

Embryo Tx | C Dorna

4/28/88

Embryos Decapitated, Vitelline Mem. Removed.

Dorna T7 lucifer tested as follows:

Group	Opaline	Dorna	Qty	T7lucifer	U7lucifer	
Dorna/RNA Embry	4.0L	50ml	20mg	9.5A		
RNA	↓	↓	↓	↓	↓	ST 2:53 p 3:51
Dorna	—	↓	↓	↓	↓	STOP

Group	LATE	late	SO/L	20mg	9.5A	
RNA	↓	↓	↓	↓	↓	ST 2:51 p 3:52
Dorna	—	↓	↓	↓	↓	STOP 3:52

Early - FERT @ 11:00pm → 14°C incubation LATE BLASTULA / EMBRYO GASTRUL

LATE - " @ 6:30pm → 27°C " EARLY NEURULA

Desegregated Embryos = 2% Cistaz / 100 μl MR

Proximally V.M. = 1mg/ml Proxaz in 100 μl MR 5'

C. Serum WASH

Ans: 5ml 245 FERT 4/20 11:35 AM
TAD poles 337 μl MR C.R.T.

① 3:04 TAD poles changed to 37°C MR

② 10:40

Start Neurula group # 2 (1) 3:17 pm
Stop @ 4:00

(Neurula start)

ORAL EVALUATION

WEAR Eye Pigment

TUBE FEED

SUTURE PIGMENT

Hanover A.C. 10:45 → 11:00p-

3D COUNT		37.5A	
EARLY G	N#1	N#2	STG
-	386	403	424
D	377	368	413
R	521	430	583
D/R	15556	1992	392

5/3/88

XENOPUS EMBRYO TREATMENT #2

1. 12 EMBRYOS COULD PEG WELL INTO 4 SETS OF 4 IN
2nd 100% MR.

RNA = T7LUCFRAm

TREATMENT	+ 20% RNA	+ 20% DODMA	+ 20% RNA + 20% DODMA	-	60s COUNT
EMBRYOTYPE	+	*	*	*	
WHOLE BLASTULA	5 878	6 786	7 771	8 774	
CHOPPED BLASTULA	9 16737	10 771	11 778	12 801	- INCUBATED 9h @ RT. IN SOLUTIONS.
WHOLE NEURULA	13 43354	14 727	15 748	16 765	- WHOLE BLASTULAS REMAINED LARGELY INTACT.
CHOPPED NEURULA	1 8243	2 774	3 859	4 779	- 30-50% OF NEURULAS DEVELOPED NORMALLY. OTHERS FORMED WEIRD BILobed STRUCTURES. (IN ALL GROUPS)

START 2 3:00 p.m.

HARVEST @ 12:00 MIDNIGHT.

TAKEN UP ON 100% LUC. LYSS. BUFF. AGITATED

CELLS SPUN DOWN 30' 14K

SUP. Poured.

RE悬PENDED IN 100% LUC. LYSS. BUFF. FREEZER/FRIDGE 3X

LYSATE

5/4 ANALYZE samples in standards LUC. ASSAY, 60s COUNT, 37% RL / ASSAY

STEVE HUSS X102

2 BYS OF 10L, Fuzzy

3.5"

MBC APPENDIX NTW.

Hypertension wanted.

9/13/88

Dorm Transfection into Xenopus Eye Bud & INTERIOR TISSUE,
 USING mRNA.

Groups As Follows:

