



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

Author	Hitoshi Tominaga, Noriyuki Satoh, Naoto Ueno, Hiroki Takahashi
journal or publication title	genesis
volume	56
number	8
page range	e23240
year	2018-08-16
Publisher	John Wiley & Sons, Inc.
Rights	(C) 2018 Wiley Periodicals, Inc. This is the peer reviewed version of the following article: Tominaga H, Satoh N, Ueno N, Takahashi H. Enhancer activities of amphioxus Brachyury genes in embryos of the ascidian, <i>Ciona intestinalis</i> . <i>genesis</i> . 2018;56:e23240, which has been published in final form at https://doi.org/10.1002/dvg.23240 . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.
Author's flag	author
URL	http://id.nii.ac.jp/1394/00000817/

doi: info:doi/10.1002/dvg.23240

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>CGGGTACCGAGCTCA</u> CTATGTACTACTATCATCGTCAG
<i>BfBra1</i> -0.5, -1, -2, -3kbp	<i>Bra1</i> _upstream_ R	<u>CTTTGGAGCAGTCAT</u> CTCGTTGTTGACGCTGGTCT
<i>BfBra1</i> -2kbp	<i>Bra1</i> _-2k_ F	<u>CGGGTACCGAGCTCA</u> CTGTAAGACATCCAGGATAACTTG
<i>BfBra1</i> -1kbp	<i>Bra1</i> _-1k_ F	<u>CGGGTACCGAGCTCA</u> ACTTCAGCTGATTATCCGGCACTT
<i>BfBra2</i> -2kbp	<i>Bra2</i> _-2k_ F	<u>CGGGTACCGAGCTCA</u> CTGGTAGTACATGAAATCAAGGAG
<i>BfBra2</i> -1kbp	<i>Bra2</i> _-1k_ F	<u>CGGGTACCGAGCTCA</u> TGCGCAATAAAGACCACAATAGCG
<i>BfBra1</i> -3kbp ~ -5.5kbp	<i>Bra1</i> _-5.5kbp_ F	<u>CGGGTACCGAGCTCA</u> TGTTTACAACTGCTAGTCAATAA
<i>BfBra1</i> -3kbp ~ -5.5kbp	<i>Bra1</i> _-3kbp_ R	<u>TTTCTCGGATATCTG</u> ACGATGATA
pPD1.27 + <i>BfBra</i> -3kbp	<i>Bra1</i> _-3k_ vectF	<u>CAGATATCCGAGAAAAGG</u> TATATAG
<i>BfBra1</i> -0.5kbp	<i>Bra1</i> _HCRdeletion_ F	<u>CGGGTACCGAGCTCA</u> ACAGAAAATTTATTACATTATTTATA ACAGTTACAGCTTCTT
<i>BfBra2</i> -5.7kbp	<i>Bra2</i> _-5.7kbp_ F	<u>CGGGTACCGAGCTCA</u> ATGTGGAATGTGCGGGCGATAGATT
<i>BfBra2</i> -1, -2, -5.7kbp	<i>Bra2</i> _upstream_ R	<u>CTTTGGAGCAGTCAT</u> GGTGCACGGTACGGCTGAAGTATC

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>CGGGTACCGAGCTCA</u> CCATGACCATGCCGTCCATGTA
	<i>Bra2</i> _downstream_ R	<u>CCGCGGTGGAGCTCCT</u> CACCAATGGTTTCTGACAAGTT
<i>BfBra1</i> downstream	<i>Bra1</i> _downstream_ F	<u>CGGGTACCGAGCTCA</u> GAAACGAGGTCAAACAACGTC
	<i>Bra1</i> _downstream_ R	<u>CCGCGGTGGAGCTCC</u> CCTATGACTCCACCATCGCTCTAA
<i>BfBra1</i> , <i>Bra2</i> intron1	Common_intron1_ F	<u>CGGGTACCGAGCTCA</u> GACCGAGCGGGACCTGAA
	Common_intron1_ R	<u>CCGCGGTGGAGCTCC</u> ACCTTCAGCACGGGGAACAT
<i>BfBra1</i> , <i>Bra2</i> intron2	Common_intron2_ F	<u>CGGGTACCGAGCTCA</u> CAAGGTCAAACACCAACAACT
<i>BfBra1</i> intron2	<i>Bra1</i> _intron2_ R	<u>CCGCGGTGGAGCTCC</u> GCTGACCATGCGCTGGTTAT
<i>BfBra2</i> intron2	<i>Bra2</i> _intron2_ R	<u>CCGCGGTGGAGCTCCT</u> GTGCAGGCTGTTACGATTATCT
<i>BfBra1</i> intron3	<i>Bra1</i> _intron3_ F	<u>CGGGTACCGAGCTCA</u> GCAGTTACGGGTACCAGAATGAA
	<i>Bra1</i> _intron3_ R	<u>CCGCGGTGGAGCTCC</u> GAAAGCCTTGGCGAAAGGGTTATA
<i>BfBra2</i> intron3	<i>Bra2</i> _intron3_ F	<u>CGGGTACCGAGCTCA</u> CACATTGCGCGAGACACAGTTCAT
	<i>Bra2</i> _intron3_ R	<u>CCGCGGTGGAGCTCC</u> AACGGGTTGTGCTTGATCTTCAAA
<i>BfBra1</i> intron4	<i>Bra1</i> _intron4_ F	<u>CGGGTACCGAGCTCA</u> TAAACCCTTCGCCAAGGCTTTCTT
	<i>Bra1</i> _intron4_ R	<u>CCGCGGTGGAGCTCCT</u> CCATTCCGTCCTTCCCATCACTT
<i>BfBra2</i> intron4	<i>Bra2</i> _intron4_ F	<u>CGGGTACCGAGCTCA</u> AAAAGCCTTCTTGACGCTAAAGAA
	<i>Bra2</i> -intron4_ R	<u>CCGCGGTGGAGCTCC</u> GCGAACGGGTTGTGCTTGAT

<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>

