



384/96 well Long Range Polymerase Chain Reaction (LRPCR)

Warning: *These protocols have been designed to work in 96 and 384 well formats on the BioRad Tetrad and Genomic Research Instrumentation Ltd (GRI) G-Storm thermocyclers. We have not validated the reactions for use in tubes or other thermocyclers.*

Roche Expand Long Template Protocol

1. Genotyping 15 µl reaction

- Gene specific primers (25-30mers, $T_m > 64$, 3 pmol/µl) 2 µl
- Construct specific universal primer (3 pmol/µl) 2 µl
- DNA (30-60ng) 1 – 6 µl

- **Total vol** **5 -10 µl**

- **Reaction mix:**

10 mM dNTP	0.75 µl
100% DMSO	0.15 µl
25mM MgCl ₂	0.75 µl
1M Trehalose	1.0 µl
Buf II	1.5 µl
(pre-heat to 65° C for 30 min with regular vortexing)	
Enz	0.1 µl
(remove from freezer and add to mix just before adding mix to DNA)	
PCR grade H ₂ O	<u>0.75 - 5 µl</u>

Total volume **5 - 10 µl**

Dispense DNA and primers into a 96 well or 384 well polypropylene plate and place on ice. Prepare the reaction mix at room temperature, remembering to pre-heat the Buffer II provided in the Roche kit at 65°C for 30min with regular vortexing to dissolve all the components. Place the reaction mix on ice before addition of enzyme. Add the enzyme to the reaction mix on ice and vortex for 10sec. Add the reaction mix to the DNA on ice. Maintain the LRPCR plate on ice up to the point that you place it on the thermocycler block.

Optional: Before thermocycling pre-heat the thermocycler block to 85°C for 2 min before placing the plate on the block.



Thermocycling protocol: (*validated on BioRad Tetrad and GRI G-Storm*)

96 well Cycling (Tetrad and G-Storm):

Lid temp set to 90°C constant

1 cycle

94°C 3min

10 cycles

93°C 15sec

70°C 30sec; -1°C/cycle

68°C 6min

25 cycles

93°C 15sec

60°C 30sec

68°C 6min; + 20sec/cycle

1 cycle

68°C 7min

4°C hold

384 well Cycling (Tetrad and G-Storm):

Lid temp set to 85°C constant

1 cycle

93°C 2min

10 cycles

92°C 10sec

70°C 30sec; -1°C/cycle

68°C 6min

25 cycles

92°C 10sec

60°C 30sec

68°C 6min; + 20sec/cycle

1 cycle

68°C 6min

4°C hold

After thermocycling add 5µl PCR grade water and run 8µl on a 1% agarose gel.