PCR amplicon sequencing: Elena L Peredo protocol 12/2014.

Uses GoTaq and ExoSapIt. Has generated ~1Kbp length reads.

DNA extraction (I use a modified CTAB extraction starting with 30-50 ul of liquid culture).

PCR 18S/ITS

PCR	1X
GoTaq® Green Master Mix (M712)	10 ul
Primer F 10 uM	0.4 ul
Primer R 10 uM	0.4 ul
DMSO	1 ul
Water	7.2 ul
DNA	1 ul
Total	20 ul

Run PCR program (I use a touch down PCR, starting in 63 C and reducing 0.5 C for 12 cycles and then 25 at 55 C)

I run 7 ul of amplification reaction in 1.5% agarose gels to check amplification and size. Go tag has already loading dyes so I only mix my sample with 2 ul of 1:1000 SyBr safe. Load on gel.

Exosap-it

Dilute 1:4 exosap-it enzyme in molecular grade H20 (0.25 ul enzyme in 0.75 ul water).

Add 1 ul of exosap-it diluted mix to 1-2 ul of PCR product (I use 2 ul of product and 1 ul of diluted enzyme mix and it works ok for me). I usually place 1 ul in the side of each of the wells of a sequencing plate and then I add and mix by pipetting my PCR reaction. Then I briefly spin it down to check that all wells are ok. As the PCR is green visual inspection is pretty fast.

Seal the plate. Run the reaction protocol 30' 37C 15' 80C hold at 4C.

Sequencing

Add to each of the wells the appropriate sequencing reaction mix. I follow BPC reaction mix, but using 10 uM primer instead of 15 uM (I don't keep a second stock for sequencing). Also I assume some evaporation of my template after the exosap-it reaction, so instead of counting it as 3 ul (1ul Exosapit+2ul reaction) I assume the template volume as 2 ul.

Sequencing PCR	X1
BDT	0.5 ul
5X buffer	0.4 ul
Primer 10 uM	0.4 ul
DMSO	0.1 ul
H2O	2 ul
Template	2 ul (already in well)