

Sep 30, 2024

## Barcoding PCR for MiSeq sequencing

DOI

**dx.doi.org/10.17504/protocols.io.kqdg3jb7l25z/v1**

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DOI: [dx.doi.org/10.17504/protocols.io.kqdg3jb7l25z/v1](https://dx.doi.org/10.17504/protocols.io.kqdg3jb7l25z/v1)

**Protocol Citation:** Eva Petrova, Roey Angel 2024. Barcoding PCR for MiSeq sequencing. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.kqdg3jb7l25z/v1>

**Manuscript citation:**

Rudi K, Rud I, Holck A. A novel multiplex quantitative DNA array based PCR (MQDA-PCR) for quantification of transgenic maize in food and feed. Nucl Acids Res. 2003;31:e62–e62.

<http://nar.oxfordjournals.org/content/31/11/e62>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** May 24, 2018

**Last Modified:** September 30, 2024

**Protocol Integer ID:** 12420

## Abstract

Barcoding PCR for MiSeq sequencing

Barcode head 8mer-GCT ATG CGC GAG CTG C Modified from Rudi et al. (2003)



## PCR mixture

1	Reagent	Final. conc.	1 tube (50µl)	1 tube(25µl)	96 tubes (50µl x100)
	PCR H <sub>2</sub> O		36.15	18.075	3615
	10X DreamTaq Green Buffer	1x	5	2.5	500
	dNTP mixture (2.0 mM each)	0.2mM	5	2.5	500
	MgCl <sub>2</sub> (25 mM)	2mM	0*	0	0
	BSA (20 µg/µl)	0.08 µg/µl	0.2	0.1	20
	Barcode (50 µM)	0.4 µM	0.4	0.2	0.4 x 100
	Dream Taq green (5U/µl)	1.25 U/50µl	0.25	0.125	25
	Template		3	1.5	3 x 100
	<b>Final volume</b>		<b>50</b>	<b>25</b>	<b>5000</b>

\* Buffer already contains MgCl<sub>2</sub> at a final conc. of 2.0 mM.

## PCR program

- 2
1. 94°C – 4'
2. x 4 – 9 {
  - a. 52°C – 30"
  - b. 72°C – 45"
  - c. 94°C – 30"}
3. 52°C – 30"
4. 72°C – 10'