**Names, addresses, and telephone numbers of the persons who developed**

**and/or supplied the regulated article.**

Wayne Parrott, 111 Riverbend Road Athens, GA 30606. 706-542-0928

**A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism (e.g., morphological or structural characteristics, physiological activities and processes, number of copies of inserted genetic material and the physical state of this material inside the recipient organism (integrated or extrachromosomal), products and secretions, growth characteristics).**

The enclosed *Agrobacterium* strains have been modified to knock out each the *recA* and/or thymidylate synthase (*thyA*) genes using CRISPR-mediated base-editing. The intended effects of each *recA* and *thyA* gene knockout are recombination deficiency/DNA damage sensitivity, and thymidine auxotrophy, respectively. CRISPR-mediated base-editing was used to introduce a premature stop codon within the coding sequence of each target gene. Introduction of premature stop codons was confirmed via sanger sequencing encompassing the target region. For *thyA* knockout validation, strains were also cultivated in replicated fashion on M9 [2] minimal bacterial medium with and without supplemented thymidine to verify induced auxotrophy. For *recA* knockout validation, strains were cultivated in replicated fashion on YP [1] medium with and without supplemented methyl methanesulfonate to verify increased sensitivity to DNA damage of the mutants. The introduced mutations are permanent, and the genetic material used to introduce them have been eliminated from the strains.

[1] Ishida, Y., Saito, H., Ohta, S., Hiei, Y., Komari, T., & Kumashiro, T. (1996). High efficiency transformation of maize (Zea mays L.) mediated by Agrobacterium tumefaciens. *Nature biotechnology*, *14*(6), 745-750.

[2] Miller, J. H. (1972). Assay of β-galactosidase. *Experiments in molecular genetics*.

**A detailed description of the molecular biology of the system (e.g., donor-recipient-vector) which is or will be used to produce the regulated article.**

A detailed description of the molecular biology techniques used to develop these strains can be found in doi.org/10.1101/2024.08.04.606528.

**Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced.**

All materials were developed in Athens, GA, USA.

**A detailed description of the processes, procedures, and safeguards which have been used or will be used in the country of origin and in the United States to prevent contamination, release, and dissemination in the production of the: donor organism; recipient organism; vector or vector agent; constituent of each regulated article which is a product; and, regulated article.**

The introduced *thyA* gene knockout serves as an additional biocontainment safeguard as the mutation renders the strains auxotrophic for thymidine, requiring supplementation for growth.

**A detailed description of the intended destination (including final and all intermediate**

**destinations), uses, and/or distribution of the regulated article (e.g., greenhouses,**

**laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location).**

THIS WILL DEPEND ON THE PERMIT

**A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations.**

This will depend on the permit/your discretion

**A detailed description of the proposed method of final disposition of the regulated article.**

The strains should be disposed of and handled in accordance with established microbiological disposal protocols. Devitalization procedures vary by institution, but typically entail either soaking materials that have come in contact with the bacterium in a bleach solution, or autoclaving at 121°C and 15psi pressure for a minimum of 30 minutes.