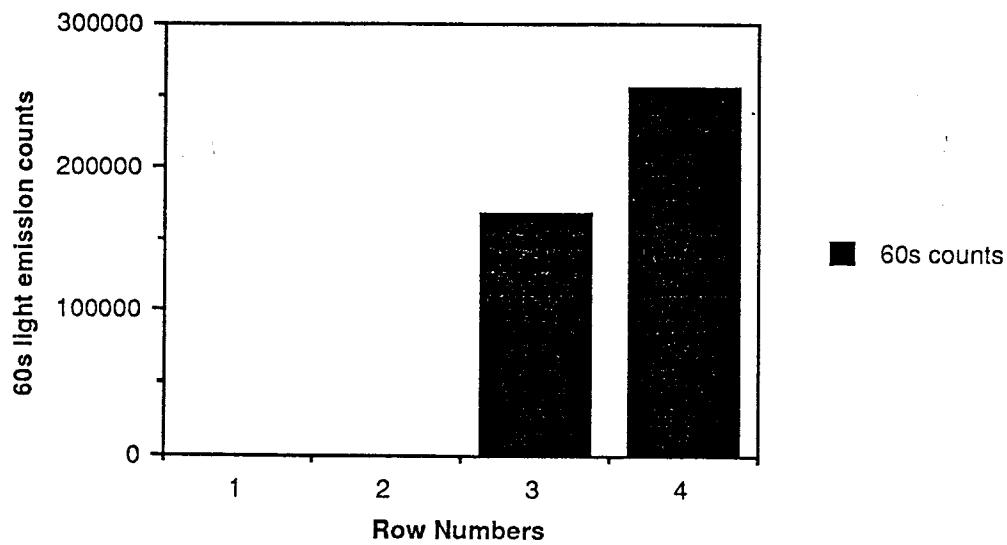
Sample	Tissue	Qty	(1/4)	RNA	DNA	100% ME	100% sub	
1	1/2 BRAIN	≈ 35 mg	-	10λ (20λ)	-	1490 λ	800?	Add tissue to
2	1/2 BRAIN		50 mg ✓	10λ	-	1390 λ	✓	SOLN. in 3cm dish.
3	1/2 BRAIN		50 mg ✓	-	-	(150) 400	✓	
4	EYES (SQUARE) ≈ 80	50 mg ✓	10λ	-	-	1390 λ	✓	
5	EYES (FOR XPLANT)		100 λ ✓	-	55 λ	1345 λ		
6	EYES (FOR XPLANT)		50 mg ✓	-	-	1400 λ		
7	EYES (FOR XPLANT)		-	-	-	(500)		

INCUBATE 1-4 8.5L

LYS E 1% Triton, COUNT SURE

1	691
2	168546
3	608
4	255087

DOTMA RNA TRANSFECTION - XENOPUS EYES



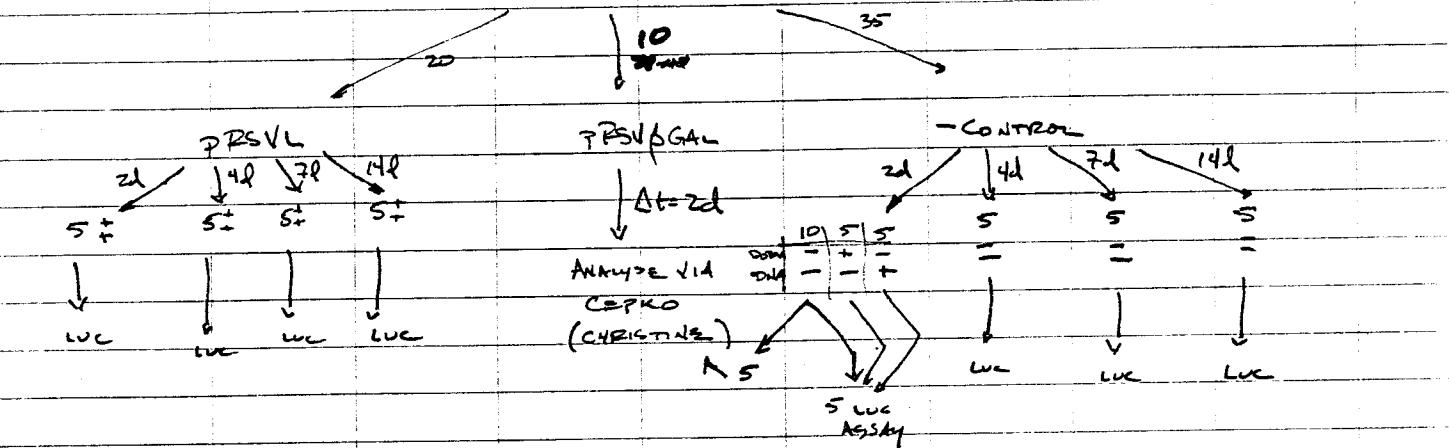
	treatment	60s counts
1	RNA 1/2 Br.	691
2	DOTMA 1/2 B	608
3	D+R 1/2 Br.	168546
4	D+R Eyes	255087

6/7/88

~ 70 embryos (post gastrula)

Prontas

Early Neurula - J.M.



OBTAINED FERT EMBRYOS FROM C. CONTROLLER (~ 80) BROUGHT TO STATE OF GASTRULATION (YAN PUA COUNT.) IN 332 MR.

DISCUSSION J.M. IN 1mg/l Prontas IN 332 MR.

