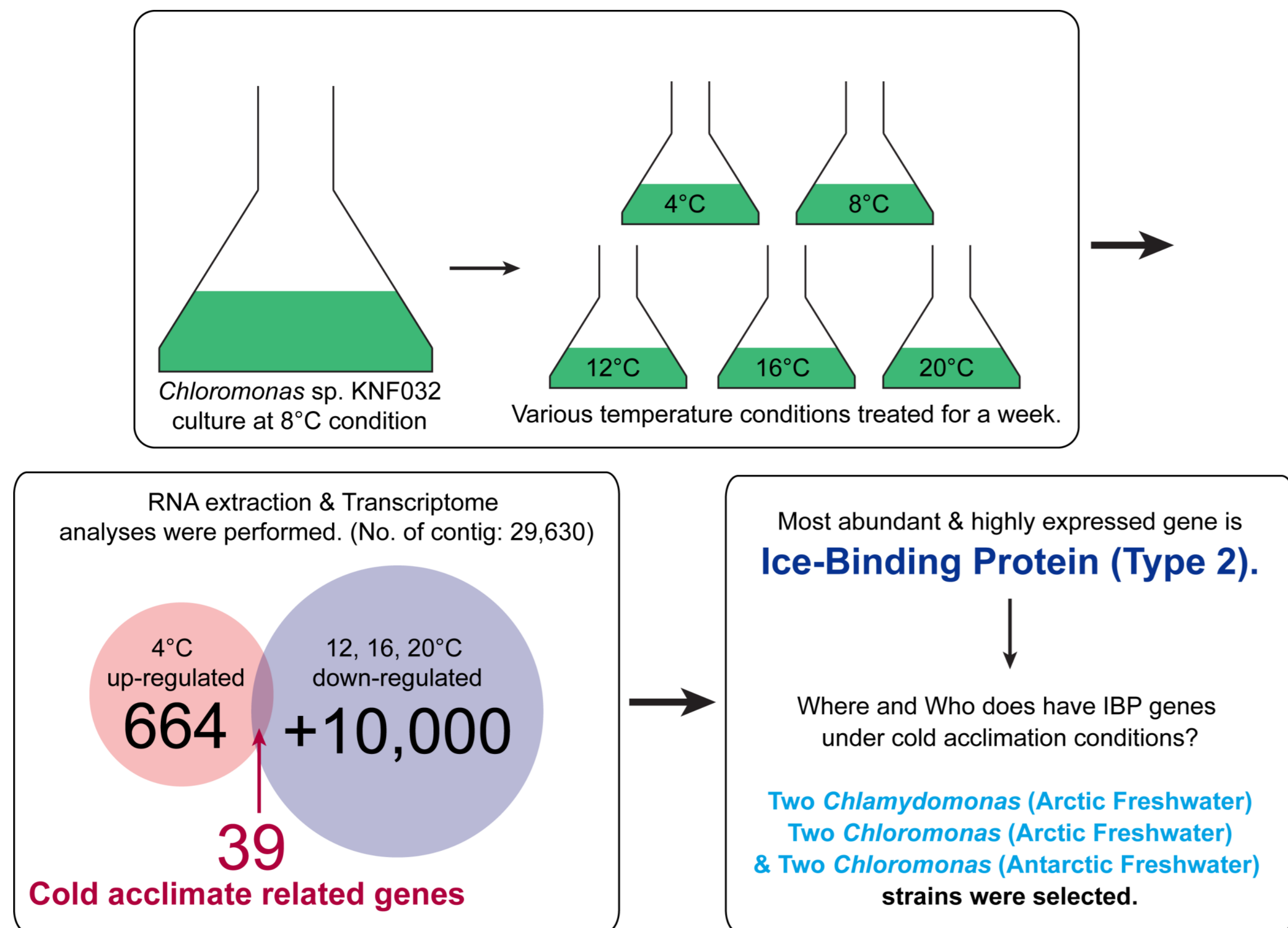


Identification of Ice-Binding Proteins of Arctic/Antarctic Chlorophyceae using Transcriptome Analyses

