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.*

Sanger modified Invitrogen SequalPrep LRPCR kit Protocol

1. **Genotyping 10 µl reaction**

• Gene specific primers (25-30mers, Tm >64, 2-3 pmol/µl)	2 µl
• Construct specific universal primer (1.5 pmol/µl)	2 µl
• DNA (20-100ng)	1-2 µl
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• **Total vol**	**5-6 µl**

- **Reaction mix:**

100% DMSO (Brown tube)	0.1 µl
10x Enhancer A (Red tube)	0.5 µl
10x Enhancer B (Yellow tube)	0.5 µl
10x Buffer (Green tube)	1.0 µl
Enzyme (Black)	0.2 µl
(remove from freezer and add to mix just before adding mix to DNA)	
PCR grade H_2O	1.7 – 2.7 µl
Total vol	**4-5 µl**

Dispense DNA and primers into a 96 well or 384 well polypropylene plate and place on ice. 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
93˚C 3min
8 cycles
93˚C 15sec
68˚C 30sec; -1°C/cycle
68˚C 9min
30 cycles
93˚C 15sec
60˚C 30sec
68˚C 9min; + 20sec/cycle
1 cycle
68˚C 9min
4˚C hold

384 well Cycling (Tetrad and G-Storm):
Lid temp set to 85˚C constant
1 cycle
93˚C 3min
8 cycles
93˚C 15sec
68˚C 30sec; -1°C/cycle
68˚C 9min
30 cycles
93˚C 15sec
60˚C 30sec
68˚C 9min; + 20sec/cycle
1 cycle
68˚C 9min
4˚C hold

After thermocycling add 10µl PCR grade water and run 8µl on a 1% agarose gel.