INSTRUCTIONS:

qRSV β GAL	SDM ₁	25 >	- DOT - DNA	1.52
DOTMA	100 μl	100 >		
100% MR	70.15 μl	1375 >	+ DOT - DNA	
			SDM ₁ DOTMA	
			700 μl 100% MR	
PBSVL	100 μl	52.6 >		
DOTMA	200 μl	200 > /	-DNA - DOTMA	
100% MR	70.15 μl	2747 >	26.3 > qRSV	
		(1375.5 = ~)	723.7 > 100% MR	

Embryos @ END OF GASTRULATION 8:30 pm

St Tr 9:20

Stop 7:15 Am 6/8/88

3:30pm 6/8 LY52D = 5 embryos OR + PBSVL + DOTMA - 42444 cpm / 37.92
(100 μl) - control - 881 "

6/9	LY52D C NP/10	5 + DNA + Prontas	INDUCTION IN SDM ₁ , TOOK 37.5 TO COUNT.
		2 + -	
		2 - +	

6/9

COUNTS FROM 40 h LY5' /
30s COUNT 60s counts

^{+DNA} ^{-Dorm}	1	68657	137314
	2	44232	88464
	3	172059	344118
	4	290282	580564
	5	243702	487404
^{+DNA} ^{-Dorm}	1	435	870
	2	406	812
^{-DNA} ^{+Dorm}	1	399	798
	2	390	780
=	1	413	826
	2	404	808

6/12 8:00 pm \rightarrow 8.4 mos @ Site 42

COUNTS FROM 5d 24h. (30s & 60s counts)

		<u>30s counts</u>	<u>60s counts</u>
^{+DNA}	1	44466	88932
^{+DNA}	2	14455	28910
	3	19390	38780
	4	148177	296354
	5	36808	73616
^{-DNA} ^{-Dorm}	1	317	634
	2	38	638
^{-DNA} ^{+Dorm}	1	311	622
	2	305	610
=	1	310	620
	2	355	710

6/15 9:30 pm (Ed)

