Members of the α-tubulin gene family in wheat (*Triticum aestivum L.)* have differential expression during cold acclimation

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ABSTRACT

Members of the α-tubulin gene family in wheat (*Triticum aestivum L.)* have differential expression during cold acclimation

Mohammed Ridha

The α-tubulins and β-tubulins are the major constituents of microtubules which have long been recognized as important structural elements in cell growth and morphogenesis and more recently have been recognized for their role in regulation and signal transduction. They are known to serve as a template for protein-protein interactions and to facilitate or retard the movement of signaling proteins within the cell. Small gene families encode tubulins in plants and animals and the composition of microtubules with respect to the relative abundance of tubulins encoded by different gene family members is thought to change in response to various stimuli including environmental stress. We have identified 15 full-length cDNAs for the members of the α-tubulin gene family in hexaploid bread wheat (*Triticum aestivum L.*). The genes can be clustered into five homeologous groups of three genes each with high similarity to one the five members of the gene family in barley (*Hordium vulgare L.*) a closely related diploid species. Five members of the gene family, representing each of the five homeologous groups were found to be regulated at the level of mRNA during cold acclimation, each with a different pattern of expression. Representatives of five homeologous groups were mapped to chromosome arms using wheat chromosome deletion stocks.

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LIST OF ABBREVIATIONS

AD:	activation domain
BD:	DNA-binding domain
BLAST:	Basic Local Alignment Search Tool
bp:	base pair
CA:	Cold Acclimated
cDNA:	Complementary deoxyribonucleic acid.
DNA :	Deoxyribonucleic acid.
FGAS:	Functional Genomics of Abiotic Stress
GAL4:	transcriptional activator
LB:	Luria-Bertani
LEA:	Late Embryogenesis Abundant
LT:	Low Temperature
LUC:	Luciferase
mRNA:	Messenger ribonucleic acid.
NA:	non-cold acclimated
NPM:	nucleotide polymorphism
PAPS:	3'-phosphoadenosine-5'-phosphosulfate
PCR:	Polymerase Chain Reaction.
PK:	Protein kinase.
QTL:	quantitative trait loci

RLKs: Receptor-like protein kinases

RNA: Ribonucleic acid.

TA: transcriptional activator

TIGR: The Institution of Genomic Research

UTR: Untranslated region

UV light: Ultraviolet light.

PART I. INTRODUCTION

Plants are important for their role as primary producers and their essential role in human life. They have been studied extensively from different aspects. One of these aspects is the effect of abiotic stresses on the growth and production of plants. Such stresses include low temperature, heat, drought, salinity, soil mineral toxicity and soil mineral deficiency. Since plants cannot avoid harsh environmental conditions by changing their location, they have mechanisms evolved to respond to environmental stresses that include genetic, physiological, biochemical, and morphological changes. Exposure to sub-optimal environmental conditions leads to large losses in crop productivity every year. That is why many institutions study these mechanisms to increase the yields of crops.

One of the most important adaptations in plants is the tolerance of low temperature (LT) including freezing and chilling. LT is an important factor that can cause plant injury, limit the geographical distribution of crop cultivation, cause significant losses in plant productivity, and affect the storage ability of fruits. Characterizing the mechanisms, genes, and signaling pathways involved in cold tolerance will help in the effort to improve this trait in cultivated crop species (Graham and Patterson, 1982; Thomashow MF, 2001). There is a wide variation among plants for cold tolerance. Some of the cereals like barley, wheat, oats and rye can survive temperatures in the range of -15 °C to -30 °C if they are fully acclimated while other important crops such as rice, sweet potato, sorghum, maize, tomato and citrus fruits are cold sensitive and can be damaged between 0 °C and 15 °C. (Lyons and Raison, 1970). Insight in to the understanding of abiotic stress resistance and specifically LT tolerance at the level of molecular biology can help in crop improvement by identifying traits that can be selected for in breeding programs or by identifying genes that could increase tolerance through genetic engineering. Improvement in cold tolerance will extend the range of cultivation of crops and increase yields by avoiding the damage of low temperatures.

1. COLD TOLERENCE AND COLD ACCLIMATION AS ADAPTIVE PROSSES

Cold tolerance in crops is a multigenic trait and an important factor for crop cultivation. Winter wheat is one of the most cold-tolerant crop species and is an excellent model to study freezing tolerance (Fowler et al., 1999). High levels of cold tolerance are achieved only after a period of acclimation which requires growth at low, above-freezing temperatures (Thomashow et al., 1999). During cold acclimation the expression levels of many genes are known to change and a number of these have been reported to be differentially regulated in cold tolerant and less tolerant cultivars (Thomashow et al., 1998 , Gulick et al., 2005, Tremblay et al., 2005 and Oono et al., 2006). The study of the regulatory and signaling elements that control the changes of gene expression are essential to the understanding of cold acclimation and the genetic basis of cold tolerance.

1.1. Cold acclimation prevents freeze-thaw damage and cell lyses

The membranes of non-acclimated plants undergo a freeze induced transition from lamella to hexagonal-II phase lipid structure when subjected to temperatures between -4 and -10°C. The hexagonal-II phase lipid structures cause the fusion of cellular membranes. Cold acclimated (CA) plants do not suffer such injury. The CA rye did not show the hexagonal-II structure even when it was frozen to -35 °C (Steponkus and Webb, 1992). The Non-acclimated (NA) winter rye goes through cycles of osmotic contraction and expansion and also freezing-thawing cycles that begin when the temperature reaches -2 °C. The freeze-thaw stress induces the lysis of the cells (Steponkus et al., 1993). Plasma membrane isolated from NA rye leaves (*Secak cereal* L. cv Puma) undergo endocytosis, with a large surface reduction of the plasma membrane because of the expansion-induced lysis. In contrast, plasma membrane from CA leaves undergo exocytosis, and the surface area is conserved such that expansion-induced lysis doesn't occur (Dowgert and Steponkus, 1984; Gordon-Kamm and Steponkus, 1984).

1.2. Damage of chilling temperature

Chilling injury is the physiological changes that are induced by exposure to chilling temperatures which is above freezing but below about 15°C. The physiological changes may be considered primary or secondary. The primary injury is the initial rapid response that causes a dysfunction in the plant, but is readily reversible if the temperature is raised to non-chilling conditions. Secondary injuries are dysfunctions that occur as a consequence of the primary injury and that may not be reversible. The characteristic visual symptoms are the consequence of secondary chilling injuries. Most symptoms require time to appear, and we can summarize these symptoms as follow: The loss of chlorophyll, (apparent as leaf yellowing), water-soaked appearance, plasmolysis, failure to maintain cellular compartments and surface lesions (Vezina et al., 1997). More severe chilling stress promotes cellular autolysis and senescence (Saltveit and Morris, 1990). A plant is considered chilling-sensitive if the primary chilling event occurs below a threshold temperature, usually in the range of 10 °C to 4°C, depending on the species. In contrast, a plant is considered chilling resistant if a primary chilling event does not occur at any temperature above 0°C (Hetherington et al., 1989).

2. CHANGES IN GENE EXPRESSION LEVELS THROUGH COLD ACCLIMATION

The expression levels of many genes are known to change during cold acclimation and a number of these genes have been reported to be differentially regulated in cold tolerant and less tolerant cultivars. Many hundreds of genes have been shown by microarray analysis to be cold regulated and strong efforts have been directed to determine the nature of cold-inducible genes and whether they have roles in freezing tolerance (Gulick et al., 2005, Tremblay et al., 2005 and Oono et al., 2006). The functions of many of the proteins encoded by cold induced genes were predicted from DNA sequence comparisons. They include lipid transfer proteins, antifreeze proteins, fatty acid desaturases, molecular chaperones and proteins involved in signal transduction (Thomashow et al., 1999). Proteins like late embryogenesis abundant (LEA) with unknown activities also demonstrate a contribution to freezing tolerance. Western analysis of three different proteins (3-L1, 3-L2, and 3-L3), which represent a new class of proteins in cereals related to group 3 LEA proteins, showed that the accumulation of 3-L2 proteins is correlated with the capacity of different wheat and rye cultivars to develop freezing tolerance (Ndong et al., 2002).

3. PERCEPTION OF LOW TEMPERATURE

Studies with *Synechocystis sp.* suggest that the primary sensor that perceives the cold signal is the plasma membrane. Plasma membrane rigidification might be the event that initiates the downstream signaling cascade (Vigh et al., 1993). Actin filament reorganization is caused by cold perception that opens Ca^{2+} channels in the plasma membrane by loose-tension forces. The physical alteration of the membrane contributes to the influx of Ca^{2+} in to the cytoplasm (Orvar et al., 2000). Secondary sensors of low temperature are proposed to include histidine kinases, phospholipases, receptor-like kinases and calcium sensors, which are all located in the plasma membrane (Sharma at al., 2005).

