

دستورالعمل جمع آوري، انتقال، آماده سازی و نگهداري نمونه ها برای آزمایشات مولکولي

این راهنمای منظور ارایه اطلاعات علمی و فنی لازم در ارتباط با نمونه هایی که برای آزمایشات ملکولی تهییه شده است. این مجموعه ترجمه پروتکل MM13-A مبوده و که در کمیته ویروس شناسی و بیولوژی ملکولی مورد بررسی و تایید قرار گرفته است.

متجمیین به ترتیب الفبا:

اعضا کمیته ویروس شناسی و بیولوژی ملکولی به ترتیب حروف الفبا عبارتند از:

()

() /

()

Linkage analysis

(blood-borne)

(HPV)

/

() ()
() ()
() ()

DNA RNA

¹ Buccal Cell

DNA

DNA RNA

DNA RNA

()

DNA RNA

DNA RNA

(DNA RNA)

DNA RNA

RNA

DNA RNA

pH

CVS CVS

DNA

) CVS . . . CVS (endometrial decidual

DNA

2 Prenatal

Prenatal 3 Chorionic Villus Sampling

CVS

(Polyester tipped)

(Rayon)

HPV

⁴ Inverted Microscope

(RNA DNA)

(home brewed)

DNA

RNA

mRNA

<http://www.cdc.gov/od/ohs/biosfty/biosfty.htm>

<http://www.cdc.gov/od/ohs/pdffiles/DOTHAZMAT8-14-02.pdf>

http://www.who.int/csr/resources/publications/WHO_CDS_CSR_LYO_2004_9/en/

DNA

DNA

DNA

DNase

DNA

DNA

DNA

(Tris-EDTA) TE

DNA

(DNase

)

DNA "frost-free"
 DNA

 :RNA
 RNA

 RNA
(RNases)
 RNA "

DNA RNA)
(

/

^

EDTA

() EDTA

HCV HIV RNA

DNA

RNA

RNA

(mRNA)

RNA

DNA RNA

RNA

RNA

RNA

HCV HIV

با توجه به عدم گزارش هایی که مورد پذیرش بین المللی باشد⁶

⁶ Frost-free
⁷ Viral load

"heme"

Acid Citrate Dextrose (ACD) EDTA

: ()

DBS DNA

DBS

(glassine)

DBS

PCR DNA

()

() EDTA :DNA

⁸ Dried Blood Spots

⁹ Bronchoalveolar Lavage (BAL)

EDTA

PCR

'Heme'

RNA

RNA

RNA

RNA

(Buccal Cells)

DNA RNA

RNA

RNA

DNA

DNA

RNA

RNA

:Buffy Coat

DNA

Buffy Coat

DNA

RNA

RNA

RNA

eosinophilia

Buffy coat

:(CSF)

CSF :**DNA**

CMV EBV VZV

DNA

HSV

RNA

CSF

:**RNA**

RNA

CSF

:(Cultured cells)

