

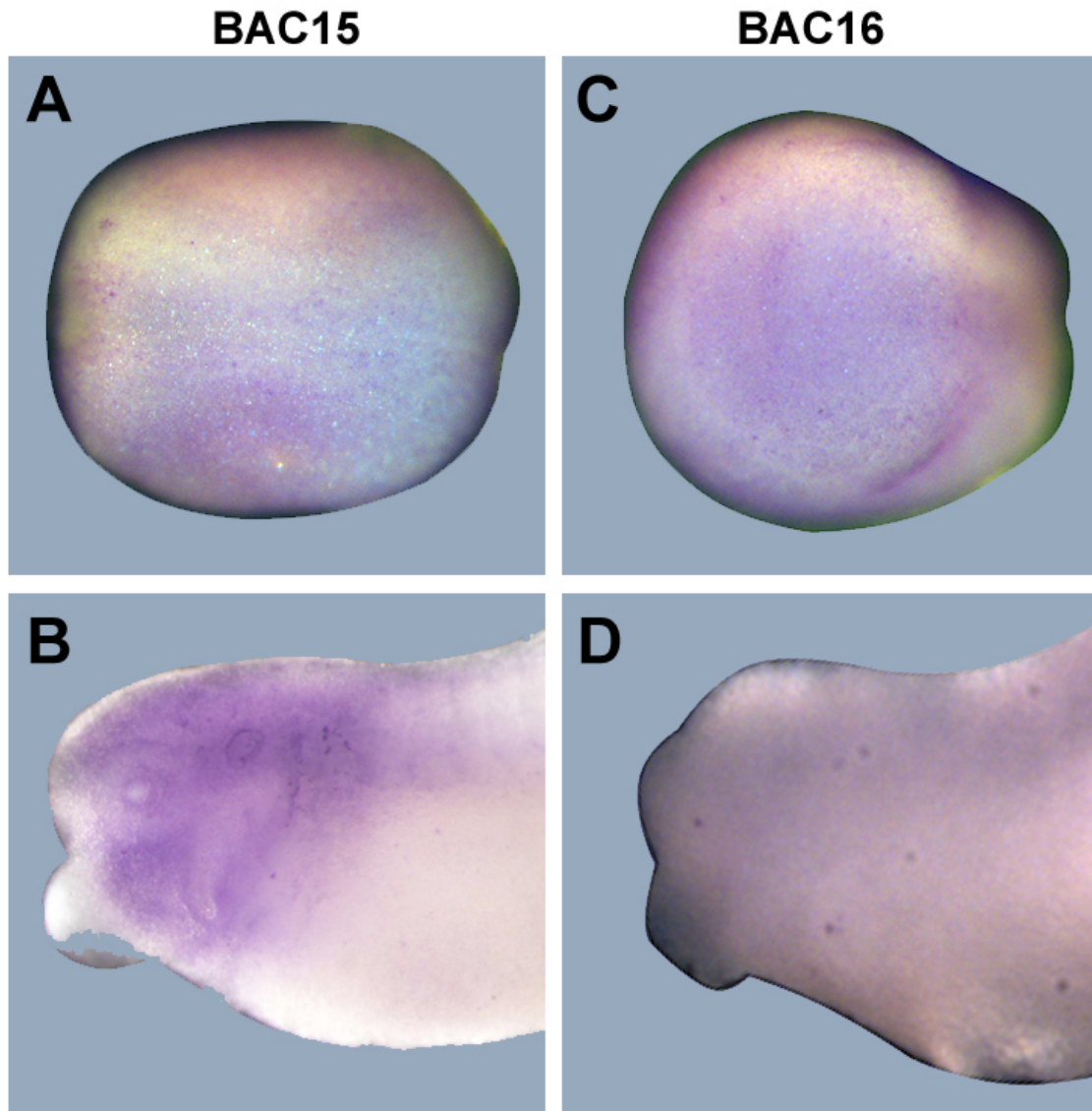
**Identification of novel cis-regulatory elements of *Eya1* in *Xenopus laevis*
using BAC recombineering**

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Supplemental material



Suppl. Fig. 1. Alignment of various BACs with the *Eya1* genomic region in *X. tropicalis*. Screenshot from *Xenopus tropicalis* Version 9.0 Genome browser (<http://www.xenbase.org/>) using the community track named xthr.out.parsed.ALLBAC.gff). The position of various BACs from a *X. tropicalis* BAC library³⁴ relative to *Eya1* is indicated and the two BACs analyzed here, termed BAC15 and BAC16, are highlighted. Note that the precise position of the plus end (right side) has not been reported for BAC16 (see Suppl. Table 1).



