



Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences

RCPN

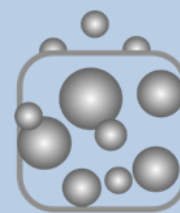
Real-Time PCR

PSR

At RCPN, we use Bio-Rad iQ5 Real Time PCR system

Description: A real-time PCR is a laboratory technique in molecular biology that it is an advanced form of the Polymerase Chain Reaction (PCR). It provides multiple copies of a rare piece of DNA. Changing temperature will control the activity of thermostable polymerase to working faster.

Golchin. A
12/30/2015



RCPN

Workflow

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

Detailed procedure

3. Real-Time PCR steps

Instruments information

QPCR system: Bio-Rad

Model: iQ5 Multicolor Real-Time PCR Detection System

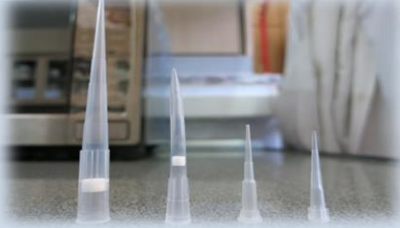
Time required

Real-Time PCR: 2.5-3 h.

Data analysis: 0.5-1 h.

Note: Wear gloves at all times to avoid RNase contamination.

Materials

Product- Cat. No.	Image	Product- Cat. No.	Image
96 well Micro plate 2239441		Aerosol-barrier filter tips PLA1	
Real-time PCR Primers -		DEPC water DW8520	
Master Mix 1507500		Bio-Rad micro seal MSB1001	

Step1:

First design your primers using the following eSource:

Find your genome from: <http://www.ncbi.nlm.nih.gov/genome/>

Principles of designing primer:

- Optimal amplicon length 18-25 bp
- Predicted primer-dimer formation
- Predicted hairpins in the amplicon
- Free tools at: <http://primer3.ut.ee/>

Step2:

Prepare materials as below and start pippeting,

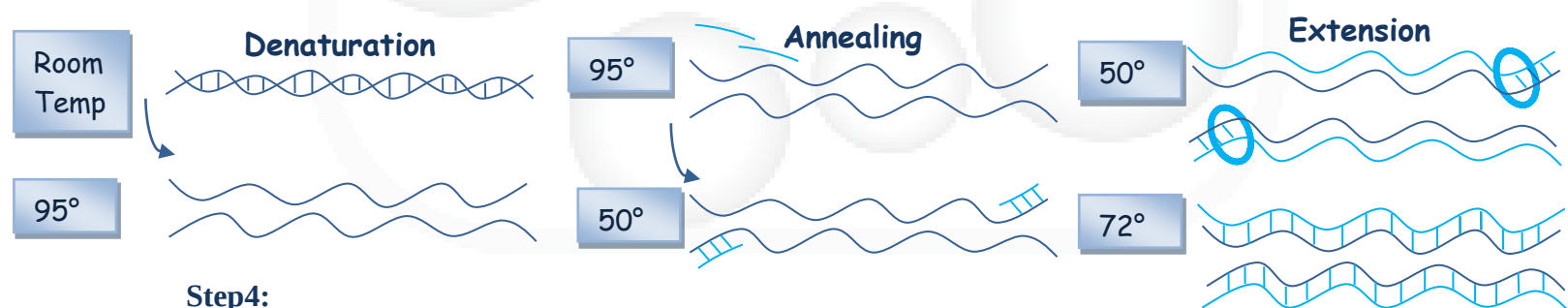
Real-time PCR reaction mixture per tube:

- | | | | |
|----------------------|-----------|--|--------------------|
| ➤ cDNA sample | → 1 μL | | ➔ Total Vol. 20 μL |
| ➤ Master mix (2X) | → 10 μL | | |
| ➤ Primer Forward | → 0.05 μL | | |
| ➤ Primer Reverse | → 0.05 μL | | |
| ➤ DEPC-treated water | → 8.9 μL | | |

Note: Place Master Mix on ice.

Step3:

- Centrifuge micro tubes.
- Make sure that all bubbles are removed.



Step4:

Set-up your plate.