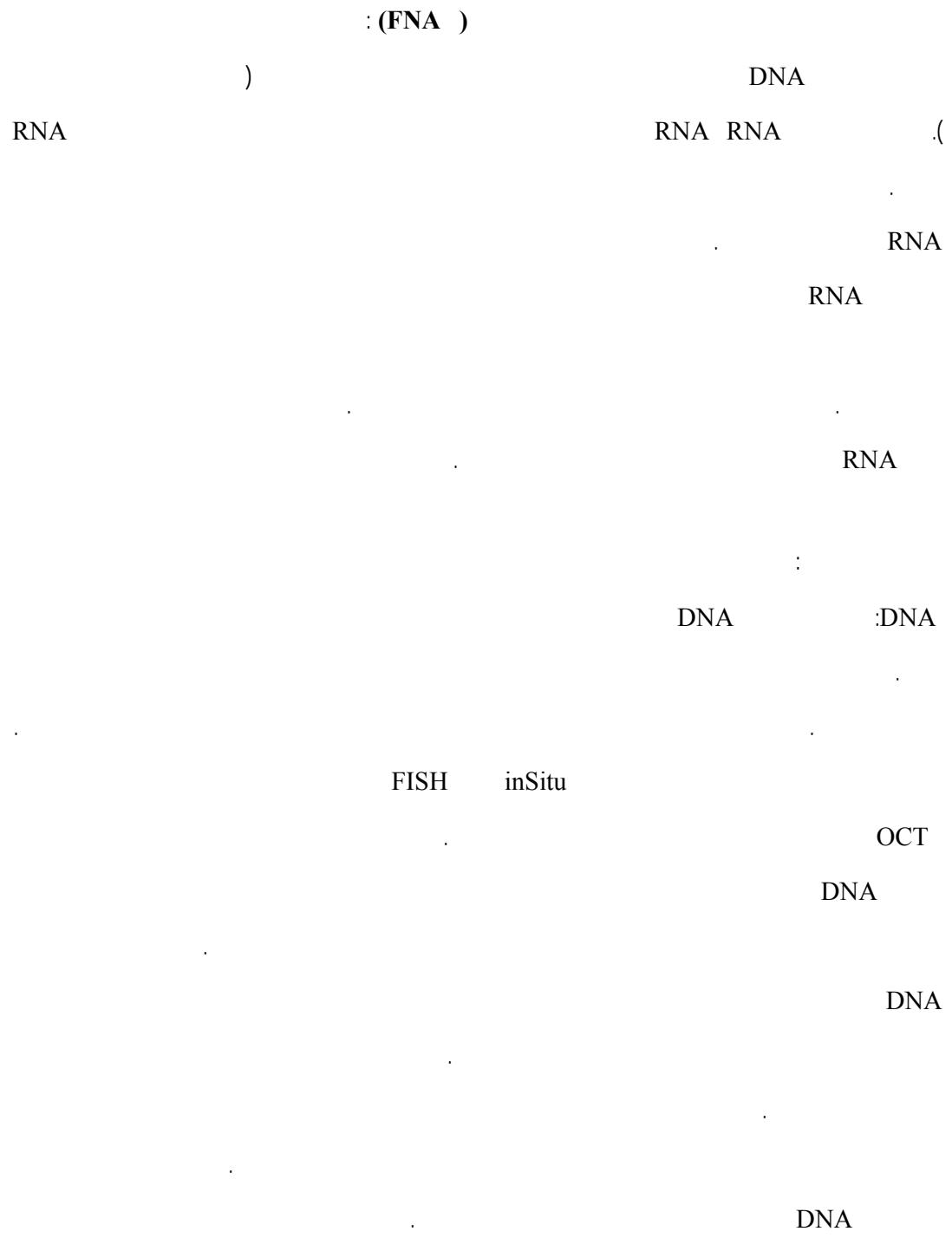
RNA DNA

RNA DNA

(EBV)

(immortalization)

Buffy Coat



¹¹ Fine Needle Aspirate
¹² Optimal Cutting Temperature

RPMI

RNA

RNA

RNA

RNA

RNA

(FISH) inSitu

OCT

RNA

RNA

()

OCT

¹³ Roswell Park Memorial Institute
¹⁴ Guanidinium Isothiocyanate

RNase RNA

" "

RNA

RNA

RNA

RNase

: **(Oral Fluids)**

RNA DNA

RNA

()

: **(FFPET)**

FFPET

FFPET

DNA

DNA

)

(B5

RNA

RNA

FFPET

FFPET

¹⁵ Formalin- Fixed Paraffin Embedded Tissue

:**Semen**

semen DNA

DNA

in situ hybridization

DNA

)

(

)

(

(Brooms)

DNA

DNA RNA

(

)

