

# Analysis of muscleblind-like protein 2

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Groups ID: 18 & 19

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# Outline

- Background
- mRNA sequence alignment
- Amino acids sequence alignment
- Analysis of zinc finger domains
- Analysis of predicted homolog structures
- Mutation analysis

# Background

MBNL

muscleblind-like

# Background

MBNL

muscleblind-like

DM1 DMPK (CUG)<sub>n</sub> hairpins candidate sequestered factors

homology to *Drosophila* muscleblind proteins

(Miller et al., 2000)

# Background

## MBNL

### muscleblind-like

DM1 DMPK (CUG)<sub>n</sub> hairpins **candidate sequestered factors**

homology to *Drosophila* **muscleblind** proteins

**muscleblind proteins**: terminal differentiation of embryonic pharyngeal, visceral and somatic muscles and ommatidial photoreceptors

**muscleblind-like proteins**: terminal differentiation of DM1-affected tissues, such as blood, eye, cardiac muscle and skeletal muscle

(Miller et al., 2000)

# Background

MBNL

muscleblind-like

MBNL & DM1

# Background

MBNL

muscleblind-like

MBNL & DM1

muscleblind proteins regulate **alternative splicing**

(Ho et al., 2004)

# Background

MBNL

muscleblind-like

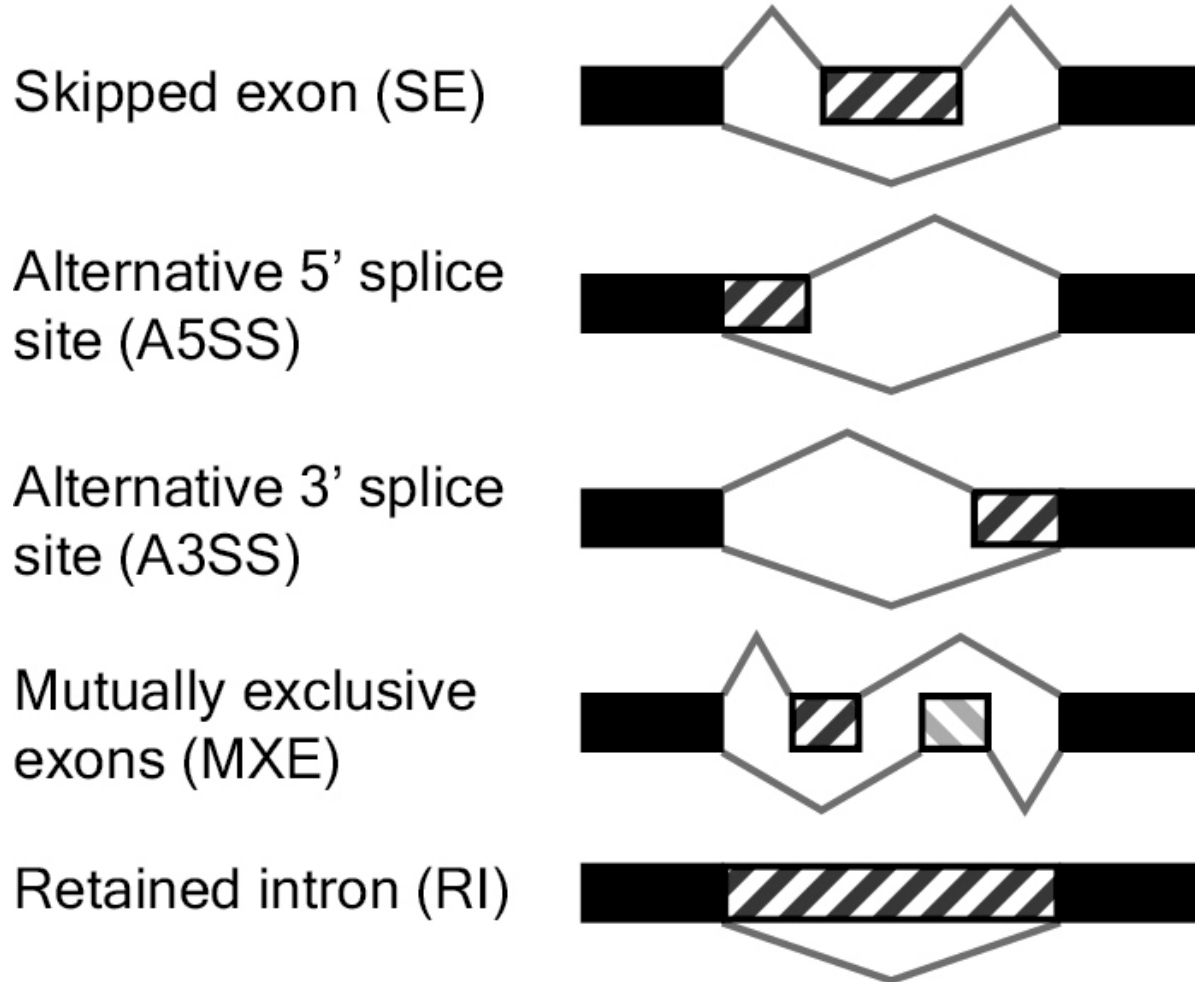
MBNL & DM1

MBNL & alternative splicing



# Background

## Alternative Splicing Events



# Background

MBNL

muscleblind-like

MBNL & DM1