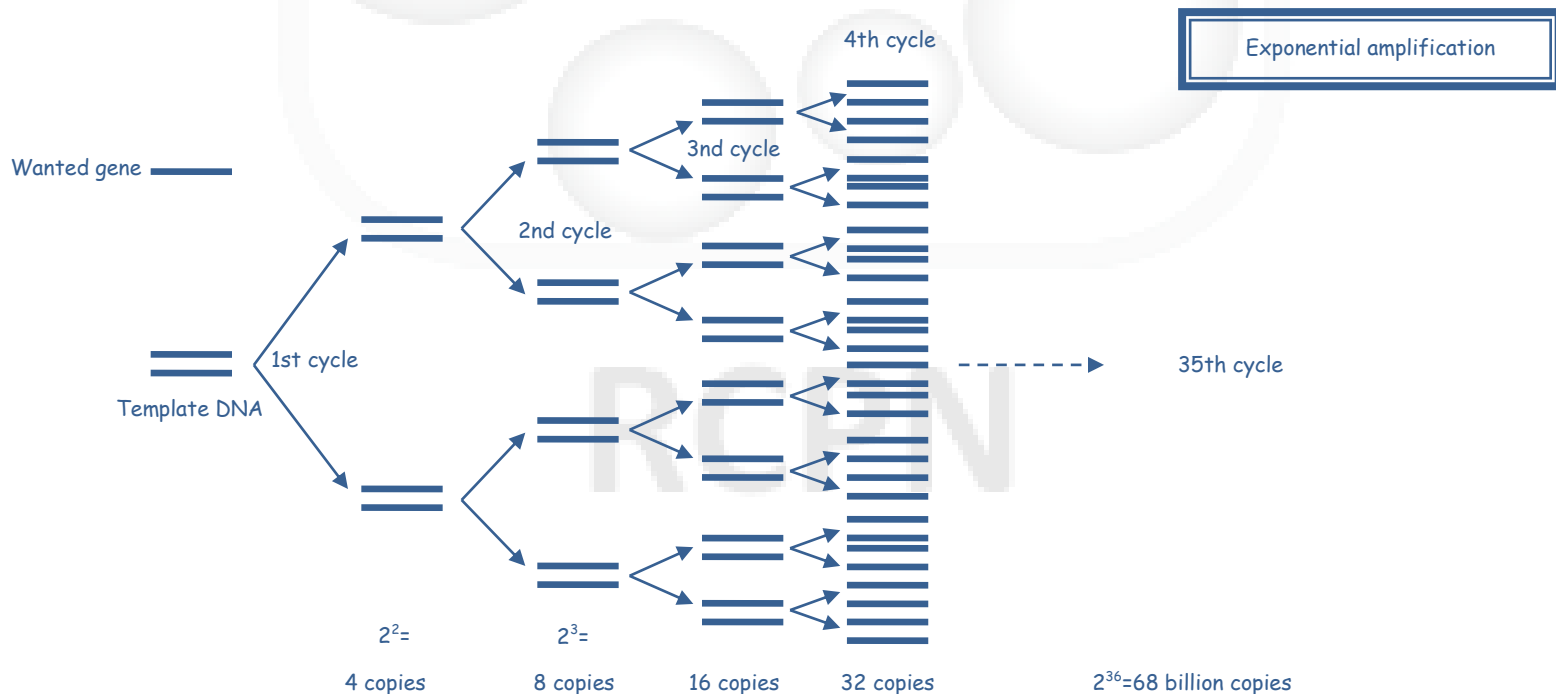
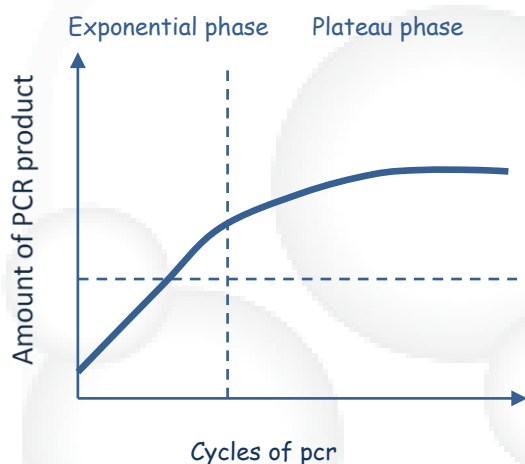
Cycles of real-time PCR:

1. 50°C 2 min, 1 cycle
2. 95°C 10 min, 1 cycle
3. 95°C 15 s → 60°C 30 s → 72°C 30 s, 40 cycles
4. 72°C 10 min, 1 cycle

Note: Annealing temperature will be different in each primer.