PCR

DNA

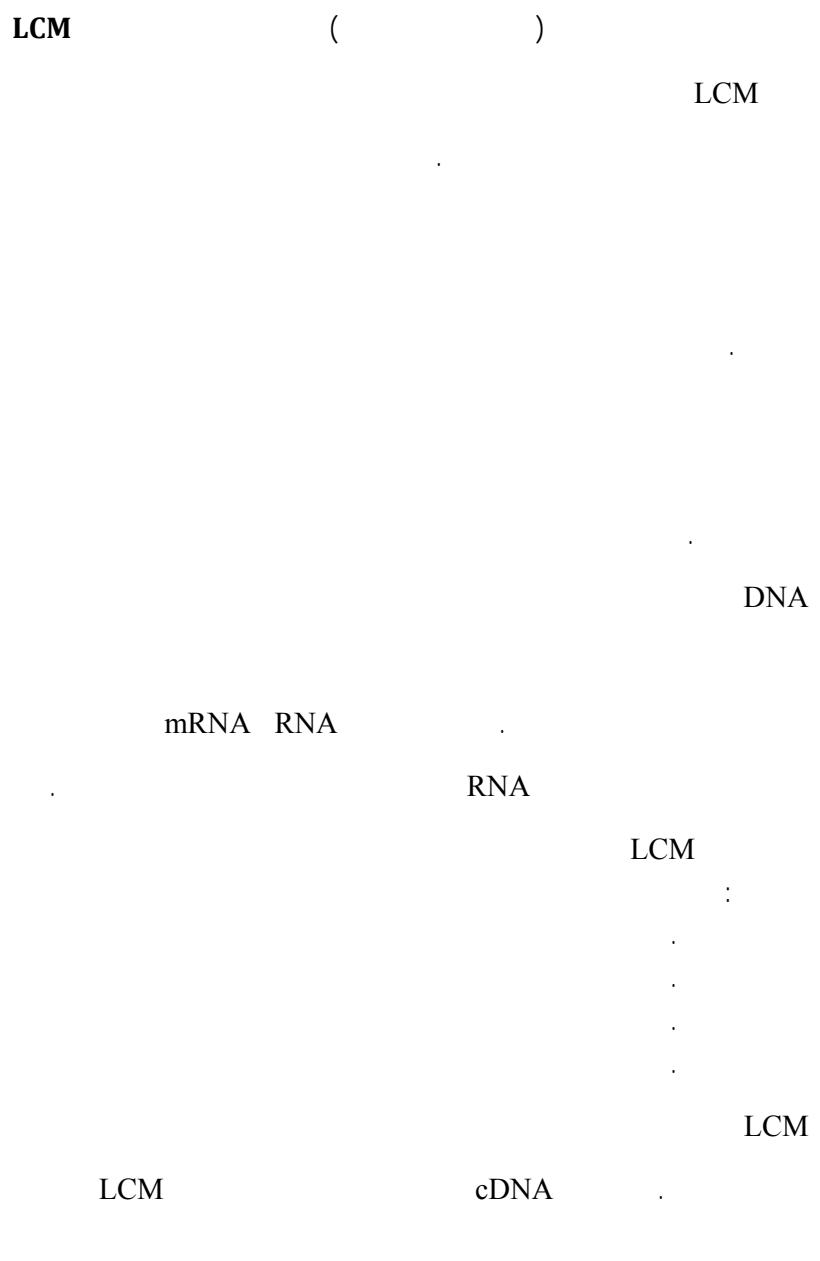
)

DNA

pH

(

RNA



¹⁶ Laser Capture Micro-dissection

Nested :

PCR

PCR

()

DNA : **(DNA/RNA)**

/ DNA

DNA ↗

DNA DNA

(g) / HBV HIV

(Vacuum)

(PH 7/4)

CSF :

CSF

bronchoalveolar lavage

NBF; Neutral) %

: ()