4. MICROTUBULES AND α-TUBULIN, A COLD-REGULATED GENE IN WHEAT

Microtubules are key elements of the cytoskeleton and have recently come to be appreciated for their role in signalling and regulation. They are critical conduits for cellular trafficking and can serve as a template for the interaction of signalling proteins (Camilleri et al., 2002). Microtubules, which are composed of α-tubulin and β-tubulin, (Figure 1), go through a transient disorganization followed by a major rearrangement in root cortical cells during cold acclimation (Jian et al., 1989). Comparison of three cultivars of winter wheat (*Triticum aestivum*) that have different degrees of freezing tolerance found that a rapid but transient partial disassembly of the microtubules and the formation of cold-stable microtubules occurred in freeze tolerant cultivars but not in sensitive cultivars (Abdrakhamanova et al., 2003). The provocation of transient microtubule disassembly induced by treatment with the herbicide pronamide, could increase freezing tolerance of the treated plants. The appearance of cold-stable microtubules was reported to be accompanied by a reduced abundance of type TUA1/2 α-tubulin isotypes (Abdrakhamanova et al., 2003). The intimate association of the microtubules with the plasma membrane, the major platform of transduction and signal perception, suggests that microtubules are targets of various switches and signals (Gilroy and Trewavas, 2001, Wasteneys and Galway, 2003). The microtubule surface is known to be associated with motor proteins, GDP/GTP binding proteins, structural microtubule-associated proteins, regulatory kinases, and phosphatases (Wasteneys, 2003). The changes in microtubules observed during cold acclimation in wheat and the extensive investigation of microtubules in mammalian systems (Dent et al., 2003, Rodriguez et al., 2003) suggest that microtubules may play an integral role in signalling that enables plants to adapt to environmental changes. The identification of a large number of RNA binding and signalling proteins that were shown to be microtubule binding proteins in Arabidopsis supports this hypothesis (Chuong et al., 2004). Microtubules in plant cells have been reported to associate with the translational elongation factor 1 alpha (Durso and Cyr 1994) and phospholipase-D (Gardiner et al 2001). Resistance to dinitroaniline herbicides is linked to a missense mutation in the coding sequence of α-tubulin of the resistant biotype (Yamamoto et al, 1998; Anthony & Hussey, 1999). The comparative analysis of α-tubulin sequences of the psychrophilic *Chloromonas* and the mesophilic *Chlamydomonas reinhardtii* showed a substitution of Met 268 to Val in the sequence of *Chloromonas* α-tubulin. This change of amino acid could explain the increased cold-resistance of psychrophilic algae, and this is another example of the role of α-tubulin in the cold adaptation (Willem et al., 1999). Microtubules are found to be stable at low temperatures in chilling tolerant species, whereas they are extremely cold sensitive in chilling sensitive species. The critical temperature that can induce microtubule disassembly is closely correlated to the species sensitivity of chilling. (Jian et al. 1989).

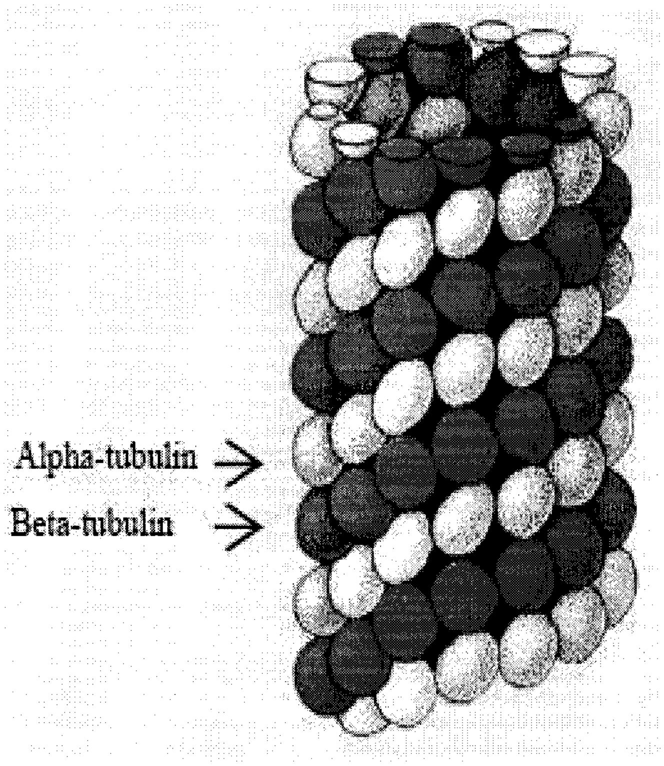


Figure 1. Microtubule. Structure of microtubule composed of α-tubulin and β-tubulin. The picture taken from the internet at [http://www.rpgroup.caltech.edu/courses/aph162/2006/webpages/Projects/Alex-JinHong-Eileen/images/mt.html.jpg]

5. MULTIGENE FAMILIES AND GENE DUPLICATION IN PLANTS

α-tubulins exist as a small gene family in higher plants and animals. Generally they are encoded by multigenic families, there are at least 6 α-tubulin members in Arabidopsis (Schroder et al., 2001), 23 α-tubulin family members in the human genome (Philip et al., 2005), and five to seven α-tubulin genes encoded in the genome of the crab *Gecarcinus lateralis* (Varadaraj et al., 1997).

A gene family is a set of genes that shares the same function, similar nucleotide sequence, similar protein sequence or common domains. Evolution of gene families occurs through a combination of segmental duplication, tandem duplication and whole genome duplication (polyploidy) events and through subsequent losses (Cannon at al, 2004). The most reliable method whereby all members of a gene family can be identified is a complete analysis of a genome sequence (Johnson et al., 2006). Gene duplication is extensively studied in the model organism Arabidopsis, which provides a complete view of chromosomal organization and evolutionary history. Arabidopsis genome analysis revealed 1528 tandem arrays of duplicated genes containing 4140 individual genes. Large segmental duplications were identified either by directly aligning chromosomal sequences or by aligning proteins coding regions and searching for tracts of conserved gene order (The Arabidopsis Genome Initiative, 2000).

According to Wen et al. (2005), members of a gene family must have similarity of amino acid sequences over 40%, and contain all amino acid signature motifs of the corresponding gene family. The size of gene families in Arabidopsis range from that of receptor-like kinases (RLKs) that have more than 600 members to the family of PAPS reductases which consists of 3 members (Gutierrez-Marcos et al., 1996). Phylogenetic techniques can be applied to recognize gene families; these techniques are also important for elucidating orthologous relationship between the members of multigenic families in different species or the paralogous relationship of the gene family members in the same organism (Doyle J., 1994).

Only one full length cDNA sequence for a wheat α-tubulin is currently available in GenBank, though EST sequences in GenBank's EST database, dbEST, indicate the existence of many other gene family members in *Triticum.* We have found the interaction between a specific wheat α-tubulin and a cold regulated receptor kinase (Tardif et al, 2006). In this study we have identified the 15 members of the α-tubulin gene family in hexaploid bread wheat (*Triticum aestivum* L.), determined that these α-tubulin genes fall into 5 paralogous groups, characterized the changes of the mRNA level of expression in response to cold acclimation for 5 genes representing the 5 paralogous groups, and determined the chromosome location for one member of each homeologous group.

Results from this study has been included in a paper accepted with revision (27th of October 2006) for publishing in Genome.

PART II. MATERIALS AND METHODS

The preparation of LB bacterial culture medium and the reagents for agarose gel electrophoresis were based on molecular biology standard techniques (Sambrook et al, 1989). Plasmid purification was done using the QIAGEN Spin Miniprep Kit (Qiagen). RNA samples and PCR products were quantified using UV-visible spectrophotometer (Cary 50Bio, VARIAN). UV images were taken by GENE GENIUS BIO IMAGING SYSTEM (SYNGENE).

1. Plant Material and Growth Conditions

Spring wheat *T. aestivum L.* cv Quantum, seeds were germinated in a 1:1 mixture of vermiculite and soil and grown for seven days in a chamber maintaining a wide spectrum fluorescent light (875 micromoles/m^2/s) with 16 hours of light and 8 hours of darkness. The temperature was maintained at 20 ± 1°C. Seven day old seedlings were cold acclimated by lowering the growth temperature to 4° C and plants were harvested after 1, 3, 6, 14 and 36 days. Control seedlings were grown for one additional day at 20 ± 1°C.

2. RNA Isolation

Pools of approximately 30 seedlings were taken as samples for each cold treatment time point and control. Each sample consisted of the aerial parts including the leaves and meristematic crown. Samples of (4-5 g) were used for the extraction of total

RNA. Tissues were ground in liquid nitrogen and 5 ml of TRIZOL reagent per gram of tissue was used to purify total RNA according to the manufacturers protocol (Invitrogen Life Technologies, Burlington, Ont).

