PCR and T-RFLP protocol for BrdU-DNA

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PCR protocol with f-Taq from Feldan Bio

Master Mix:

Water (qf 50 µl):	23.3 µl	
Buffer (10X):	5 µl	1X (1.5 mM $MgCl_2$ and 0.1 mg/ml BSA)
Band Sharpener (5X):	10 µl	1X
$MgCl_2$ (25 mM):	1.4 µl	0.7 mM
BSA (10 mg/ml):	1 µl	0.2 mg/ml
dNTP (10 mM each):	1 µl	0.2 mM each
8f-Hex:	2.5 µl	0.5 µM
1389r:	2.5 µl	0.5 µM
Taq (5U/ µl):	0.3 µl	1.5 units

47 µl Master Mix + 3 µl template DNA

Cycling:

- 95°C for 3min
- 94°C for 30s
  53°C for 1min
  72°C for 2min
  (30 cycles)
- 72°C for 10min

Supplier:
dNTP (Bioline, Tauton, MA) or Feldan Bio
Primers (Operon Technologies, Alameda, CA),
BSA (New England BioLabs)
$MgCl_2$ (Bioline)
Taq polymerase (Feldan Bio, Quebec, Canada)

8f: 5'-AGA GTT TGA TCC TGG CTC AG, (Liu et al., 1997)
1389r: 5'-ACG GGC GGT GTG TAC AAG (Osborn et al., 2000)

PCR products purification and quantification
QIAquick PCR purification kit (Qiagen), elution in 50 µl
Quant-iT dsDNA Assay Kit (Molecular Probes, Invitrogen)

T-RFLP digestion with HhaI (New England BioLabs, Ipswich, MA)

Master Mix:

Water (qf 20 µl):	X µl	
NE Buffer 4 (10X):	2 µl	1X
BSA (100X):	0.2 µl	1X
HhaI (20 U/ µl):	0.2 µl	2 units

Set up reaction in a 96-wells PCR plate
Master Mix: 2.4 µl to 11 µl
Purified PCR products: 200 ng (about 17.6 to 9 µl, depending on the DNA concentration)

Digestion:
37°C for 3h
80°C for 20 min
Cool down at 4°C and quick spin
Freeze at -80°C and send on dry ice to Michigan Sate University

Alternative: Cool down at 4°C and quick spin. Mix 2 µl of sample digest with 12 µl of formamide + 0.5 µl of Map Marker 1000 (standard), then incubate at 94°C for 5 min. It can be stored few days at 4°C before sending to UC Berkeley.

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