Suppl. Fig. 2. Control embryos injected with BACs without reporter cassettes. Embryos of *Xenopus laevis* at neural plate (A, C: dorsal view, anterior to the left) and tailbud stages (B, D: lateral view, anterior to the left) after injection with BAC15 (A,B) or BAC16 (C,D) constructs at the one-cell stage. In the absence of reporter cassettes, no expression domains are observed after in situ hybridization for *GFP*.

Suppl. Table 1

BACs covering the *Eya1* coding region and its vicinity

| BAC Identifier | Size (bp) | End Seq Genbank | End Seq orientation | Chr. 06 map ¹ | Name used here |
|----------------|-----------|----------------------|---------------------|--|----------------|
| ALN0AAA37YG13 | 79 030 | JY026638 JS989568 | minus plus | 105 650 055 105 571 025 | BAC13 |
| ALN0AAA44YC16 | 125 252 | JY073067 JY014952 | minus plus | 105 718 400 105 593 148 | BAC16A |
| ALN0AAA111YP08 | 94 084 | JS920919 JS970870 | minus plus | 105 880 209 105 786 125 | BAC08 |
| ALN0AAA145YK19 | 101 712 | JY006731 JY006221 | minus plus | 105 751 764 105 650 052 | BAC19 |
| ALN0AAA172YJ15 | 94 591 | JS939121 JS973010 | minus plus | 105 760 926 105 666 335 | BAC15 |
| ALN0AAA215YH17 | 131 335 | JS946481 JY043529 | minus plus | 105 747 150 105 615 815 | BAC17 |
| ALN0AAA212YO16 | 81 285 | JS943664 JS994048 | minus plus | 105 862 090 <105 763 626 ² | BAC16 |

¹Chr. 06 map is based on *X. tropicalis* genome assembly v9.0 available on Xenbase

²Precise position of plus end has not been reported but has to be outside of outer homology arm for our BAC16P1D1 construct which starts at position 105 763 626.

Suppl. Table 2

Primers for recombineering of insert 1 (reporter) into BAC 15

| Primer Name ¹ | Primer Sequences (5' – 3') |
|------------------------------|--|
| | (black: BAC sequence; red: restriction site; blue: insert sequence) |
| Xt_Eya1_BAC15_GFP_H1_AscI_F | CGATGGCTGGAGGGTTACACGCTCTGAAATGCACTTCTCTTA TTTCTAGGTGCGCGCCTCTAAGCGCGGACACTTGAG |
| Xt_Eya1_BAC15_Amp_H2_AsiSI_R | AATTATCAAGGCCTGTCCCACTGGGAGAATCACCCTGCCCAC TAAGTCTACGCGATCGCCGCTCAGTGGAACGAAAAC |
| Xt_Eya1_BAC15_FB | ACAGGATATGGTCAGTTTGTATTTTTG |
| Xt_Eya1_BAC15_RB | ACCCAAGAACAACACTGTCTT |
| Xt_Eya1_Amp_BAC15_FI | GCGGTATCATTGCAGCACTG |
| Xt_Eya1_GFP_BAC15_RI | TAGGTCAGGGTGGTCACGA |

¹F: Forward primer for BAC recombineering;

R: Reverse primer for BAC recombineering;

FB: Forward primer binding to BAC adjacent to insertion for verification of insertion;

RB: Reverse primer binding to BAC adjacent to insertion for verification of insertion;

FI: Forward primer binding to insert for verification of insert orientation;