3. DNA Sequencing and analysis

cDNA clones for 15 α-tubulins were identified in the EST database developed in the Genome Canada program, Functional Genomics of Abiotic Stress (FGAS). DNA sequencing was performed at the Montreal Genome Centre, McGill University. The DNA sequences for the *T. aestivum* α-tubulins reported in this article have been deposited in GenBank with consecutive accession numbers DQ435659 through DQ435673. Multiple sequence comparison was done with Clustal W (Thompson et al., 1994) to cluster gene family members in wheat and compare them to α-tubulin sequences in rice, barley and Arabidopsis. Gene specific primers were designed for individual gene family members chosen from polymorphic regions near the end of the coding region and the 3' UTR using the Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www). FGAS (https://bioinfo.uwindsor.ca/cgi-bin/abiotic/assembly.cgi) wheat ESTs databases and The Institute Genomic Research (TIGR, http://tigrblast.tigr.org/tgi/) wheat gene index were used to verify the sequence specificity of the gene specific primers. Basic local alignment search tool (BLAST, Altschul et al., 1990) services provided by the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) were widely used in this study. These services include five programs which can compare the amino acid sequences (BLASTP), nucleotide sequences (BLASTN), a given nucleotide sequence translated in six reading frames of amino acid sequence within protein databases (BLASTX), a given amino acid sequence with the nucleotide sequence in the DNA databases translated in six reading frames of amino acid sequence (TBLASTN), and the comparison of a given nucleotide sequence translated in six reading frames to amino acid with sequence of the whole nucleotide database translated in six reading frame to amino acid sequence (TBLASTX). There are also versions of these programs that can show the percentage of similarity and the identity between two individual sequences, which can be nucleotide or amino acid sequences (bl2seq).

4. RT-PCR measurement of transcript level of selected genes

Total RNA samples were treated with DNaseI (Ambion inc, Austin, Texas). Samples were quantified by absorbance at 260nm. Aliquots of 5 μg of RNA was used for reverse transcription using Invitrogen SuperscriptII and an oligo-dT primer (Invitrogen Canada, Burlington, Ont.) according to the manufacturer's recommendation. A fiftieth volume of each cDNA was used for PCR amplification by Taq DNA Polymerase (MBI Fermentas, Burlington, Ont.) under the following conditions: 95 °C, 2min, followed by 35 cycles at 94 °C, 30s; 58 °C, 30ds; 72 °C, 1min; and followed by 72 °C, 7 min and then held at 4 °C. The ubiquitin cDNA was amplified as an internal control (Yan et al., 2003). To rule out DNA contamination of the RNA samples, control PCR reactions were carried out with RNA samples that had not been used for reverse transcription. The gene specific primers for each gene are listed in Table (1):

Table 1. PCR Primers used in this project

Name	Sequence (5'-3')	Direction
Ta_TUBA-1-3	GCGCCTCTCGGTTGATTAC	Forward
Ta_TUBA-1-3	GGTTTTGATGGTTGCGACT	Reverse
Ta_TUBA-2-3	TCAGGTCATTTCATCACTGACA	Forward
Ta_TUBA-2-3	CACCAGGAGGCAGGCTTA	Reverse
Ta_TUBA-5-3	GCCAGCTCTTCCATCCA	Forward
Ta_TUBA-5-3	AGAGCGCACACTTGATCC	Reverse
Ta_TUBA-4-3	GCCGACAACTGCACTGGA	Forward
Ta_TUBA-4-3	CGTCCTCCTCGCCATCA	Reverse
Ta_TUBA-3-3	GAGGTGAGGACTGGCACCTAT	Forward
Ta_TUBA-3-3	AGGTTGGTCTGGAATTCGTTC	Reverse
Ta_TUBA-3-2	TGAATGTTGATGTGAACGAGTTT	Forward
Ta_TUBA-3-2	TAGACGAAGGGACGCTTGA	Reverse
Ta_TUBA-3-1	CTGGTGCCCTACCCAAGA	Forward
Ta_TUBA-3-1	GGCGGGGGTCACACTTT	Reverse

5. Gene mapping to chromosome arms

Cytogenetic stocks of the hexaploid cultivar Chinese Spring (*T. aestivum*) were used for chromosomal mapping of gene family members. The selected set includes 19 nullisomic-tetrasomic (NT) lines for mapping ESTs to individual chromosomes (Sears, 1954; Sears, 1966; Qi et al., 2004). These lines have one chromosome pair substituted by a homeologous pair of chromosomes. Selected ditelosomic (DT) lines, which are missing specific chromosome arms (Sears and Sears 1978) and partial chromosome arm deletion lines (Endo and Gill 1996) were used to confirm chromosome position of genes and determine the chromosomal arm for the locus. DNA from the cytogentic sotcks was screened by PCR with gene specific primers.

PART III. RESULTS

1. The α-tubulin gene family in *T. aestivum*

Full length cDNA clones for 15 members of the α-tubulin gene family were identified within the FGAS clone collection. cDNA clones for all 15 members of the gene were fully sequenced, and their coding regions were identified. There were three length variations among the genes; they encoded proteins of 449, 450 or 451 amino acids. Sequence similarity was used to group the gene family members into five groups that likely correspond to the orthologs from the ancestral genomes of hexaploid wheat (Figure 2). DNA sequence comparison indicated that the five groups of three genes each have high sequence similarity to one of the five α-tubulins identified in the diploid barley (*Hordium vulgare*) another species of the Triticae. Gene family members were assigned a two digit number, the first digit is for the homeologous group with the same number that has been used for the barley orthologs and the second digit distinguishes each member of the homeologous group (Figure 2). Nucleic acid sequence similarity within the coding region within homelogous groups was 98% to 97% and sequence similarity between homeologous groups ranged from 94% to 77%. Amino acid sequence similarity within homeologous groups was 99-100% and between groups it ranged from 98% to 87% (Table 2). Sequence similarity in the 5' and 3' UTRs among wheat α-tubulins is lower than that within the coding regions, and is characterized by a number of small deletion/insertions. There is higher sequence similarity between the wheat gene and its most similar α-tubulin gene from barley than between a wheat gene and genes from other homeologous groups within wheat indicating that the gene family arose before the evolutionary separation of wheat and barley. Rice also has 5 members in the α-tubulin family; rice tubulin 5 is most similar to group 1 in wheat. Orthologous relations between other wheat and rice α-tubulin gene family members are not suggested by sequence comparison since other α-tubulin family members in rice have higher amino acid sequence similarity among themselves than with wheat α-tubulins (Figure 2).

