



Enhancer activities of amphioxus Brachyury genes in embryos of the ascidian, *Ciona intestinalis*

Author	Hitoshi Tominaga, Noriyuki Satoh, Naoto Ueno, Hiroki Takahashi
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Tables

Table 1. Number of *Ciona* embryo *LacZ* expression

	Muscle	No signal
<i>BfBral</i> -3kbp 8h	139	160
<i>BfBral</i> -3kbp 12h	78	203
<i>BfBra2</i> -2kbp 8h	205	223
<i>BfBra2</i> -2kbp 12h	130	232

	No signal	Notochord
<i>BfBral</i> +3kbp 7h	39	24
<i>BfBral</i> +3kbp 11h	30	34
<i>BfBra2</i> +3kbp 7h	91	9
<i>BfBra2</i> +3kbp 11h	82	18

7h	No signal	Notochord	Muscle	Noto + Mus	Ectopic
<i>BfBral</i> intron1	9	7	0	0	1
<i>BfBral</i> intron2	29	35	1	2	2
<i>BfBral</i> intron3	150	53	0	1	0
<i>BfBral</i> intron4	25	24	0	2	1
<i>BfBral</i> intron5	140	0	0	2	1
<i>BfBral</i> intron6	86	0	0	0	0

11h	No signal	Notochord	Muscle	Noto + Mus	Ectopic
<i>BfBral</i> intron1	35	41	6	5	3
<i>BfBral</i> intron2	74	72	2	5	8
<i>BfBral</i> intron3	19	24	5	4	4
<i>BfBral</i> intron4	29	46	0	0	3
<i>BfBral</i> intron5	46	6	1	3	2
<i>BfBral</i> intron6	38	2	4	1	2

7h	Notochord	Muscle	Both	No signal
<i>BfBra2</i> intron1	29	91	32	32
<i>BfBra2</i> intron2	30	13	46	39
<i>BfBra2</i> intron3	0	89	28	0
<i>BfBra2</i> intron4	1	70	134	1
<i>BfBra2</i> intron5	0	154	15	0
<i>BfBra2</i> intron6	2	210	19	37

11h	Notochord	Muscle	Both	No signal
<i>BfBra2</i> intron1	17	65	24	53
<i>BfBra2</i> intron2	15	34	15	66
<i>BfBra2</i> intron3	0	107	0	0
<i>BfBra2</i> intron4	46	22	48	15
<i>BfBra2</i> intron5	24	52	15	35
<i>BfBra2</i> intron6	17	241	19	107

Table 2. Primer sequences

Product Name	Primer Name	Sequence (5'→3')
pPD1.27_ <i>LacZ</i> vector pPD1.27 + <i>BfBra</i> -3kbp	pPD1.27_F	<u>ATGACTGCTCCAAAGAAGAAG</u>
	pPD1.27_R	<u>TGAGCTCGGTACCCGGGGATC</u>
<i>BfBra1</i> -3kbp	<i>Bra1</i> _-3k_F	<u>CGGGTACCGAGCTCACTATGTACTACTATCATCGTCAG</u>
<i>BfBra1</i> -0.5, -1, -2, -3kbp	<i>Bra1</i> _upstream_R	<u>CTTTGGAGCAGTCATCTCGTTGTTGACGCTGGTCT</u>
<i>BfBra1</i> -2kbp	<i>Bra1</i> _-2k_F	<u>CGGGTACCGAGCTCACTGTAAGACATCCAGGATAACTTG</u>
<i>BfBra1</i> -1kbp	<i>Bra1</i> _-1k_F	<u>CGGGTACCGAGCTCAACTTCAGCTGATTATCCGGCACTT</u>
<i>BfBra2</i> -2kbp	<i>Bra2</i> _-2k_F	<u>CGGGTACCGAGCTCACTGGTAGTACATGAAATCAAGGAG</u>
<i>BfBra2</i> -1kbp	<i>Bra2</i> _-1k_F	<u>CGGGTACCGAGCTCATGCGCAATAAAGACCACAATAGCG</u>
<i>BfBra1</i> -3kbp ~ -5.5kbp	<i>Bra1</i> _-5.5kbp_F	<u>CGGGTACCGAGCTCATGTTTACAAACTGCTAGTCAATAA</u>
<i>BfBra1</i> -3kbp ~ -5.5kbp	<i>Bra1</i> _-3kbp_R	<u>TTTCTCGGATATCTGACGATGATA</u>
pPD1.27 + <i>BfBra</i> -3kbp	<i>Bra1</i> _-3k_vectF	<u>CAGATATCCGAGAAAAGGTATATAG</u>
<i>BfBra1</i> -0.5kbp	<i>Bra1</i> _HCRdeletion_F	<u>CGGGTACCGAGCTCAACAGAAAATTTATTACATTATTTATAACAGTTACAGCTTCTT</u>
<i>BfBra2</i> -5.7kbp	<i>Bra2</i> _-5.7kbp_F	<u>CGGGTACCGAGCTCAATGTGGAATGTCCGGCGATAGATT</u>
<i>BfBra2</i> -1, -2, -5.7kbp	<i>Bra2</i> _upstream_R	<u>CTTTGGAGCAGTCATGGTGCACGGTACGGCTGAAGTATC</u>

