犏牛DMTR7基因的生物信息学分析

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研究背景

牦牛是分布在青藏高原及其毗邻高山地区的重要畜种，是青藏高原居民赖以生存的生活资料。

野牦牛

家牦牛

犏牛是牦牛和黄牛杂交产生的后代，其具有很强的杂种优势，但是F1代公犏牛因不能正常产生精子，导致无法将杂种优势固定下来，这成为牦牛杂交改良的一大难题，犏牛雄性不育的分子机理至今仍不明确。

公犏牛

组织形态学观察发现犏牛初级精母细胞很少，无次级精母细胞，附睾内未观测到精子，而黄牛、牦牛睾丸组织中可见各级生精细胞，附睾内可见发育良好的精子，可见F1代犏牛雄性不育主要是由精子发生过程中减数分裂障碍所引起的。

雄性哺乳动物XY染色体的联会配对依赖于一种特殊的染色体结构XY body而完成。XY body在雄性生殖细胞减数分裂过程中发挥着重要作用。DMRT7蛋白选择性的集中于粗线期精母细胞XY body上。

DMRT7基因缺陷小鼠生精细胞缺乏，生精过程被阻滞在减数分裂粗线期，表现出性染色体联会调控缺陷,小鼠表现出雄性不育，雌性正常，说明DMRT7基因在减数分裂粗线期和双线期之间雄性特有的性染色体联会过程中发挥重要作用。

F1代犏牛雄性生殖细胞减数分裂停滞在粗线期阶段，表现出的染色体联会障碍表型与DMRT7基因缺陷小鼠表型非常相似。

假设：

1、犏牛DMRT7蛋白质中是否存在影响其功能的变异位点。

2、犏牛DMRT7基因表达量的变化是否影响其功能。

密码子偏好性

#Codon	AA	Fraction	Frequency	Number
GCA	A	0.121	10.782	4
GCC	A	0.364	32.345	12
GCG	A	0.030	2.695	1
GCT	A	0.485	43.127	16
TGC	C	0.615	21.563	8
TGT	C	0.385	13.477	5
GAC	D	0.800	10.782	4
GAT	D	0.200	2.695	1
GAA	E	0.429	16.173	6
GAG	E	0.571	21.563	8
TTC	F	0.500	5.391	2
TTT	F	0.500	5.391	2
GGA	G	0.375	32.345	12
GGC	G	0.281	24.259	9
GGG	G	0.250	21.563	8

牦牛

#Codon	AA	Fraction	Frequency	Number
GCA	A	0.121	10.782	4
GCC	A	0.364	32.345	12
GCG	A	0.030	2.695	1
GCT	A	0.485	43.127	16
TGC	C	0.615	21.563	8
TGT	C	0.385	13.477	5
GAC	D	0.800	10.782	4
GAT	D	0.200	2.695	1
GAA	E	0.429	16.173	6
GAG	E	0.571	21.563	8
TTC	F	0.500	5.391	2
TTT	F	0.500	5.391	2
GGA	G	0.375	32.345	12
GGC	G	0.281	24.259	9
GGG	G	0.250	21.563	8