Emery 2/3 C. S. G. ~~47~~ 43/44

Stanza Dotma DNA 3rd Count 6th Count

1 + + 5927 11854

2 + + 11633 23266

3 + + 28268 56536

4 + + 1462 2924

5 + + 888 1776

6 + - 356 712

7 + - 356 712

8 - + 359 718

9 - + 357 714

10 - - 358 716

11 - - 354 708

6/21 Emery C. Stanza = 47/43 (14d) All Remaining w/20, COUNTED

+ Dotma
+ DNA

3rd Count

1 7129 60 1018 2036

2 4596 5 9170 4877 9754

3 2744 5489 947 13941

4 47255 94510 531 1062

5 6771 13542 5567 11134

6 37878 75756

7 4056 8112

8 28152 56304

9 32627 65254

10 6455 12910

11 4229 9458

12 7793 15586

13 163270 326570

14 111082 222164

15 3683 7366

16 44216 88432

17 16582 33164

18 10712 21424

19 31796 63552

+ DNA
- Dotma

1 602 1200

2 56492 984

3 530 1060

4 514 1028

5 515 1030

- DNA
- Dotma

1 530 1060

2 551 1102

3 561 1122

4 488 976

5 498 996

6 491 992

7 560 1120

8 482 964

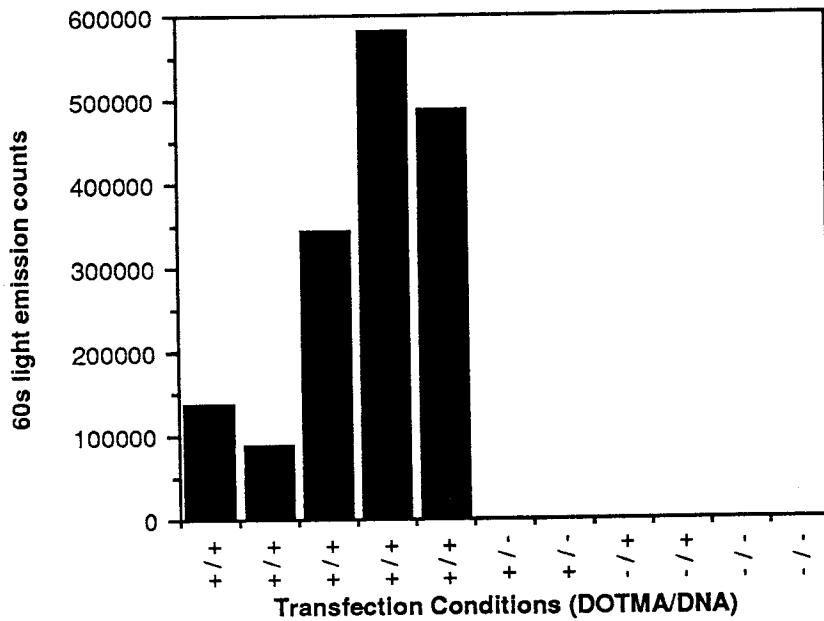
9 569 1138

+ DNA
- DNA

500 1000

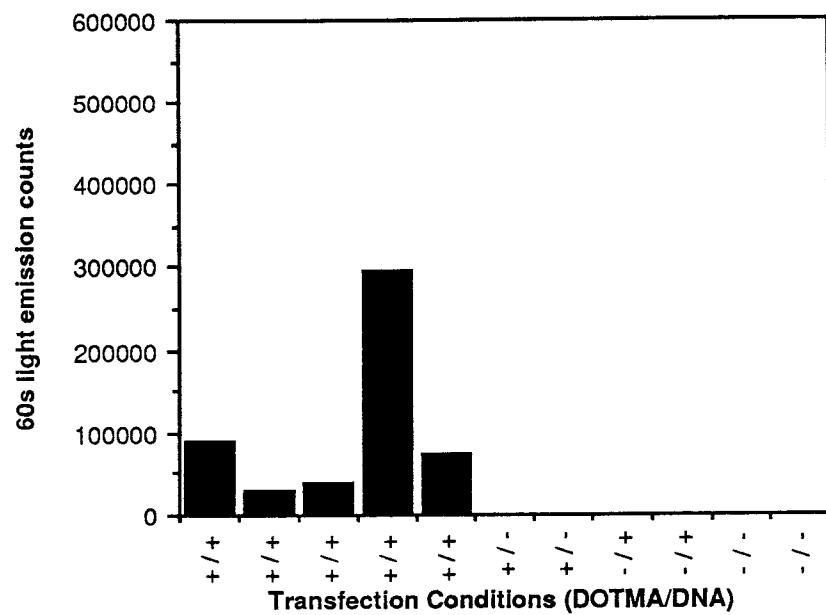
516 1032

Luciferase Assay 48h Post Transfection



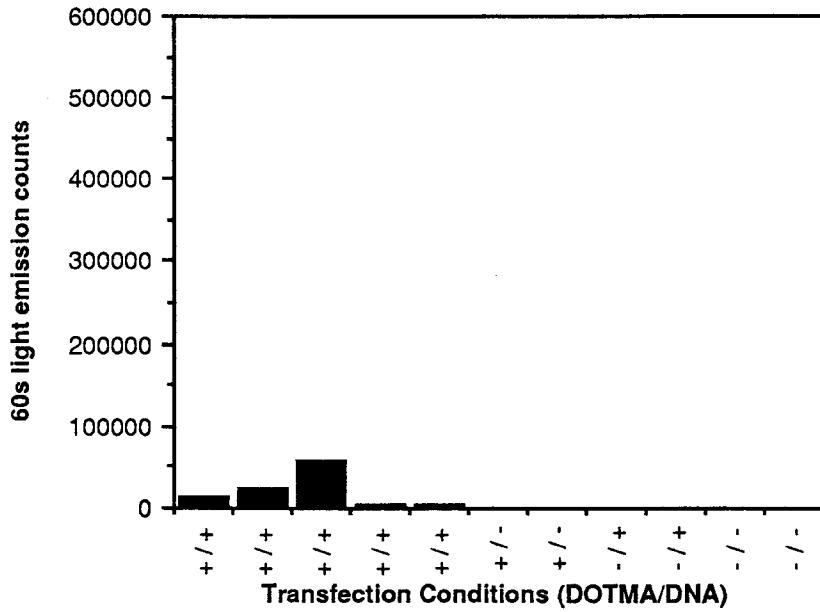
	time post tx.	DOTMA/DNA	60s counts
1	48h	+ / +	137314
2	48h	+ / +	88464
3	48h	+ / +	344118
4	48h	+ / +	580564
5	48h	+ / +	487404
6	48h	+ / -	798
7	48h	+ / -	780
8	48h	- / +	870
9	48h	- / +	812
10	48h	- / -	826
11	48h	- / -	808

