desired targeted modification has been achieved. In the absence of unwanted, random integration of the repair template, we believe that only the components and phenotype of the targeted modification should be considered when determining whether the resultant plant or plant product is a regulated article.

Delivery of the meganuclease and repair template as double-stranded DNA. Finally, targeted genome modification of plants can be achieved by delivering both the meganuclease construct and the repair template as double-stranded DNA. These DNA molecules could be delivered by biolistics, thereby allowing the meganuclease to be transiently expressed to create the targeted break; the repair template would then fix the break and incorporate the desired sequence change. Both events could be achieved without integration of the DNA constructs into the plant genome.

Cellectis believes that a plant modified by MGN2 as described above, wherein neither the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, nor is an unclassified organism or organism whose classification is unknown, is not a regulated article under 7 CFR 340 because it does not satisfy any of the regulatory criteria that would subject it to APHIS oversight.

Cellectis requests that USDA-APHIS confirm that a plant modified by MGN2 as described above without plant pest components is not a regulated article within the meaning of the current regulations.

Please contact us if you have questions or require additional information. Thank you for your assistance in this matter.

Very truly yours,
THENELL & ASSOCIATES LLC

Scott Thenell

J. Scott Thenell