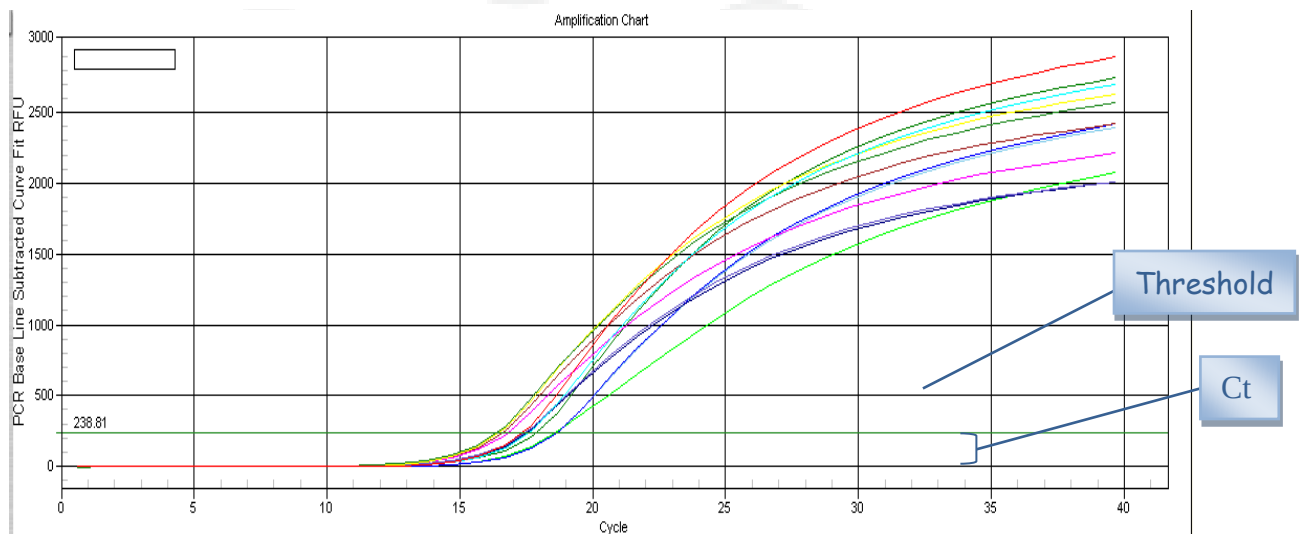
1. Data analysis

The amount of DNA theoretically doubles with every cycle of PCR (exponential reaction). The reaction finally tails off and reaches a plateau. After two cycles we have 2×2 times as much. After N cycles we shall have 2^N times as much. (Real-time graphs are for Miss Malekian, pharmacy student at Tabriz University of medical sciences)