Luciferase Assay 120h Post Transfection



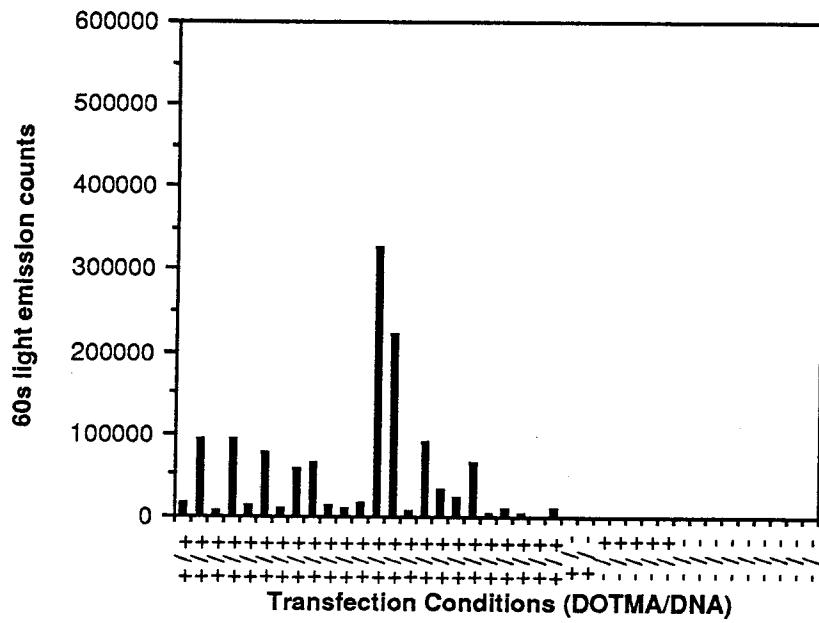
	time post tx.	DOTMA/DNA	60s counts
1	120h	+ / +	88932
2	120h	+ / +	28910
3	120h	+ / +	38780
4	120h	+ / +	296354
5	120h	+ / +	73616
6	120h	+ / -	622
7	120h	+ / -	610
8	120h	- / +	634
9	120h	- / +	638
10	120h	- / -	620
11	120h	- / -	710

Luciferase Assay 192h Post Transfection



	time post tx.	DOTMA/DNA	60s counts
1	192h	+ / +	11854
2	192h	+ / +	23266
3	192h	+ / +	56536
4	192h	+ / +	2924
5	192h	+ / +	1776
6	192h	+ / -	712
7	192h	+ / -	712
8	192h	- / +	718
9	192h	- / +	714
10	192h	- / -	716
11	192h	- / -	708

