

# Quantitative determination of transgene copy number in transformed CHO cell lines by real-time PCR

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## INTRODUCTION

Chinese Hamster Ovary cells (CHO cells) are often used as expression system for the production of certain glycoprotein recombinant drugs. The reason for that is that CHO system has a few favorable properties such as known selection markers, low protein secretion, good glycosilation patterns of glycoproteins and substantial knowledge about long-term culture systems and potential large scale cultivation.

In our case an expression vector containing cDNA of our glycoprotein was transfected into CHO cells. As a part of the molecular characterization of the recombinant cell-line the number of construct insertions had to be determined. We have developed a real-time PCR quantification for this purpose.

## RESULTS and CONCLUSIONS

Table 1: Rm5 was used to create a standard curve, all other RM and samples (data not shown) were treated as unknown samples.

Sample Name	Endogenous gene					Transgene					transgene/endogene
	Ct	Log (copy number)	Copy number	AVG (Copy number)	stdev (copy number)	Cv	AVG (Copy number)	stdev (copy number)	Cv	transgene/endogene	
RM10 (900c)	26,88	2,95	895,00								
RM10 (900c)	29,02	2,84	870,85								
RM10 (900c)	29,00	2,84	860,02	881,96	12,19	0,01	9218,67	554,87	0,06	10,45	
RM10 (500c)	30,32	2,55	355,42								
RM10 (500c)	30,30	2,55	360,63	244,73	196,25	0,46	3640,86	98,20	0,03	14,88	
RM10 (100c)	32,54	1,89	77,82								
RM10 (100c)	32,86	1,80	62,49								
RM10 (100c)	32,91	1,78	60,42	66,91	9,50	0,14	674,85	46,62	0,07	10,09	
RM10 (20c)	35,00	1,16	14,31								
RM10 (20c)	35,74	0,84	8,64								
RM10 (20c)	35,73	0,84	8,69	10,54	3,26	0,31	167,80	19,94	0,12	15,91	
RM10 (10c)	35,93	0,88	7,59								
RM10 (10c)	35,91	0,89	7,70	6,24	2,42	0,46	86,75	6,31	0,07	13,89	
RM5 (1000c)	26,95	2,99	979,72								
RM5 (1000c)	28,74	3,02	1055,66								
RM5 (1000c)	28,91	2,97	938,50	991,29	59,43	0,06	4272,73	269,99	0,06	4,91	
RM5 (500c)	29,82	2,70	503,52								
RM5 (500c)	29,80	2,71	510,42								
RM5 (500c)	29,88	2,68	481,30	486,41	15,21	0,03	3270,42	1852,44	0,10	6,58	
RM5 (100c)	32,16	2,00	101,05								
RM5 (100c)	32,24	1,98	95,47								
RM5 (100c)	32,41	1,93	84,92	93,81	8,19	0,09	457,61	15,12	0,03	4,88	
RM5 (20c)	34,00	1,46	25,55								
RM5 (20c)	34,36	1,35	22,27								
RM5 (20c)	34,38	1,34	21,99	24,27	3,71	0,15	95,60	12,37	0,13	3,94	
RM5 (10c)	35,57	0,99	9,73								
RM5 (10c)	35,88	0,89	7,84								
RM5 (10c)	35,60	0,98	9,53	9,03	1,04	0,12	56,84	8,14	0,14	6,29	
RM2 (1000c)	26,88	2,98	954,74								
RM2 (1000c)	26,96	2,96	903,00								
RM2 (1000c)	26,88	2,98	956,85	938,20	30,50	0,03	2417,51	429,61	0,18	2,58	
RM2 (500c)	29,83	2,70	498,30								
RM2 (500c)	29,77	2,71	517,84								
RM2 (500c)	29,81	2,70	505,92	507,35	9,85	0,02	949,50	91,29	0,10	1,87	
RM2 (100c)	32,02	2,04	110,69								
RM2 (100c)	31,88	2,08	121,07								
RM2 (100c)	31,97	2,06	114,71	115,49	5,23	0,05	246,84	20,52	0,08	2,14	
RM2 (20c)	34,74	1,23	17,16								
RM2 (20c)	34,03	1,45	27,97								
RM2 (20c)	34,04	1,44	27,67	24,27	6,16	0,25	50,32	15,70	0,31	2,07	
RM2 (10c)	34,91	1,18	15,23								
RM2 (10c)	35,60	0,98	9,52								
RM2 (10c)	35,17	1,11	12,77	12,51	2,86	0,23	31,82	5,60	0,18	2,54	
RM1 (1000c)	26,90	2,98	944,27								
RM1 (1000c)	26,90	2,98	945,82								
RM1 (1000c)	26,98	2,95	891,21	927,03	31,03	0,03	1022,85	117,90	0,12	1,10	
RM1 (500c)	29,89	2,68	478,26								
RM1 (500c)	29,93	2,67	465,86								
RM1 (500c)	29,95	2,66	458,47	467,46	10,02	0,02	551,34	16,98	0,03	1,18	
RM1 (100c)	32,07	2,03	107,42								
RM1 (100c)	32,24	1,98	95,29								
RM1 (100c)	32,27	1,97	93,19	98,63	7,68	0,08	108,01	4,72	0,04	1,10	
RM1 (20c)	34,55	1,29	19,49								
RM1 (20c)	34,60	1,28	18,93								
RM1 (20c)	34,54	1,30	19,72	19,38	0,41	0,02	22,85	7,84	0,46	1,17	
RM1 (10c)	36,08	0,83	6,63								
RM1 (10c)	35,12	1,12	13,22								
RM1 (10c)	Undetermined	#VALUE!	#VALUE!	10,02	4,52	0,46	11,58	5,41	0,40	1,16	

