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Barcoding PCR for MiSeq sequencing

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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

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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 ₂ 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 ₂ (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 × 100
	Dream Taq green (5U/µl)	1.25 U/50µl	0.25	0.125	25
	Template		3	1.5	3 × 100
	Final volume		50	25	5000

* Buffer already contains MgCl₂ 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'