

3-primer PCR for testing markers or small studies

(see Boutin-Ganache et al 2001, Schable et al. 2002):

Sigma Jump Start Taq (0.25U):	0.1 ul
10X Reaction Buffer (1X):	1.0 ul
25 mM Magnesium Chloride (1.5mM):	0.6 ul
1.25mM dNTPs (125uM of each base):	1.0 ul

10uM Unlabelled, Untagged Primer (0.5uM):	0.5 ul
10uM Tagged Primer (0.05uM):	0.05 ul
10uM Labelled M13R/CAG tag (0.45uM):	0.45 ul

Double distilled, purified water (ddH ₂ O):	4.3 ul
Template DNA (want ~15ng/ul):	<u>2.0 ul</u>

Run reactions with 60C touchdown protocol. Some may need a 55C or 65C touchdown to allow amp./clean up non-specific product.