(Buffered Formalin

DNA

DNA

DNA

DNA

%

pH= %

DNA	%	:	
			RNA
% v/v			DNA
			DNA

(%) : **Carnoy's fixative**

(%)	: Methacarn	(%)
	(%)	(%)
RNA		RNA

AMeX	:	
DNA	AMeX	
RNA		mRNA
		AMeX

			RNA
			RNA
			RNA

: **Hope**

/ / pH

¹⁷ Acetone- Methylbenzoate- Xylen

¹⁸ HEPES-glutamic acid buffer- mediated Organic solvent Protection Effect

RNA DNA

RT PCR PCR

inSitu

inSitu

()

(artifact)

DNA

PBS

Agar-Saline-Giemsa

FFPET

FFPET

FFPET

DNA

FFPET

FFPET

FFPET

FFPET

/

)

(

/

B5

DNA

)

RNA DNA

FFPET

FFPET

DNA

(Southern Blotting

DNA

DNA

FFPET

PCR

RT-PCR

RNA

FFPET

¹⁹ Formalin Fixed Paraffin- Embedded Tissue

FFPET

()
DNA PCR

%

DNA "K"
L.C.M. DNA

DNA RNA ,

DNA DNA
DNA RNA

()

RNA Chomezynski & Sacchi

RNA

RNA

RNA

Target Capture

()

DNA

RNAase , DNAase

42^oC

Silica Technology

Boom

RNAase

(lysis and binding buffer)

pH

)

(

()

(Micro Concentrator)

PBS

RNA

DNA

0.22

(Micro Concentrator)

() **DNA**

DNA DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA

() DNA ()

DNA ()

