EXPAR - DNA Protocol (65oC)

Introduction

An isothermal amplification method for microRNAs.
End Product: DNA
Reaction Temperature: 65^{o}C

The protocol is based on Ellie Mok *et al.*; Comprehensive evaluation of molecular enhancers of the isothermal exponential amplification reaction.

Materials

› Materials

- Template DNA (instead of primers) for each microRNA
- microRNAs
- Nb.BtsI (nicking enzyme)
- rCutSmart™ Buffer (buffer for Nb.BtsI)
- Bst 2.0 DNA Polymerase
- Isothermal Amplification Buffer (buffer for Bst 2.0 DNA Polymerase)
- Deoxynucleotide (dNTP) Solution Mix
- SYBR Green I
- PCR H_2O

› Equipment

- Pipettes & tips
- Eppendorfs
- Centrifuge
- Incubator
- Real Time PCR machine

›

Procedure

1. Prepare Mix A (for each microRNA):

Mix A

	A	B	C
1	**Material**	**Final Concentration**	**Volume**
2	PCR H2O		up to 20 ul final volume (for both Mix A & Mix B)
3	Template DNA	150 nM	0.5 ul
4	microRNA	10 nM	0.5 ul
5	dNTPs	250 mM	0.5 ul
6	rCutSmart Buffer	0.5x	1 ul

2. Prepare ix B (for each microRNA):

Mix B

	A	B	C
1	**Material**	**Final Concentration**	**Volume**
2	Isothermal Amplification Buffer	1x	2 ul
3	Bst 2.0 DNA Polymerase	0.4 U/ul	1 ul
4	Nb. BtsI	0.5 U/ul	1 ul
5	SYBR Green	0.5x	1 ul

3. Incubate mix A to 56^{o}C for 10 minutes (hybridisation of template and microRNA)
4. Add mix B to mix A while on ice
5. Prepare the necessary negative controls by following the above-mentioned procedure but without adding microRNA to Mix A and by adjusting the right volume of water
6. Centifiguration of the samples for 3 sec. at max speed
7. Load 20 ul of the samples to the wells of the q-PCR plate while on ice
8. Put the plate on the q-PCR machine and run the designed protocol (65^{o}C for 20 minutes)