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Workflow



Take home messages,

- Three Arctic *Chloromonas* sp. (KNF012, KNF030 and KNF032) and two Antarctic *Chloromonas* sp. (KSF057 and KSF063) are closely related to *Chloromonas* sp. CCCryo273-66 strain isolated from Antarctic region.
- Temperature dependent transcriptome analysis of KNF032 results in 39 cold acclimate-related genes. Five among twelve annotated transcripts are Ice-Binding Protein (IBP). All of 17 IBP transcripts are denoted as type 2 IBPs which has not a DUF3494 domain.
- Type 2 IBP genes are found in Arctic/Antarctic freshwater *Chloromonas* strains. It is remarkable that type 2 IBP has not shared any domains and genetic structures of type 1 IBPs.
- Most of motifs are highly conserved as TFT. First Thr(T) of some motifs is often substituted to Lys(K), Asp(D) and Ser(S) less than second Phe(F) and third Thr(T).
- Predicted TF binding sites give a hint that these ibp genes are able to function as cold acclimation response like in plants.

PART II. Transcriptome analyses

Table 1. Description of 12 annotated transcripts among 39 cold acclimate-related genes. Five ice-binding proteins were remarkably expressed at low temperature condition.

Contig ID	RPKM score					Description	E-Value
	4°C	8°C	12°C	16°C	20°C		
ArF32Contig690	358.41	136.89	54.62	17.87	15.03	ice-binding protein-4	0
ArF32Contig2971	269.44	88.97	45.55	13.52	4.57	ice-binding protein-3	0
ArF32Contig24913	147.25	41.66	24.42	32.45	31.50	predicted protein	1.08E-10
ArF32Contig6739	138.63	50.19	40.04	23.75	24.78	ice-binding protein-3	2.04E-158
ArF32Contig14333	129.43	59.49	52.95	3.56	0.43	hypothetical protein VOLCADRAFT_106879	1.45E-09
ArF32Contig6901	89.86	38.28	18.18	9.38	8.99	ice-binding protein-4	1.42E-81
ArF32Contig11683	40.66	19.42	13.21	10.22	12.81	acetyl-coa synthetase	0
ArF32Contig28905	30.54	14.58	2.52	1.19	2.48	hypothetical protein VOLCADRAFT_121013	5.12E-48
ArF32Contig21934	27.16	10.36	6.81	6.68	5.11	flagellar associated protein	1.02E-21
ArF32Contig26817	20.21	6.54	1.82	0.64	0.38	polysaccharide deacetylase family protein	1.86E-18
ArF32Contig26619	15.41	2.44	0.94	0.99	1.58	ice-binding protein-4	6.89E-33
ArF32Contig28814	6.35	2.83	1.45	2.55	1.22	hypothetical protein VOLCADRAFT_92225	8.75E-23