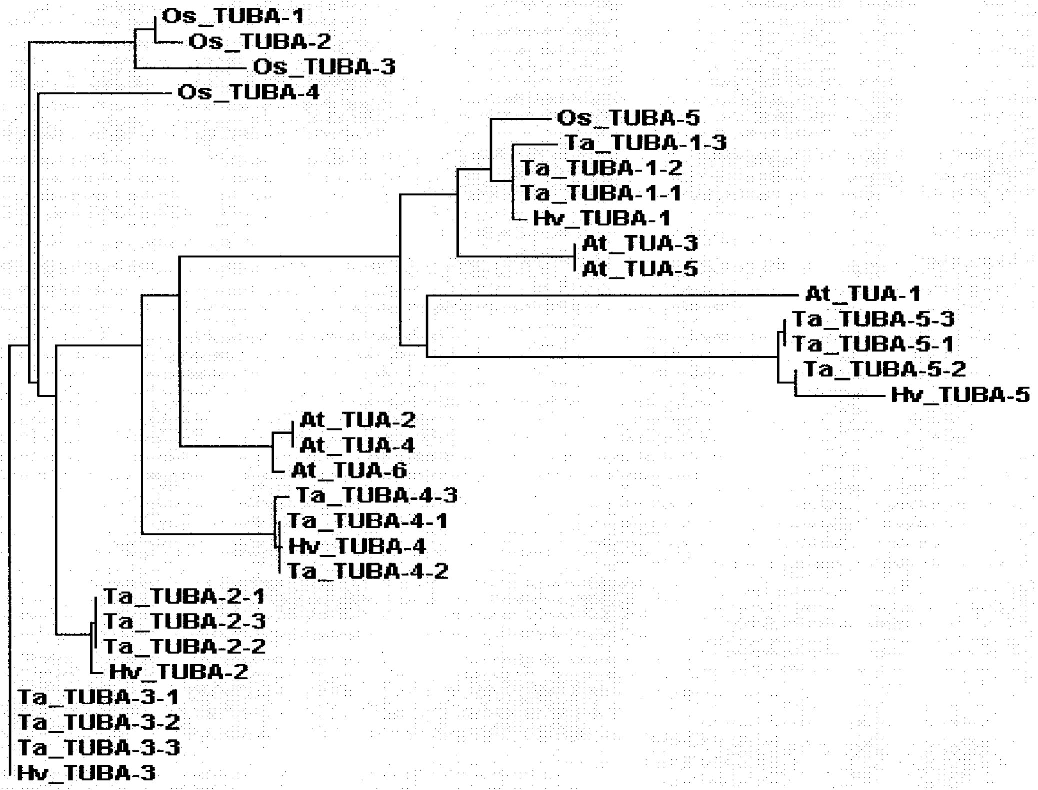
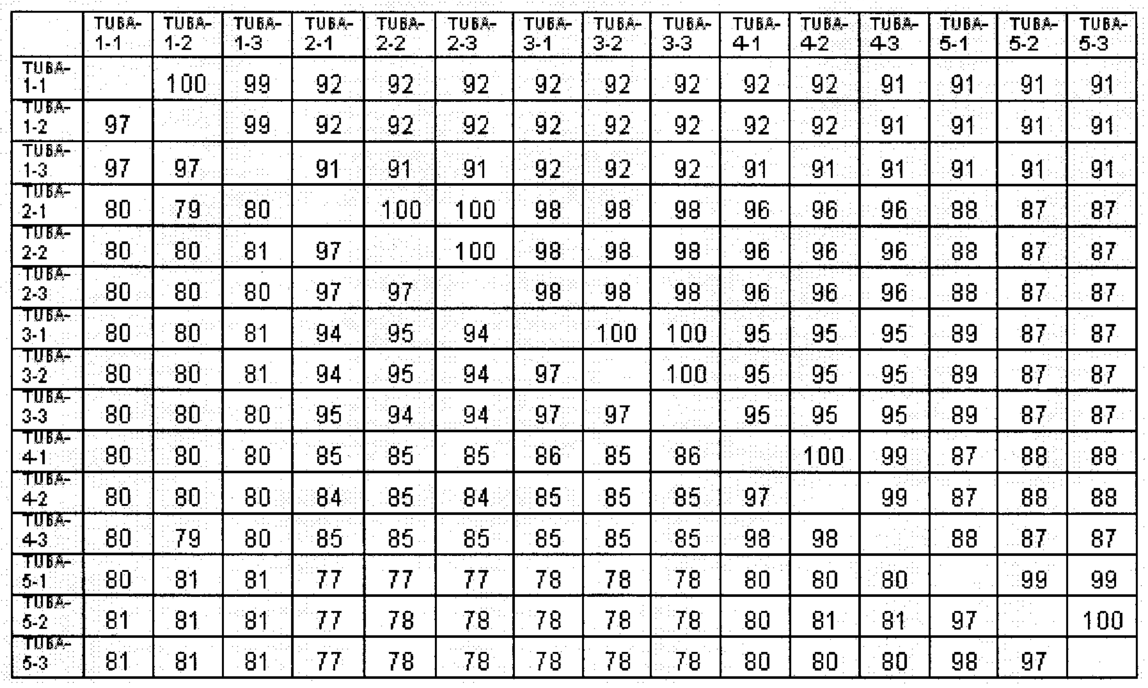


Figure 2. **Phylogram for α-tubulin genes from wheat (Ta), barley (Hv), rice (Os) and Arabidopsis (At).** The phylogram was produced with clustal W using the amino acid sequences. Horizontal branch lengths are proportional to the degree of divergence between genes.

Table 2. **Amino acid and nucleotide sequence percentage of identity between the α-tubulin gene family members of T. aestivum.**



	TUBA-1-1	TUBA-1-2	TUBA-1-3	TUBA-2-1	TUBA-2-2	TUBA-2-3	TUBA-3-1	TUBA-3-2	TUBA-3-3	TUBA-4-1	TUBA-4-2	TUBA-4-3	TUBA-5-1	TUBA-5-2	TUBA-5-3
TUBA-1-1		100	99	92	92	92	92	92	92	92	92	91	91	91	91
TUBA-1-2	97		99	92	92	92	92	92	92	92	92	91	91	91	91
TUBA-1-3	97	97		91	91	91	92	92	92	91	91	91	91	91	91
TUBA-2-1	80	79	80		100	100	98	98	98	96	96	96	88	87	87
TUBA-2-2	80	80	81	97		100	98	98	98	96	96	96	88	87	87
TUBA-2-3	80	80	80	97	97		98	98	98	96	96	96	88	87	87
TUBA-3-1	80	80	81	94	95	94		100	100	95	95	95	89	87	87
TUBA-3-2	80	80	81	94	95	94	97		100	95	95	95	89	87	87
TUBA-3-3	80	80	80	95	94	94	97	97		95	95	95	89	87	87
TUBA-4-1	80	80	80	85	85	85	86	85	86		100	99	87	88	88
TUBA-4-2	80	80	80	84	85	84	85	85	85	97		99	87	88	88
TUBA-4-3	80	79	80	85	85	85	85	85	85	98	98		88	87	87
TUBA-5-1	80	81	81	77	77	77	78	78	78	80	80	80		99	99
TUBA-5-2	81	81	81	77	78	78	78	78	78	80	81	81	97		100
TUBA-5-3	81	81	81	77	78	78	78	78	78	80	80	80	98	97	

The upper right half lists the amino acid sequence identity and the lower left half lists the nucleotide sequence identity within the coding region.

2. Chromosome assignment of α tubulin genes

One member from each of the five α tubulin paralogous groups (α-tubulin 1-3, 2-3, 3-3, 4-3 and 5-3) were mapped to a chromosome using gene specific primers for PCR screening of nulli/tetrasomic lines. Figure 3 shows the chromosomal configuration of nulli/tetrasomic lines. The Ta_TUBA-1-3 is on chromosome 2D, Ta_TUBA-2-3 is on 1D, Ta_TUBA-3-3 is on 4D, Ta_TUBA-4-3 is on 5D and Ta_TUBA-5-3 is on 4D (Figure 4). The chromosomal locations of these α-tubulin genes were confirmed in the ditelosomic and deletion lines and found to be on 2DS, 1DL, 4DS, 5DL and 4DL respectively (Figure 5).

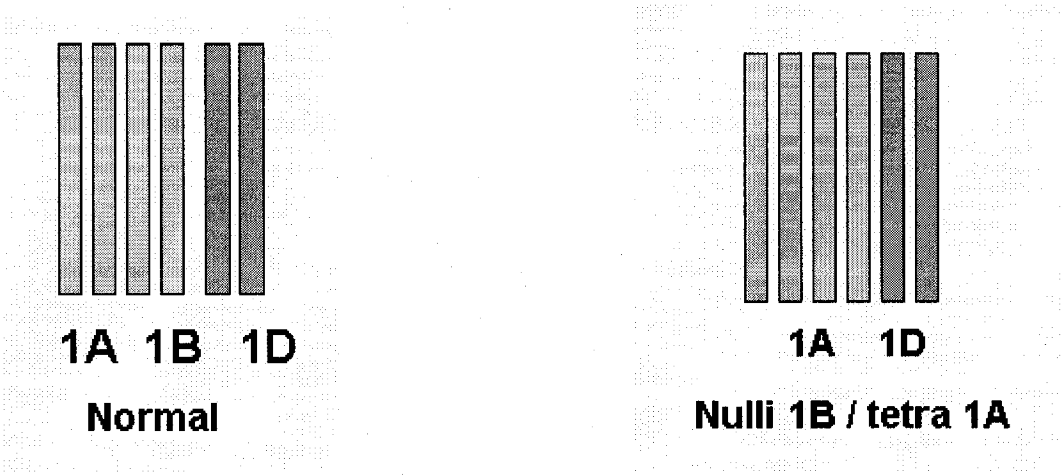


Figure 3. **nulli/tetrasomic line.** In these lines there is one pair of chromosomes deleted and replaced by an additional pair of homeologous chromosomes.