Luciferase Assay 336h Post Transfection



lynn 336h Tx. Data

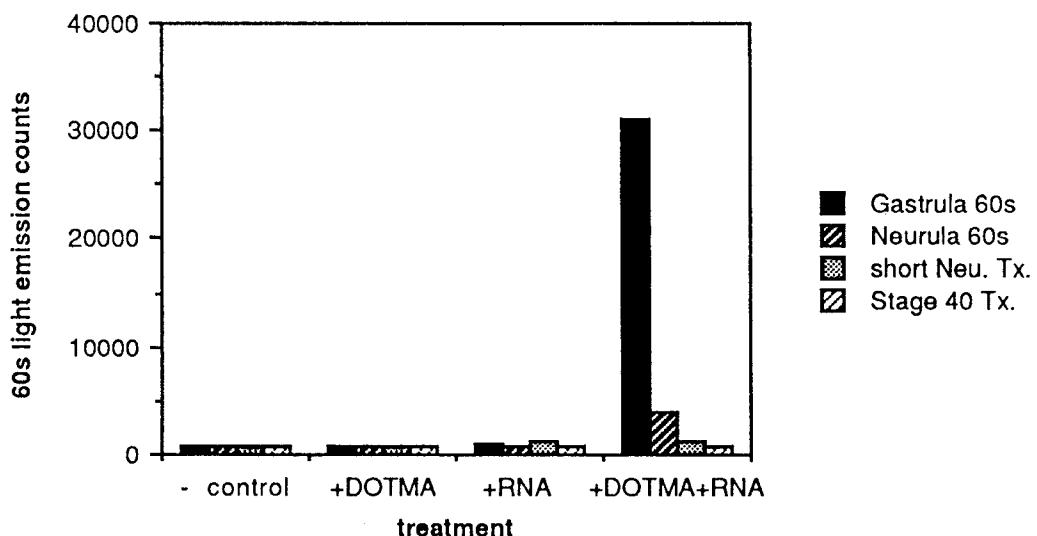
Tue, Jun 21, 1988 3:34 PM

time post tx.	DOTMA/DNA	60s counts
1	336h	+ / +
2	336h	+ / +
3	336h	+ / +
4	336h	+ / +
5	336h	+ / +
6	336h	+ / +
7	336h	+ / +
8	336h	+ / +
9	336h	+ / +
10	336h	+ / +
11	336h	+ / +
12	336h	+ / +
13	336h	+ / +
14	336h	+ / +
15	336h	+ / +
16	336h	+ / +
17	336h	+ / +
18	336h	+ / +
19	336h	+ / +
20	336h	+ / +
21	336h	+ / +
22	336h	+ / +
23	336h	+ / +
24	336h	+ / +
25	336h	+ / -
26	336h	+ / -
27	336h	- / +
28	336h	- / +
29	336h	- / +
30	336h	- / +
31	336h	- / +
32	336h	- / -
33	336h	- / -
34	336h	- / -
35	336h	- / -
36	336h	- / -
37	336h	- / -
38	336h	- / -
39	336h	- / -
40	336h	- / -

xenopus embryo data

	treatment	Gastrula 60s	Neurula 60s	short Neu. Tx.	Stage 40 Tx.
1	- control	772	816	848	790
2	+DOTMA	754	736	826	802
3	+RNA	1,042	860	1166	738
4	+DOTMA+RNA	31112	3984	1130	784

xenopus embryo transfection



Embryo Transfection with Dotma

Embryos degelled with 2% cystine/100% MR

Vitelline membrane removed with 1mg/ml Pronase in 100% MR

Lipofectin/T7LUCFRAn RNA transfection tested as follows:

GROUP	Opti-Mem	DOTMA	Qty. RNA	Vol. RNA
DOTMA/RNA	4ml	50ug	20ug	9.5ul
RNA	4ml	-	20ug	9.5ul
DOTMA	4ml	50ug	-	-
-	4ml	-	-	-

Approx. 10 embryos per group.

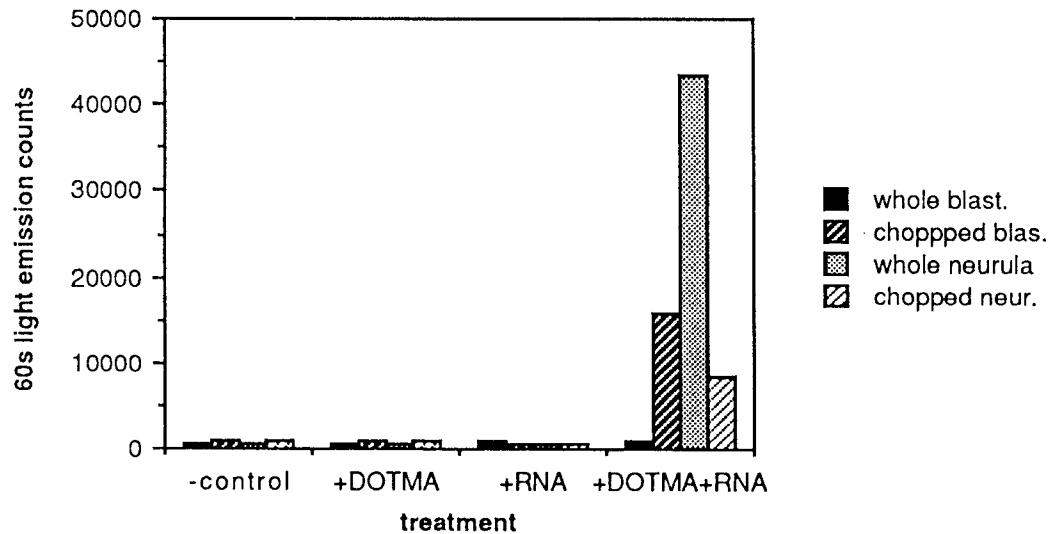
Late blastula/early gastrula transfected for 1hr 21min.

Early neurula transfected for 1hr 3min (long), 43min (short).

Cells harvested by washing in 1mM DTT 0.1M KPO4 pH 7.8 with aspiration, removal of floating lipids with wash, resuspension in 100ul of same buffer, freeze/thaw 3 times (liquid N2, 37 degrees C), spun out cell debris 14k, 5min.