MBNL & alternative splicing

DM1 differentiation cancer

# Background

MBNL

muscleblind-like

MBNL & DM1

MBNL & alternative splicing

DM1 differentiation cancer

cardiovascular disease

# Background

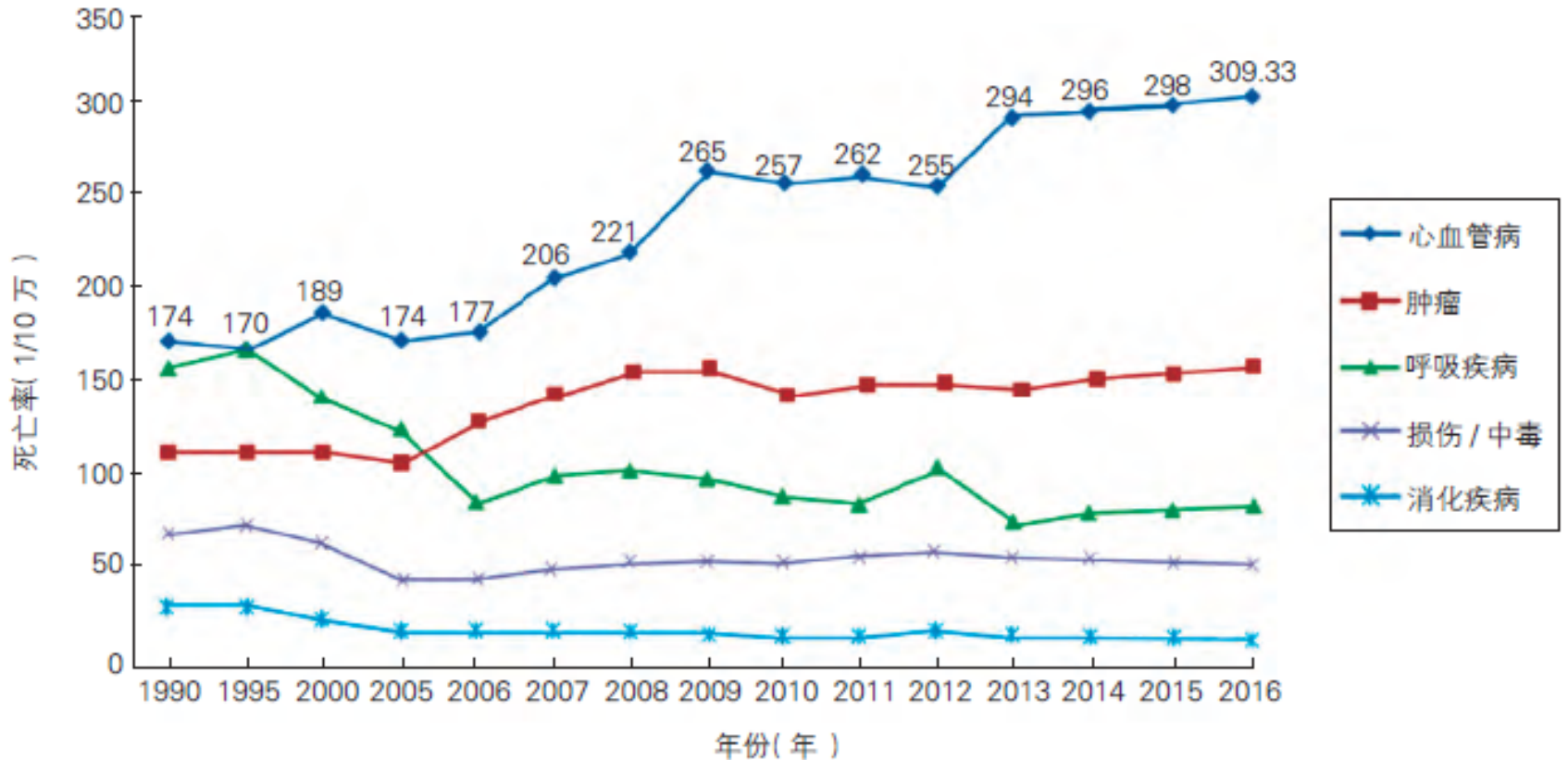


图 1 1990~2016 年中国农村居民主要疾病死亡率变化

# Background

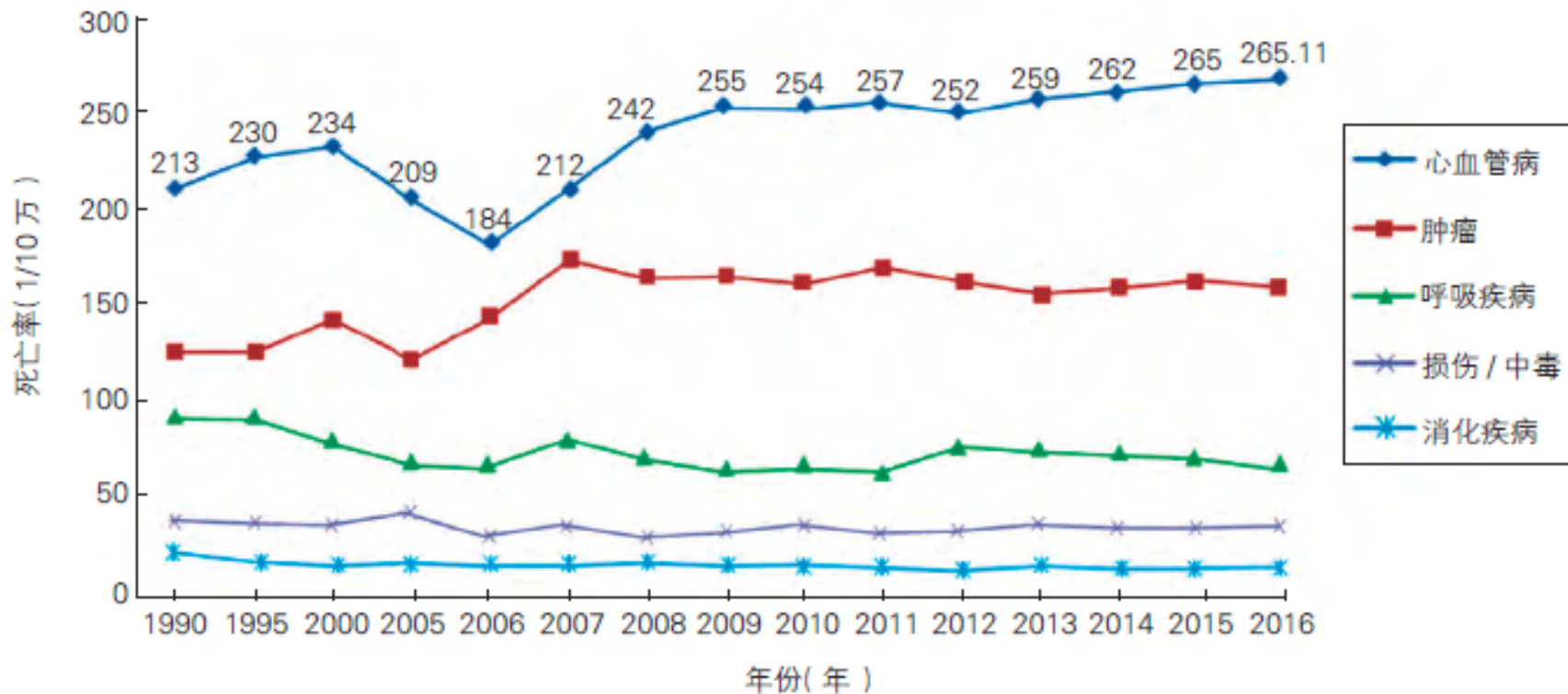


图2 1990~2016年中国城市居民主要疾病死亡率变化

# Background

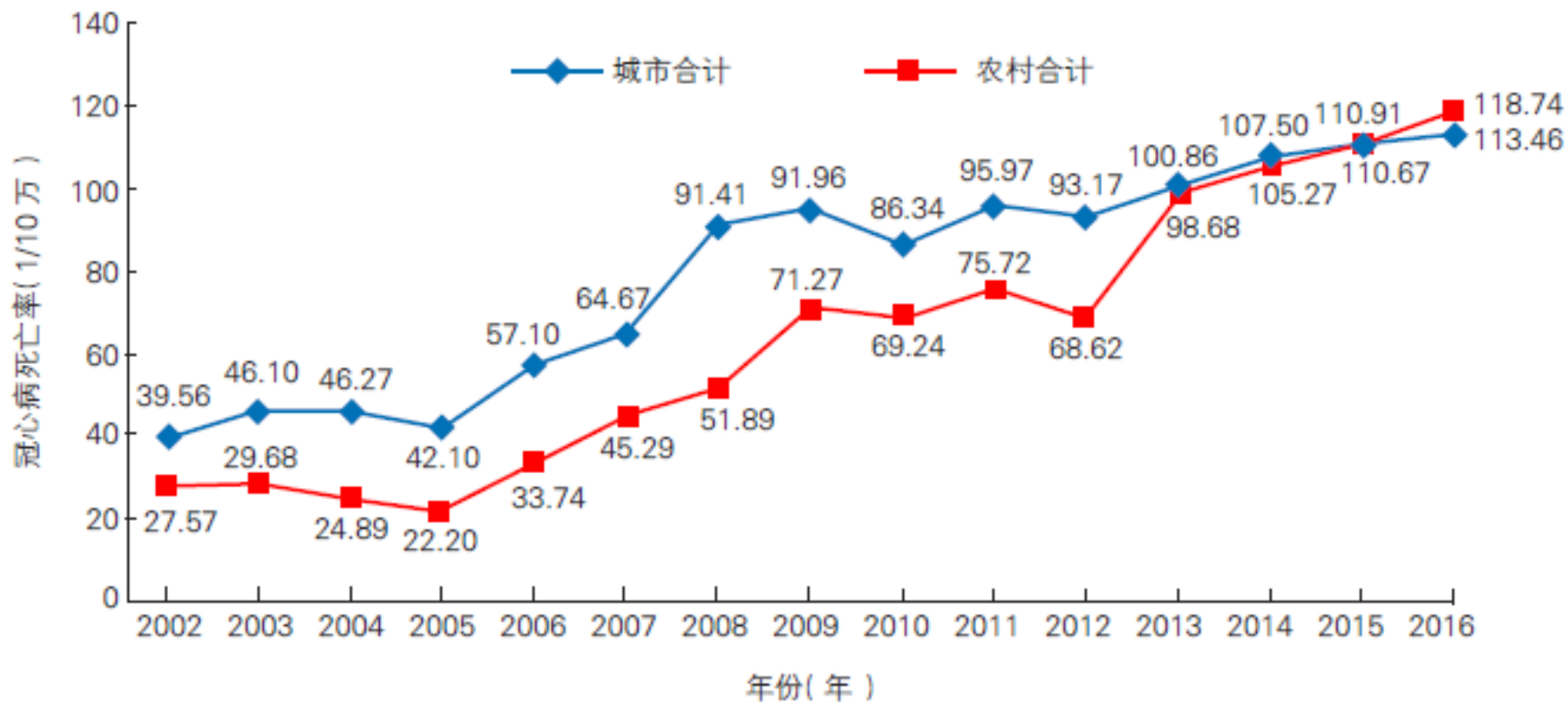


图 12 2002~2016 年中国城乡地区冠心病死亡率变化趋势

# Background

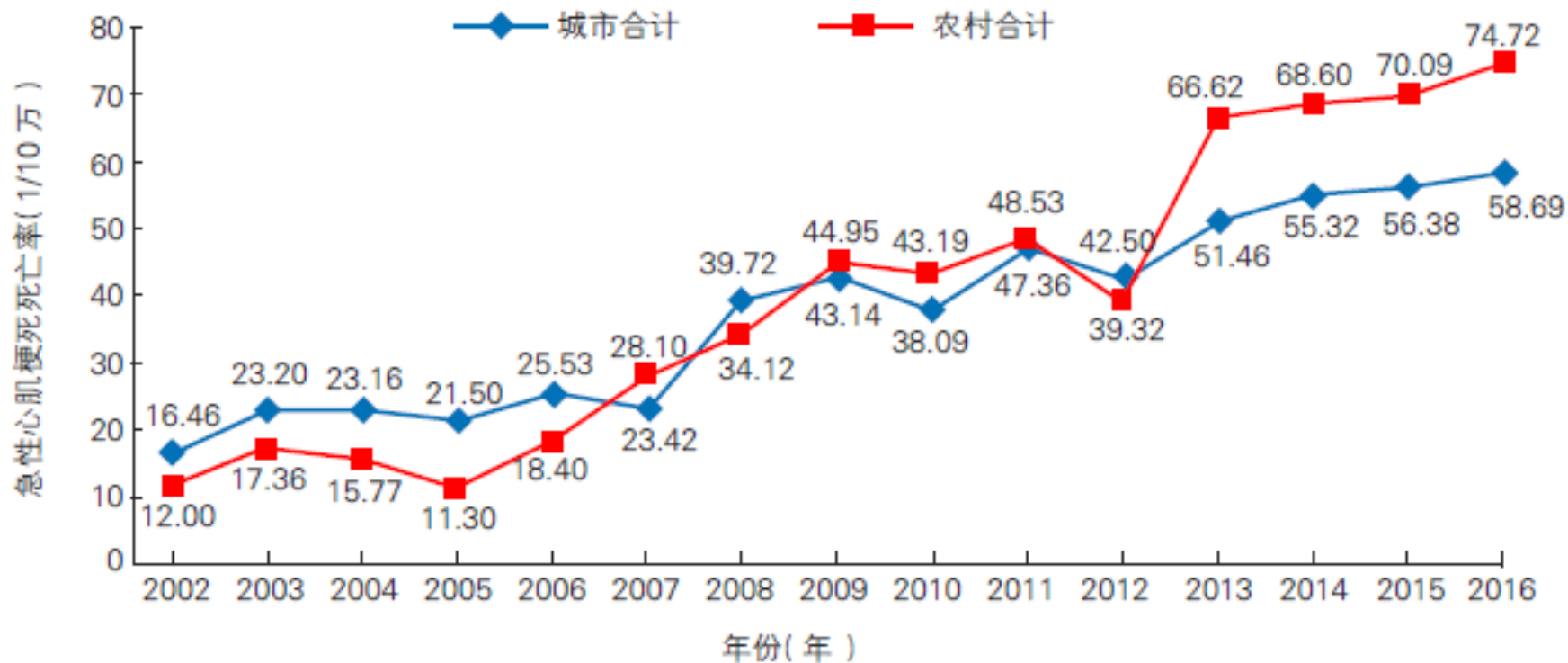
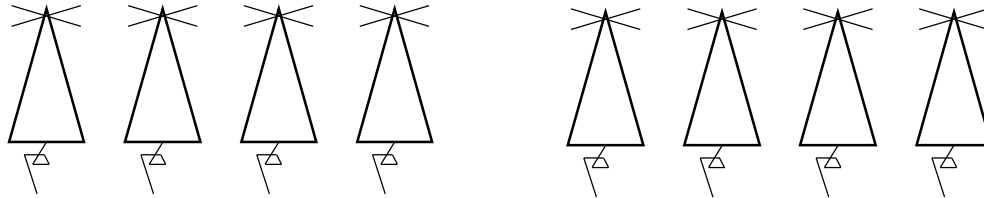


图 13 2002~2016 年城乡地区急性心肌梗死死亡率变化趋势

# Background



SHAM

IR

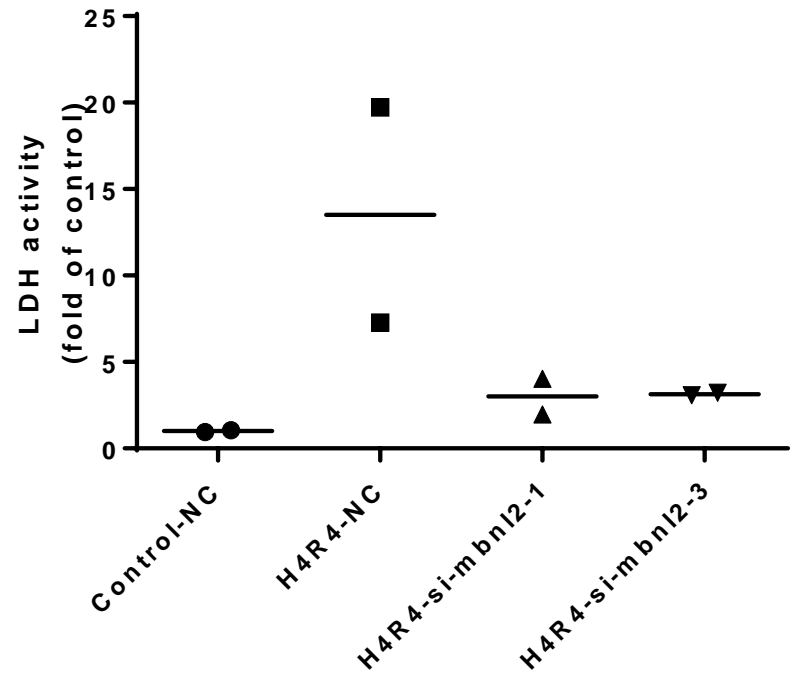
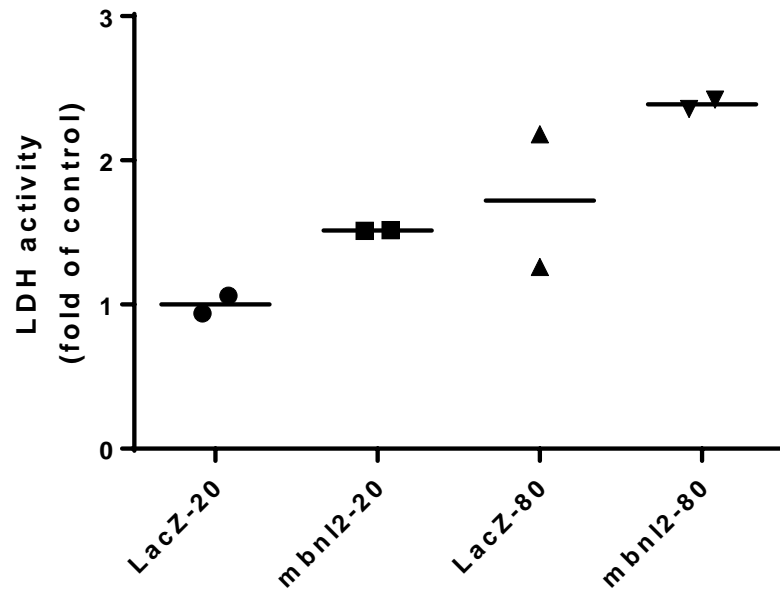
MBNL2↑