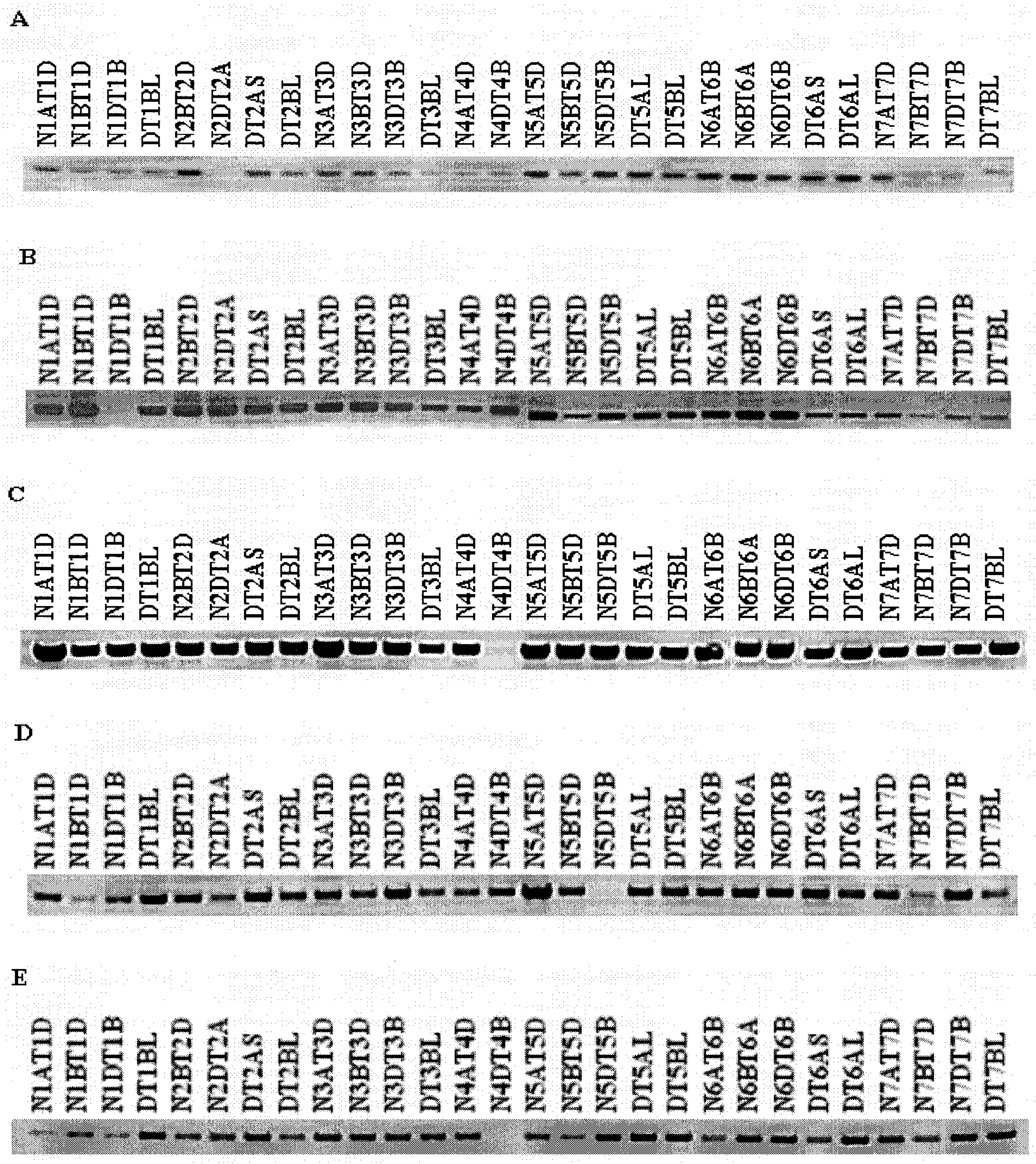


Figure 4. **Mapping of the α-tubulin genes to chromosomes.** PCR amplification of DNA from nullitetrasomic lines with gene specific primers are used to map the genes to a chromosome: (**A**) Ta_TUBA-1-3 on chromosome 2D (**B**) Ta_TUBA-2-3 on chromosome 1D (**C**) Ta_TUBA-3-3 on chromosome 4D (**D**) Ta_TUBA-4-3 on chromosome 5D (**E**) Ta_TUBA-5-3 on chromosome 4D.

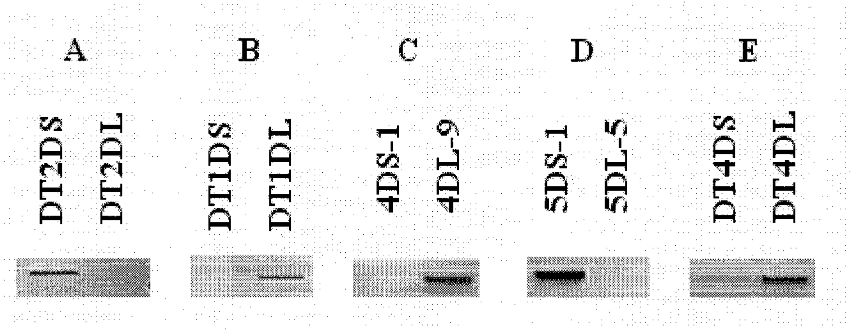


Figure 5. **Mapping of the α-tubulin genes to a chromosomal arm.** Ditelosomic (A,B,E) and chromosome segment deletion (C,D) lines were used to determine the chromosome arm location of five genes from each of the paralogous groups of α-tubulins by PCR with gene specific primers: (A) Ta_TUBA-1-3 (B) Ta_TUBA-2-3 (C) Ta_TUBA-3-3 (D) Ta_TUBA-4-3 (E) Ta_TUBA-5-3

```
TUBA-3-1         AGATGAGTTTG-CGACCTGATGTACGTCAAGCGTCCCTTCGTCTACTACTATCCTGTGAT 1559
gi|20314067      AGCTGAGTTTG-CGACCTGATGTACGTTAAGCTTGCCTTCGTCTACTACTATCCTGTGAT 379
TUBA-3-2         AGCTGAGATTG-CGACCTGATG------------------------------------AT 1498
TUBA-3-3         TGCTGAGTTCAACGACCTGATGTACGCCAAGCGTTCCTTCGT-------------GTGAT 1525
                  * **** *   **********                                    **

TUBA-3-1         CTGCCCAAGCGGCTTTATCTGTTGTCTGTCTGTTTGAAT-----GTTTGCTGTGTGGTGT 1614
gi|20314067      CTGCCCAAGCGGGACTATCTGTTGTCTGTCTGTTTGAAT-----GTTTGCTGTGTGGTGT 434
TUBA-3-2         CTGCCCGAGTGGCTTTATCTGTTTTCTGTCTGTTTGAATTGAATGTTTGCTGTGGGGTGT 1558
TUBA-3-3         CTGCCCGAGTGGCTTTATCTGCTGTCTGTCTGTTTGAAT-----GTTTGCTGTGTGGTGT 1580
                 ****** ** **   ****** * ***************     ********** *****

TUBA-3-1         TTGGTTTACAACCTGTTGTGTTGTATGAACCTGTGGTATGTTTGAACCTGCTTCGCACCT 1674
gi|20314067      TTGGTTTACAACCTGTTGTGTTGTATGAACCTGTGGTATGTTTGAACCTGCTTCGCACCT 494
TUBA-3-2         TTGGTTTACAACCTGTTGTGTT-----------------GTATGAACCTGCTTCGCACCT 1601
TUBA-3-3         TTGGTTTACAACCTGTGGTAT------------------GTTTGAACCTGCTTCGCACCT 1622
                 **************** ** *                  ** ******************

TUBA-3-1         TGGTCAATATGCATGTTATCTGGTTTGCCTAAAAAAAAAAAAAAA------------- 1719
gi|20314067      TGGTCAATATGCATGTTATCTGGTTTGCCTAAAAAAAAAAAAAAAAAAAAAAA----- 547
TUBA-3-2         TGGTCAATATGCATGTTATCCGGTTTGCCTACACCATAAAAAAAAAAAAAAAAAAAAA 1659
TUBA-3-3         TGGTCAATATGCATGTTATCTGGTTTGCCT------TAAAAAAAAAAAAAAA------ 1668
                 ******************** *********       ********
```

Figure 6. Deletion / Insertion region in the 3' UTR. Multiple alignment of *Triticum monococum* EST, gi 20314067, for an α-tubulin with 3 α-tubulin members from group 3 of *T. aestivum* show that TUBA-3-1 is the most similar to the *monococum* EST based on deletions in the 3'UTR.

Experimental gene ID	Genome	Gene name	Mapping assignment	Identity to *T. monococcum* EST	Identity to *T. turgidum* EST
Ta_TUBA-1-1	A	Ta_TUBA-1A		98%	99%
Ta_TUBA-1-2	B	Ta_TUBA-1B		97%	99%
Ta_TUBA-1-3	D	Ta_TUBA-1D	chromosome 2D	97%	97%
Ta_TUBA-2-1	A	Ta_TUBA-2A		99%	99%
Ta_TUBA-2-2	B	Ta_TUBA-2B		98%	99%
Ta_TUBA-2-3	D	Ta_TUBA-2D	chromosome 1D	97%	97%
Ta_TUBA-3-1	A	Ta_TUBA-3A		98%	99%
Ta_TUBA-3-2	B	Ta_TUBA-3B		98%	99%
Ta_TUBA-3-3	D	Ta_TUBA-3D	chromosome 4D	98%	97%
Ta_TUBA-4-1	A	Ta_TUBA-4A		99%	99%
Ta_TUBA-4-2	B	Ta_TUBA-4B		97%	97%
Ta_TUBA-4-3	D	Ta_TUBA-4D	chromosome 5D	97%	97%
Ta_TUBA-5-1	A	Ta_TUBA-5A	chromosome 4A	83%	100%
Ta_TUBA-5-2	B	Ta_TUBA-5B	chromosome 4B	83%	97%
Ta_TUBA-5-3	D	Ta_TUBA-5D	chromosome 4D	83%	97%

Table 3. **Assignment of the gene family members to the ancestral A, B or D genome.** Ancestral genome assignments were based on mapping to chromosome arms in chromosome cytogenetic stocks, and by sequence identity or the common presence of small deletions in the 3'UTRs seen in EST sequences in *T. monococcum* or *T. turdum.*

3. α-Tubulin gene expression

The mRNA expression levels for three homeologous members from group 3 were estimated by RT-PCR. The expression of all 3 member showed a similar pattern of expression (Figure 7A). Five members of the α-tubulin gene family were assayed over a time course of 36 days of cold acclimation by RT-PCR. The five genes (α-tubulin 1-3, 2-3, 3-3, 4-3 and 5-3) represent the five paralogous groups. Each of the 5 representative genes had altered levels of mRNA in response to cold treatment (Figure 7B), with four principle patterns of expression. The level of Ta_TUBA-2-3 mRNA decreased at day 1, increased at day 3, declined at days 6 and 14 and was strongly increased at day 36.

