

Double PCR Protocol

1. Purify single reactions by PCR kit or gel purification. I usually use qiaquick kit.
2. Do a 10 minute high speed spin to get rid of the matrix
3. Isopropanol precipitate
 - a. Add 1 ul of glycogen
 - b. Add 1/2 volume of 7.5M NH₄OAC
 - c. Add 1 volume isopropanol
 - d. Vortex well and let stand at RT for 15 min
 - e. Pellet 15 min RT max. speed
 - f. Decant isopropanol and wash pellet with 70% ETOH
 - g. Pellet 10 min RT max. speed
 - h. Decant ETOH and dry on bench
 - i. Resuspend in 10ul of water
4. Use 0.5ul of the ppt for a 100ul secondary pcr reaction