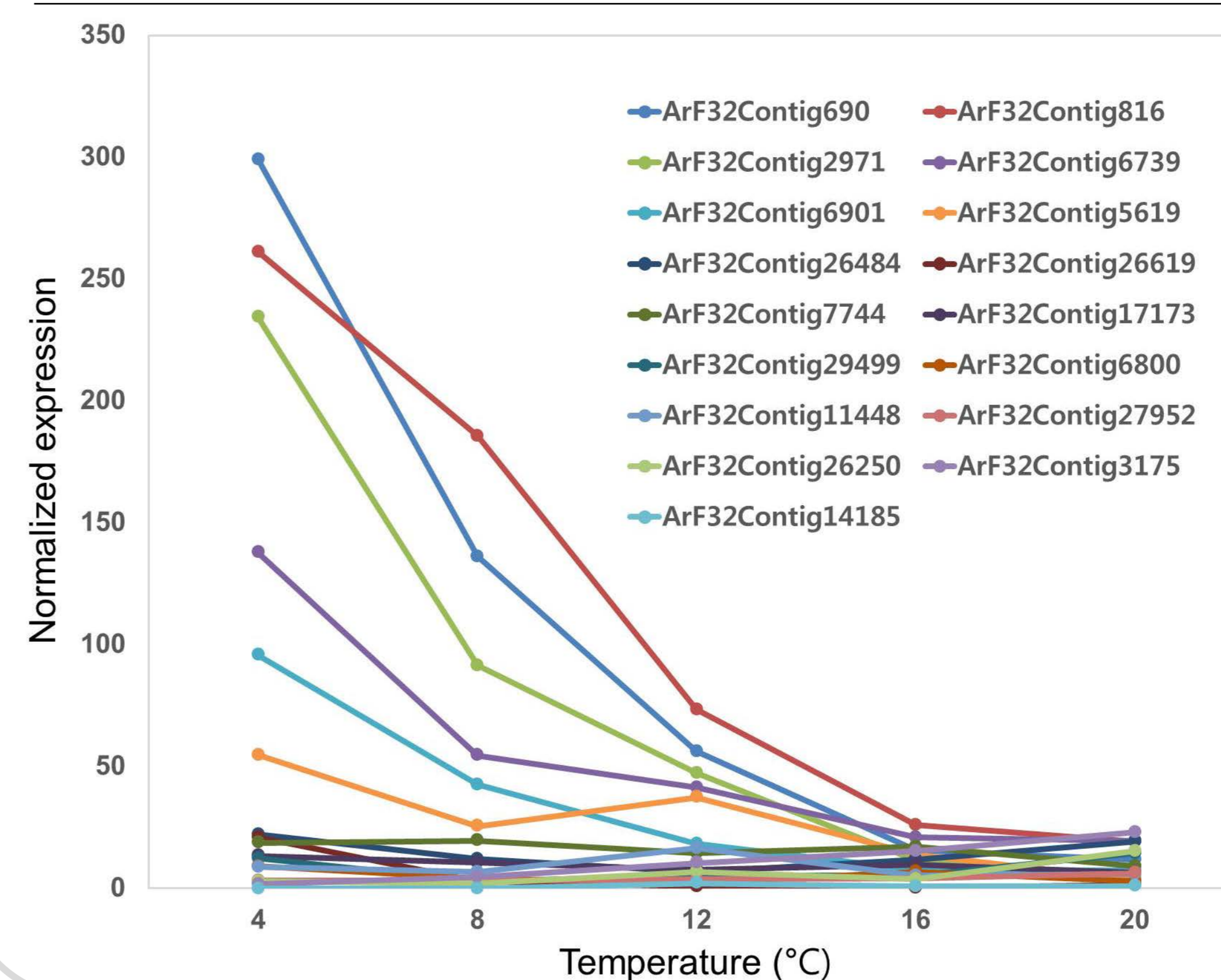


Figure 2. Normalized expression level of all 17 ice-binding protein (IBP) transcripts according to temperature changes. All transcripts was highly expressed at 4°C condition and then decreased according to temperature increasing. Most strongly expressed contig690, 816 and 2971 were selected to further study.