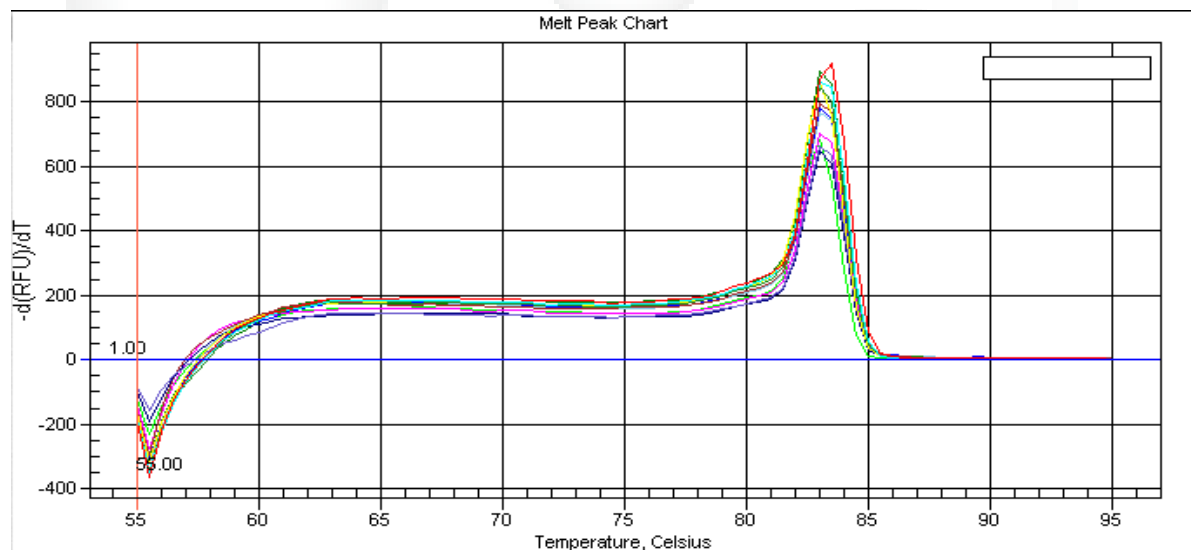


Parameters of Real Time PCR

Ct (Threshold cycle): The cycle number at which the fluorescence signal crosses threshold.

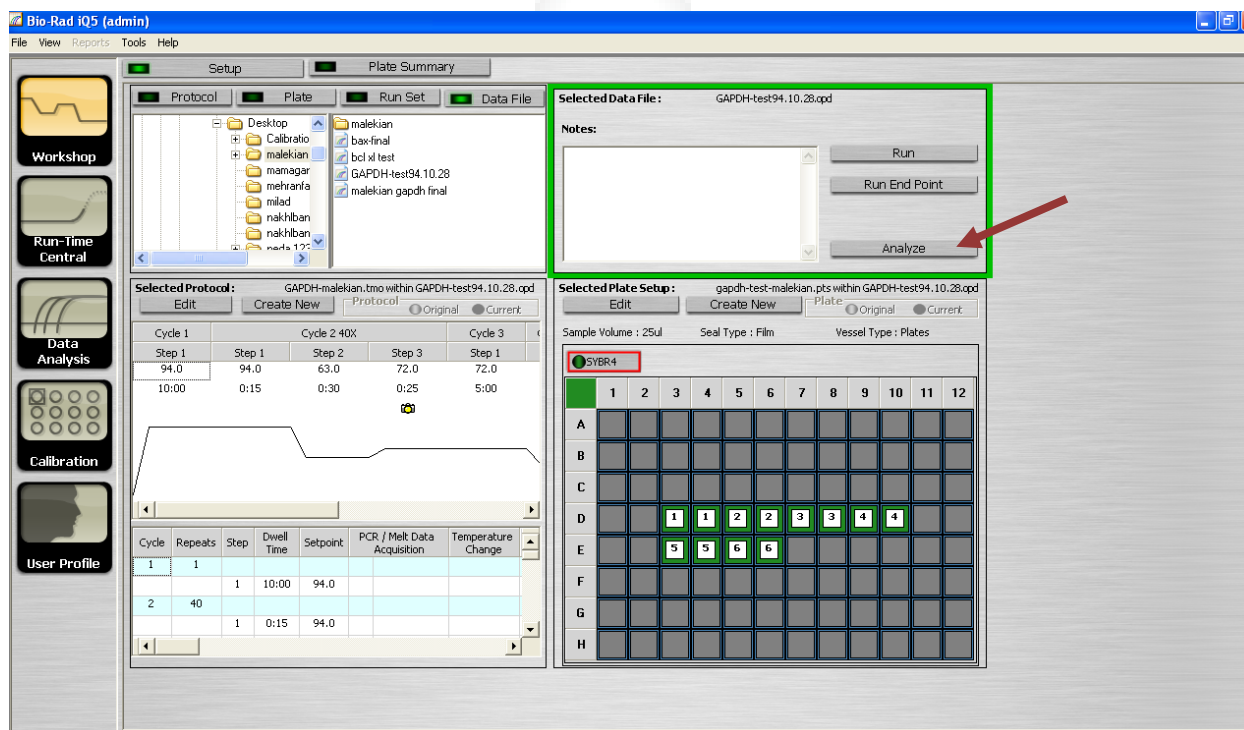


Melt Curve: Dissociation-characteristics of double-stranded DNA during heating.

