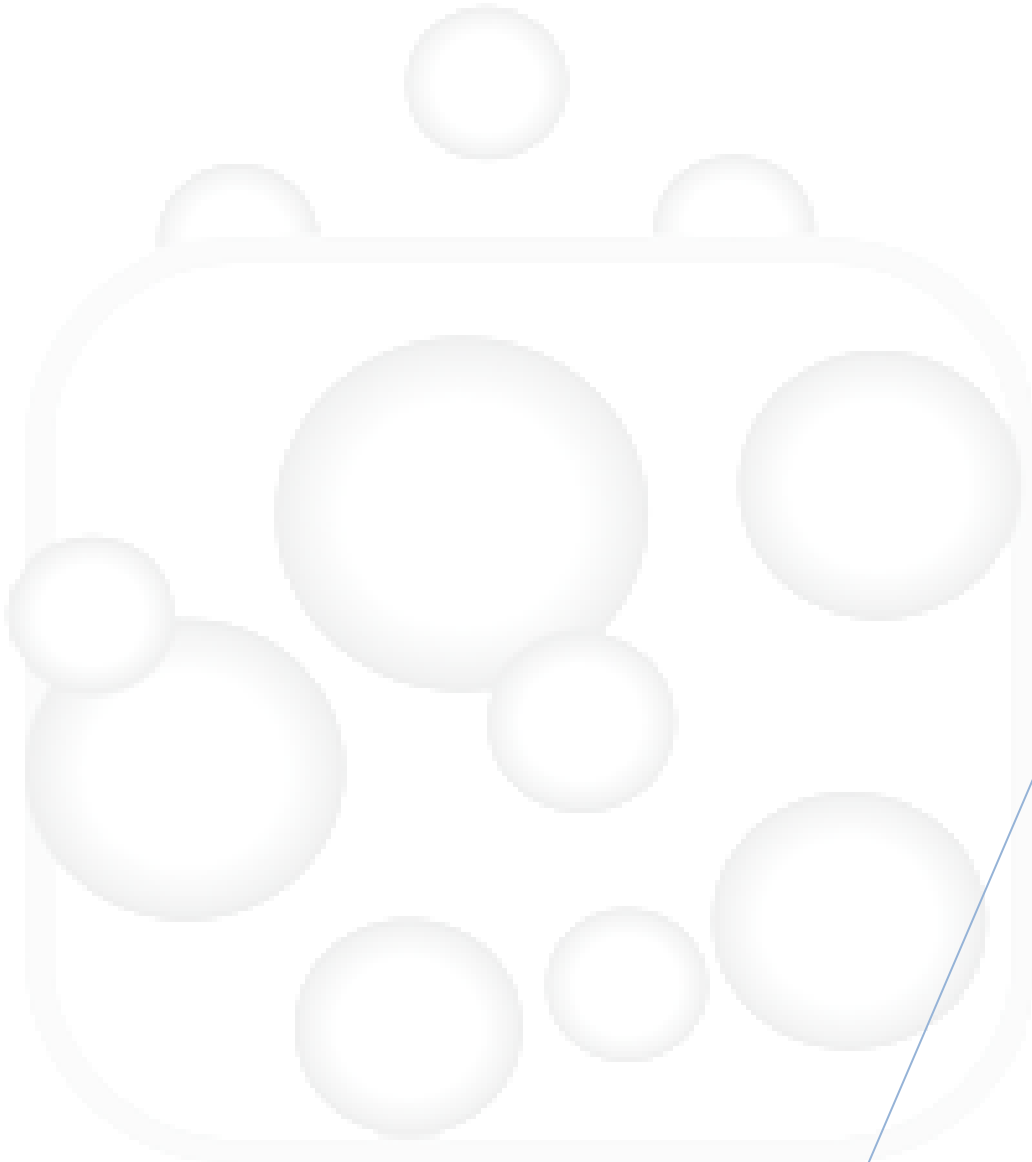




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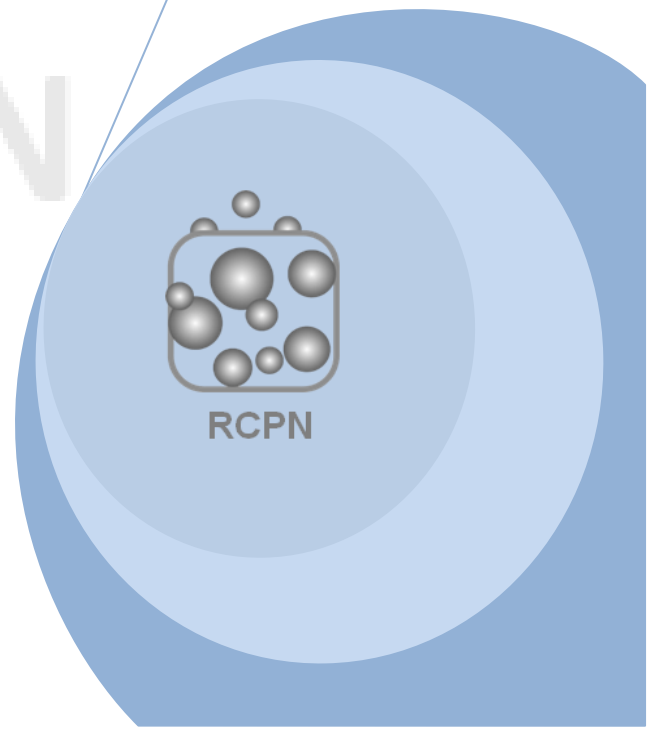
RCPN



RCPN

**cDNA synthesis (RT-PCR)**  
PSR

Golchin. A  
12/30/2015



## Workflow

RNA extraction → cDNA synthesis (RT-PCR) → Real-Time PCR → Data analysis

# Detailed procedure

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## 2. cDNA synthesis (RT-PCR)

### Materials

product	Cat. No.
Random hexamer primer	-
RNase inhibitor	00219287
dNTP	10297-018
Reverse transcriptase	18064-022
0.2 mL microfuge tube	-
Ultrapure Dnase/Rnase-Free Distilled water	DW8520

### Step 1:

#### Primer/RNA Mix:

- Template RNA → 1  $\mu\text{g}$
- Random hexamer primers → 0.5  $\mu\text{M}$  or 20 pmol
- DEPC-Treated water up to → 12.5  $\mu\text{L}$

**Optional:** if RNA template is GC rich or is known to contain secondary structure, mix gently; incubate at (65°C for 5 min).

### Step 2:

Chill on ice for 1 minute. Centrifuge briefly in a micro centrifuge.

### Step 3:

#### Buffer/Enzyme mix, add on ice:

- 10X reaction buffer → 2  $\mu\text{L}$
- RNase inhibitor → 0.5  $\mu\text{L}$
- dNTP mix, 10 mM each → 2  $\mu\text{l}$  (1mM final concentration)
- Reverse transcriptase (mM LV) → 1  $\mu\text{L}$
- DEPC-treated water up to → 20  $\mu\text{L}$

Mix by pipetting up and down.

### Step 4:

Add Buffer/Enzyme mix to the first tube, mix gently and transfer in a 0.2 mL microfuge tube.

**Step 5:**

Place the tube in the PCR machine programmed as follows:

{ 10 min 25°C  
60 min 42°C → for GC reached → 45°C  
10 min 70°C

**Step 6:** Analyze the PCR products by agarose gel electrophoresis

**Step 7:** Store cDNA at -20°C.

Good luck!



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Document ID: RCPN2	
Document name	cDNA synthesis (RT-PCR)
Document arrangement	Mohanna Osali, Rahimeh Mousavi
Date	12/30/2015
Full file name	cDNA synthesis (RT-PCR)
Document Description	This handbook is intended as an introduction to cDNA synthesis
Author / translator	Golchin. Asal