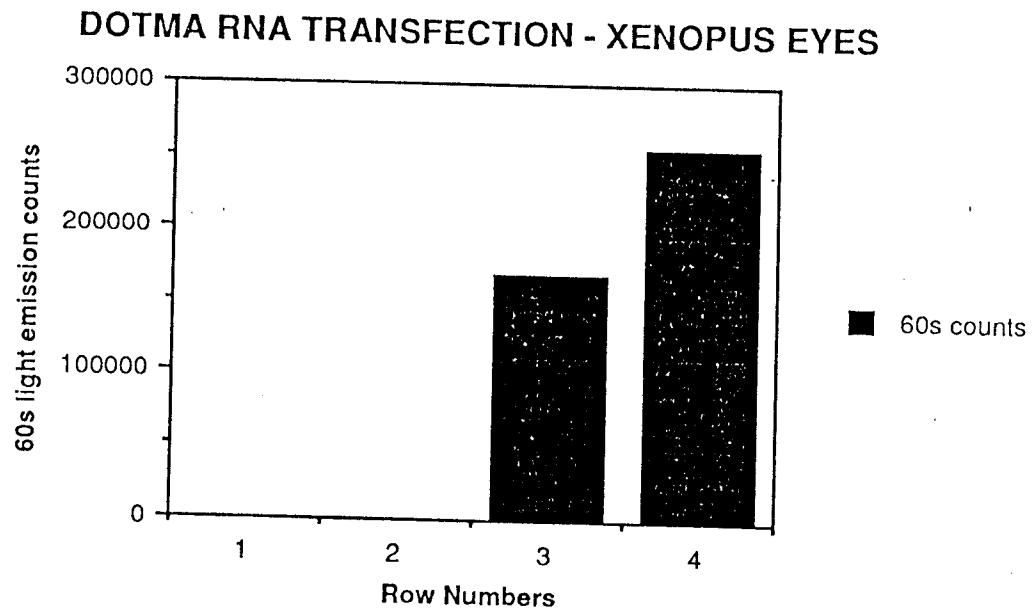
37.5ul aliquots assayed for luciferase activity.

Xenopus embryo transfection #2 5-4-88



Xenopus embryo tx. 2

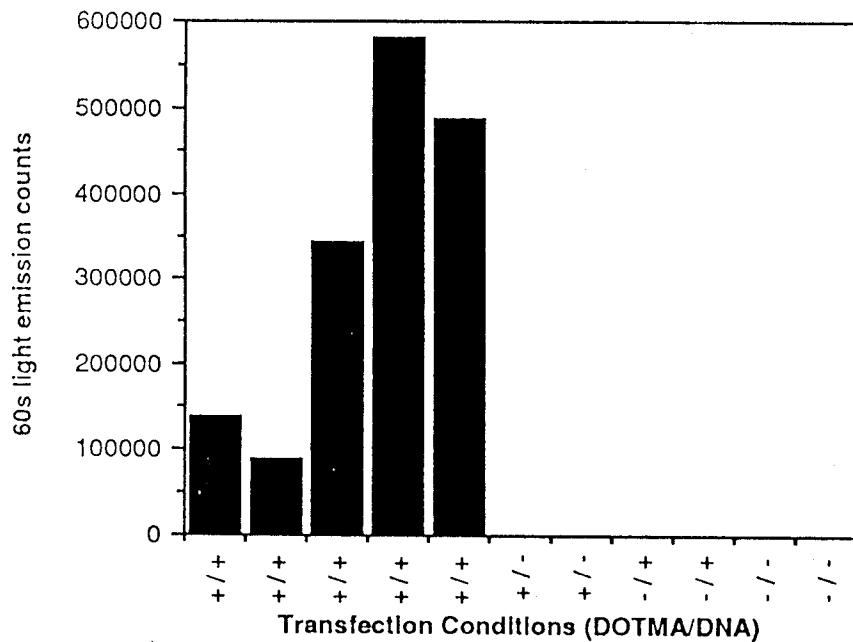
	treatment	whole blast.	chopped blast.	whole neurula	chopped neur.
1	-control	774	801	765	779
2	+DOTMA	771	778	748	859
3	+RNA	786	771	727	774
4	+DOTMA+RNA	878	15737	43354	8243



treatment	60s counts
1 RNA 1/2 Br.	691
2 DOTMA 1/2 B	608
3 D+R 1/2 Br.	168546
4 D+R Eyes	255087

