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Isothermal Reaction and Troubleshooting

LAMP is an extremely sensitive detection method and care should be taken to avoid contamination of set-up areas and equipment with DNA of previous reactions. A common problem with LAMP reactions is amplification in no-template controls due to

1. carry-over contamination or
2. amplification of unspecifically annealed primers or primer dimer formations.

Protocols

Preparation of a 10 x conc. Primer Mix

primer	stock conc.	final conc. in 10 x	100 µl	500 µl
F3	100 µM	2 µM	2 µl	10 µl
B3	100 µM	2 µM	2 µl	10 µl
FIP	100 µM	8-16 µM	8-16 µl	40-80 µl
BIP	100 µM	8-16 µM	8-16 µl	40-80 µl
LoopF	100 µM	4-10 µM	4-10 µl	20-50 µl
LoopB	100 µM	4-10 µM	4-10 µl	20-50 µl
PCR-grade Water			fill up to 100 µl	fill up to 500 µl

Set-up of the isothermal amplification assay

component	stock conc.	final conc. in assay	20 µl	50 µl
Sapphire Bst Buffer *	5 x	1 x	4 µl	10 µl
dNTP Mix	10 mM	1.4 mM	2.8 µl	7 µl
Primer Mix	F3/B3 2 µM FIP/BIP 8-16 µM LoopF/LoopB 4-10 µM	F3/B3 0.2 µM FIP/BIP 0.8-1.6 µM LoopF/LoopB 0.4-1.0 µM	2 µl	5 µl
Sapphire Bst Polymerase	8 units/µl	0.32 units/µl	0.8 µl	2 µl
$MgCl_2$ Stock Solution (#PCR-266) *	25 mM	0-2 mM	0-1.6 µl	0-4 µl
SYBR Green Fluorescent DNA Stain (#PCR-378)	100 µM	SYBR Green: 1-2 µM	0.2 - 0.4 µl	0.5 - 1.0 µl
PCR-grade Water			fill up to 20 µl	fill up to 50 µl

* Sapphire Bst Buffer already contains 6 mM $MgSO_4$ in 1 x concentration. A total Mg^{2+} of 6-8 mM in the final assay is recommended.

- Use a LAMP detection instrument or a real-time PCR cycler to run the assays
- Set the instrument to a constant incubation temperature between 60 to 65°C (depending on the primer annealing temperature)
- Measure the fluorescence intensity at an interval of 1 min for up to 30 min.

Troubleshooting

If amplification in no-template controls occurs the following points should be reviewed.

Cross contamination from environments

- Clean equipment and areas with “DNA Away” solution
- Replace reagent stocks and pre-mixes with new components
- Stop reactions at an earlier point of time before non-template amplification occur

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Carry-over contamination from previous reaction products

- Avoid opening reaction vessels after amplification
- Use separate preparation area and equipment if post-reaction processing is necessary
- Use a master mix with UNG and dUTP instead of dTTP or add both components separately to the assay to prevent carry-over contamination

Non-template amplification from primers

- Increase incubation temperature stepwise by 1-2°C
- Design a new set of primers for the target sequence

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