IR: ischemia reperfusion

unpublished data

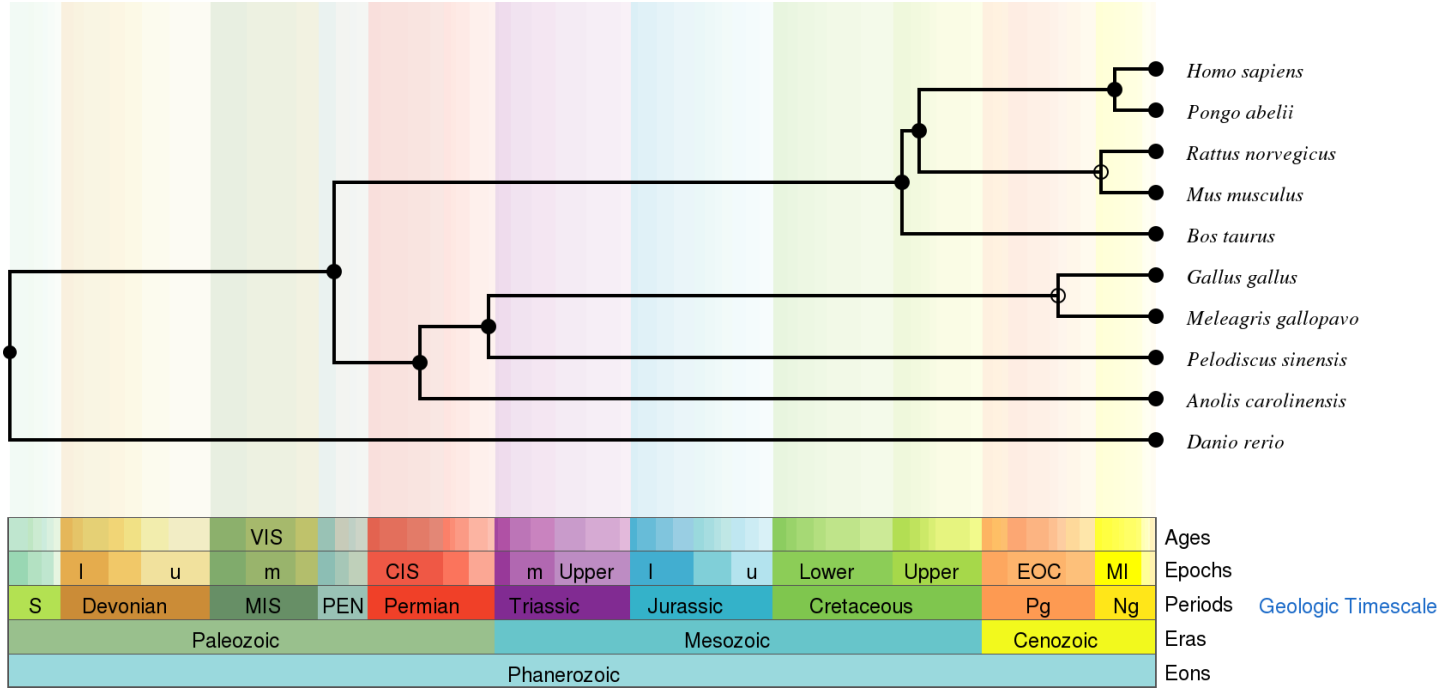


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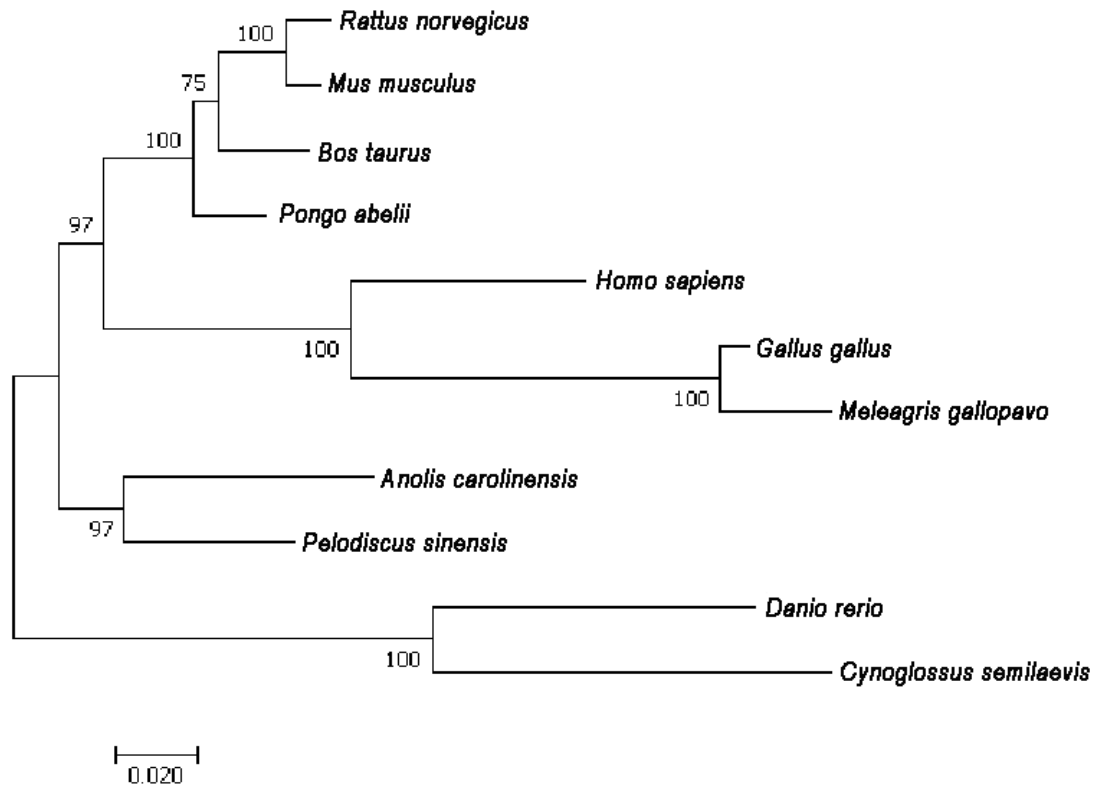


pre-experimental data

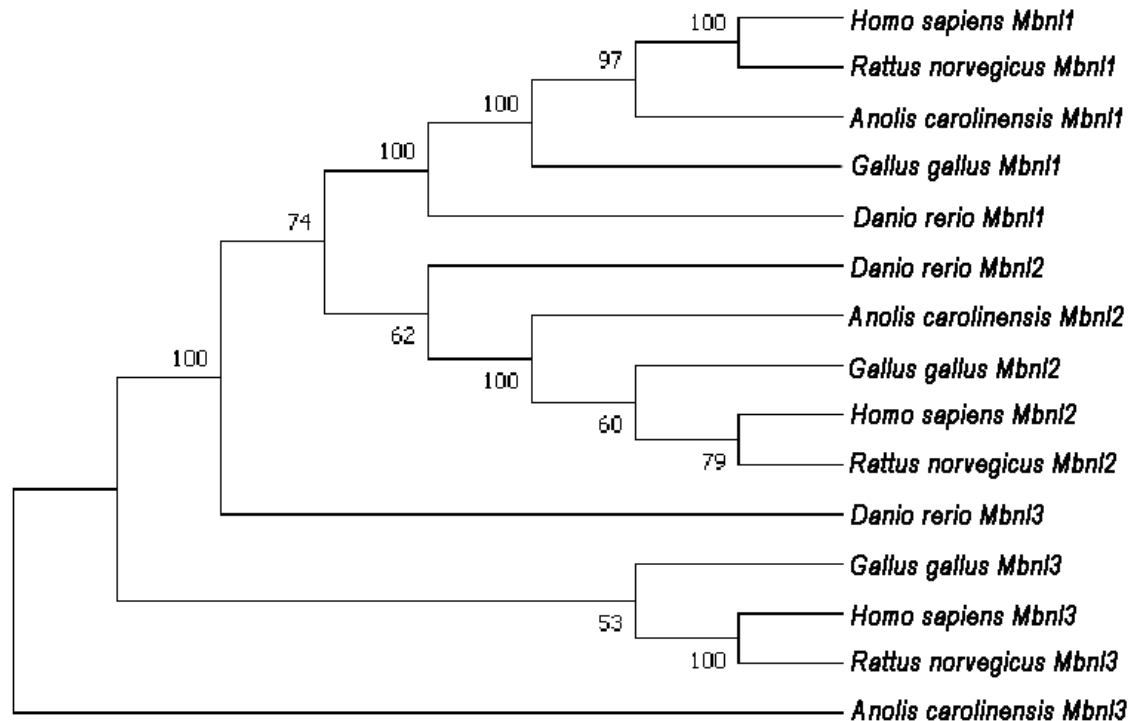
# Evolutionary timescale of selected species



