

First PCR

Thermocycler Settings

CLeDNA Metabarcoding Wet Lab Protocol V.4.0

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ALWAYS CROSS CHECK SETTINGS ON THERMOCYCLER BEFORE USING!

Primers:	Vert12S or MiFish12S			
Step	# of Cycles		Temperature	Time
Activation	1x		95°	5 min
Touchdown	13x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	69.5° (-1.5 C /cycle)	30 sec
		<i>Extension</i>	72°	90 sec
Amplification	35x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	50°	30 sec
		<i>Extension</i>	72°	45 sec
Final Extension and hold	1x		72°	10 min
			10°	infinity

Primers:	trnL, 16S, 18S, Fungal ITS1			
Step	# of Cycles		Temperature	Time
Activation	1x		95°	5 min
Touchdown	13x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	69.5° (-1.5 C /cycle)	30 sec
		<i>Extension</i>	72°	60 sec
Amplification	35x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	50°	30 sec
		<i>Extension</i>	72°	60 sec
Final Extension and hold	1x		72°	10 min
			10°	infinity

Primers:	Plant ITS2			
Step	# of Cycles		Temperature	Time
Activation	1x		95°	5 min
Touchdown	13x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	70° (-2.0° C /cycle)	30 sec
		<i>Extension</i>	72°	60 sec
Amplification	40x	<i>Denaturation</i>	95°	30 sec
		<i>Annealing</i>	55°	30 sec
		<i>Extension</i>	72°	60 sec
Final Extension and hold	1x		72°	10 min
			10°	infinity

Primers:	CO1			
Step	# of Cycles		Temperature	Time
Activation	1x		95°	5 min
Touchdown	13x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	69.5° (-1.5 C /cycle)	90 sec
		<i>Extension</i>	72°	90 sec
Amplification	40x	<i>Denaturation</i>	94°	30 sec
		<i>Annealing</i>	50°	30 sec
		<i>Extension</i>	72°	60 sec
Final Extension and hold	1x		72°	10 min
			10°	infinity