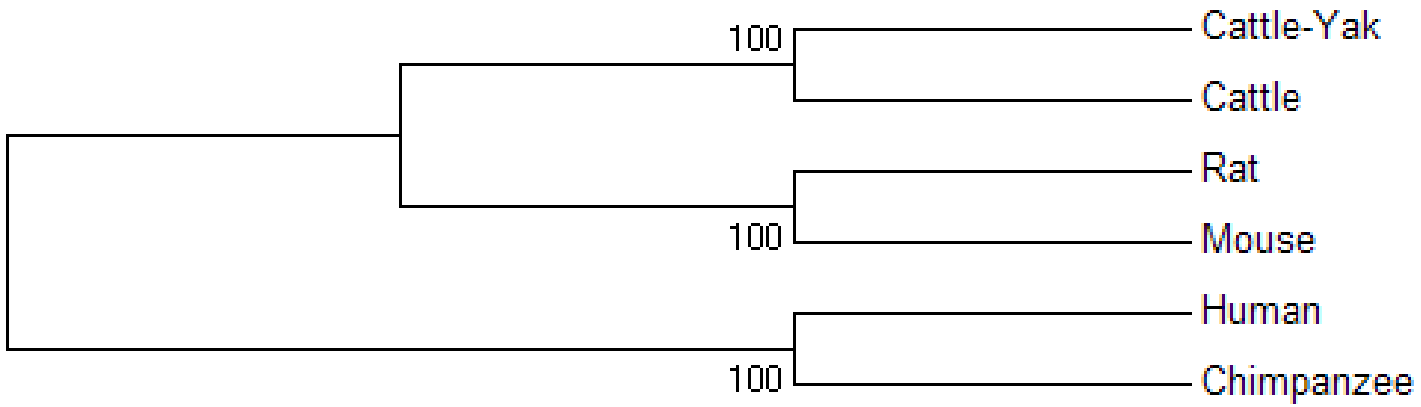
犏牛

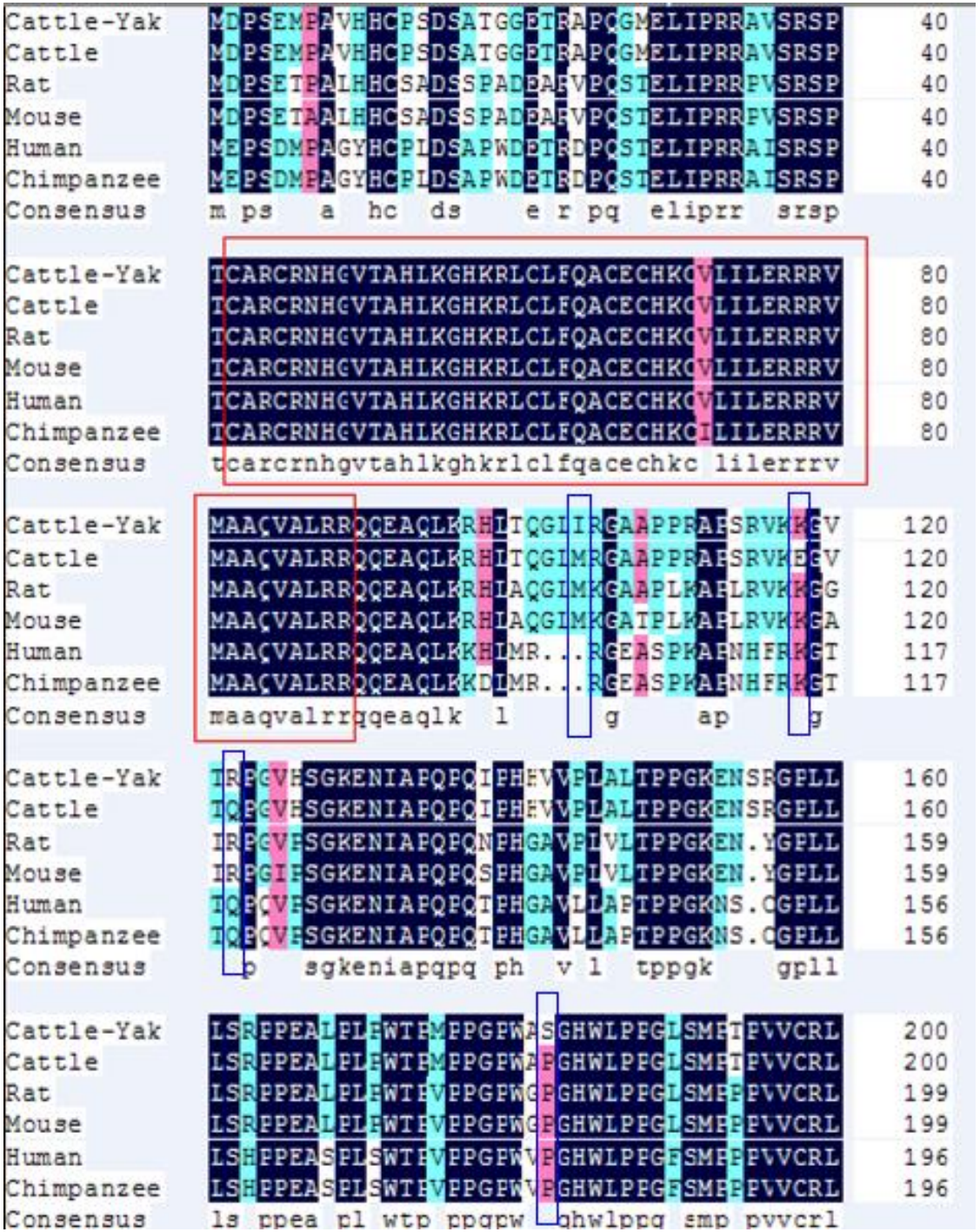
用GENSCAN将犏牛DMRT7 mRNA序列翻译成氨基酸序列

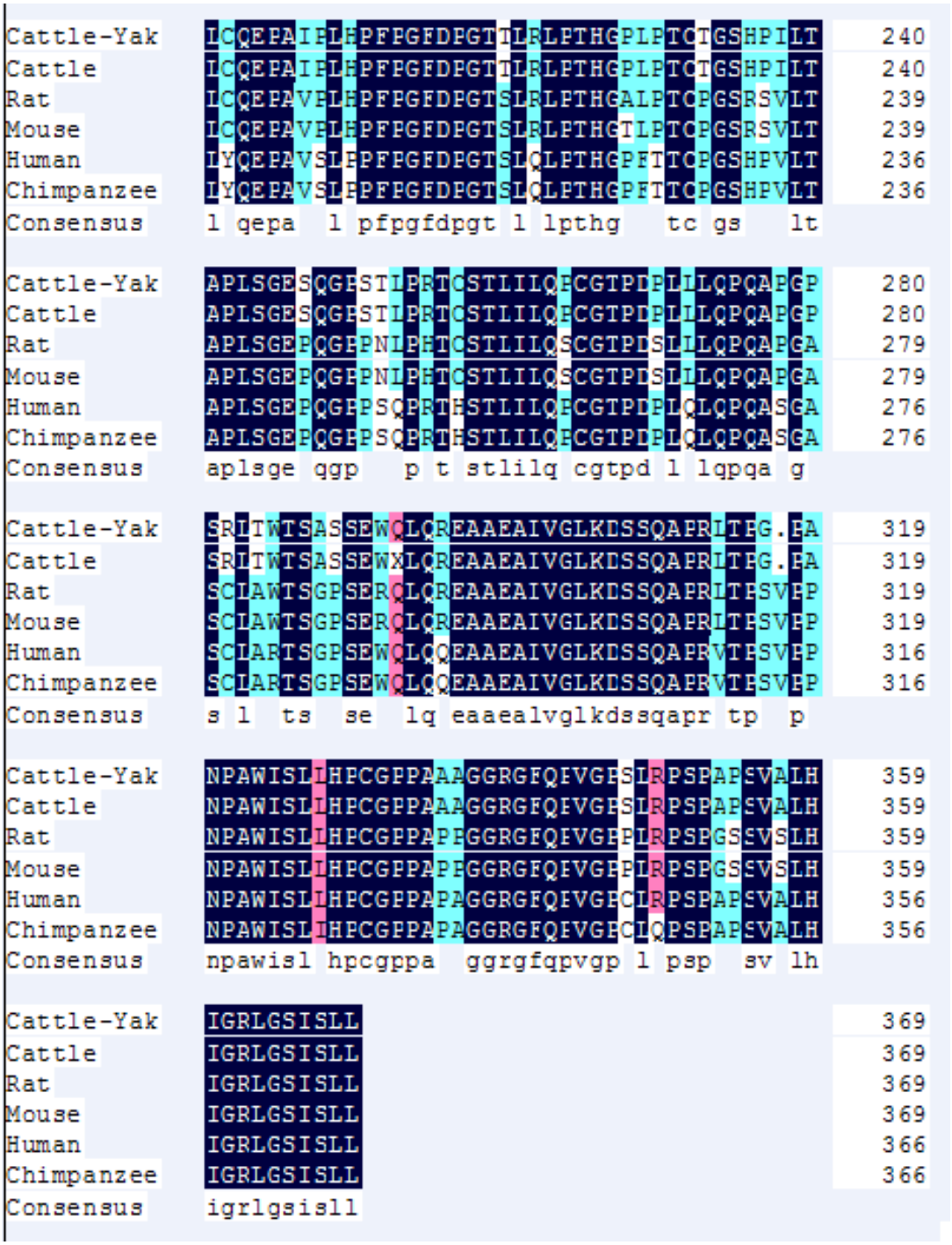
>DMRT7 Cattle-yak 370_aa
MDPSEMPAVHHCPSDSATGGETRAPQGMELIPRRAVS
RSPTCARCRNHGVTAHLKGHKRLCLFQACECHKCVLIL
ERRRVMAAQVALRRQQEAQLKRHLTQGLIRGAAPPRAP
SRVKKGVTRPGVHSGKENIAPQPQIPHHVVPLALTPPGK
ENSRGPLLLSRPPEALPLPWTPMPPGPWASGHWLPPG
LSMPTPVVCRLLCQEPAIPLHPFPGFDPGTTLRLPTHGP
LPTCTGSHPILTAPLSGESQGPSTLPRTCSTLILQPCGTP
DPLLLQPQAPGPSRLTWTSASSEWQLQREAAEALVGLK
DSSQAPRLTPGPANPAWISLLHPCGPPAAAGGRGFQPV
GPSLRPSPAPSVALHI GRLGSISLLS