RI: Reverse primer binding to insert for verification of insert orientation

Suppl. Table 3

Primers for recombineering of insert 1 (reporter) into BAC 16

| Primer Name ¹ | Primer Sequences (5' – 3') |
|------------------------------|--|
| | (black: BAC sequence; red: restriction site; blue: insert sequence) |
| Xt_Eya1_BAC16_Amp_H1_AscI_F | TTGGTTGTTTAAAGCACAGTTGACATAATATTGGTGTGTAACC CTCCAGTGGCGCGCCGGCACTTTTCGGGGAAATGT |
| Xt_Eya1_BAC16_GFP_H2_AsiSI_R | TATATTGTCATGAGGAAGGAAATATGTGTACATTGCAGTCTA CATTAAACGGATCGCTTCAGGTTTCAGGGGGAGGTG |
| Xt_Eya1_BAC16_FB | GGACTTGCCTGAGATGCTGA |
| Xt_Eya1_BAC16_RB | GTGGTTCTCCCAGAGAGCAA |
| Xt_Eya1_GFP_BAC16_FI | ATGGCCGACAAGCAGAAGAA |
| Xt_Eya1_Amp_BAC16_RI | TAATACCGCGCCACATAGCA |

¹F: Forward primer for BAC recombineering;

R: Reverse primer for BAC recombineering;

FB: Forward primer binding to BAC adjacent to insertion for verification of insertion;

RB: Reverse primer binding to BAC adjacent to insertion for verification of insertion;

FI: Forward primer binding to insert for verification of insert orientation;

RI: Reverse primer binding to insert for verification of insert orientation

Suppl. Table 4

Primers for recombineering of insert 2 (deletion) into BAC15

| Primer Name ¹ | Primer Sequences (5' – 3') |
|---------------------------|--|
| | (black: BAC sequence; blue: insert sequence) |
| Xt_Eya1_BAC15_P1H1_F | AACTTAACAGCTGGGAAAGAAAAATCACTCTCAAAGTTGT CTAAAATGTAGCTTGCAGTGGGCTTACATG |
| Xt_Eya1_BAC15_P1H2_R | ATAGAAGTTAATAAAAAAGCAAGATCCTATATTATCATTAG CATAAGACA CGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P1H3_R | AATAAGTGTCCGATAAATAATTAATTTAATCTCATTATAGA CTTTTTCAGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P1H4_R | CATTTTACTTCAAGTCTCCCACCAGAACCGTACACTCTCC ATACGGAAC CGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P1H5_R | CTGAGACGTTTACAGCAATGAAAACTGGTTAATTGAAAT CCATAAAGCT CGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P1H1_FB | ACAGCTGGGAAAGAAAAATCACT |
| Xt_Eya1_BAC15_P1H2_RB | ACAGCTGGGAAAGAAAAATCACT |
| Xt_Eya1_BAC15_P1H3_RB | TGGCATTGCAAACATTCTGG |
| Xt_Eya1_BAC15_P1H4_RB | GTTTTACCAACGCAGGGCAA |
| Xt_Eya1_BAC15_P1H5_RB | ACCATGAAAAGCAAACATTGGT |
| Xt_Eya1_BAC15_KanaP1H1_RI | CACGGCATACTTTACGCAGC |
| Xt_Eya1_BAC15_KanaP1H2_RI | CGGACCGCTATCAGGACATAG |
| Xt_Eya1_BAC15_P2H1_F | CACATAACAGCAATTCCATGACACCAAATGGCACAGAAG GTGAGTCCCAAGCTTGCAGTGGGCTTACATG |
| Xt_Eya1_BAC15_P2H2_R | GGGAAATGTTTTTCAGAGAAAAGGGATAACTCAAGCA CATAGCATGT CGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P2H3_R | GCTATAACATTGTATTGAGCCTTTACACATTCTCATAAAGA GTTTGTTCA CGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P2H4_R | CAACGCGCCATAAATTAATTAGCAACTACATATATGATGG CTCCCTGTG CGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P2H5_R | AATTTGCTAAGCAACCAGCCACAGTGTTCACTAGACTCA TGCTGTGCCCGGCCGCTCTAGA ACTAGTG |
| Xt_Eya1_BAC15_P2H1_FB | CACAGGATATGGTCAGTTTGTATTTTT |
| Xt_Eya1_BAC15_P2H2_RB | GGGAAATGTTTTTCAGAGAAAAGGGA |
| Xt_Eya1_BAC15_P2H3_RB | AGTAAGATGAAGTGTATGCAAAGGTG |
| Xt_Eya1_BAC15_P2H4_RB | GTTGGCAACCTTACACCCTT |
| Xt_Eya1_BAC15_P2H5_RB | AGCGCCGACTATACCTGATG |

