Tissue PCR

Introduction

The purpose of this protocol is to confirm correct insertion/deletion of DNA fragments after transformation in *A.niger*.

Info about controls:

Negative control:

Everything but the template (which is the plasmid with bioblocks).
If we have a band then we have contamination.

Positive control:

The genomic DNA for insertion and the insertion plasmid for a knockout.
The bands should be smaller than the ones we get from the bioblocks (the size of a protospacer) because there is only one protospacer/sgRNA in this one.

Materials

- MyTaq plant PCR kit
- Forward Primer (10 µM)
- Reverse Primer (10 µM)
- MyTaq DNA Polymerase
- MilliQ water

Procedure

All reactions are set up on ice!!

1. Make a master mix solution of Mytaq plant PCR kit and each Forward and Reverse primer.

Reactant	Per reaction (10 µL) [µL]
MyTaq plant PCR kit	5
10 µM Forward Primer	0.5
10 µM Reverse Primer	0.5

2. In another PCR tube add 15 µL MilliQ water. Using an inoculation loop or toothpick poke the fungi from the plate and transfer the loop to the PCR tube with water.
3. Transfer 4 µL of the solution from PCR with water to the PCR tube with the master mix. Use the rest of the solution for making a stock.
4. Run reaction in a thermocycler using the following settings.

Step	Temperature	Duration	Number of Cycles
Initial denaturation	95 °C	3 minutes	1 cycle
Amplification	95 °C	15 seconds	25-30 cycles
	45-68°C	15 seconds	
	72 °C	around 45 seconds/kb*	
Final extension	72 °C	10 minutes	1 cycle
Hold	12 °C	-	1 cycle

*Not recommended to have more than 3 min extension.

5. The PCR products can then be stored at -20 °C, used directly, or purified using PCR purification or gel extraction.