RT-PCR with the positive control MHCII primers showed no PCR product. However, a product was detectable when further precipitations of the RNA with LiCl (method A) were included in the extraction procedure (Figure 1, lanes 1 and 2). A comparison of RNA obtained by the Chomczynski method and by LiCl precipitation showed no obvious differences (data not shown). Cytokine mRNA, including IL-1α and IFNγ (Figure 1, lanes 3 and 4) were detected, both of which contain 3' AUU- UA sequences (4,8). These results strongly suggest that some form of inhibitory factor is preventing either the RT or PCR process and that there is no detectable mRNA degradation in tissues taken from animals up to 30 min after death.

Variations of method A were then used to identify the optimal procedure of extracting RNA from lung tissue to give a consistent RT-PCR product. Both methods B and C were not reliable for generating RNA ideal for use in RT-PCR. Upon repeated extractions of various lung tissues, some extractions provided RNA suitable for RT-PCR. The variable RT-PCR results are most likely due to varying amounts of the inhibitory substance(s) present in the tissues, suggesting that these extraction methods are insufficient and inadequate in removing all the inhibitory substances. RNA from over 30 lung specimens has been obtained that is consistently suitable for cytokine RT-PCR. Generation of RNA from lung parenchyma suitable for RT-PCR thus requires an additional two precipitations of the RNA with LiCl following RNA extraction using guanidinium thiocyanate.

Method A was used to quantitate differences in the efficiency of the RT and PCR reactions of RNA samples obtained with and without LiCl precipitation by inclusion of radiolabeled [33P]dATP in the dNTP solutions. This showed that the RT and PCR process were 80% and 310% more efficient, respectively, following LiCl precipitations. Thus, the total accumulated increase in efficiency was approximately 400% more than that obtained when using RNA not precipitated with LiCl.

We believe that this method provides a simple but effective modification to a widely used method for the extraction of RNA for use in RT-PCR. This method has been developed for the isolation of RNA from lung tissue but is an approach that should also be applicable to other tissues where RT-PCR inhibitors may be present.

REFERENCES

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Oven Cooking Bags Allow an Inexpensive Alternative in Oil-Free Competitive RT-PCR


Plastic oven baking bags (Pingvin™; Hoechst, Wiesbaden, Germany) are made of a tough heat-resistant plastic that is readily adapted to any application involving moderate heat and pressure. We have discovered that this plastic readily forms a vapor-proof seal with the polycarbonate plastic of the vacuum-formed plastic plates that are commonly used in 96-well polymerase chain reaction (PCR) thermal cyclers. They therefore provide a simple, rapid and inexpensive means for carrying out high-volume semiquantitative competitive reverse transcription (RT)-PCR without the need for expensive accessories using any 96-well format thermal cycler equipped with an after-market heated lid such as the HL-1 (Techno, Princeton, NJ, USA). Our method involves using this heated lid with only minor modifications in combination with sheets of cut oven baking bag plastic to enable oil-free thermal cycling on standard thermal cyclers.

Heated lids are designed to enable oil-free thermal cycling by maintaining the upper part of the reaction vessels at a constant temperature. They are necessary to prevent any evaporation, which may alter the relative concentration of the reaction components in the PCR and therefore significantly affect the efficiency of the cycling protocol (1,4). Even though any heating element that can simply maintain a constant temperature above the highest cycling temperature of the PCR over an area the size of the upper portion of the reaction vessels can be classified as a heated lid, this valuable accessory is missing from many older thermal cyclers. Many thermal cyclers purchased in the early 1990’s were not outfitted with heated lids as original equipment, leaving operators faced with the dismal choice of either continuing to use oil overlays, which are messy and extend sample handling time, or having to purchase new thermal cyclers (3). Recently, a
A brand new market has emerged with many manufacturers offering expensive so-called after-market heated lids, which are optionally designed to fit specific thermal cyclers and can readily be bolted in place. These heated lids are relatively simple appliances containing a heating element and have many similarities to a common portable steamless electric "travel" iron.

The thermal cycler used in this study has been specifically designed to accept the HL-1 accessory heated lid, but any thermal cycler with so-called "hard-spots" can be modified to accept this sort of heated lid, which can subsequently be adjusted to fit at a precise height over the heating block, where a plastic clamp is used to maintain a constant pressure between the heating block and reaction vessel(s). Figure 1 illustrates how easily a thin-walled vacuum-formed 96-well plate (TS-2; Techne) can be modified by cutting away the side panels to facilitate a proper fit. The plate is then cut to the appropriate size, depending on the exact number of PCR amplifications to be performed, and covered with either a carefully cut-to-fit layer of commercially available plastic sealant film (TS-1; Techne) or, as we have discovered, oven baking plastic offers an inexpensive and fully functional alternative for PCR. Plastic oven baking bags provide an inexpensive substitute to the commercially available sealant plastic and, in practice, either one of these functions equally as well as the other in PCR. The sheet of oven baking plastic is cut, applied and followed by a doubled-layer of heavy-duty aluminum foil (Flora, Radolfzell, Germany). The pressure-induced deformation of the plastic sheet caused by the clamped heating block forces wedges of plastic into the upper voids of the reaction vessels. This combination forms a tight molded-fit upon heating, thus providing a vapor-proof fit between the polycarbonate surfaces of the vacuum-formed reaction plate and wedge(s) of the sealant plastic sheet.