pSP1.72 <i>CiBra</i> basal promoter > <i>LacZ</i> vector	<i>CiBra</i> _promotor_F	<u>GGAGCTCCACCGGGCTGTATAAACTTGCACCCGAGTGT</u>
	pSP1.72_R	<u>TGAGCTCGGTACCCGCTTCAGCTGCTCGAGTTCTATAGT</u>
<i>BfBra2</i> downstream	<i>Bra2</i> _downstream_F	<u>CGGGTACCGAGCTCACCATGACCATGCCGTCCATGTA</u>
	<i>Bra2</i> _downstream_R	<u>CCGCGGTGGAGCTCCTCACCAATGGTTTCTGACAAGTT</u>
<i>BfBra1</i> downstream	<i>Bra1</i> _downstream_F	<u>CGGGTACCGAGCTCAGAACGAGGTCAAACAACGTC</u>
	<i>Bra1</i> _downstream_R	<u>CCGCGGTGGAGCTCCCCTATGACTCCACCATCGCTCTAA</u>
<i>BfBra1</i> , <i>Bra2</i> intron1	Common_intron1_F	<u>CGGGTACCGAGCTCAGACCGAGCGGGACCTGAA</u>
	Common_intron1_R	<u>CCGCGGTGGAGCTCCACCTTCAGCACGGGGAACAT</u>
<i>BfBra1</i> , <i>Bra2</i> intron2	Common_intron2_F	<u>CGGGTACCGAGCTCAAGGTCAAACACCAACAACT</u>
<i>BfBra1</i> intron2	<i>Bra1</i> _intron2_R	<u>CCGCGGTGGAGCTCCGCTGACCATGCGCTGGTTAT</u>
<i>BfBra2</i> intron2	<i>Bra2</i> _intron2_R	<u>CCGCGGTGGAGCTCCTGTGCAGGCTGTTACGATTATCT</u>
<i>BfBra1</i> intron3	<i>Bra1</i> _intron3_F	<u>CGGGTACCGAGCTCAGCAGTTACGGGTACCAGAATGAA</u>
	<i>Bra1</i> _intron3_R	<u>CCGCGGTGGAGCTCCGAAAGCCTTGGCGAAAGGGTTATA</u>
<i>BfBra2</i> intron3	<i>Bra2</i> _intron3_F	<u>CGGGTACCGAGCTCACATTCCGCCGAGACACAGTTCAT</u>
	<i>Bra2</i> _intron3_R	<u>CCGCGGTGGAGCTCCAACGGGTTGTGCTTGATCTTCAAA</u>
<i>BfBra1</i> intron4	<i>Bra1</i> _intron4_F	<u>CGGGTACCGAGCTCATAACCTTTCCGCCAAGGCTTTCTT</u>
	<i>Bra1</i> _intron4_R	<u>CCGCGGTGGAGCTCCTCCATTCCGTCCTTCCCATCACTT</u>
<i>BfBra2</i> intron4	<i>Bra2</i> _intron4_F	<u>CGGGTACCGAGCTCAAAGCCTTCTTGACGCTAAAGAA</u>
	<i>Bra2</i> -intron4_R	<u>CCGCGGTGGAGCTCCGCGAACGGGTTGTGCTTGAT</u>

<i>BfBra1</i> intron5	<i>Bra1_intron5_F</i>	<u>CGGGTACCGAGCTCAGGAAGATTGCAAGATCAACCACAAT</u>
<i>BfBra2</i> intron5	<i>Bra2_intron5_F</i>	<u>CGGGTACCGAGCTCAGAGTGGACATGACGACTTGACTGA</u>
<i>BfBra1, Bra2</i> intron5	Common_intron5_R	<u>CCGCGGTGGAGCTCCGGGCAGATGGGGCCTGTA</u>
<i>BfBra1, Bra2</i> intron6	Common_intron6_F	<u>CGGGTACCGAGCTCACCGCACCCGTACCAGAGA</u>
<i>BfBra1</i> intron6	<i>Bra1_intron6_R</i>	<u>CCGCGGTGGAGCTCCCATGGCTGACATGGACAGCATGTT</u>
<i>BfBra2</i> intron6	<i>Bra2_intron6_R</i>	<u>CCGCGGTGGAGCTCCCATGGCTGACATGGACAGCATGTT</u>