同源性比较

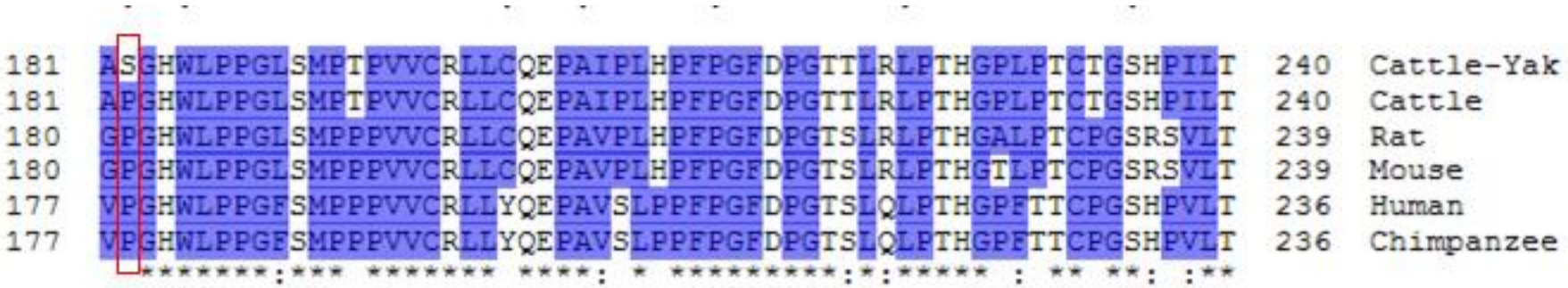
犏牛DMRT7蛋白与黄牛、大鼠、小鼠、人和猩猩DMRT7蛋白的同源性分别为：99%、84% 、83%、80%和79%。