Ta_TUBA-3-3 and Ta_TUBA-5-3, mRNA levels decreased at day 1, and also showed cycles of induction and repression during the 36 day time course. The Ta_TUBA-4-3 gene had a distinct pattern of expression; it had low levels of expression in the control plants and had a strong induction at day 1, modest declines at day 3 and 6 and another increase at day 14 and low levels at day 36. The Ta_TUBA-1-3 had gradually declining levels of mRNA throughout the time course of cold acclimation.

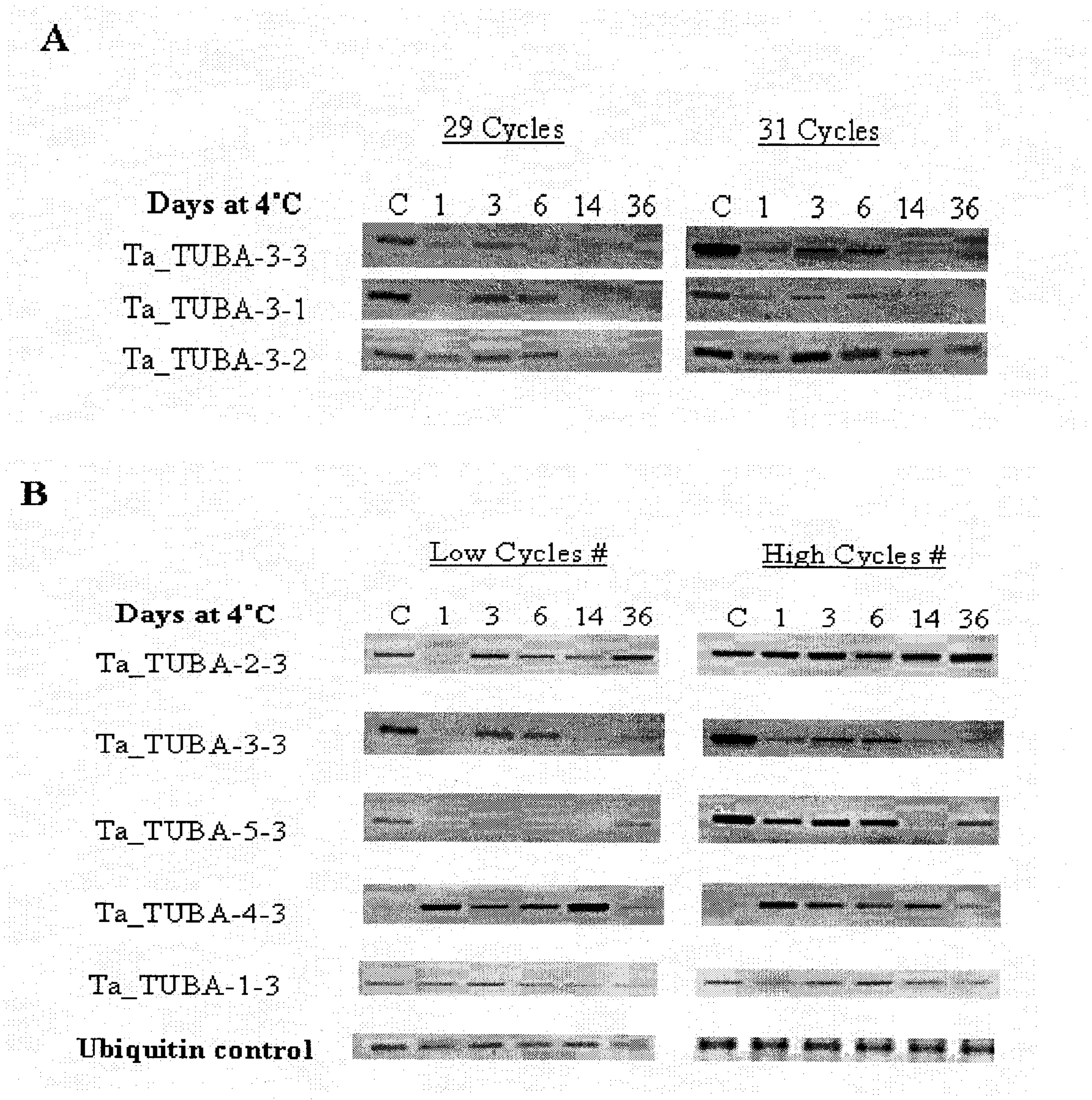


Figure 7. **The expression of α-Tubulin genes through cold acclimation.** (**A**) Three homeologous members from the same subgroup showing the same pattern of expression through cold acclimation (**B**) The pattern of mRNA level of expression for five alpha tubulin genes representing the five paralogous groups. RNA levels were measured by reverse transcription-PCR. Two sets of reactions were done to demonstrate that the PCR reaction had not saturated in the case of the lower number of cycles used. The high cycle set had two cycles of application more than the low cycle set. Ubiquitin was used as internal control.

PART IV. DISCUSSION

1. The α-tubulin gene family in wheat

The search for members of the α-tubulin gene family in the wheat FGAS EST data base for *T. aestivum* indicated that there were sequences for 15 members of the family. The five α-tublin gene family members identified in barley predict that hexaploid wheat would have 15 gene family members. Multiple sequence alignment and construction of a similarity dendrogram grouped the gene family members into 5 clusters with three members each, corresponding to the homeologous genes from each of the donor genomes of *T. aestivum* (Figure 2). When the barley α -tubulin genes were included in the comparison, each cluster contained one member of the α-tubulin gene family from barley. The members of the homelogous groups share 97-98% nucleotide sequence identity within the coding region. The highest degree of sequence identity between the groups is between the groups for Ta_TUBA-2 and Ta_TUBA-3 which share 95% identity. The most diverged groups are for Ta_TUBA-2 and Ta_TUBA-5 which share 77% identity. Amino acid sequence identity within homeologous groups is between 99% and 100%. Between homeologous groups, the highest degree of amino acid identity is 98% between the Ta_TUBA-2 and Ta_TUBA-3 group and the most diverged groups, Ta_TUBA-2 and Ta_TUBA-5, have 87% identity. The summary of gene identity is given in Table 2. The 97-98% nucleotide sequence identity within homeologous sets is similar to the degree of similarity between the most similar gene family members in wheat and barley. Rice and Arabidopsis, whose genomes have been fully sequenced, have five and six members of the α-tublin gene family, respectively. Sequence similarity among members of the gene family within each of those species is high, and though rice α-tubulin 3 is most similar to wheat α-tublin group 1, DNA sequence similarity does not give insight into orthology relationships between the other gene family members in the Triticae and those in rice and Arabidopsis. Indeed, sequence similarity alone would suggest that gene duplication occurred independently in the three lineages or that events such as gene conversion (Huang et al., 2003, Lassner et al., 1986) have led to conservation or homogenization of sequence among duplicated genes within different lineages (Figure 2).

2. Chromosome assignment of α tubulin genes

The members of the α tubulin family genes that showed changes in expression during cold treatment were mapped to chromosomes by using gene specific primers to amplify DNA from a series of nulli-tetrasomic lines (Figure 4), which have one pair of chromosomes deleted and replaced by a pair of homeologous chromosomes. Gene specific primers for individual gene family members were used to assign genes to specific chromosome arms (Figure 5) by identifying specific chromosome deletion stocks that did not yield a PCR product. The study of (Qi et al., 2004) identified chromosomes containing members of the tubulin family that are in agreement with the work reported here, though that work did not map individual gene family members to specific chromosomes.

