CCDB
Canadian Centre for DNA Barcoding

Advances
Methods Release No. 4
December 1st, 2006

Pre-made frozen PCR and sequencing plates

Application

PCR and sequencing reaction setups are time-consuming steps in DNA barcoding. This Methods Release describes an approach that saves time and aids quality assurance by allowing the production of large numbers of premixed and pre-dispensed PCR and sequencing plates. Theses methods are useful for adoption by facilities operating at any production level.

Method Overview

Trehalose is widely used as a cryoprotectant[1]. It also acts as a potent PCR enhancer by both lowering the DNA melting temperature and stabilizing *Taq* polymerase[2]. As a result, trehalose stabilizes frozen PCR mixes and can overcome the effect of inhibitors that may be present in crude DNA extracts, resulting in improved PCR success.

We have further developed the use of trehalose as a core reagent in both standard PCR and sequencing reactions[3,4]. Aliquoted 'ready to use' PCR and sequencing mixes containing 5% trehalose[a] can be stored at -20ºC for 3 months and do not degrade even after several freeze-thaw cycles. Therefore, large numbers of 96- or 384-well PCR plates can be filled with standardized mix at a single point in time and held frozen until use.

The combination of a thermostable polymerase with trehalose ensures high performance even after multiple freeze-thaws. This technique has been proven effective for freezing master mixes with Platinum *Taq* (Invitrogen™), DYEnamic™ ET Terminator Cycle Sequencing Kits (Amersham Biosciences) and BigDye® Terminator Cycle Sequencing Kits (Applied Biosystems). Results with regular *Taq* polymerases may be less satisfactory.

More Information:

1. Franks F (1990). Freeze drying: from empiricism to predictability. Cryoletters 11:93-110.
2. Spiess AN, Mueller N, Ivell R (2004). Trehalose is a potent PCR enhancer: Lowering of DNA melting temperature and thermal stabilization of Taq polymerase by the disaccharide trehalose. Clinical Chemistry 50:1256-1259
3. Hajibabaei M, deWaard JR, Ivanova NV et al. (2005). Critical factors for assembling a high volume of DNA barcodes. Philosophical Transactions of the Royal Society: Biological Sciences 360:1959 – 1967.
4. Ivanova NV, deWaard JR, Hajibabaei M, Hebert PDN (2005). Protocols for high-volume DNA barcoding. Draft submission to: DNA working group Consortium for the Barcode of Life. Published online at http://www.dnabarcoding.ca/

At a glance

» Trehalose protects enzymes during freeze- thaw cycles, and enhances PCR success by lowering DNA melting temperature and by stabilizing *Taq*

» Premixed PCR and sequencing plates can be stored at -20ºC for months

» Tested on more then 50,000 samples

» Adapted for both manual and automated systems

Materials:

a. D-(+)-Trehalose dehydrate, Sigma-Aldrich Catalogue Number 90210

By Nataly Ivanova and Chris Grainger

BIODC:ccdb.adv.mr4.01122006.2