Protocol cDNA synthesis in oligo $(dT)_{25}$ magnetic beads (S1419)

Overview

Introduction

This protocol can be used with 50 - 500 µg of beads (100 µl to 1ml). The following information is for 50 µg of oligo (dT)25 magnetic beads:

Protocol

1. Following the Low-salt buffer wash step, remove and discard low-salt buffer.
2. Quickly wash the beads once with cold 1X RT reaction buffer.
3. Prepare the following reaction mixture separately and add to the beads immediately following the wash with cold RT buffer.

   In a microcentrifuge tube add:
   10 µl - 10X RT buffer
   16 µl - 10mM dNTP mix
   5 µl - 50 U RNase Inhibitor
   1µl - 200 U M-MuLV Reverse Transcriptase
   68 µl - cold nuclease-free dH2O
   100 µl - Total reaction volume

   Please note: This reaction can be scaled up (to use a higher number of beads) based on these ratios.
4. Incubate at 42°C for at least 1 hour. Gently agitating the beads by hand periodically.
5. Wash the beads three times with cold (DNase-free) TE.
6. Store immobilized cDNA beads at 1µg/µl in TE at 4°C. Use 10 µl of beads suspension per 50 µl PCR reaction.