

Specific luciferase activity during development expressed as LU/ μ g of total protein

Construct (clone#)	Analysis #	Repetitions	LU/ μ g	Mean LU/ μ g for construct	Percentage of WT (%)
WT (55)	1	15	6.84×10^5	1.2×10^6	100
WT (56)	1	8	1.35×10^6		
WT(58)	1	8	6.49×10^5		
WT(58)	2	29	2.11×10^6		
C1M (1)	1	3	3.53×10^4	4.16×10^4	3.47
C1M (1)	2	6	2.51×10^4		
C1M (2)	1	4	9.53×10^4		
C1M (3)	1	9	1.99×10^4		
C1M (3)	2	6	1.08×10^4		
C1M (53)	1	22	6.33×10^4		
C2M (6)	1	8	5.12×10^4	4.49×10^4	3.75
C2M (6)	2	6	4.46×10^4		
C2M (7)	1	1	6.97×10^4		
C2M (56)	1	6	1.42×10^4		
C12M (1)	1	17	4.28×10^4	4.48×10^4	3.74
C12M (51)	1	20	5.14×10^4		
C12M (52)	1	21	3.66×10^4		
C12M (53)	1	17	4.67×10^4		
C12M (54)	1	14	5.21×10^4		
C12M (55)	1	12	3.95×10^4		
A55M (1)	1	7	3.6×10^5	2.93×10^5	24.4
A55M (10)	1	3	1.01×10^5		
A55M (12)	1	4	1.34×10^5		
A55M (14)	1	3	5.76×10^5		

Table 1. Effects of C-Box Mutations on Reporter Gene Expression.

Stable transformants of mutant (or wild-type) *gp2* promoter / pVTDH constructs, were selected as described in Materials and Methods. Some transformant clones were analyzed on multiple occasions. Results from each analysis are shown in Tables 1 and 2. The number of replicates made for each clone on each occasion has been indicated as replication #. Cells from 2 developmental stages were analyzed, undifferentiated stage (0 hours) (data not shown) and second finger stage (20 hours). Cell lysates from harvested cells were analyzed for luciferase activity (luciferase units per microliter of lysate, LU/ μ l) and total protein content (μ g of protein / μ l of lysate) (see Materials and Methods for detailed description of procedures). These data were used to determine the specific luciferase activity for each clone calculated as the luciferase units per microgram of total protein. The mean specific luciferase activity was calculated for each construct. The mean value of the wild-type construct was used as basis (100%) to calculate the percentage value of all other constructs. Data from wild-type clones and mutants involving the C-boxes have been shown in this table. Constructs C1M, C2M and C12M show a drop of nearly 96% from wild-type. Construct A55M resulted in a roughly 75% decrease from wild-type levels.