DNA

DNA

pH

DNA

DNA

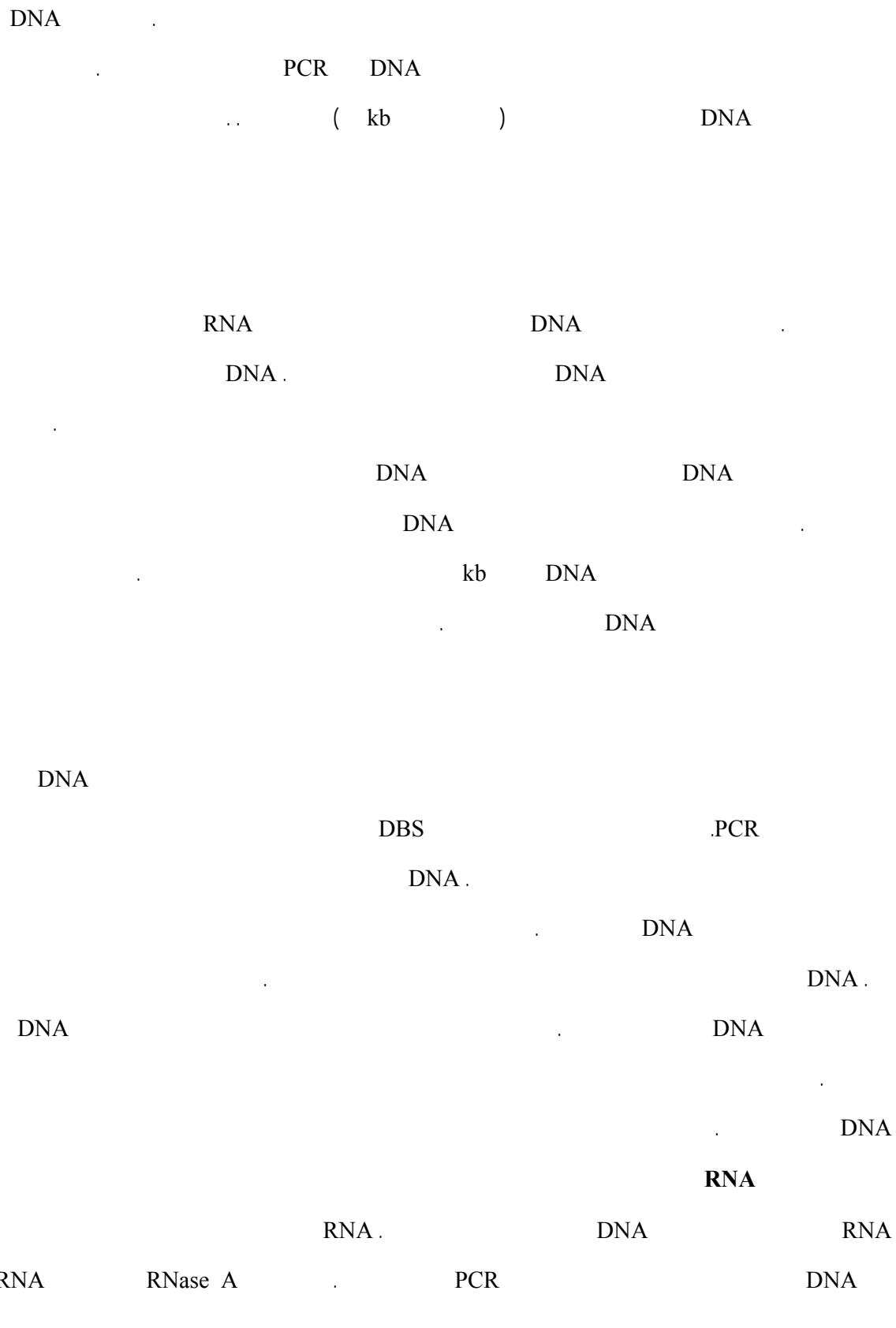
DNA

PCR

Salting - out



²⁰ Cesium Chloride Density Gradients
²¹ Silica-Based Methods



²² Anion – Exchange Method

RNase DNA DNA
 |
 DNase

 : DNA
K |

K

: **Rotor Stator Homogenizer**

/

DNA

EDTA DNA DNA
 |
 DNA

DNA

DNA

g

K

DNA

DNA

(Home Made)

DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA

DNA . DNA
DNA . DNA
DNA . CSF
(Carries)DNA
RNA DNA
DNA DNA
DNA DNA

DNA

K
() K
K /

/

RNA

RNA
RNA
RNase RNA
RNase (Chaotropic)
RNase RNA

Rotor-Stator Homogenizer

)

(. . . .

Bead Mills

/

/

()

)

(

²³ Beads

Spin-Column

spin-column

spin-column

(cleanup) RNA

DNA

(RNA mRNA RNA RNA) RNA

RNA

K RNA

() K RNase (SDS)

DNase I DNA

K

RNase RNA

RNA (K)

DNase DNase I DNA

) DNase

RNA DNase I (silica

cDNA DNase I

RT-PCR

²⁴ Chaotropic

(denaturant) K
pH

RNA DNA pH
 RNA

RNA

RNase

RNA

RNA salting out

RNA

RNA

RNA

()

