

## TGRADIENT Thermocycler



The Biometra Thermocycler is used for running:

- PCR
- RT-PCR

### Optimization of annealing temperatures:

With the TGradient, annealing temperatures of new primers can be optimized in a single run. Due to a temperature gradient across the block during the annealing step, each row represents an individual temperature. Thus 12 different temperatures can be tested in a single run. After the program is completed and samples have been analyzed in a gel, the individual annealing temperatures for tubes in each column can be retrieved from the instrument's gradient calculator.

### Thermal Cycler conditions:

1-step RT-PCR cycling		
Reverse Transcription	30 min	50°C

<b>Initial PCR activation step</b>	15 min	95°C
<b>3-step PCR cycling</b>		
<i>Denaturation</i>	0.5-1 min	94°C
<i>Annealing</i>	0.5-1 min	50-68°C
<i>Extension</i>	1 min	72°C
<b>Number of Cycles</b>	25-40 min	
<b>Final Extension</b>	10 min	72°C

**NOTE**

The steps for creating, starting and running the program are given in the short manual beside the instrument in the Multi-user lab (223 Coles Hall).