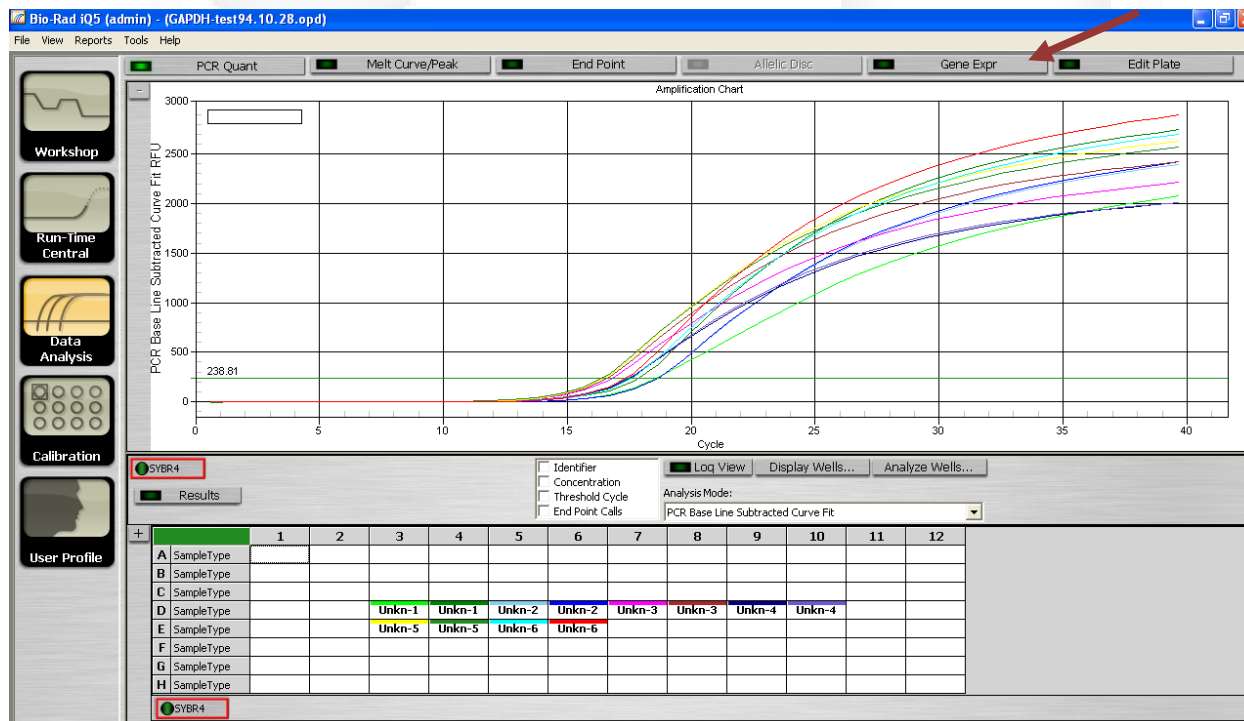


Now you can export results to Excel within the iQ5 software and start analyzing your data.