RNA DNA

RT-PCR

RNA RNA

() 36000 -40000 x g

RNA DNA

 RNA

 RNA

5s rRNA tRNA RNA

DNA RNA

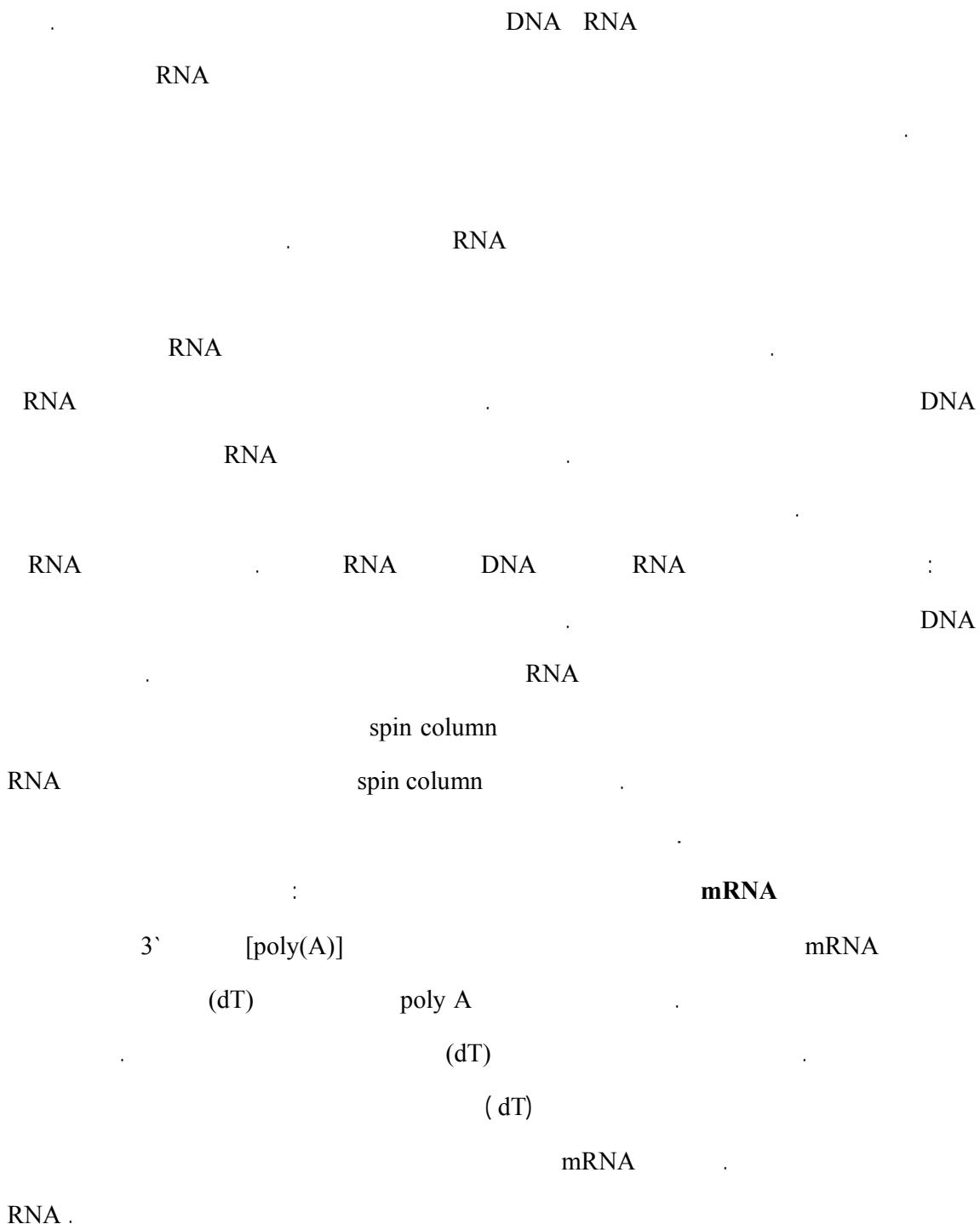
DNA RNA ()

() RNA

 RNA

RNA

RNA



²⁵ Multiwell units

²⁶ Oligo Affinity Chromatography

mRNA

mRNA (dT)

RNA

RNA

RNA

RNA

K

RNA

(. . .) RNA

EDTA

RNA

RNA

(. . .)

(. . .)

RNA

(globin RNA) mRNA

RNA

RNA

)

(

RNA

²⁷ Centrifugal vacuum concentrator
²⁸ Density -Gradian Centrifugation

rRNA mRNA

mRNA . mRNA mRNA
mRNA A 5'
cDNA (dT) (dT)

RNA
DNA RNA RNA
RNA RNA RNA
RNA RNA RNA

RNA , spin column
RNA)
GTC (RNA

²⁹ Random Primer

³⁰ Free Circulating RNA

³¹ Guanidium Thiocyanate