



APPLICATION NOTE NO 1

mRNA purification kit

High quality mRNA purification from paraffin embedded tissues using the KingFisher for molecular finger-printing

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Introduction

To study the cellular and molecular mechanisms underlying synaptic and dendritic reorganization following various brain injuries, human postmortem brain tissues are used for regional and single cell mRNA amplification techniques and cDNA microarray "DNA chip" technology (1-3). These studies enable a "molecular fingerprint" of the affected region, and provide information regarding the regulation of multiple mRNAs in individual neurons and dendrites.

One of our standard procedures is to extract RNA from small micropunches of fixed postmortem paraffin embedded human hippocampus using labor intensive organic extraction procedures that are toxic to the investigator and can introduce variability in terms of increased experimenter error due to the several centrifugation and pipetting steps that are required. We were interested in finding a cost effective, high throughput way of semi-automating our need for high quality RNA species. KingFisher provided such a means to potentially increase our throughput ability without decreasing our yield through the magnetic, rather than organic, extraction procedure.

Method

mRNA purification kit :

Modified protocol for mRNA extraction from paraffin-embedded tissue.

Small micropunches of fixed postmortem paraffin-embedded human hippocampus were used as starting material. The estimated total number of cells is between 300-600 and total RNA is in the picogram range.

1. The mRNA kit was modified by using RNase free water as the elution buffer.
2. Tissue sections were deparaffinized and the microexcised tissue was placed in a proteinase K solution (50 $\mu\text{g}/\text{ml}$) for 6 hours at 37 °C.

The proteinase K treated sample was pipeted into KingFisher wells and the protocol was followed as recommended in the kit insert.

Summary of results

With minor modifications of the standard elution procedure we were able to reproducibly extract mRNAs that were subsequently amplified and used as a probe for hybridization on a variety of array platforms.

Bioanalysis is done using two different primer sets for the subsequent linear RNA amplification procedure (1-3) and analysed using BioAnalyzer (Picture 1). Note the long-range representation of messages from high (greater than 7.5 Kb) to low (hundred bp) on the gel for both conventional and KingFisher extraction procedures on adjacent samples from the same case. Primers A and B differ in the amount of small oligonucleotide-like fragments that are generated. However little difference appears across extraction conditions.

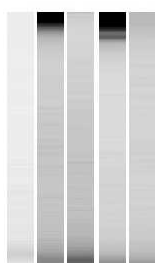


Fig.1. Bioanalysis data performed using BioAnalyzer for linear RNA amplification using two different primers for mRNA extracted with conventional and KingFisher extraction procedures 1: Conventional mRNA extraction with primer A, 2: Conventional mRNA extraction with primer B, 3 KingFisher mRNA extraction with primer A, 4: KingFisher mRNA extraction with primer B, 5: Control

Two identical customer-designed arrays were hybridized (Picture 2.) with P33 UTP labeled probes generated using amplified RNA from adjacent samples from the same case (and corresponding the bioanalysis data of the picture 1.) using conventional and KingFisher extraction methods. Remarkably, no quantitative differences have been observed between extraction conditions as can be evidenced by the relative hybridization signal intensity of the 32 cDNAs depicted per array.

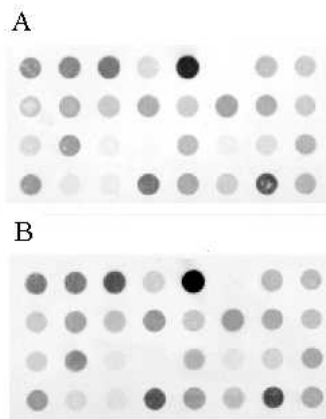


Fig.2. Two identical arrays were hybridized with amplified RNA extracted with conventional extraction method (panel A) and with KingFisher (panel B).

Conclusion

The results with KingFisher mRNA kit together KingFisher instrument have been highly reproducible. RNA can be extracted in less than an hour without any labor intensive or hazardous procedures. Comparing adjacent samples extracted by our standard laboratory organic method and the KingFisher method no differences have been detected by bioanalysis or by cDNA microarray analysis on custom-designed arrays. Thus the KingFisher system is now being incorporated into our standard procedure for mRNA isolation for subsequent downstream amplification and genetic analysis procedures.

References

- Ginsberg, S.D. (2001) Gene expression profiling using single cell microdissection combined with cDNA microarrays. In D.H. Geschwind (Ed.), *DNA Microarrays: The New Frontier in Gene Discovery and Gene Expression Analysis.*, Society for Neuroscience Press, Washington, pp. in press.
- Ginsberg, S.D. *et al.* (2002) Expression profile of transcripts in Alzheimer's disease tangle-bearing CA1 neurons, *Ann. Neurol.*, 48 (77-87).
- Ginsberg, S.D. *et al.* (1999) Molecular pathology of Alzheimer's disease and related disorders. In A. Peters and J.H. Morrison (Eds.), *Cerebral Cortex, vol. 14. Neurodegenerative and Age-related Changes in Structure and Function of Cerebral Cortex*, Kluwer Academic/Plenum, New York, pp. 603-653.

Plastics

| Code | Name |
|----------|---|
| 97002090 | KingFisher 100 μ l 8-pack, 8 plates & 8 tip combs / box |
| 97002094 | KingFisher 200 μ l 8-pack, 8 plates & 8 tip combs / box |
| 97002131 | KingFisher mL Combi 60, tubes and tip combs for 60 |

Kits

| Code | Name | Amount of samples |
|---------|---|------------------------|
| 6300020 | KingFisher mRNA purification kit | 24 (includes plastics) |
| 6300021 | KingFisher mRNA purification kit | 96 |
| 6300022 | KingFisher mL mRNA purification kit | 60 |
| 6300031 | KingFisher PCR and Gel cleanup kit | 96 |
| 6300032 | KingFisher mL PCR and Gel cleanup kit | 30 |
| 6300041 | KingFisher Genomic DNA purification kit | 30 (KF mL) 96 (KF) |
| 6300042 | KingFisher Genomic DNA purification kit | 120 (KF mL) 300 (KF) |
| 6300051 | KingFisher Total RNA purification kit | 30 (KF mL) 96 (KF) |
| 6300061 | KingFisher mL Blood DNA kit | 30 |
| 6300062 | KingFisher mL Blood DNA kit | 120 |

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