PART I. Phylogeny of KOPRI strains

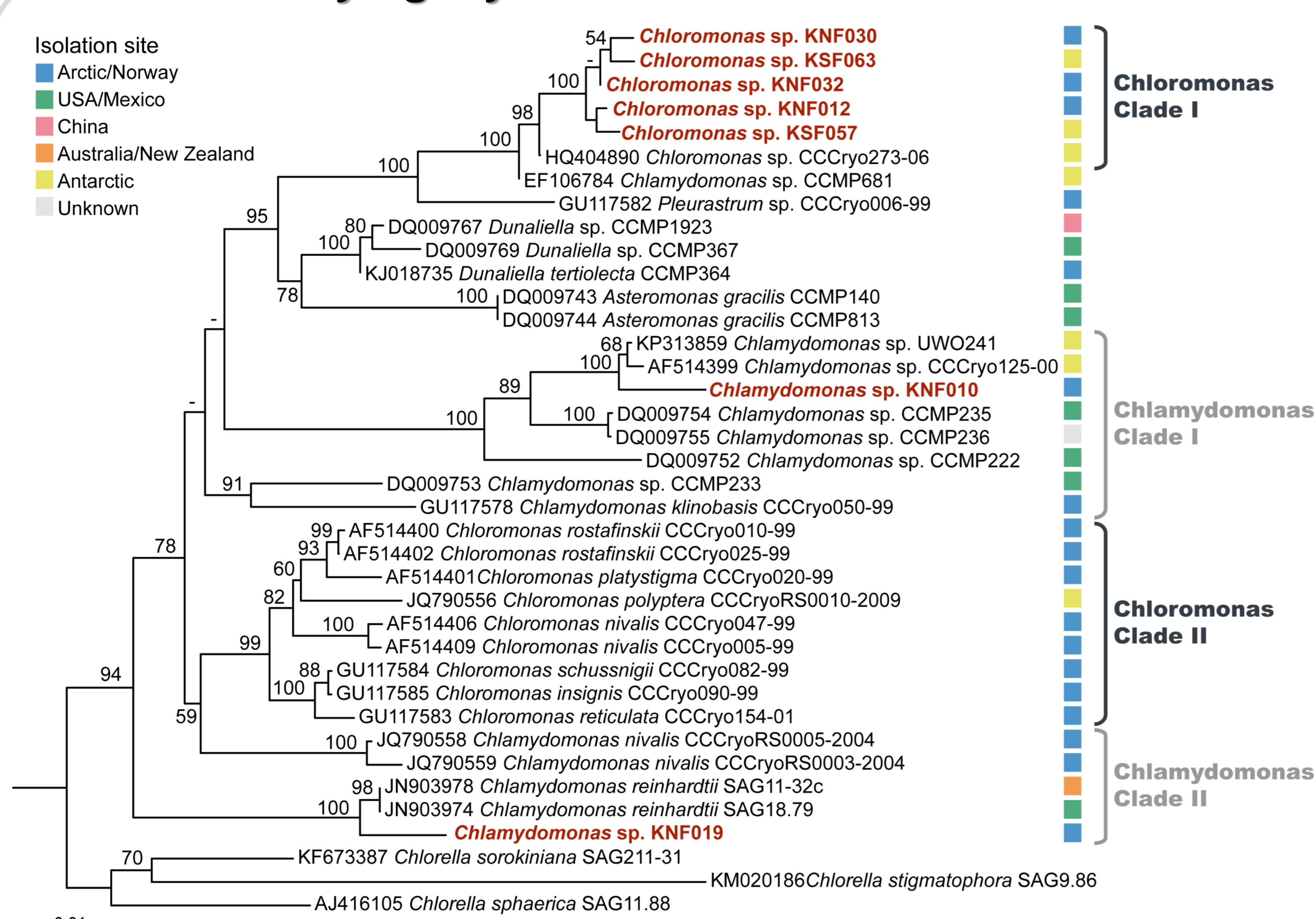


Figure 1. Neighbor-Joining tree of six KOPRI culture collection strains (two *Chlamydomonas* sp. and four *Chloromonas* sp.) inferred from 18S rRNA sequences (1190 nt, 38 taxa). Numbers above branches indicated NJ bootstrap value (>50%). Genus *Chlororella* (Trebouxiophyceae) served as outgroup taxa. Color boxes indicates isolation site of each strain.

