

g23 T4-type (myovirus) PCR Protocol

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Abstract

Citation: Matthew Sullivan g23 T4-type (myovirus) PCR Protocol. **protocols.io**

dx.doi.org/10.17504/protocols.io.dfq3mv

Published: 21 Jan 2016

Guidelines

Primers (Filée et al, 2005):

MZIA1bis primer F: GAT ATT TGI GGI GTT CAG CCI ATG A

MZIA6 primer R: CGC GGT TGA TTT CCA GCA TGA TTT C

Reaction Mix (using LA Takara):

Water	8.25µl
10x LA Buffer	2.5µl
dNTP mix, 2.5mM each	5.0µl
Primer A, 10µM	4.0µl
Primer B, 10µM	4.0µl
LA Takara HS 2.5U/µl	0.25µl
DNA (or viral lysates)	1.0µl
Total	25µl

Reaction Mix (using GoTaq):

Water	3.5µl
Primer A, 10µM	4.0µl
Primer B, 10µM	4.0µl
GoTaq	12.5µl
DNA (or viral lysates) ¹	1.0µl
Total	25µl

Cycling

94°C 3.5min

94°C	15 sec	
38°C + 0.3°C	60 sec	30 cycles
72°C	45 sec	

72°C	5 min
4°C	Hold

Products in the range of 450-650 bp

¹When using CsCl purified samples (i.e. not dialyzed), must dilute the sample 1:2 to 1:10; undiluted CsCl will inhibit PCR reactions

Protocol