Direct isolation of poly-A$^{(+)}$ mRNA from tissue culture cells

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ABSTRACT

The conventional methods for isolating cytoplasmic poly-A$^{(+)}$ mRNA from small amounts of cells are laborious, inconvenient and very time-consuming. Indeed, the isolation of mRNA for RT-PCR may take up to two days. Using the PickPen™ magnetic particle transfer device together with optimized reagents shortens the protocol from two days to 10 minutes while the fastest magnetic rack technique shortens the protocol to 15 minutes. However, the simultaneous processing of six samples with PickPen or a magnetic rack requires 30 minutes or 90 minutes, respectively. Using PickPen technology we have isolated poly-A$^{(+)}$ mRNA with a size-range from 0.5-kb to 7-kb from cell lysates. The isolated mRNA is of high quality, as it supports the amplification of long (1.7-kb) β-*actin*-specific cDNA as well as target RNA that can be used for preparing cDNA libraries.

INTRODUCTION

The conventional methods for isolating cytoplasmic poly-A$^{(+)}$ mRNA from small numbers of cells are inconvenient and labor-intensive, particularly when total RNA is first extracted with toxic organic compounds followed by poly-A$^{(+)}$mRNA enrichment using oligo(dT)-cellulose chromatography. These methods include multiple steps, they are unreliable and result in low yields of an already limited amount of RNA. Furthermore, the methods are very time-consuming. The isolation of mRNA for RT-PCR from cells may take up to two days.

Recent developments in mRNA isolation techniques, such as spin-column and biomagnetic separation techniques using oligo(dT) resin or paramagnetic particles coated with oligo(dT) together with magnetic rack separation make previously time-consuming mRNA isolations attractive and practical. Indeed, the most commonly used oligo(dT) resin-technique or the most rapid magnetic rack method have significantly decreased the time required for isolating mRNA, from two days to 60 or 15 minutes respectively. Unlike magnetic rack techniques, resin-based methods utilizing e.g. column filtration are not very suitable for working with less than 100 cells, as substantial amounts of mRNA are lost in the resin-bed. Thus biomagnetic separation techniques appear to be the method of choice when poly-A$^{(+)}$ mRNA is to be isolated from small amounts of cells, for example microdissected cells. However, the processing of parallel samples significantly increases the isolation time. Indeed, simultaneous processing of six samples with spin-column or magnetic rack techniques require two hours or 90 minutes respectively, and for obvious reasons it is desirable to shorten this time.

RNA ISOLATION WITH PICKPEN –THE IDEAL METHOD?

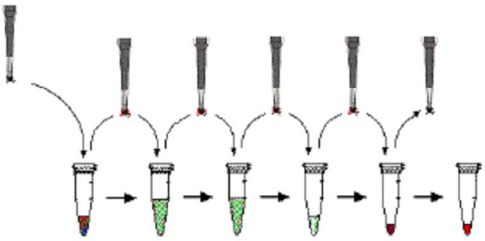
An ideal method for isolating poly-A$^{(+)}$ mRNA would be a technique that is rapid, convenient, reliable and equally suitable for a few cells as for a million cells.

Unlike magnetic rack techniques, which are based on transferring liquids, PickPen technology moves particles instead. This is both faster and more convenient than the presently used magnetic rack techniques. The oligo (dT)-coated particles, with the isolated poly-A(+) mRNA bound to them are carried through the process using the PickPen, simplifying pipetting and eliminating decanting steps. Therefore the PickPen technology is very close to the ideal method for isolating mRNA rapidly.

MATERIALS & METHODS

For isolating mRNA, human U937 or K562 cells were lysed in lysis/annealing buffer, mixed with washed oligo(dT)30 coated magnetic particles (Fig.1; step a), incubated for 4 min at ambient temperature for hybridizing oligo(dT) to mRNA.

Particle-bound mRNA was washed twice in Wash buffer 1 (b,c), once in Wash buffer 2 (d) and finally resuspended in Elution buffer (e) for eluting mRNA at +65 ^{0}C (e). The resulting mRNA (f) was subjected to downstream applications (RT-PCR, ss-cDNA, ds-cDNA synthesis).



(a) (b) (c) (d) (e) (f)

Fig 1. mRNA isolation protocol

The QuickPick™ mRNA mini- reagents from Bio-Nobile™ were used in the isolation.

RESULTS

PickPen shortens a mRNA isolation from up to two days to 10 minutes. In a method comparison, the most rapid magnetic rack technique can be carried out in 15 minutes per isolation, and commonly used spin-column methods take 60 minutes. However, the *simultaneous* processing of six samples with PickPen, magnetic rack or spin-column technique requires 30 minutes, 90 minutes or two hours respectively, making work with the PickPen considerbaly more efficient.

With PickPen technology we have isolated from crude cell lysates about 0.1 pg poly-A$^{(+)}$ mRNA per cell that has a smear-like appearance on the gel from about 0.5-kb to 7-kb. To show that the isolated mRNA is of high quality, it should support the amplification of very long *actin* cDNA, (fig.2).

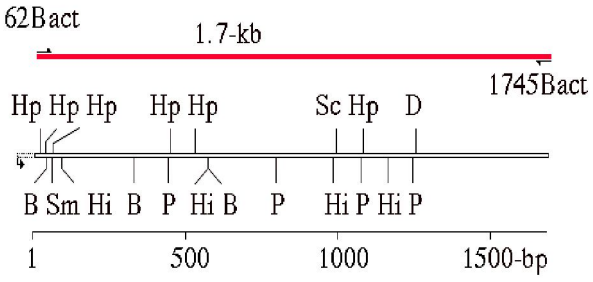


Fig 2. Gene map of human β-*actin* gene

Indeed, the isolated mRNA is of high quality, as 4 ng of mRNA supports the amplification of very long cDNA, almost full-length (1.7-kb) β-*actin*-specific cDNA (Fig. 3; lanes 1 and 2).

Mr 1 2

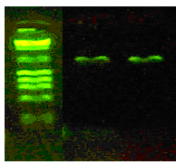


Fig 3. β-*actin* RT-PCR

We have used small amounts (85 ng) of mRNA isolated from crude cell lysates for constructing double-stranded cDNA libraries.

CONCLUSIONS

PickPen, using unique magnetic particle transfer technology, shortens the mRNA isolation to 10 minutes, and is faster than the other biomagnetic separation methods. PickPen technology speeds up the otherwise time-consuming washing steps in mRNA isolation. Thus, parallel sample preparation using PickPen is significantly faster and more convenient than that of magnetic rack methods. The quality of mRNA isolated directly from crude cell lysates is of high quality, as it serves as template for RT-PCR and cDNA library synthesis.