PCR with iPROOF High Fidelity DNA Polymerase (BIORAD)

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Where

On the bench, PCR machine on the bench left to the door

Material needed

Quantity	What
for 50 μl of PCR mixture 10μl	5x iProof HF buffer
1μl	of each primer
1 μl	dNTP mix of 10mM (Roche tube in the -20° freezer with purple cap)
0.5 μl	High Fidelity Polymerase
0.5 μl	template
	calculate dH_2O quantity needed to have 50μl

Steps

1. Add everything together.
2. Put in the PCR thermal cycler (PCR machine), Place carefully and use green plastic rack if required.
3. Program the PCR thermal cycler: **1.** 98°C for 30s **2.** 98°C for 7.5 seconds **3.** 60°C for 20 sec (this depends on the primers, therefore look up the annealing temperature of the primers used) **4.** 72°C you have to calculate the time required. This polymerase has an efficiency of 15-30s/kb. (calculate for the longest fragment.) **6.** 72°C for 5 min **7.** 4°C "forever
4. Save the file in the MAIN folder → OK→ SAVE→RUN → Select the block you use → RUN → Change sample volume to 50μl, LidT should be 105°C and the box just below ("hotlid") should ALWAYS be checked
5. You can use STATUS to check the progress of the PCR.

Warnings

- Sign in for PCR on the White Board just above the machine (write iGEM and from when to when you will use it.)
- It is possible to save polymerase and only do a 10 μl mix if you only want to check if your insert is there and don't actually want to amplify it.