3. Genome assignment of α-tubulins

T. aestivum has one of the largest genomes among major cereal crops, due to its hexaploid nature and a high content of intergenic space largely derived from retroposons. The assembly and annotation of the genome is a major challenge since most genes are expected to have at least three highly similar copies. The α-tublin gene family can serve as a model for the characterization of genes in homeologous series within wheat. The wheat EST sequence collection is one of the largest among plant species with over 580,000 sequences at the The Institute for Genomic Research (TIGR) wheat gene index release 10.0 (14 January 2005). The assembly of these sequences into contigs gives an important insight in to the nature of the wheat genome, however the assembly of wheat sequences is especially tentative due to the presence of highly similar sequences originating from homeologous genes. The comparison of homeologs within the α-tublin gene family, which show consistent sequence identity of approximately 97% within the coding region and gives insight into parameters for gene sequence assembly, especially when quality values for individual base calls in the sequences are not available.

The 97% sequence identity between homeologs in *T aestivum* also facilitates the identification of the genome of origin for individual gene family members. Several members of the α-tublin gene family are relatively highly expressed genes, thus for many of the gene family members homologs can be found among the ESTs from progenitor species, even though the size of the EST data sets for related species are modest. The genomes derived from the progenitor species are designated as A, B and D. The A genome progenitor, *T. urartu*, is very closely related to *T. monococcum* (Huang et al., 2002) for which approximately 11,190 EST sequences are available in GenBank. *T.*

turgidum is the tetraploid donor of genomes A and B, and *Aegilops tauschi.* is the D genome donor. There are approximately 10,658 *T. turgidum* and 116 *Aegilops tauschii* EST sequences in GenBank (July 2006). The sequences of many of the members of the α-tublin gene family members have 99% sequence identity with EST sequences from *T. monococcum*, and/or *T. turgidum*, which allows the assignment of the most likely genome of origin for these genes. For example, Ta α-tublin 2-1 has 99% identity with *T. monococcum* and *T. turgidum* ESTs, indicating it is likely the A genome copy. Ta α-tubulin 2-2 has 96% identity with *T. monococcum* ESTs and 99% identity with *T. turgidum*, Ta_TUBA- 2-3 has 97% identity with both *T. monocuccum* and *T. turgidum*, thus the likely assignment of the tubulins 2-2, and 2-3 are to the B and D genomes respectively. For three homeologous groups there was one of the three members of the group with a higher sequence identity with a *T. monococcum* EST than the others. In the case of tubulin 3-1 and 3-2 both had 99% identity with *T. turgidum*, but neither had 99% identity with a *T. monococcum* EST. However, the alignment of the three *T. aestivum* group 3 homeologs with the EST gi20314067 from *T. monococcum* showed perfect colinearity between Ta_tubulin 3-1 and the *T. monococcum* EST, whereas the other genes differed by presence of three large insertions/deletions as well as several SNPs (Figure 6) . Ta_TUB-3-1 was assigned to the A genome and the other two genes 3-2 and 3-3 assigned to B and D genome respectively. For the group 5 it was not possible to distinguish the gene family members from the A and B genomes due to the limitation of the EST databases for the progenitor species. The summary of gene similarity to ESTs from the two progenitor species and genome assignment is given in Table 3. Based on this analysis the genes are named in accordance with the genome of origin. The second digit in the experimental gene ID will be changed to the genome letters. The digits 1, 2, 3 will be changed to A, B, D respectively. For example Ta_TUBA-1-1 will be referred to as Ta_TUBA-1-A. All gene names are listed in Table 3.

4. The expression of α-tubulin genes during cold acclimation

Genes representing each of the 5 homeologous groups were assayed, by RT-PCR analysis, for changes in mRNA levels during cold acclimation using gene specific primers. These five genes were chosen for two reasons: first, they gave good gene specific primers based on regions with many unique bases, and secondly, due to the invalidity of the other gene specific primers for the other genes based on the primer 3 software used to design the primers. Each α-tubulin gene was found to be affected by cold treatment, and each gene had a distinct pattern of expression. Two of the genes, Ta_TUBA-3-3 and 5-3, showed a somewhat similar pattern of expression, which included reasonably high levels of expression under control conditions, an initial decrease in mRNA at day 1 of cold treatment followed by a rise at day 3, and decrease at day 14 with a second increase at day 36 of cold acclimation. The Ta_TUBA-4-3 gene had a distinct pattern of expression that also included increases and decreases over the time course of the treatment. The Ta_TUBA-1-3 had gradually declining levels of mRNA throughout the time course of cold acclimation (Figure 7B). The differential expression of α-tubulins during cold acclimation indicates that they may play a role in cold acclimation and low temperature tolerance. The mRNA levels for the wheat protein kinase (PLK) F29 has been shown to be induced during cold acclimation (Gulick et al., 2005) and in work being reported elsewhere, PLK-F29 was shown to interact specifically with Ta_TUBA-2-3. This tubulin gene also had cycles of induction and repression and had the most complex expression pattern among the family members tested. The rearrangement of microtubules in root cortical cells associated with the degree of cold tolerance and differential expression of α-tubulin proteins has been reported (Abdrakhamanova et al., 2003), however, the amino acid sequence of the α-tubulin genes in this study, indicates that the antibodies used in that study were not clearly gene-family-member specific. We hypothesize that the changes of gene expression of the members of the α-tublin family are associated with changing composition of the microtubules and that this subsequently can influence the specific protein-protein interaction on the surface of microtubules. The study of protein-protein interaction on the microtubules during cold acclimation warrants further investigation.

Conclusion

Fifteen α-tubulin genes were identified in wheat. Sequence analysis clusters these into five homeologous groups that correspond to the five gene family members identified in another member of the Triticae, *Hordium vulgare*. Each of the five representatives of the paralogous groups were found to be cold regulated and were mapped to 4 chromosomes in wheat. The α-tubulin-2-2 which interacts with the cold regulated protein kinase F29 has a unique pattern of expression in that it cycles through induced and repressed levels of mRNA. This presents the novel possibility that one level of regulation of F29 could be mediated by regulation of its association with microtubules and its movement in the cell. This hypothesis is currently being investigated by localization studies and the confirmation of the specificity of the interaction of the cold regulated protein kinase F29 with α-tubulin family members.