¹F: Forward primer for BAC recombineering;

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FB: Forward primer binding to BAC adjacent to insertion for verification of insertion;

RB: Reverse primer binding to BAC adjacent to insertion for verification of insertion;

FI: Forward primer binding to insert for verification of insert orientation;

RI: Reverse primer binding to insert for verification of insert orientation

Suppl. Table 5

Primers for recombineering of insert 2 (deletion) into BAC16

| Primer Name ¹ | Primer Sequences (5' – 3') (black: BAC sequence; blue: insert sequence) |
|--------------------------|---|
| Xt_Eya1_BAC16_P1H3_F | CGAATTAATAAAGTTGTGTGCATCAATAAAAAATGGTT AATGATAGAATAGCTTGCAGTGGGCTTACATG |
| Xt_Eya1_BAC16_P1H4_R | AGGGCAGCACTCCTAGGATTAAGAGAAAAATCGTAAA CTAAAAGATAAAACGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P1H5_R | ATTTGTACAAGGTAATCTTGGAACTACTCTGTAAGCAT GTCCATAATGGCTCGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P1H6_R | TACACCTGAAAGTCCACCTTGAGGGTCAGTTAGGATG AGATTTGTGAAATAAGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P1H7_R | TCGTGACTCGTGACTGACTATTGTTTTTTTGTCCATCA GGAAACCATTGTAGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P1H3_FB | ACTGTCATCCTGTGCAAAGGT |
| Xt_Eya1_BAC16_P1H4_RB | AGGGCAGCACTCCTAGGAT |
| Xt_Eya1_BAC16_P1H5_RB | AAAAAGGGGGCTCAGGGAAA |
| Xt_Eya1_BAC16_P1H6_RB | TCATTTGGCATTTCGTATCTTTAGAGA |
| Xt_Eya1_BAC16_P1H7_RB | ATGGGAGACTTTCCTTGGGC |
| Xt_Eya1_BAC16_P2H1_F | AATAAAACCAGGACATTCATAGGAAAGTGTGGCCATTT GGGATTGCCCTTAGCTTGCAGTGGGCTTACATG |
| Xt_Eya1_BAC16_P2H2_R | AAGGTCGCAGGCATGTAAAAAAGTTGCAGTTGTGTCA AAAAAGTTACGGCGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P2H3_R | TTCTGCAGCCCGCCTCCCTCAACTTGCTTCATTGAATC CCTGTATAAGACCGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P2H4_R | AAGTTTGGGTCACTATAGCATAAGTATCTTTAACCATG AGAAGTGTGTTCCGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P2H5_R | TACTCTGTATGTCCAATGTATGAAACCCACTTATTGTAC AGCGCTGCGGACGGCCGCTCTAGAACTAGTG |
| Xt_Eya1_BAC16_P2H1_RB | CTCAAGGGGCTGAGAAGTGA |
| Xt_Eya1_BAC16_P2H2_FB | AAAGGTTGGGATCCCTATGC |
| Xt_Eya1_BAC16_P2H3_FB | TCACAACCTCTGGTCATGCC |
| Xt_Eya1_BAC16_P2H4_FB | CTGCCCTCAGTGTAGCCAA |
| Xt_Eya1_BAC16_P2H5_FB | CCAAGGTAATATAAGAGCTCTCAGC |

¹F: Forward primer for BAC recombineering;
R: Reverse primer for BAC recombineering;
FB: Forward primer binding to BAC adjacent to insertion for verification of insertion;
RB: Reverse primer binding to BAC adjacent to insertion for verification of insertion;