1. Does your knock out mutant have a distinct phenotype compared to the control strain and is it consistent?

   No significant phenotypic differences were observed between the knock-out mutants and the control strain. The mutants exhibited consistent characteristics across all transformation plates as well as plates following transformant amplification. These characteristics include dark green septate hyphae with a woolly colony texture and mycelia. When viewed from the back of the plate, the color appears to be almost black.

2. If you have a distinct phenotype is there any obvious explanation of the function your gene has or that it affects. Does this relate to the information you gained from bioinformatic analysis?

   There is no unique explanation. Due to the lack of significant differences in macroscopic characteristics between the experimental group (Aspergillus nidulans with constructed nests) and the control group, we cannot determine the function of the AN8549 gene. The function of AN8549 is most likely related to its impact on biochemical pathways, which may not result in observable phenotypic changes. In such cases, it is necessary to use more detection methods to determine the molecular-level changes in cell phenotype. Bioinformatics analysis suggests that this gene encodes a type of O-methyltransferase, but it does not specify which substance the enzyme methylates. Further experiments are needed to refine these findings.

3. Do your transformants require pyridoxine – why is this important?

   The transformants require pyridoxine, also known as vitamin B6, for normal cell growth. While fungi can typically synthesize this vitamin themselves, providing pyridoxine becomes essential if the pyroA gene is mutated. In our strain of pyrA4 auxotrophic mutants, there is a scarcity of an enzyme necessary for pyridoxine biosynthesis due to the loss of its gene. Therefore, pyridoxine supplementation is necessary. Pyridoxine may act as a coenzyme for orotidine 5’-phosphate decarboxylase, which catalyzes the conversion of orotidylic acid to uridine acid. Without pyridoxine, the mutant do not readily survive on uridine-selective medium.

4. What experiments would you conduct in order to extend the work and test any conclusions?

   Firstly, it is necessary to optimize and repeat the PCR experiments, improve the PCR primers, and repeat the experiments to reduce the appearance of non-specific bands, and determine whether the occurrence of false positives is random.

   Secondly, the metabolomics of Aspergillus nidulans need to be analyzed. Gas chromatography-mass spectrometry (GC-MS) and other methods can be used to analyze small molecules in cells, compare the differences in small molecule products between the experimental group and the control group, and identify which molecules are not methylated in the experimental group but are methylated in the control group. This will help determine the targets of O-methyltransferase modification.