Cattle-Yak
Cattle
Rat
Mouse
Human
Chimpanzee
Consensus

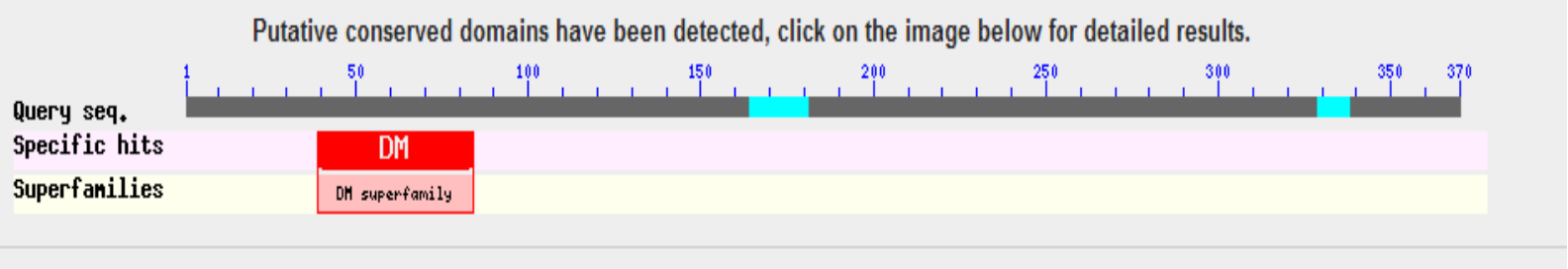

Cattle-Yak LCQEPAIPLHPFPGFDPGTTLRLPTHGPLPTCTGSHPILT 240
Cattle LCQEPAIPLHPFPGFDPGTTLRLPTHGPLPTCTGSHPILT 240
Rat LCQEPAVPLHPFPGFDPGTSLRLPTHGALPTCPGSRSVLT 239
Mouse LCQEPAVPLHPFPGFDPGTSLRLPTHGTLPTCPGSRSVLT 239
Human LYQEPAVSLPPFPGFDPGTSLQLPTHGPFTTCPGSHPVLT 236
Chimpanzee LYQEPAVSLPPFPGFDPGTSLQLPTHGPFTTCPGSHPVLT 236
Consensus l qepa l pfpgfdpgt l lpthg tc gs lt
Cattle-Yak APLSGESQGPSTLPRTCSTLILQPCGTPDPLLLQPQAPGP 280
Cattle APLSGESQGPSTLPRTCSTLILQPCGTPDPLLLQPQAPGP 280
Rat APLSGEPQGPPNLPHTCSTLILQSCGTPDSLLLQPQAPGA 279
Mouse APLSGEPQGPPNLPHTCSTLILQSCGTPDSLLLQPQAPGA 279
Human APLSGEPQGPSQPRTHSTLILQPCGTPDPLQLQPQASGA 276
Chimpanzee APLSGEPQGPSQPRTHSTLILQPCGTPDPLQLQPQASGA 276
Consensus aplsge qgp p t stlilq cgtpd l lqpqa g
Cattle-Yak SRLTWTSASSEWQLQREAAEAIVGLKDSSQAPRLTPG.PA 319
Cattle SRLTWTSASSEWXLQREAAEAIVGLKDSSQAPRLTPG.PA 319
Rat SCLAWTSGPSERQLQREAAEAIVGLKDSSQAPRLTPSVPP 319
Mouse SCLAWTSGPSERQLQREAAEAIVGLKDSSQAPRLTPSVPP 319
Human SCLARTSGPSEWQLQQEAAEAIVGLKDSSQAPRVTPSVPP 316
Chimpanzee SCLARTSGPSEWQLQQEAAEAIVGLKDSSQAPRVTPSVPP 316
Consensus s l ts se lq eaaealvglkdssqapr tp p
Cattle-Yak NPAWISLLHPCGPPAAAGGRGFQFVGPSLRPSPAPSVALH 359
Cattle NPAWISLLHPCGPPAAAGGRGFQFVGPSLRPSPAPSVALH 359
Rat NPAWISLLHPCGPPAPPGGRGFQFVGPPLRPSPGSSVSLH 359
Mouse NPAWISLLHPCGPPAPPGGRGFQFVGPPLRPSPGSSVSLH 359
Human NPAWISLLHPCGPPAPAGGRGFQFVGPCLRPSPAPSVALH 356
Chimpanzee NPAWISLIHPCGPPAPAGGRGFQFVGPCLQPSPAPSVALH 356
Consensus npawisl hpcgppa ggrgfqpvgp l psp sv lh
Cattle-Yak IGRLGSISLL 369
Cattle IGRLGSISLL 369
Rat IGRLGSISLL 369
Mouse IGRLGSISLL 369
Human IGRLGSISLL 366
Chimpanzee IGRLGSISLL 366
Consensus igrlgsisll

疏水性分析


181 ASGHWLPPGLSMPTPVVCRLLCQEPAIPLHPFPGFDPGTTLRLPTHGPLPTCTGSHPILT 240 Cattle-Yak
181 APGHWLPPGLSMPTPVVCRLLCQEPAIPLHPFPGFDPGTTLRLPTHGPLPTCTGSHPILT 240 Cattle
180 GPGHWLPPGLSMPPPVVCRLLCQEPAVPLHPFPGFDPGTSLRLPTHGALPTCPGSRSVLT 239 Rat
180 GPGHWLPPGLSMPPPVVCRLLCQEPAVPLHPFPGFDPGTSLRLPTHGTLPTCPGSRSVLT 239 Mouse
177 VPGHWLPPGFSMPPPVVCRLLYQEPAVSLPPFPGFDPGTSLQLPTHGPFTTCPGSHPVLT 236 Human
177 VPGHWLPPGFSMPPPVVCRLLYQEPAVSLPPFPGFDPGTSLQLPTHGPFTTCPGSHPVLT 236 Chimpanzee

