Megan's PCR troubleshooting guide
Suggestions are not in any particular order

Case of the missing (previously present) PCR product

1) Use new reagents
dNTPs, primers, Taq are prone to degradation

2) Decrease annealing temp. by 4-8C

3) Increase or decrease template concentration

4) Increase primer concentration

5) Try different thermal cycle machine

Product is longer than expected

1) Length increased by intron

2) Wrong product being amplified (e.g. plant instead of fungus)

Multiple bands (<50 bp or primer dimers)

1) Use less primer, DNA template or Taq
Add more DNA if primer dimers

2) Decrease annealing time

3) Increase annealing temp.

4) Decrease extension time

5) Decrease extension temp.

6) Increase KCl concentration

7) Increase MgCl2 concentration

8) Add up to 5% DMSO

Multiple bands (>50 bp)

1) Use less primer, DNA template or Taq

2) Increase annealing time

3) Increase annealing temp.

4) Increase extension time

5) Increase extension temp.

6) Decrease KCl concentration

7) Increase MgCl2 concentration

Weak PCR product

1) Optimize annealing temperature

2) Increase primer, DNA template, Taq concentrations (separately, to isolate problem)

3) Increase primer length (if oligo is very short)

PCR product smearing (on-line tips)

1) Dilution series of template

2) Reduce number of cycles

3) Use new reagents and clean pipettes

4) Try suggestions for multiple bands >50 bp

5) Lower enzyme concentration

6) Optimize MgCL2 concentration

Final Mg2+ concentration in reaction (mM)	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**
Required volume of 25 mM MgCl2 per reaction (ul)	0	2	4	6	8	10	12	14

6) Optimize primer concentration or use fresh primers

7) Optimize primer design