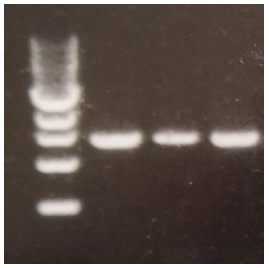
Nov 16, 2018 Version 3

Bounce PCR V.3



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Sam Mugford[1], Saskia Hogenhout[1]
[1]John Innes Centre

OpenPlant Project

Sam Mugford

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We use this protocol and it's working

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Abstract

Bounce PCR: optimisation free PCR for synthetic biology cloning applications.

Synthetic biology projects require the cloning of multiple DNA components, and increasingly this is done though in-vitro gene synthesis. This remains an expensive alternative to traditional cloning by PCR. However, PCR often requires time-consuming optimisation. Bounce PCR is a largely optimisation-free PCR method suitable for the rapid cloning of multiple targets. Bounce PCR is a modified version of Touchdown PCR that takes advantage of primer-extension sequences -commonly used in molecular cloning – to successfully amplify most DNA fragments without time consuming optimisation. Touchdown (and related methods) employ a sequential lowering of the annealing temperature over successive cycles to minimise non-specific primer-binding during the early amplification cycles, but maximise amplification efficiency in later cycles when the target amplicon is more abundant (Don et al. 1991; Rowther et al. 2012). However, the lower annealing temperature in later cycles may still allow for non-specific amplification. Bounce PCR takes advantage of the increased primer melting temperature caused by the addition to the template of primer-extension sequences (for example: restriction enzyme recognition sites, Gateway recombination sites, or vector overlaps for Gibson cloning) to maintain the specificity in later cycles- without sacrificing efficiency- by increasing the annealing temperature again after an initial round of touchdown.

Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K., & Mattick, J. S. (1991). 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic acids research*, *19*(14), 4008.

Rowther, F. B., Kardooni, H., & Warr, T. (2012). TOUCH-UP gradient amplification method. *Journal of biomolecular techniques : JBT*, *23*(1), 1-3.

Guidelines

This protocol describes only the thermal cycling parameters of the PCR, and should work with your preferred DNA polymerase and protocol for setting up the reaction mixture. The advantage of Bounce PCR relies on the presence of primer-extension sequences, if your primers are an exact match to your template, without 5′ extension sequences, then it is unlikely to offer an advantage over standard Touchdown PCR.

Materials

PCR mixture in suitable reaction tube.

Thermal cycler.

Troubleshooting

Initial denaturation

1 94 °C for 00:02:00

Denaturation

2 94 °C for 00:00:30

Annealing

3

	Temperature	Duration
	60ºC	40 seconds
	59ºC	40 seconds
	58ºC	40 seconds
	57ºC	40 seconds
	56ºC	40 seconds
	55ºC	40 seconds
	54ºC	40 seconds
	53ºC	40 seconds
	52ºC	40 seconds
	51ºC	40 seconds
	50ºC	40 seconds

	49ºC	40 seconds
	48ºC	40 seconds
	47ºC	40 seconds
	46ºC	40 seconds
	45ºC	40 seconds
	45.6ºC	40 seconds
	46.2ºC	40 seconds
	46.8ºC	40 seconds
	47.4ºC	40 seconds
	48ºC	40 seconds
	48.6ºC	40 seconds
	49.2ºC	40 seconds
	49.8ºC	40 seconds
	50.4ºC	40 seconds
	51ºC	40 seconds
	51.6ºC	40 seconds
	52.2ºC	40 seconds
	52.8ºC	40 seconds
	53.4ºC	40 seconds
	54ºC	40 seconds

	54.6ºC	40 seconds
	55.2ºC	40 seconds
	55.8ºC	40 seconds
	56.4ºC	40 seconds
	57ºC	40 seconds
	57.6ºC	40 seconds
	58.2ºC	40 seconds
	58.8ºC	40 seconds
	59.4ºC	40 seconds
	60ºC	40 seconds

60 degree C for 40 seconds (decreasing by 1 degree C per cycle for the first 15 cycles (“Touchdown”), then increasing by 0.6 degree C per cycle for the next 25 cycles (“Bounce”))

Extension

4 72 °C for 00:01:00 per kb of target length

Repeating

5 Repeat steps 2-4 for 40 cycles, varying the temperature of step 3 as described

go to step #2 undefined

Final extension

6 72 °C for 00:06:00