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Working

Genomic mapping of transformed DNA fragments

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Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

Mapping of transformed DNA within mutated diatoms. A stepwise specific PCR reaction that allow for identification of genome location of transformed fragments such as antibiotic resistance genes or GFP constructs.

Degenerate primer 1: 5' - GCCGGACTTAGCGAGCA TGTGTAGTACCTTGCCG ATCCTNASATANSATANT TC - 3'
Degenerate primer 2: 5' - GCCGGACTTAGCGAGCA TGTGTAGTACCTTGCCG ATCCCNTSABGNACYTN CTG - 3'
Degenerate primer 3: 5' - GCCGGACTTAGCGAGCA TGTGTAGTACCTTGCCG ATCCNGACGARWGANA WGAC - 3'
Degenerate primer 4: 5' - GCCGGACTTAGCGAGCA TGTGTAGTACCTTGCCG ATCCTAHATGDAGKACN TAC - 3'

Specific primer 1: 5' - GCCGGACTTAGCGAGCA TGTGTAG - 3'
Specific primer 2: 5' - CATGTGTAGTACCTTGC CGATCC - 3'

Internal primers must be designed with regard to each construct that are to be mapped. They should not overlap but instead cover consecutive stretches of known DNA sequence of the transformed construct.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Gel electrophoresis equipment		
SuperFi Polymerase	View	Thermo Fisher Scientific

SAFETY WARNINGS

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BEFORE STARTING

Make sure to have good quality DNA

1 Primary, 25uL PCR reaction

Vol.	Component
5uL	5x SuperFi Buffer
0.8uL	dNTP - Mix (10mM Each)
3uL	Internal Primer (10uL)
0.2uL	SuperFi
12.5uL	Water
2.5uL	Degenerate Primer (40uM)

1uL	gDNA (100ng/uL)

Primary PCR program

Step	Temp	Time
1	96	5 min
2	98	10 sec
3	70	15 sec
4	72	3 min
5	Goto 2 - 30 more times	
6	98	10 sec
7	25	1 min
8	Ramp to 72 0.5C per sec	
9	72	3 min
10	Goto 6 - 1 more time	
11	98	10 sec
12	70	15 sec
13	72	3 min
14	98	10 sec
15	70	15 sec
16	72	3 min
17	98	10 sec
18	70	15 sec
19	72	3 min
20	98	10 sec
21	40	45 sec
22	72	3 min
23	Goto 11 - 10 More times	
24	72	5min
25	4	Forever

2 Dilute Primary PCR reaction 40x times (5uL + 195uL water)

3 **Secondary, 25uL PCR reaction**

Vol.	Component
5uL	5x SuperFi Buffer
0.5uL	dNTP - Mix (10mM Each)
1.5uL	Internal Primer 2 (10uM)
0.2uL	SuperFi
1.5uL	Specific primer 1 (10uM)
15.3uL	Water
1uL	Diluted primary PCR reaction

Secondary PCR program

Step	Temp	Time
1	98	30 sec
2	98	10 sec
3	70	15 sec
4	72	3 min
5	98	10 sec
6	70	15 sec
7	72	3 min
8	98	10 sec
9	57	30 sec
10	72	3 min
11	Goto 2 - 12 More times	
12	72	5min
13	4	Forever

4 Dilute Secondary PCR reaction 40x times (5uL + 195uL water)

5 **Tertiary, 50uL PCR reaction**

Vol.	Component
10uL	5x SuperFi Buffer
0.8uL	dNTP - Mix (10mM Each)
2uL	Internal Primer 3 (10uM)
0.3uL	SuperFi
2uL	Specific primer 2 (10uM)
25.4uL	Water
2uL	Diluted Secondary reaction

Tertiary PCR program

Step	Temp	Time
1	98	30 sec
2	98	10 sec
3	57	30 sec
4	72	3 min
5	Goto 2 - 14 More times	
6	72	5min
7	4	Forever

6 Run a 1% agarose gel, excise bands and cleanup using a gel cleanup kit (Ex. Promega Wizard)

7 Clone 2uL of purified bands into Blunt TOPO vector (Thermo Fisher Scientific) and transform competent E.coli dH5a (Thermo Fisher Scientific) by 30 sec heat shock at 42C. Grow 5mL overnight cultures of picked colonies and extract vectors.

8 Sanger sequence using the TOPO vectors internal primers.