To demonstrate the reproducibility and accuracy of this method, we have performed a series of semiquantitative competitive RT-PCRs using a constant amount of cDNA. The cDNA is synthesized by RT of mRNA isolated from a flash-frozen normal control biopsy of a patient undergoing surgery for colorectal cancer (2). At the completion of thermal cycling, the heated lid is opened with a snap, and the combination plastic-foil seal is easily peeled away. The vacuum-formed plate can then be removed from the heating block and placed into a conventional 96-well microplate (MicroWell™; Nunclon, Roskilde, Denmark), which can serve as a convenient sample handling tray. Besides readily serving as a holder, the standard 96-well plate can also serve as a receptacle for the direct transfer and mixing of PCR solutions with pre-dispensed loading buffer (30% [vol/vol] glycerol, 0.25% [wt/vol] bromophenol blue, 0.25% [wt/vol] xylene cyanol) using an eight-channel pipet for the subsequent agarose gel (3% [wt/vol] NuSieve® agarose; FMC BioProducts, Rockland, ME, USA) with 1× TAE (40 mM Tris-acetate, pH 8.0, 1 mM EDTA) and ethidium bromide (5 µL/mL) for electrophoretic separation. Figure 2 illustrates the results of the semiquantitative RT-PCR followed by quantitative digital image analysis (Image-Pro™ Plus Version 1.3; Media Cybernetics, Silver Spring, MD, USA) of the resultant agarose gel(s).

The dimensions of the Pingvin oven cooking bag used in this study are 3 m × 35 cm. It can easily be cut to fit any size reaction vessel depending on the number of samples to be sealed. Over the last few years, we have successfully used this material for performing hundreds of PCRs and have not observed any difference between using it and

Figure 1. Trimming and sealing of the 96-well, thin-walled polycarbonate plastic plate to accommodate a specific number of PCR samples to be analyzed.
using the sealant film intended for this purpose. Even though we still occasionally use the later, the oven cooking bags have the advantages of being much more economical, accessible at any local supermarket, easier to cut and handle and have worked well for both standard and semiquantitative PCR again and again, proving to be functional and reliable.

To obtain a good seal between the plate and sheet of oven baking plastic, it is absolutely necessary that the entire upper portion of the plate be completely unsoiled and oil-free before placing the sheet. Once the sheet is carefully aligned, the sheets of aluminum foil can be placed on top of it, and the whole arrangement can even be taped in place if desired before transport. At this point, it is safe to transport the entire arrangement including all of the PCR components on ice to a pre-heated (95°C) thermal cycler for PCR amplification. Next, the heated lid is quickly clamped into place, transferring heat to the sealant plastic, which is thus molded into the shape of the upper dimensions of the reaction vessels of the plate forming a vapor-proof seal.

With the exception of radiation heating, heat transferred from the heated lid is restricted to the upper boundaries of the plate containing the reaction vessels. We have observed little affect on PCR cycling time or quality following the attachment and use of the heated lid with the thermal cycler (data not shown). Clamping of the heated lid to the block assures a good seal by retaining an even pressure across the reaction vessels of the plate forcing the plastic sealant-lined aluminum foil into the openings of each reaction vessel on the plate (Figure 1C). The initial sealing process usually takes less than 30 s and is completed at least 1–2 min before the first cycle of PCR actually begins. We have had very few problems with leakage using this method, and if leakage does occur, it is usually observed at the edges of the plate and can usually be prevented by allowing some of the plastic sealant sheet to overlap the edges. In practice, we have found that it is better to simply leave those areas void and carry out reactions in sets of six across using only the inner-columns rather than all eight columns, since it is very seldom that any problems exist with broken seals in the inner-columns.

In closing, we feel that this is an inexpensive alternative method to the myriad of commercially available confirmations that are currently on the market, yet it is still just as easy to use in the laboratory. The use and re-use of these plastic vessels result in both savings in our laboratory and spares the environment. Over the past few years, this method has saved a great deal of plastic waste, time and expense in our laboratory. In our hands, it has proven to be a valuable and time-saving technique that has helped to eliminate many of the difficulties associated with performing the high volume of multiple PCRs, which are necessary for semi-quantitative competitive RT-PCR in a clinical laboratory.

REFERENCES


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