结构域预测

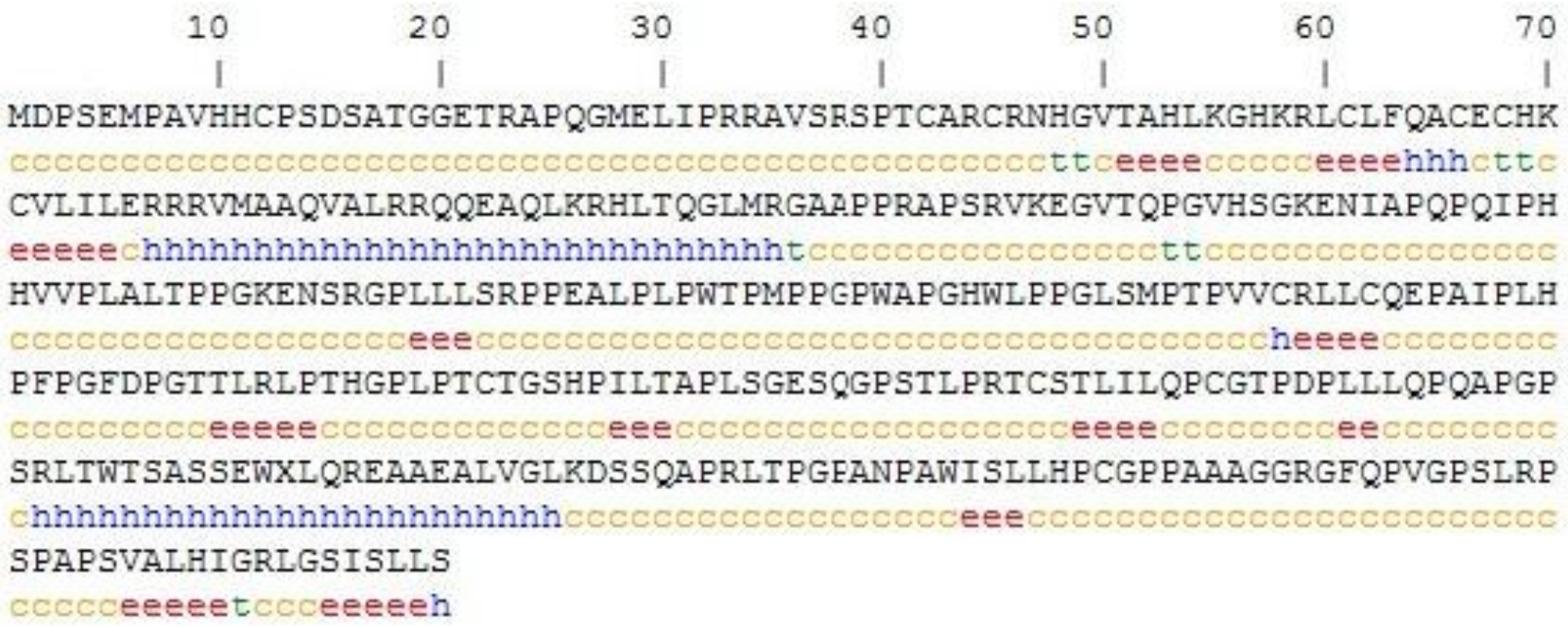
犏牛DMRT7蛋白含有DM超家族的结构域。



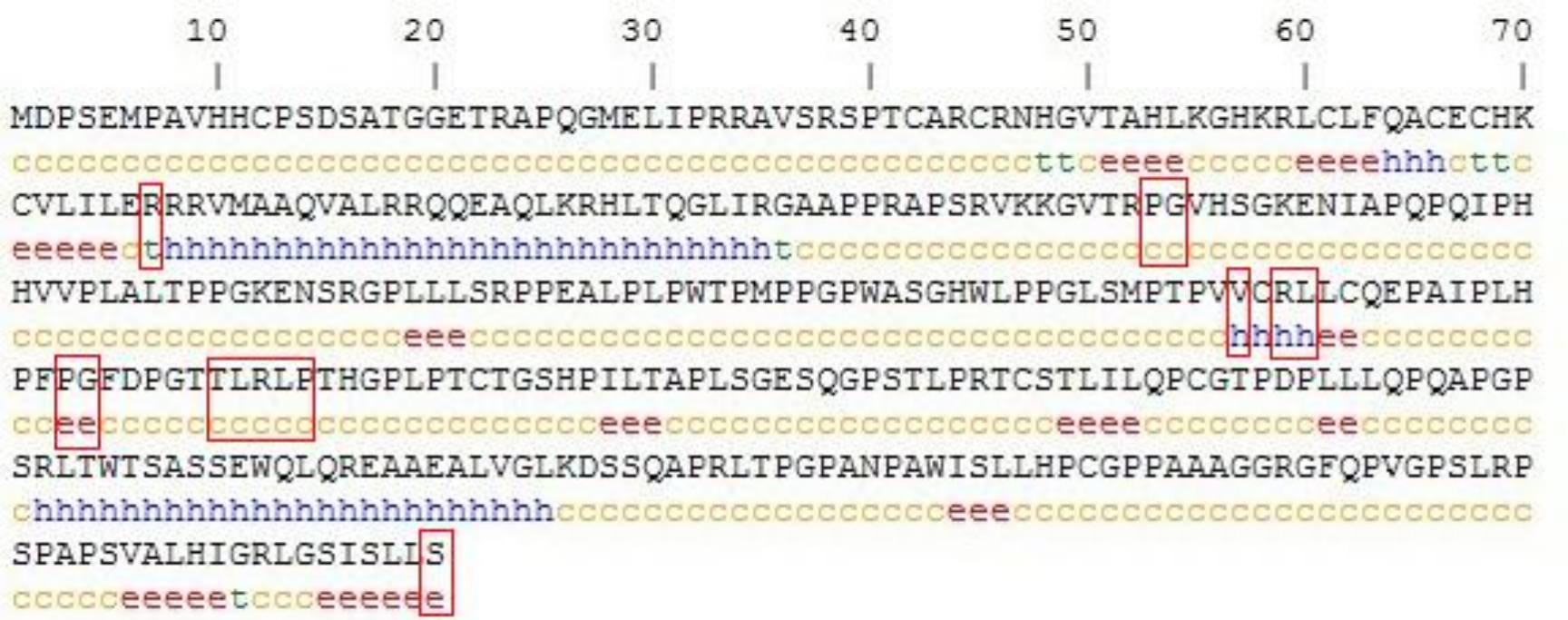
亚细胞定位

Subcellular location	Cytoplasmic	nuclear	Cytoskeletal	Plasma membrane	Mitochondrial
Cattle-Yak	45.3%	39.1%	8.7%	4.3%	4.3%
Cattle	34.8%	47.8%	8.7%	4.3%	4.3%

