Molecular Ecology Laboratory

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Laboratory Protocol

Protocol number: 1
Protocol description: Dloop Amplification for Cetacean Spp.
Original reference: none
Original entry: Carrie LeDuc
Last updated: 23 October 23, 2003
Updated by: Carrie LeDuc

Required materials:
Primers:
~400bp D-Loop control region (used for species ID)
D: 5' CCT GAA GTA AGA ACC AGA TG 3'
TRO: 5' CCT CCC TAA GAC TCA AGG AAG 3'
~600bp additional D-Loop control region
DL3: 5' TGG CCG CTC CAT TAG ATC ACG AGC 3'
12S: 5' AAG GCT AGG ACC AAA CCT 3'

Controls:
1. A positive, same species and known to amplify.
2. A negative, no template control.

Additional reagents
mQH_2O
10XPCR Buffer (15mM $MgCl_2$)
dNTPs
Taq
DNA

Required equipment:
1. PCR machine

Required materials:
starred (*) reagents are described in the 'standard laboratory solutions' document)
1. 0.1X T.E., pH 8.0 *
2. Ultrapure human placental DNA from Sigma (catalog No. D4642)
3. CMYC taqman assay reagents
   - forward primer = cMYC_E3_F1U1

Required equipment:

1. Spectrophotometer (for 1st estimate of DNA concentration; optional)
2. 7700 Sequence Detector
3. DNA nebulizer (or other DNA sheering method, e.g. sonicator or sheering with small gage syringe needle)

Procedure:

1. Set up all PCR reactions in a designated pre PCR area
2. Wipe down area and pipets with 10% bleach followed with 70% EtOH
3. Prepare enough mix for your samples, controls, and ~10% extra
4. Always prepare at least one negative/no template control and one positive control

Mix for one reaction:

Reagent	Concentration	Volume in ul
mQH_2O		37.75
10XPCR Buffer	20mM MgCl2	5.0
dNTPs	10mM (2.5 ea)	3.0
Primer 1	10uM	1.5
Primer 2	10uM	1.5
Taq		0.25
DNA		1.0

Cycling Parameters:

* 90C 2min 30sec
* 35 cycles of:
    94C 0:45
    48C 1:00
    72C 1:30
* 72C 5:00