PART III. IBPs of Arctic/Antarctic Chlorophyceae

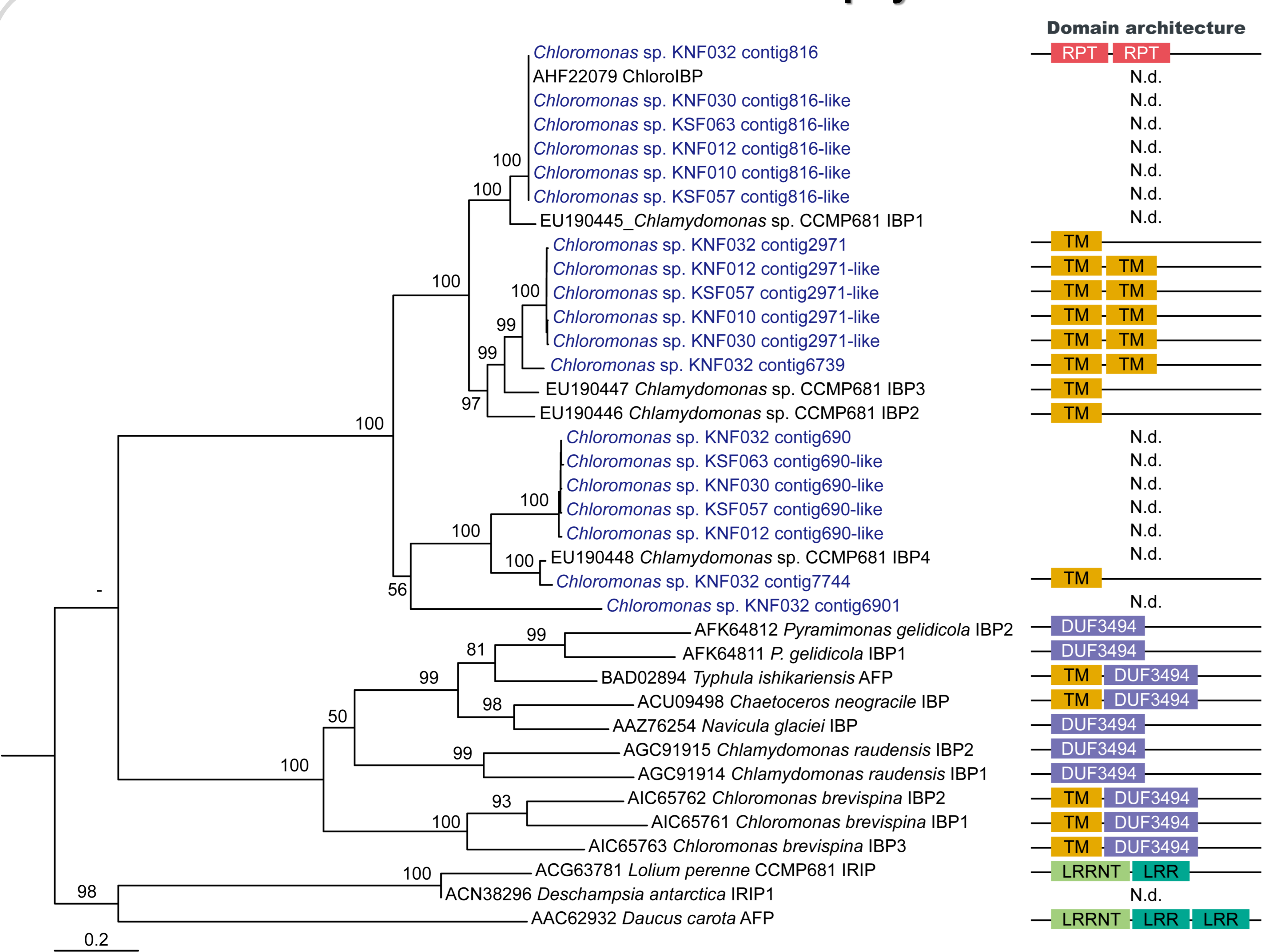


Figure 3. Neighbor-Joining tree of IBP amino acid sequences including 13 newly analyzed IBPs from six KOPRI strains. Numbers above branches indicated NJ bootstrap value (>50%). Anti-freezing protein (AFP) and ice-recrystallization inhibiting protein (IRIP) from Planta served as outgroup. Domain architecture was predicted using SMART homepage with PFAM database. Type 2 IBP was clearly distinguished from type 1 IBPs containing DUF3494 domain. Some proteins have not been detected any domains. Domain name was abbreviated as follow: RPT, repeated domain in UCH-proteine; TM, transmembrane; DUF3494, unknown domain; LRRNT, leucine rich repeat N-terminal domain; LRR, leucine rich repeat.