FIGURE 4

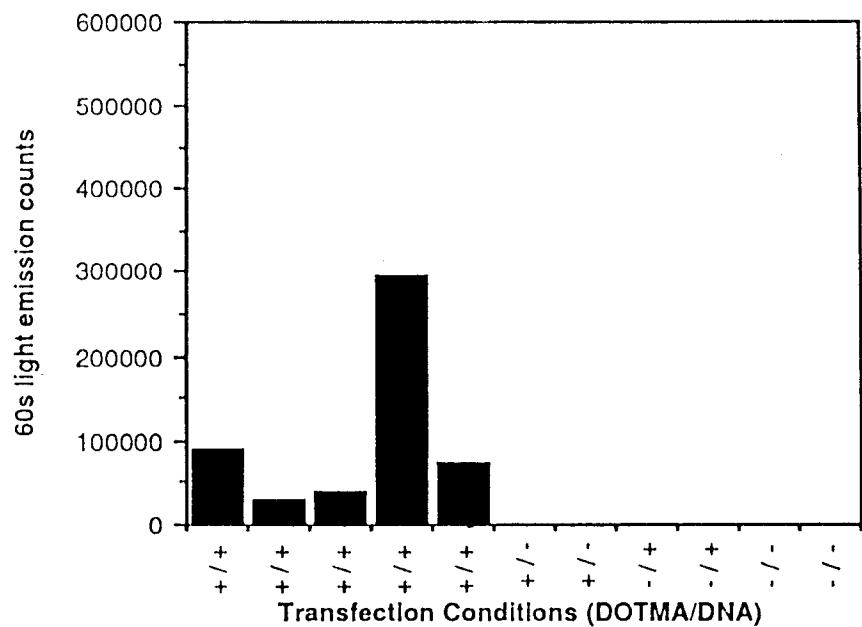
Luciferase Assay 48h Post Transfection



	time post tx.	DOTMA/DNA	60s counts
1	48h	+ / +	137314
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8	48h	- / +	870
9	48h	- / +	812
10	48h	- / -	826
11	48h	- / -	808

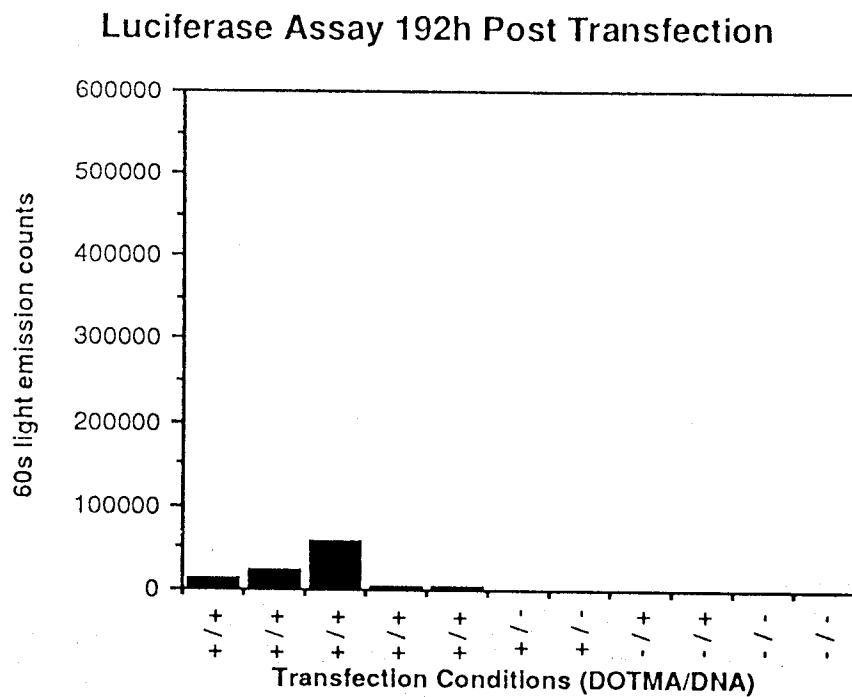
FIGURE 5

Luciferase Assay 120h Post Transfection



	time post tx.	DOTMA/DNA	60s counts
1	120h	+ / +	88932
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3	120h	+ / +	38780
4	120h	+ / +	296354
5	120h	+ / +	73616
6	120h	+ / -	622
7	120h	+ / -	610
8	120h	- / +	634
9	120h	- / +	638
10	120h	- / -	620
11	120h	- / -	710

FIGURE 6



	time post tx.	DOTMA/DNA	60s counts
1	192h	+ / +	11854
2	192h	+ / +	23266
3	192h	+ / +	56536
4	192h	+ / +	2924
5	192h	+ / +	1776
6	192h	+ / -	712
7	192h	+ / -	712
8	192h	- / +	718
9	192h	- / +	714
10	192h	- / -	716
11	192h	- / -	708

Luciferase Assay 336h Post Transfection

