Colony PCR

After overnight incubation of the transformed cells, pick colonies from the ligation and the control plates for colony PCR to quickly test if the ligation was successful in any of the colonies.

7.1 Compare your positive ligation plate to your negative ligation plate to estimate the number of background negative colonies. Plan to screen an appropriate amount so that it is highly probable at least one of the positive colonies will have the correct insert size given the amount of background negative colonies. Screen at least one negative colony also.

7.2 To conduct PCR, you will need a forward and reverse primer specific to the plasmid being screened. The primers should amplify the region where the insert was added.

7.3 For each colony to be picked, prepare a PCR tube with the following mixture: 12 μL 2× Green Master Mix, 10 μL dH2O, 1 μL (20 pmol) forward primer, 1 μL (20 pmol) reverse primer.

7.4 Use a micropipette tip to pick a single colony off of the culture plate. Place the tip into a labeled PCR tube and mix by pipetting up and down.

7.5 Remove 1 μL of the PCR mixture and place it in a labeled test tube containing 200 μL culture media with antibiotic to reserve some of the cells from the colony to grow later. Incubate these cultures at 37° C.

7.6 Conduct the following PCR cycle: 95° C for 10 min, followed by 20 cycles of 95° C for 15 s, 46° C (or appropriate annealing temperature for your primers) for 15 s, 72° C for 60 s/kb of DNA of the expected size for a successful DNA ligation.

7.7 Run the reaction products on an agarose gel appropriate for the size of the amplified product. You can use our gel optimization tool to choose the appropriate percent agarose. Colonies containing unsuccessful ligations will have the same insert size as the negative control colony. Successful ligations will be bigger than the negative control insert size. For colonies that show the expected insert size, save the corresponding culture and discard all unsuccessful colonies.

7.8 The BioBrick part can be further verified by miniprepping and digesting and gel electrophoresis, or by sequencing. After successful ligation and transformation, the part can be used or manipulated more to construct more new parts.