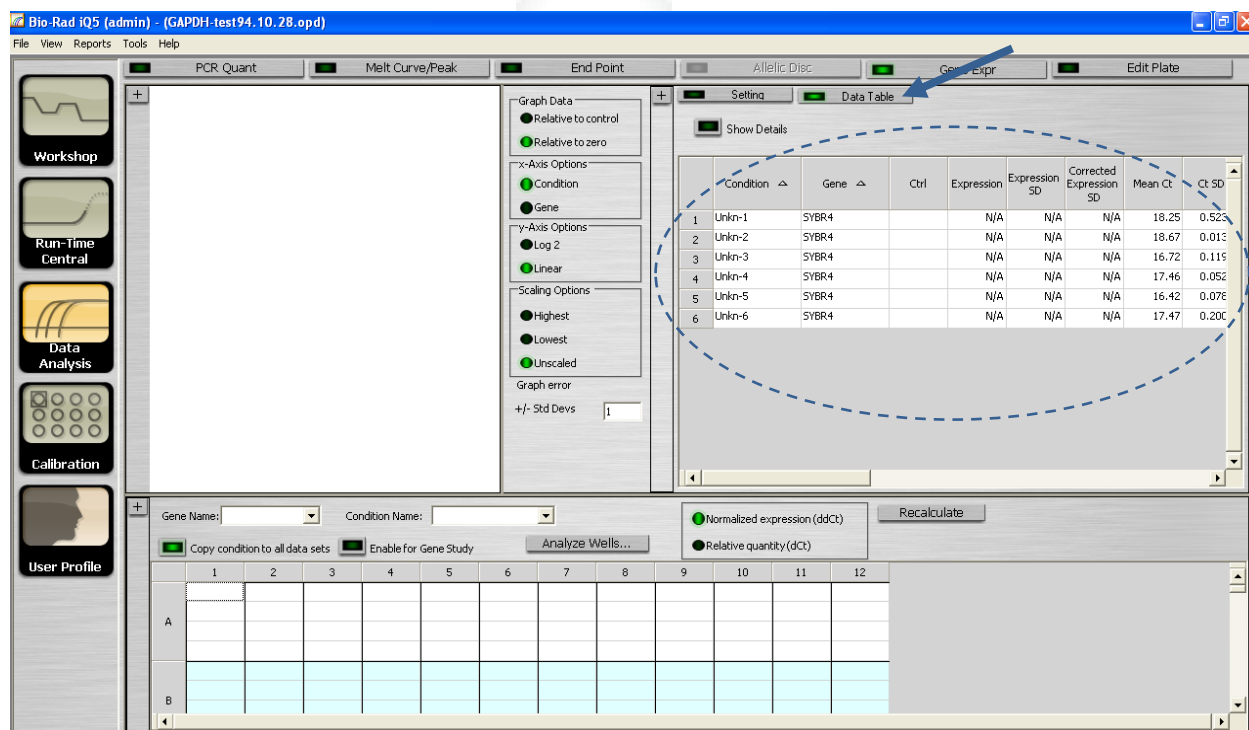
Open your result and click on Analyze button:



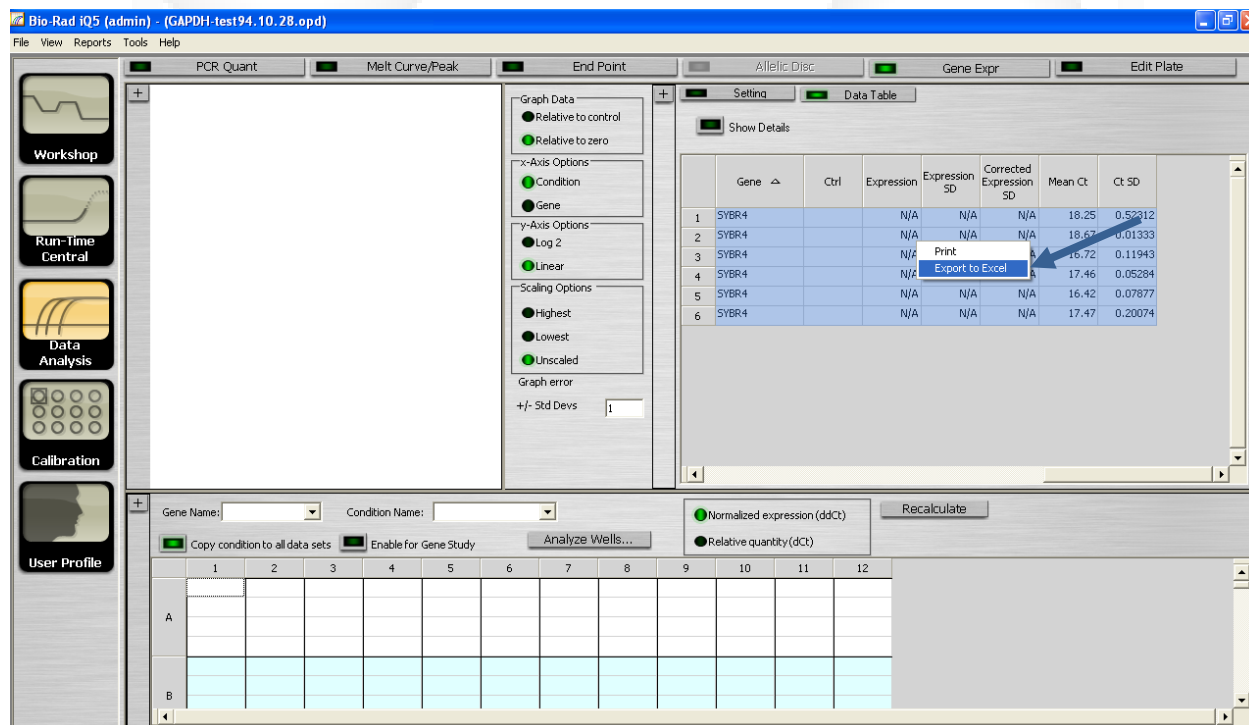
Click on Gene Exp button:



Click on Data Table button and see your result:



Right-click on selected data and Export to Excel:



ANNEX

How to convert Centrifuge RPM to RCF or G-force?

The relationship between **RPM** and **RCF** is as follows:

$$g = (1.118 \times 10^{-5}) R S^2$$

g is the relative centrifugal force, R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in revolutions per minute.

As an example, centrifugation of a sample at 5,000 RPM in a micro centrifuge that has a rotor with a radius of 7 cm will deliver a centrifugal force of $1,957 \times .*$

	Rotor Radius (from center of rotor to sample) in centimeters											
Speed RPM	4	5	6	7	8	9	10	11	12	13	14	15
1000	45	56	67	78	89	101	112	123	134	145	157	168
1500	101	126	151	176	201	226	252	277	302	327	352	377
2000	179	224	268	313	358	402	447	492	537	581	626	671
2500	280	349	419	489	559	629	699	769	839	908	978	1048
3000	402	503	604	704	805	906	1006	1107	1207	1308	1409	1509
3500	548	685	822	959	1096	1233	1370	1507	1643	1780	1917	2054
4000	716	894	1073	1252	1431	1610	1789	1968	2147	2325	2504	2683
4500	906	1132	1358	1585	1811	2038	2264	2490	2717	2943	3170	3396
5000	1118	1398	1677	1957	2236	2516	2795	3075	3354	3634	3913	4193
5500	1353	1691	2029	2367	2706	3044	3382	3720	4058	4397	4735	5073
6000	1610	2012	2415	2817	3220	3622	4025	4427	4830	5232	5635	6037
6500	1889	2362	2834	3306	3779	4251	4724	5196	5668	6141	6613	7085
7000	2191	2739	3287	3835	4383	4930	5478	6026	6574	7122	7669	8217
7500	2516	3144	3773	4402	5031	5660	6289	6918	7547	8175	8804	9433
8000	2862	3578	4293	5009	5724	6440	7155	7871	8586	9302	10017	10733
8500	3231	4039	4847	5654	6462	7270	8078	8885	9693	10501	11309	12116
9000	3622	4528	5433	6339	7245	8150	9056	9961	10867	11773	12678	13584
9500	4036	5045	6054	7063	8072	9081	10090	11099	12108	13117	14126	15135
10000	4472	5590	6708	7826	8944	10062	11180	12298	13416	14534	15652	16770
10500	4930	6163	7396	8628	9861	11093	12326	13559	14791	16024	17256	18489
11000	5411	6764	8117	9469	10822	12175	13528	14881	16233	17586	18939	20292
11500	5914	7393	8871	10350	11828	13307	14786	16264	17743	19221	20700	22178
12000	6440	8050	9660	11269	12879	14489	16099	17709	19319	20929	22539	24149
13000	7558	9447	11337	13226	15115	17005	18894	20784	22673	24562	26452	28341
13500	8150	10188	12225	14263	16300	18338	20376	22413	24451	26488	28526	30563
14000	8765	10956	13148	15339	17530	19722	21913	24104	26295	28487	30678	32869

*Data adopted from © 2009 Thermo Fisher Scientific Inc for educational purpose.

Good luck!



Was this document helpful? Tell us on protocol_rcpn@outlook.com