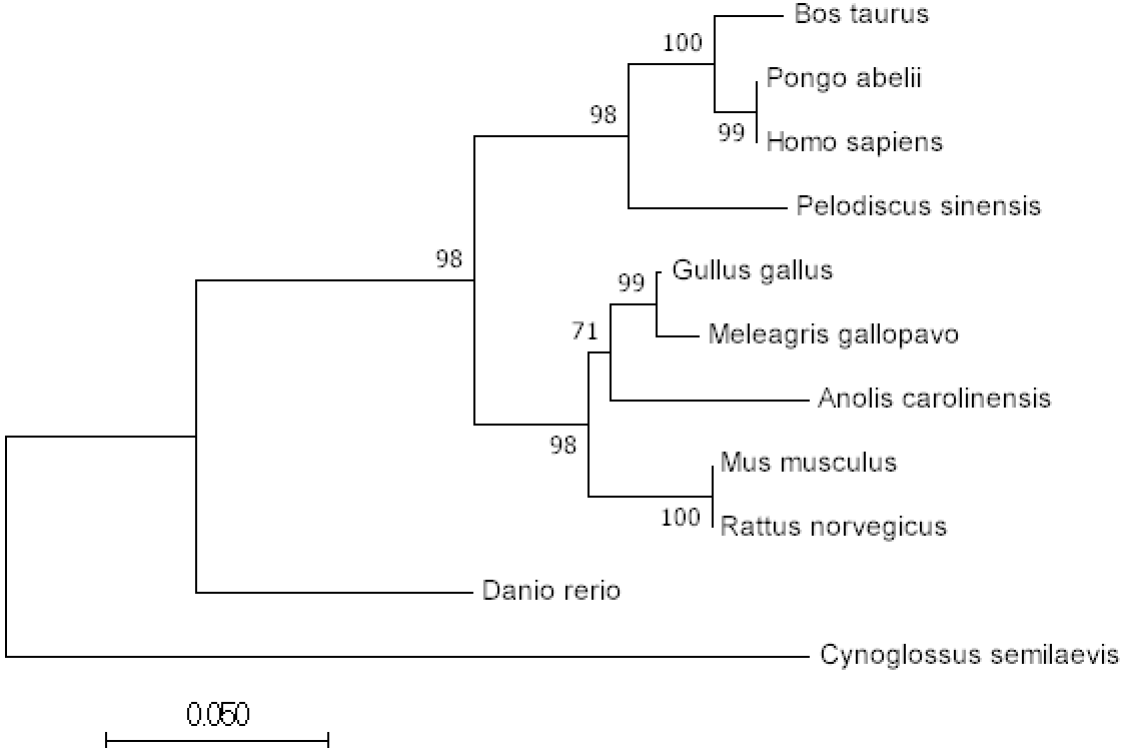
# Neighbor joining tree constructed by MEGA(mRNA) of *Mbnl2* mRNA



# Neighbor joining tree constructed by MEGA of *Mbnl* family mRNA



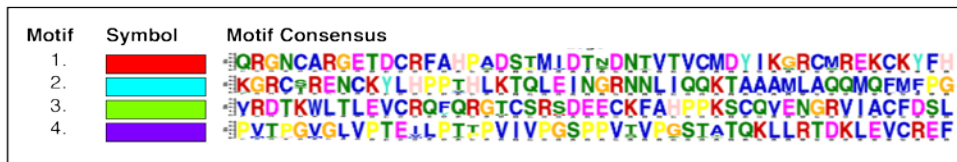
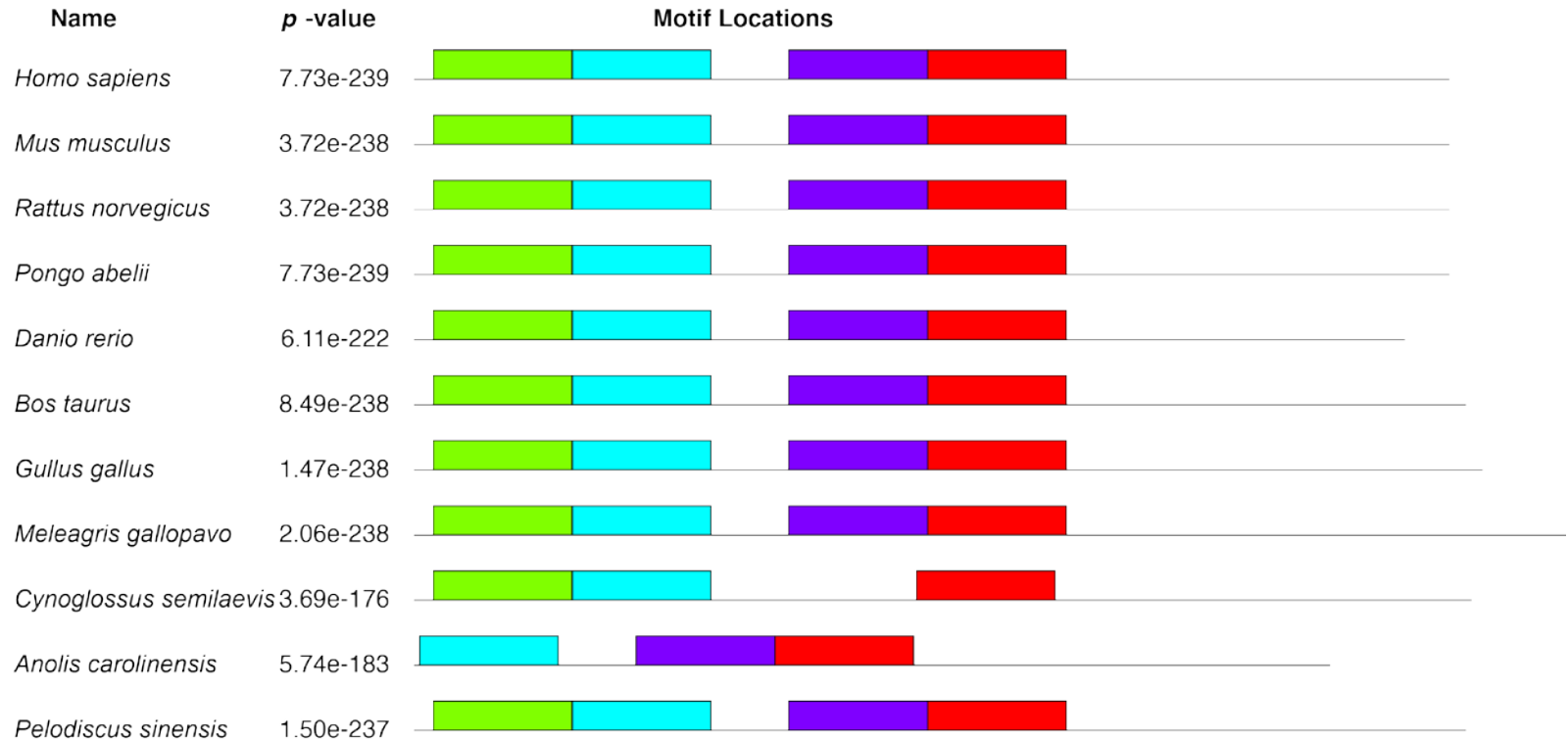
# MBNL2 neighbor joining tree constructed by MEGA



