M13 Check PCR: Screening of insert size in clones (protocol according to general procedures from e.g. Invitrogen):

M13 PCR Primers for Invitrogen clones:

Primer	5´-3
M13F	TGT AAA ACG ACG GCC AGT
M13R	CAG GAA ACA GCT ATG ACC

Length of desired amplificate should correspond to the length that was obtained in the PCR reaction for the cloning reaction.

Example of general PCR reaction for M13 PCR:

Components	Amount (µl)
5x Buffer with 20 mM Magnesium Promega Green GoTaq reaction buffer + Mg_2^+ M791A	10
2,5 mM Nucleotide mix Promega U151B	1
Taq Polymerase Promega M830B	0,25
Primer F 100 pmol	0,226
Primer R 100 pmol	0,226
DNA … ng/µl	10
MQ total volume 50 µl	28,3

The PCR reaction can be scaled down to 25 µl.

Example of standard M13 PCR Programme:

Step	Temperature (° C)	Time (sec)	
Lid	95	∞	
Pre-Denaturation	95	180 or 600 (better)	
Denaturation	94	40	
Annealing	50	40	Cycles: 35
Elongation	72	90	
Final elongation	72	600	
Cooling	4	∞	