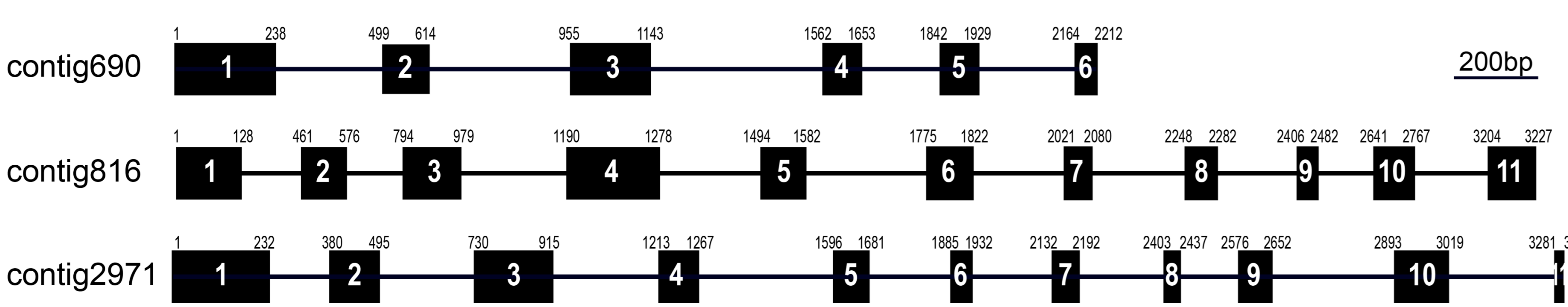


Figure 5. Gene structures of contig690, 816 and 2971 CDS region. A total of 11 exons were found in contig816 and 2971.

	I	II	III	IV	V	VI	VII
KNF032 contig6739	TFT	TFT	TFT	TFT	TFT	TFT	TWT
CCMP681 IBP3	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KNF032 contig2971	TLT	TFT	TFT	TFT	TFT	TFT	TWT
KNF010 contig2971-like	TLT	TFT	TFT	TFT	TFT	TFT	TWT
KNF012 contig2971-like	TLT	TFT	TFT	TFT	TFT	TFT	TWT
KNF030 contig2971-like	TLT	TFT	TFT	TFT	TFT	TFT	TWT
KSF057 contig2971-like	TLT	TFT	TFT	TFT	TFT	TFT	TWT
KNF032 contig6901	TWT	TFT	TFT	TFT	TFT	TFT	TFT
CCMP681 IBP2	TFT	TFT	TFT	VFT	TFT	TFT	TWT
KNF032 contig816	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KNF010 contig816-like	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KNF012 contig816-like	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KNF030 contig816-like	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KSF057 contig816-like	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KSF063 contig816-like	TFT	TFT	TFT	TFT	TFT	TFT	TWT
CCMP681 IBP1	TFT	TFT	TFT	TFT	TFT	TFT	TWT
ChlorolBP	TFT	TFT	TFT	TFT	TFT	TFT	TWT
KNF032 contig690	TFT	TFT	SFT	TFT	TFT	DFT	KWT
KNF012 contig690-like	TFT	TFT	SFT	TFT	TFT	DFT	KWT
KNF030 contig690-like	TFT	TFT	SFT	TFT	TFT	DFT	KWT
KSF057 contig690-like	TFT	TFT	SFT	TFT	TFT	DFT	KWT
KSF063 contig690-like	TFT	TFT	SFT	TFT	TFT	DFT	KWT
KNF032 contig7744	KFT	TFT	SFT	TFT	TFT	DFT	KWT
CCMP681 IBP4	KFT	TFT	SFT	TFT	TFT	DFT	KWT

Figure 4. Highly conserved TXT motifs discovered in all of type 2 IBP proteins. Multiple alignments were performed by ClustalW program. According to previously studied IBP proteins of *Chlamydomonas* sp. CCMP681 was used to select TXT motifs. Most motifs are expressed as TFT but some motifs at I, III, VI and VII positions were changeable. First Thr(T) is often substituted to Lys(K), Asp(D) and Ser(S) than second Phe(F) and third Thr(T).

Table 2. Predicted transcription factor binding at N-terminus of IBP transcript. Number of transcription factor binding site was reported in parenthesis

Contig	B3	bZIP	bHLH	ERF	MYB	Trihelix	WRKY
690	ABI3(1), VP1(1)	DPBF(2), OBF4(1), RSG(1)	N.d	CBF(2)	GAMYB(1)	N.d	WRKY18(1)
816	ABI3(1), VP1(2)	OBF4(4)	N.d	CBF(2)	N.d	GT-1(1)	WRKY1,2,3(3)
2971	ABI3(1)	N.D	PIF3(3)	CBF(1)	N.d	GT-2(2)	WRKY1,2,3(2), WRKY18(1)