Laboratory for Conservation Genetics, Leipzig

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Laboratory Protocol

Protocol number: 1
Protocol description: DNA standard dilution series for qPCR
Original reference: none
Original entry: Phil Morin, May 2000
Last updated: 14 December, 2000
Updated by: Phil Morin

Required materials:
starred (*) reagents are described in the 'standard laboratory solutions' document)

1. 0.1X T.E., pH 8.0 *
2. DNA standards from the PE Biosystems TaqMan DNA template Reagents kit (Part Number 401970)
3. PE optical PCR plates for the 7700 sequence detector
4. Plastic stick-on plate sealers (for prepared plates with standard DNA)

Required equipment:

1. 7700 Sequence Detector

Protocol:

1. Dilute the PE Biosystems standard tubes 1-5 as shown below. The color codes correspond to dilutions from one tube to the next with the same color code (e.g., 10 µl of tube 3 is used to make the 1:10 dilution for the 6th tube).

   One PE standard kit is enough for 6 assays (in triplicate):

Standard Tube Number	PE tube No.	concentration (pg/ul)	amount/ reaction (pg/5 µl)	starting volume (uL)	0.1X T.E. pH 8.0 vol. (uL)	remove for next (uL)	final volume (ul)	dilution factor	total before removal
1	5	12,000	60,000	100	0	0	100	0	100
2	4	6000	30,000	100	0	0	100	0	100
3	3	3000	15,000	100	0	10	90	0	100
4	2	1440	7,200	100	0	10	90	0	100
5	1	600	3,000	100	0	10	90	0	100
6	3 (1:10)	300	1,500	10	90	10	90	10	100
7	2 (1:10)	144	720	10	90	10	90	10	100
8	1 (1:10)	60	300	10	90	10	90	10	100
9	3 (1:100)	30	150	10	90	10	90	10	100
10	2 (1:100)	14.4	72	10	90	10	90	10	100
11	1 (1:100)	6	30	10	90	0	100	10	100
12	NTC	0			100				

2. Make replicate plates with triplicate standards (rows A, B, C), seal them with plastic plate seals, label them with the standard name, date, and your name, and freeze them at –20°C.