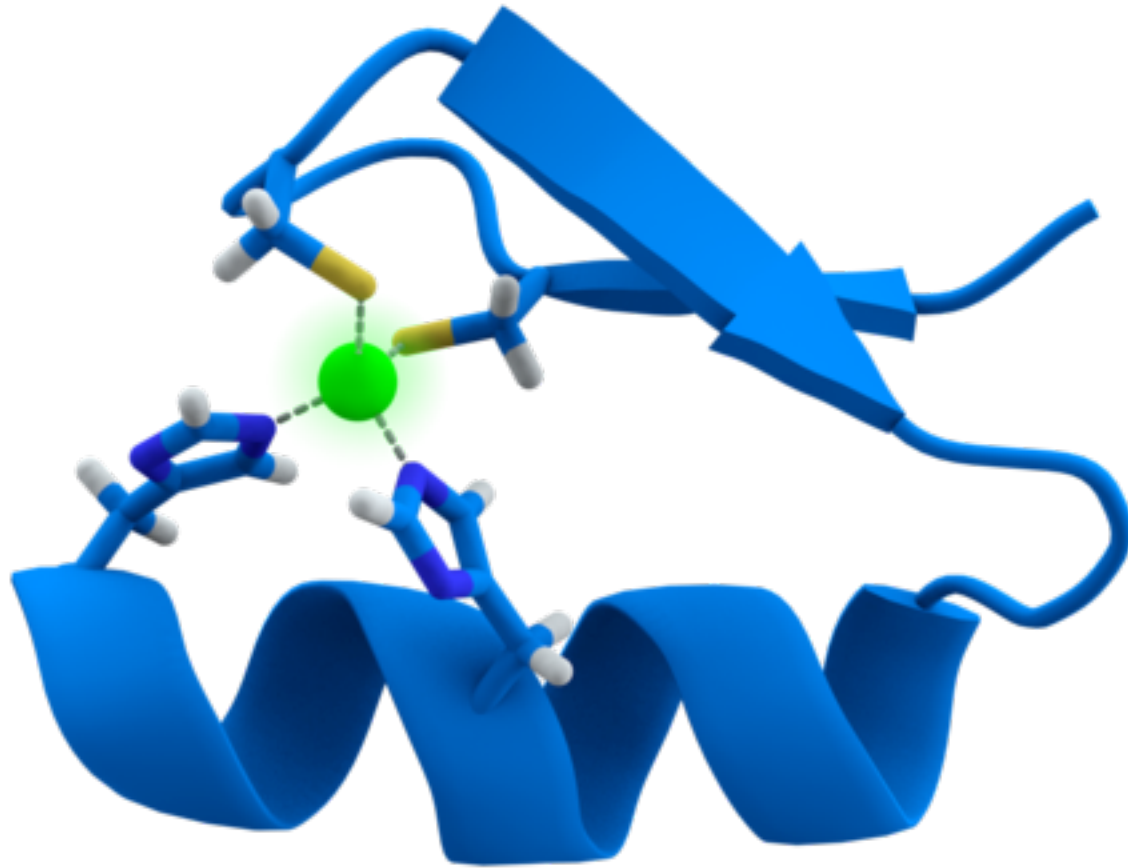
# Multiple protein sequence alignment of MBNL2

	1	10	20	30	40	50	60	70	80	90	100	110	120
Homo	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Pongo	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Bos	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Pelodiscus	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Gullus	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Anolis	.....	.....	.....	.....	.....	.....	.....	TFQGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA
Meleagris	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Mus	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Rattus	MALNVAPVRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	THLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Danio	MALN IASIRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPP	AHLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
Cynoglossus	MALNVSSIRD	TKWLTLEVCRQ	FQFGTCSRSD	EECKFAHPPK	SCQVENGRVI	ACFDSLKGR	C	SRENCKYLHPPS	AHLKTQLEI	NGRNNLIQ	OQTAAAMLA	QQMFMFPG	TPLHPVP
	130	140	150	160	170	180	190	200	210	220	230		
Homo	AIGTNTAISF	FAPYLAPVTP	GVGLVPT	EILPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Pongo	AIGTNTAISF	FAPYLAPVTP	GVGLVPT	EILPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Bos	AIGTNTAISF	FAPYLAPVTP	GVGLVPT	EILPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Pelodiscus	TIGTNTAISF	FAPYLAPVTP	GVGLVPT	EILPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Gullus	TIGTNTAISF	FAPYLAPVTP	GVGLVPT	EILPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Anolis	TIGSNTAISF	FAPYLAPVTP	GVGLVPT	EIVPTTPV	IIVPGSP	PVTVPGST	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Meleagris	TIGTNTAISF	FAPYLAPVTP	GVGLVPT	EILPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Mus	TIGTNTAISF	FAPYLAPVTP	GVGLVPT	EIVPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Rattus	TIGTNTAISF	FAPYLAPVTP	GVGLVPT	EIVPTTPV	IIVPGSP	PVTVPGSTA	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Danio	GLGSNPGLS	YAPYLTPMSH	GMGLVPT	EMLPS	TPVIVP	GSPVTVPGSSS	...	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
Cynoglossus	LANNTGLG	YGSYISPS	LSHGMSL	VPSDILPS	SPLLVS	ASPPITVQS	SSSSP	IQKLLRTD	KLEVCRE	FORGNCA	RGETDCR	FAHPADS	TMIDT
	240	250	260		270	280	290	300	310	320	330		
Homo	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Pongo	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Bos	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Pelodiscus	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Gullus	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Anolis	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Meleagris	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Mus	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Rattus	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AATVM	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Danio	AHLQAKIKAA	QHOANQRA	VAAQAAAA	AMTQS	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	
Cynoglossus	AHLQVQIKI	SGQQATQ	TAVATQ	AAAATV	.....	AFPPGAL	HLPLPKR	QALEKSN	NGS	AVFNPSV	LHYQQAL	TS	

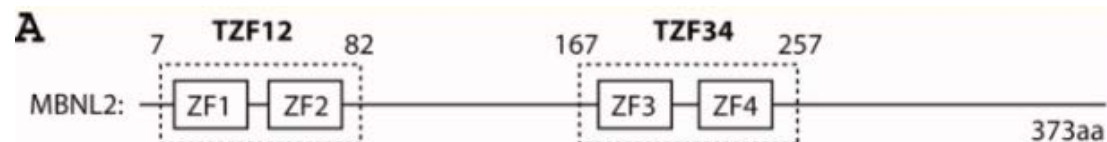
# Motif discovery in MBNL2 protein



# Structure of a single zinc finger





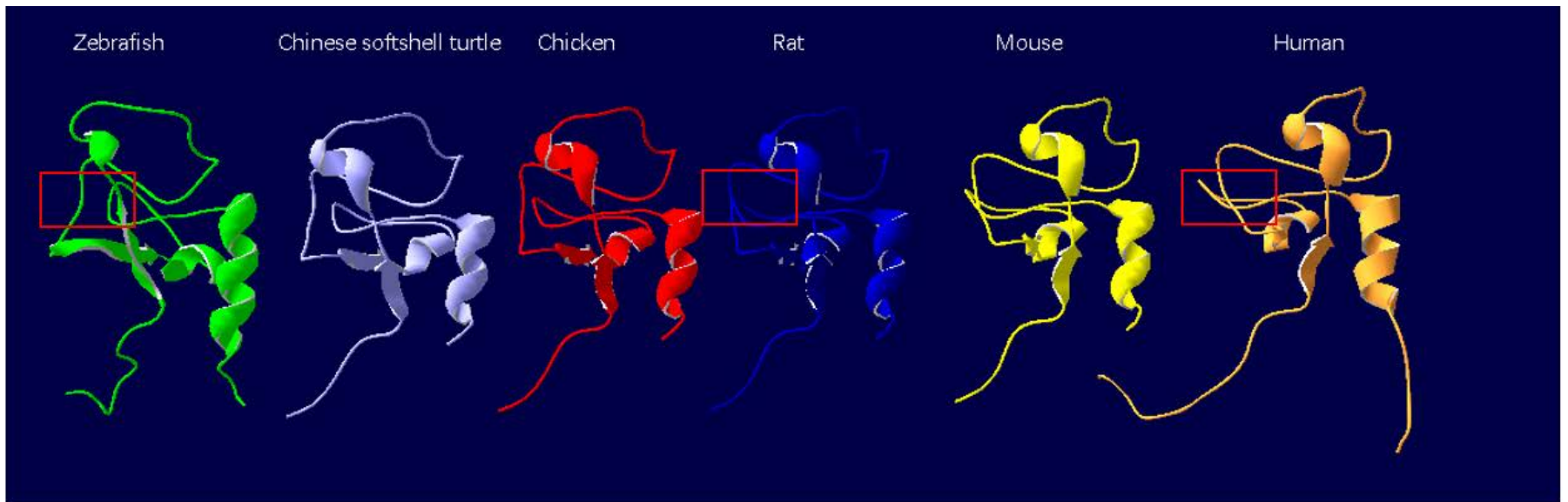


Feature key	Position(s)	Description	Actions	Graphical view	Length
Zinc finger <sup>i</sup>	13 - 41	C3H1-type 1	Add  BLAST		29
Zinc finger <sup>i</sup>	47 - 73	C3H1-type 2	Add  BLAST		27
Zinc finger <sup>i</sup>	176 - 204	C3H1-type 3	Add  BLAST		29
Zinc finger <sup>i</sup>	212 - 238	C3H1-type 4	Add  BLAST		27

