Solutions to 7.012 Recombinant DNA/Cloning

a) Plasmid p7013 would give a single band at 3000 bp and the tet gene fragment would give a single band at 200 bp.

b) When you mix the DNA and bacteria in the transformation protocol, most of the bacteria in the transformation reaction do not take up a plasmid. The presence of ampicillin prevents all these other bacteria from growing and allows you to select only those containing the ampicillin resistance gene.

c)

![Diagram of Plasmid 1, 2, and 3 with EcoRI, SalI, StuI, Ori, Amp^r, and Tet^r genes]

d) A selection for tetracycline and ampicillin resistance would produce only plasmids 2 and 3, molecules that contain both the genes for ampicillin and tetracycline resistance.

e) Use EcoRI and SalI to cut both the p7013 plasmid and the tet gene fragment. This procedure generates two different "sticky" ends on each molecule, which will allow the tet gene to insert in only one orientation.

You could also cut p7013 with StuI and SalI, and cut the tet gene fragment with PvuII and SalI. StuI and PvuII both generate "blunt" ends that can be ligated together.