• In the last column we calculated the ratio between endogenous gene and transgene in a transfected CHO cell-line. These numbers can be compared to the theoretically calculated numbers (given in the figure 1). Since similar results were obtained with all of the reference materials, we were able to use them in the quantification of the transgene copy number in samples.

• Sample of non transfected CHO cell-line was quantified for its endogene we determined one copy of the gene per haploid genome. This was in concordance with the literature data.

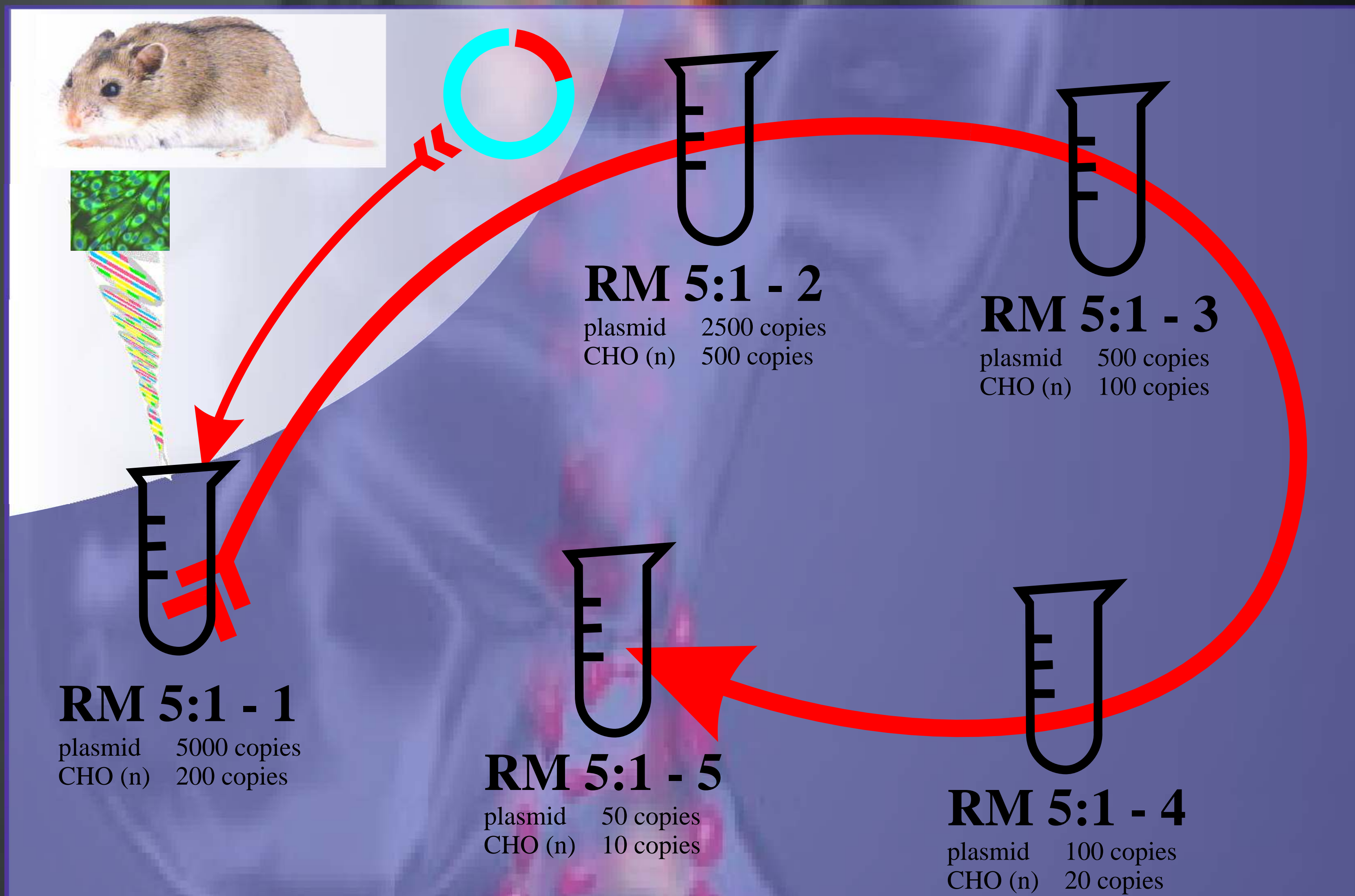


Figure 1: dilutions of reference material 5 (RM5)

## OUTLINE OF THE EXPERIMENT

- Construction of two amplicon for the 5'-3' exonuclease real-time PCR assay: one in the endogenous gene of the CHO cells and the other in the gene coding our glycoprotein (transgene).
- Concentration of the DNA from recombinant CHO cell-lines, untransfected CHO cell-line and the linearised plasmid with the insert was measured spectrophotometrically and then verified by real-time PCR.
- Preparations of reference materials (RM). We have prepared RM by mixing nontransformed CHO DNA and plasmid DNA since no CHO cell-line with a known copy number of the transgene was available. Based on the spectrophotometrically measured concentrations of DNA, on the size of CHO genome and of the plasmid (containing the insert), we were able to mix certain amounts of both DNA in order to produce different RM with ratios between the plasmid (with insert) and CHO genome (haploid, n) 10:1, 5:1, 2:1 and 1:1. Several dilutions of these RM were prepared each of them contained (at this point only theoretically estimated) a known copy number of both DNA at the mentioned ratios.
- Absolute quantification with real-time PCR was used to verify the applicability of these reference materials for the determination of the transgene copy number in transfected CHO cell lines. Each RM was used to create a standard curve and all the other RM were applied as the unknown samples. In this way we compared all RM among themselves to check the efficiency of the quantification.
- Finally the estimation of the copy number of the inserted gene in transfected CHO cell-lines by absolute quantification with real-time PCR using prepared reference materials.