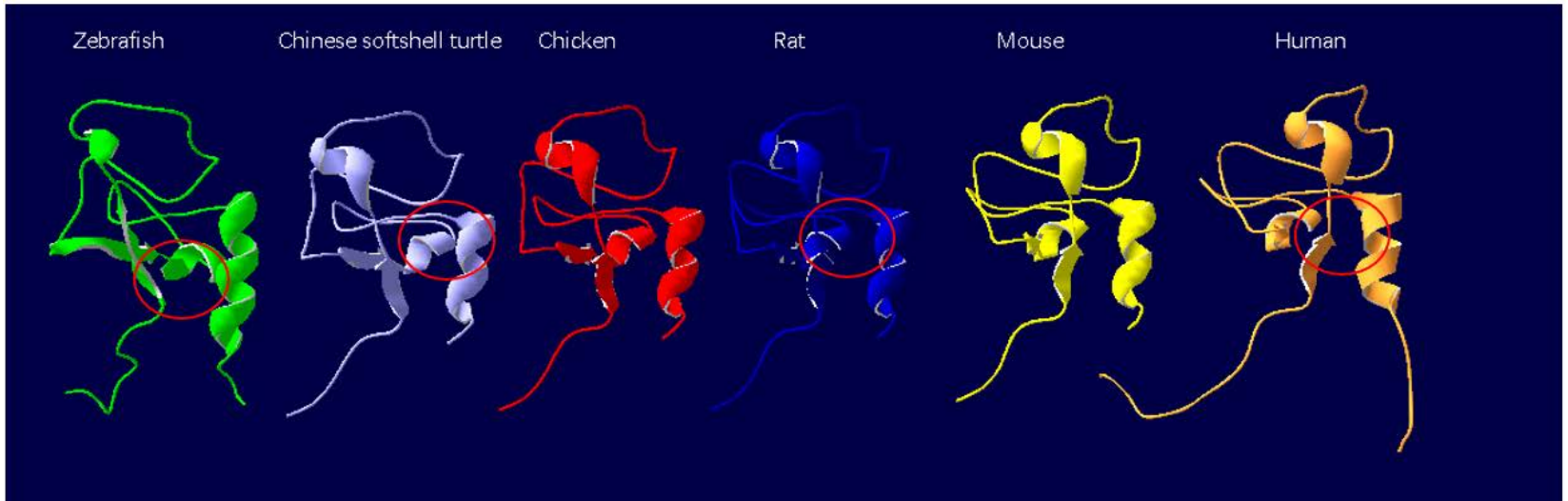
Species/Abbrev	Group Name						★					*	★	*			★			★	*									
1. Homo sapiens MBNL1 ZF1		W	L	T	L	E	V	C	R	E	F	Q	R	G	T	C	S	R	P	D	T	E	C	K	F	A	H	P	S	K
2. Homo sapiens MBNL2 ZF1		W	L	T	L	E	V	C	R	Q	F	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
3. Homo sapiens MBNL3 ZF1		W	L	T	L	E	V	C	R	E	F	Q	R	G	T	C	S	R	A	D	A	D	C	K	F	A	H	P	P	R
4. Homo sapiens MBNL1 ZF2		-	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	P
5. Homo sapiens MBNL2 ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	T
6. Homo sapiens MBNL3 ZF2		-	G	R	V	V	A	C	F	D	S	L	K	G	R	C	T	R	-	-	E	N	C	K	Y	L	H	P	P	P
7. Homo sapiens MBNL1 ZF3		T	D	R	L	E	V	C	R	E	Y	Q	R	G	N	C	N	R	G	E	N	D	C	R	F	A	H	P	A	D
8. Homo sapiens MBNL2 ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
9. Homo sapiens MBNL3 ZF3		S	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	T	R	G	E	N	D	C	R	Y	A	H	P	T	D
10. Homo sapiens MBNL1 ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	S	R	-	-	E	K	C	K	Y	F	H	P	P	A
11. Homo sapiens MBNL2 ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A
12. Homo sapiens MBNL3 ZF4		D	N	T	V	T	I	C	M	D	Y	I	K	G	R	C	S	R	-	-	E	K	C	K	Y	F	H	P	P	A

Species/Abbrv	Group Name			★			★	*		★		★*																		
1. Homo sapiens MBNL2 ZF1		W	L	T	L	E	V	C	R	Q	F	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
2. Homo sapiens MBNL2 ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	T
3. Homo sapiens MBNL2 ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
4. Homo sapiens MBNL2 ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A
5. Mus musculus ZF1		W	L	T	L	E	V	C	R	Q	Y	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
6. Mus musculus ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	T
7. Mus musculus ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
8. Mus musculus ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A
9. Rattus norvegicus ZF1		W	L	T	L	E	V	C	R	Q	Y	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
10. Rattus norvegicus ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	T
11. Rattus norvegicus ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
12. Rattus norvegicus ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A
13. Pongo abelii ZF1		W	L	T	L	E	V	C	R	Q	F	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
14. Pongo abelii ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	T
15. Pongo abelii ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
16. Pongo abelii ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A
17. Danio rerio ZF1		W	L	T	L	E	V	C	R	Q	F	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
18. Danio rerio ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	T	R	-	-	E	N	C	K	Y	L	H	P	P	A
19. Danio rerio ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	S	D
20. Danio rerio ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	S	R	C	S	R	-	-	E	K	C	K	Y	F	H	P	P	A
21. Bos taurus ZF1		W	L	T	L	E	V	C	R	Q	F	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
22. Bos taurus ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	S	R	-	-	E	N	C	K	Y	L	H	P	P	T
23. Bos taurus ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
24. Bos taurus ZF4		D	N	S	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A
25. Gallus gallus ZF1		W	L	T	L	E	V	C	R	Q	F	Q	R	G	T	C	S	R	S	D	E	E	C	K	F	A	H	P	P	K
26. Gallus gallus ZF2		N	G	R	V	I	A	C	F	D	S	L	K	G	R	C	T	R	-	-	E	N	C	K	Y	L	H	P	P	T
27. Gallus gallus ZF3		T	D	K	L	E	V	C	R	E	F	Q	R	G	N	C	A	R	G	E	T	D	C	R	F	A	H	P	A	D
28. Gallus gallus ZF4		D	N	T	V	T	V	C	M	D	Y	I	K	G	R	C	M	R	-	-	E	K	C	K	Y	F	H	P	P	A