二级结构预测



黄牛DMRT7蛋白的二级结构

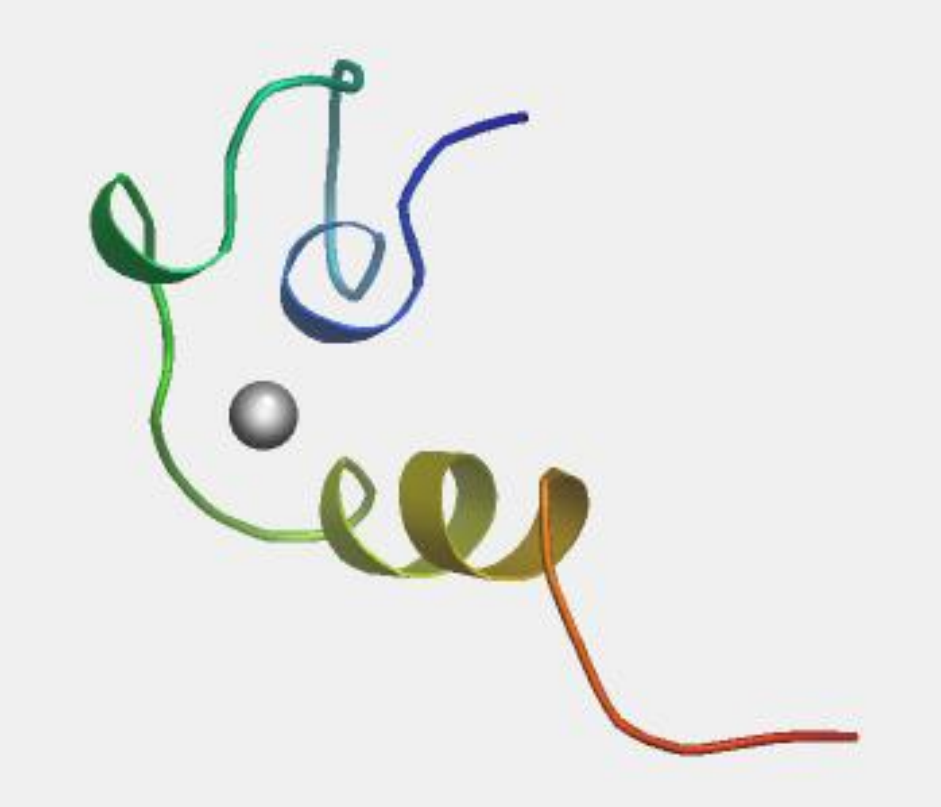


蝙牛DMRT7蛋白的二级结构

h:Alpha helix t: Beta turn

e: Extended strand c:Random coil

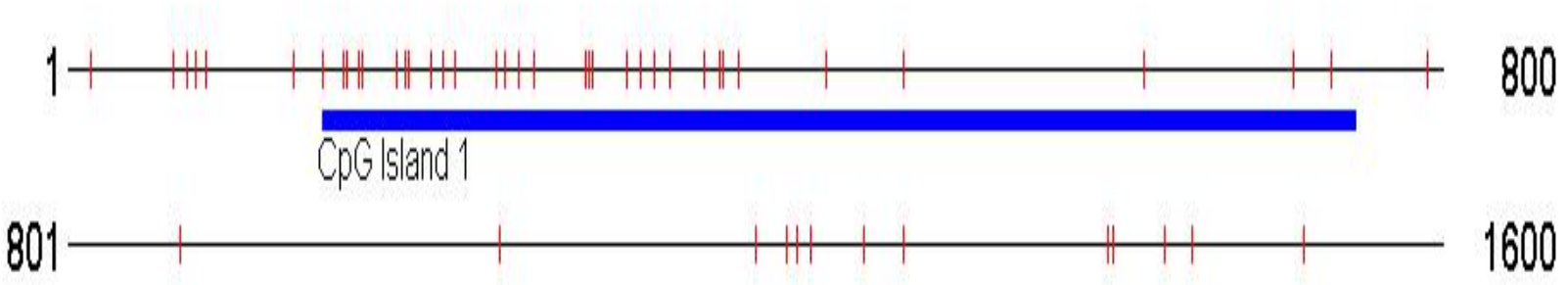
三级结构预测



犏牛DMRT7蛋白质中变异位点可能会对其功能产生影响，后续将克隆牦牛DMTR7基因的mRNA序列，通过比对进行验证。

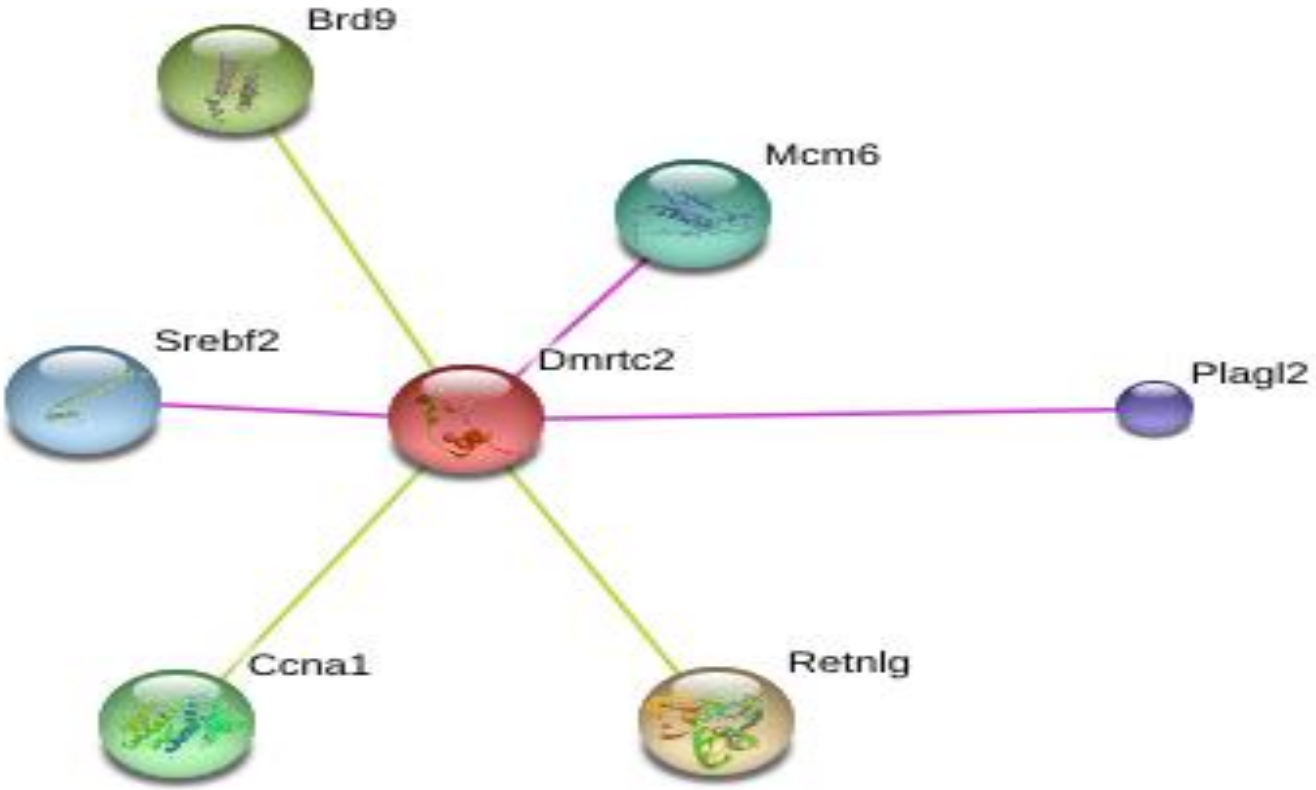
CpG岛预测

用CpG Island Searcher软件分析黄牛DMRT7基因启动子区的CpG岛，发现CpG岛在148-750bp间，GC=55%。



用Proscan软件分析DMRT7基因启动子区的转录因子结合位点，发现DMRT7基因启动子区存在SP１、ＳＰ２、AP-2、CREB、STAT5等多个转录因子结合位点。其中SP1、CREB等位点为甲基化敏感位点，CREB为生殖和胚胎发育相关的转录因子结合位点。

蛋白互作分析



下一步将分析犏牛、牦牛和黄牛DMRT7基因表达量的差异和该基因启动子区的甲基化程度。

同时也将分析DMRT7互作基因的特征及表达量。

参考文献

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Thank you