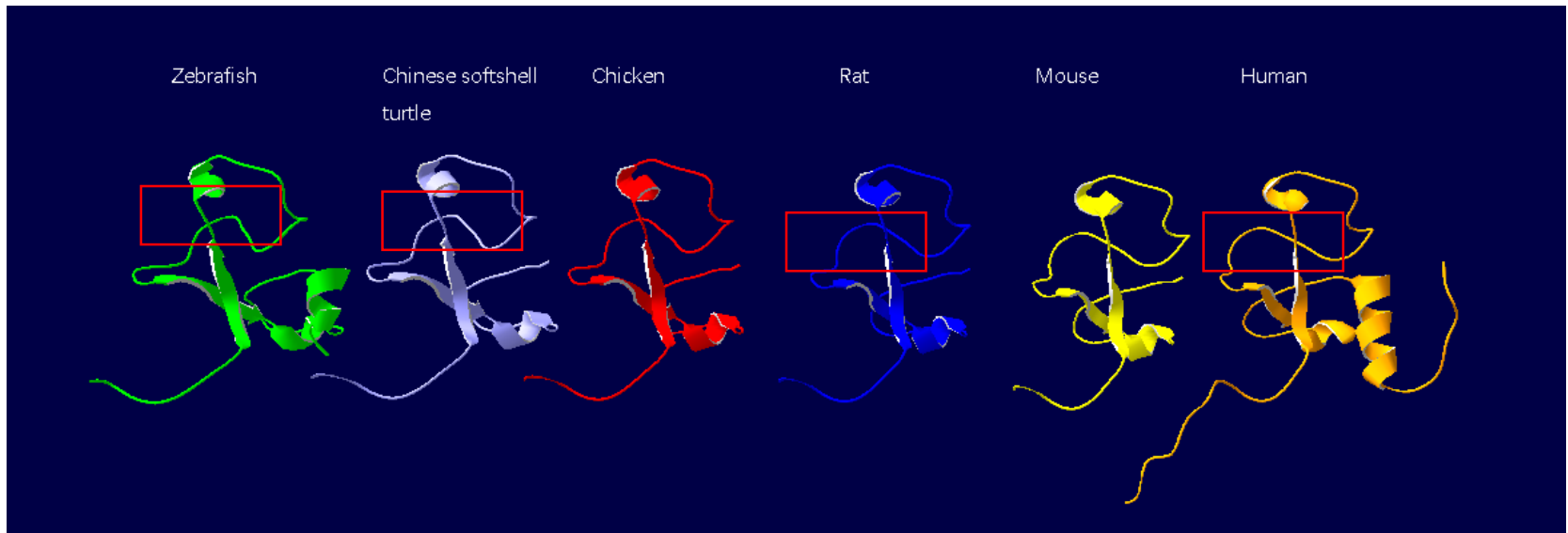
# Predicted MBNL2 zinc fingers 1 and 2



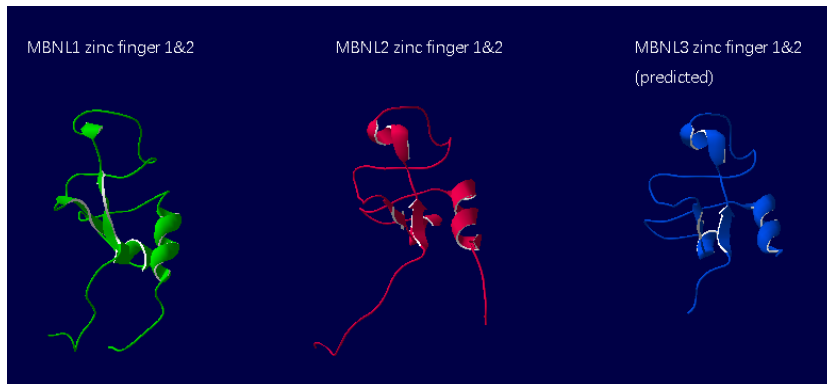
# Predicted MBNL2 zinc fingers 1 and 2



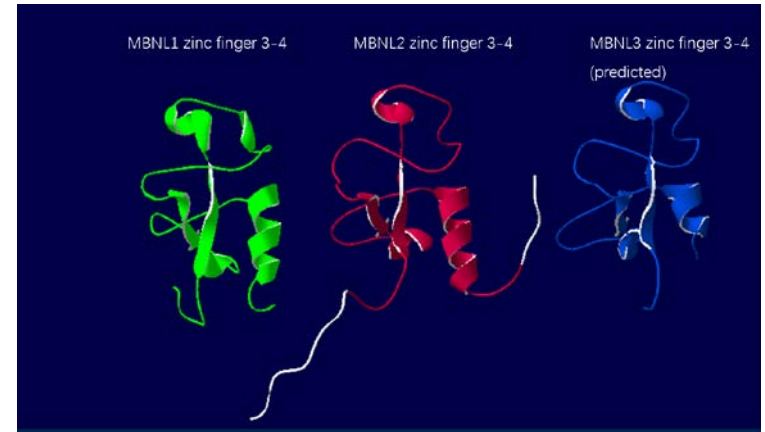
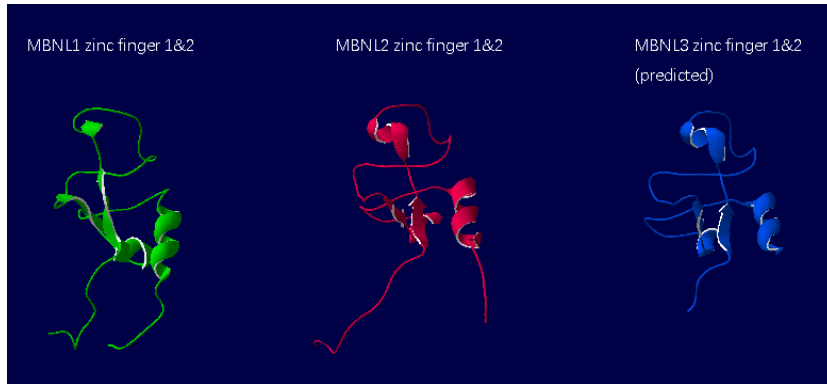
# Predicted MBNL2 zinc fingers 3 and 4



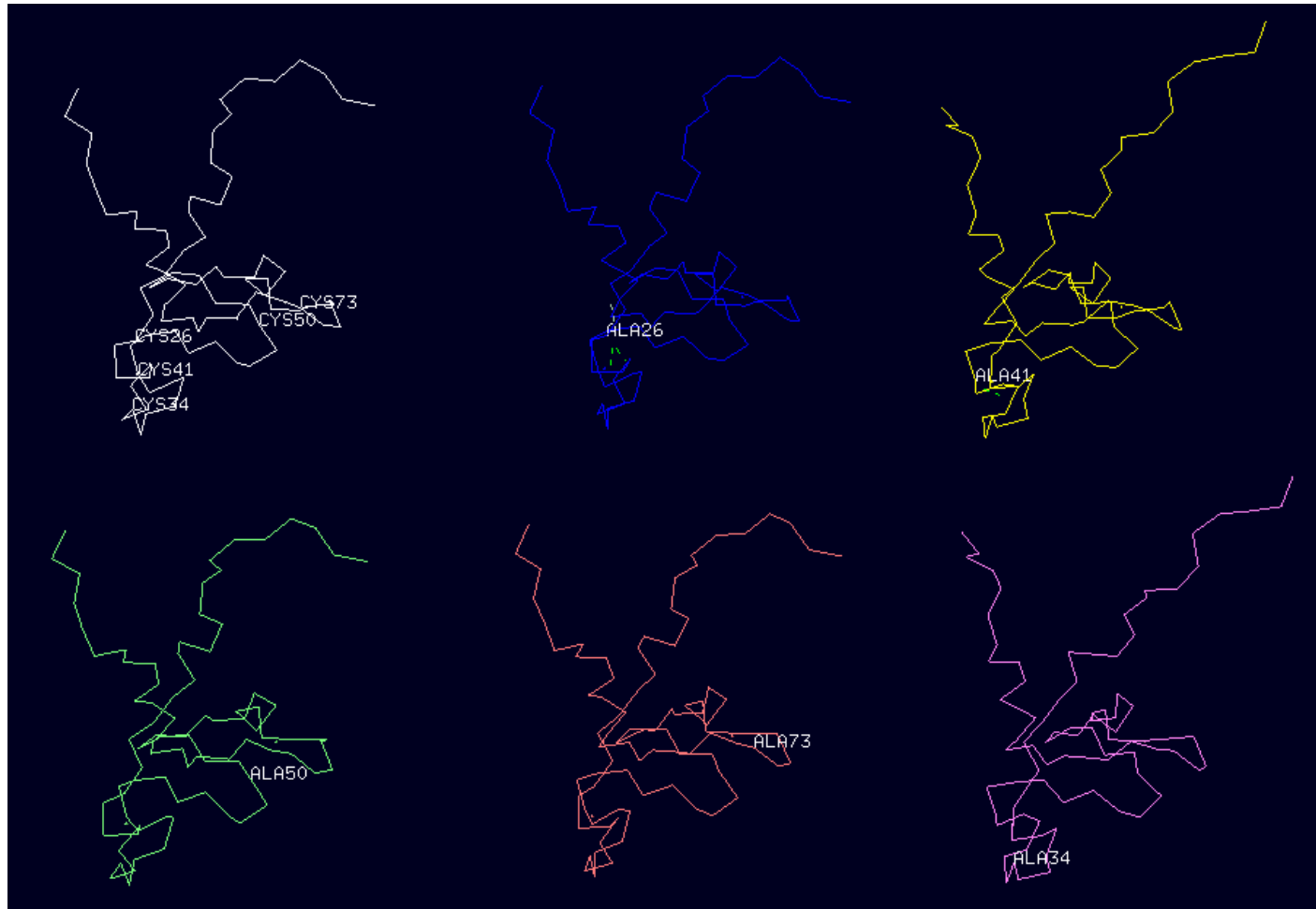
# Human MBNL1, MBNL2 and MBNL3



# Human MBNL1, MBNL2 and MBNL3



# Mutation of MBNL2 tandem zinc finger domain 1&2





# Summary and prospects

- With similar structures and sequences, we can perform genetic animal disease model experiment based on MBNL2.
- We can try to design some mutations of MBNL2 with different efficiency.

# 小组分工

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Thanks

**Happy new year**