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Appendix I

Nucleic acid sequences for alpha tubulin gene family in wheat.

```
>TUBA-1-1 (TUBA-1A)
CGCTTTTATCTCCCGATCTCACGCATTTTGGGAAGACAAGGGAGAGAGAAACAGAGGAGG
CCATTCTTTACACCTTCCTGGGAAAAGAACCGGGAGGAGGAGAAGGCGAGCGAGACGGAA
GGGAGCGAGGAGGAGGAAGGCGCGACGAGATGAGGGAGATCATCAGCATCCACATCGGCC
AGGCCGGGATCCAGGTCGGCAACGCCTGCTGGGAGCTCTACTGCCTCGAGCACGGCATCC
AGCAAGATGGCACCATGCCCAGTGACACCACGGTCGGGGTTGCACACGATGCGTTCAACA
CGTTCTTCAGTGAGACCGGTGCGGGCAAGCACGTGCCGAGGGCCATCTTCGTCGACCTTG
AGCCCACTGTCATCGATGAGGTGCGCACCGGTGCCTACCGCCAGCTCTTCCACCCGGAGC
AGCTCATCTCTGGGAAGGAGGATGCCGCTAACAACTTCGCTCGTGGCCACTACACTGTTG
GAAAGGAGATTGTAGATCTATGTCTGGATCGTGTACGCAAATTGGCAGACAATTGCACCG
GCCTGCAGGGATTCTTGGTGTTCAATGCTGTTGGTGGTGGAACTGGATCAGGACTGGGCT
CTCTGTTGTTGGAGCGCCTCTCGGTTGATTATGGCAAGAAATCTAAGCTTGGTTTCACCA
TTTACCCTTCCCCGCAGGTCTCAACAGCTGTTGTAGAACCATACAACAGTGTCCTCTCCA
CTCACTCTTTGCTTGAGCACACCGATGTTGCGGTCCTCCTAGATAACGAGGCTATCTATG
ACATATGCCGGAGGTCTCTTGACATTGAGAGGCCAACCTACACCAACTTGAACAGGCTGA
TATCACAGATCATATCCTCACTTACCACCTCCCTGAGGTTTGATGGTGCCATCAATGTGG
ATGTCACAGAGTTCCAGACCAACCTCGTCCCTTACCCACGTATCCATTTCATGCTTTCGT
CGTATGCCCCTGTTATCTCTGCGGAGAAGGCTTACCATGAGCAGCTCTCTGTGCCTGAAA
TCACCAACGCTGTGTTTGAGCCCTCAAGCATGATGGCCAAGTGTGACCCTAGGCATGGCA
AATATATGGCCTGCTGCTTGATGTACCGTGGTGATGTTGTTCCCAAGGACGTCAATGCTG
CGGTAGCAACCATCAAAACCAAGAGAACTGTCCAGTTCGTCGACTGGTGCCCTACCGGGT
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TCGACCACAAGTTCGACTTGATGTACGCCAAGCGCGCGTTCGTGCACTGGTACGTCGGCG
AGGGCATGGAGGAAGGTGAGTTCTCAGAAGCCCGTGAGGACTTGGCCGCTCTGGAGAAGG
ACTACGAGGAGGTTGGCGCCGAAGGCGCGGACGACGAGGGCGATGAGGGGGATGACTATT
AAGTAGCTGGTTAATAAGTAGTTGGCTGGTTAATGATTGGCTTTGATCTGTATACTCAGT
AAGTATGGTTCCATTGCGTTACATATTGATTGCTGTTATGCGTGTTTCCTTTCCTGTAAT
GTACTGAAGATGTTGTTAGGGG
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>TUBA-1-2 (TUBA-1B)
AGGCCGTTCTTTACGCCTTCCTGGGAAAAGAGCCGGGAGGAGGAGGAGGAGAAGGCGAGC
AAGACGGAGGAAAGCGAGACGAGATGAGGGAGATCATCAGCATCCACATCGGCCAGGCCG
GGATCCAGGTCGGCAACGCCTGCTGGGAGCTCTACTGCCTCGAGCACGGCATCCAGCAGG
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TCAGTGAGACCGGTGCGGGCAAGCATGTGCCGAGGGCCATCTTTGTCGACCTTGAGCCCA
CTGTCATCGATGAGGTGCGCACCGGTGCGTACCGCCAGCTCTTCCACCCGGAGCAGCTCA
TCTCTGGCAAGGAGGATGCCGCTAACAACTTCGCCCGTGGCCACTACACTGTTGGAAAGG
AGATTGTAGATCTATGTCTGGATCGTGTACGCAAATTGGCAGACAATTGCACCGGGCTGC
AGGGATTCTTGGTGTTCAATGCTGTCGGTGGTGGAACTGGATCAGGACTGGGCTCTCTGT
TGTTGGAGCGCCTCTCGGTTGATTATGGCAAGAAATCTAAGCTTGGTTTCACCATTTACC
CTTCCCCGCAGGTCTCGACAGCTGTTGTGGAACCATACAACAGTGTCCTCTCCACTCACT
CTTTGCTTGAGCACACCGACGTTGCGGTCCTCCTAGATAACGAGGCTATCTATGACATAT
GCCGGAGGTCTCTTGACATCGAGAGGCCAACCTACACCAACTTGAACAGGCTGATATCAC
AGATCATATCCTCACTTACCACCTCGCTGAGGTTCGATGGTGCCATCAATGTGGATGTCA
CAGAGTTCCAGACCAACCTCGTCCCTTACCCACGTATCCATTTCATGCTTTCGTCGTATG
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CAACCATCAAAACCAAGAGAACTGTCCAGTTCGTTGACTGGTGCCCTACCGGGTTCAAGT
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GGGCCGTGTGCATGATCAGCAACAACACTGCCGTTGCCGAAGTGTTTTTGCGCATCGACC
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TGGAGGAAGGTGAGTTCTCGGAGGCCCGTGAGGACTTGGCCGCTCTCGAGAAGGACTACG
AGGAGGTTGGCGCCGAAGGCGCAGACGATGAGGGCGACGAGGGGGATGACTACTAAGTAG
CTGGTTAATAAGTAGTTGGCTGGTTAATGATTGGCTTTGATCTGTATACTCAGTAAGTAT
GGTTCCATTGCGTTACATATTGATTGCTGTTATGCGTGTTTCCTTTTCTTGTAATGTACT
GAAGTTGTTGTTAGGGTGGCCATGATTGTTGATACCCCATTTCCTCATTTTGGCTTTCAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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>TUBA-1-3 (TUBA-1D)
CCACGCGTCCGGACTGGAGCACGAGGAAGACAAGGGAGAGAGAAGCAGAGGAGGCCATTC
TTTACAACCGGGAGGAGGAGAAGGCGAGAGAGACGGAAGGGAGCGAGGAGACGAGATGAG
GGAGATCATCAGCATCCACATCGGCCAGGCCGGGATCCAGGTCGGCAACGCCTGCTGGGA
GCTCTACTGCCTCGAGCACGGCATCCAGCAGGATGGCACCATGCCCAGTGATACCACGGT
TGGGGTTGCACACGATGCGTTCAACACGTTCTTCAGTGAGACTGGTGCGGGCAAGCACGT
GCCGAGGGCCATCTTCGTCGACCTTGAGCCCACTGTCATTGATGAGGTGCGCACCGGTGC
CTACCGCCAGCTCTTCCACCCGGAGCAGCTCATCTCCGGGAAGGAGGATGCCGCTAACAA
CTTCGCCCGTGGTCACTACACTGTTGGGAAGGAGATTGTAGATCTATGTCTGGATCGTGT
ACGCAAATTGGCAGACAACTGCACCGGGCTGCAGGGATTCTTGGTGTTCAATGCTGTCGG
TGGTGGAACTGGATCAGGACTGGGCTCTCTGTTGTTGGAGCGCCTCTCGGTTGATTACGG
CAAGAAATCTAAGCTTGGTTTCACCATTTACCCTTCCCCACAGGTCTCGACAGCTGTTGT
AGAGCCATACAACAGTGTCCTCTCCACTCACTCTTTGCTTGAGCACACCGATGTTGCGGT
CCTCCTAGATAATGAGGCTATCTATGACATATGCCGGAGGTCTCTTGACATTGAGAGGCC
AACCTACACCAACTTGAACAGGCTGATATCACAGATCATATCCTCACTTACCACCTCCCT
GAGGTTTGATGGTGCCATCAATGTGGATGTCACAGAGTTCCAGACCAACCTCGTCCCTTA
CCCACGTATCCATTTCATGCTTTCGTCGTATGCCCCTGTTATCTCTGCGGAGAAGGCTTA
CCATGAGCAGCTCTCTGTGCCTGAAATCACCAATGCTGTCTTTGAGCCTTCAAGCATGAT
GGCCAAGTGTGACCCTAGGCATGGCAAATATATGGCCTGCTGCTTGATGTACCGTGGTGA
TGTTGTTCCCAAGGACGTCAATGCCGCAGTCGCAACCATCAAAACCAAGAGAACTGTCCA
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CGCCGTCGCCGAAGTGTTCTCGCGCATCGACCACAAGTTCGACTTGATGTACGCCAAGCG
CGCGTTCGTGCACTGGTACGTCGGCGAGGGCATGGAGGAAGGTGAGTTCTCAGAAGCCCG
TGAGGACTTGGCTGCTCTGGAGAAGGACTACGAGGAGGTTGGCGCCGAAGGCGCAGACGA
TGAGGGCGACGAGGGGGATGACTATTAAGTAGCTGGTTAATAAGTAGTTGGCTGGTTAAT
GATTGGCTTTGATCTGTATACTCAGTAAGTATGGTTCCATTGCGTTACATATTGATTGCT
GTTATGCGTGTTTCCTTTCCTGTAATGTACTGAAGATGTTGTTAGGGTGGCCATGATTGT
TGATACCCCATTTCCCCATTTTGGCTTTCGATGCTACTCGTCCCAAGTTTGGGGGGTGTG
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CGGGGGTTTTTTTTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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>TUBA-2-1 (TUBA-2A)
CTAAGCAGAGTGGGGAAAGGCGTCTTCGTACTCGCCTCTCTCCGCGCAACCGAGCCTTCC
GCCCACCTCCTCCCCATTTCGCCAGCGGCGCACCCACCAACCACCCCCACCCGCCGCCAT
GAGGGAGTGCATCTCGATCCACATCGGCCAGGCCGGCATCCAGGTCGGGAACGCGTGCTG
GGAGCTGTACTGCCTCGAGCATGGCATTCAGCCTGATGGCCAGATGCCCGGTGACAAGAC
CGTTGGGGGAGGTGATGATGCTTTCAACACCTTCTTCAGCGAGACTGGGGCTGGGAAGCA
CGTCCCCCGTGCTGTCTTCGTAGATCTCGAGCCCACTGTGATTGATGAGGTGAGGACTGG
CGCTTACCGCCAGCTCTTCCACCCTGAGCAGCTTATCAGTGGCAAGGAGGATGCAGCCAA
CAACTTCGCCCGTGGTCATTACACCATTGGCAAGGAGATTGTTGATCTGTGCCTTGACCG
TATCAGGAAGCTTTCAGACAACTGCACTGGTCTCCAGGGATTCCTTGTCTTCAACGCTGT
TGGAGGTGGAACTGGCTCTGGCCTTGGTTCTCTTCTGCTGGAGCGCCTCTCTGTTGACTA
TGGAAAGAAGTCCAAGCTTGGGTTCACAGTGTACCCATCACCCCAGGTCTCCACCTCTGT
TGTTGAGCCATACAACAGTGTCCTGTCCACCCACTCCCTCCTTGAGCACACTGATGTGTC
TATCCTTCTTGACAATGAGGCCATCTATGACATCTGCCGCCGCTCCCTTGACATTGAGCG
CCCAACATACACCAACCTCAACAGGCTTGTTTCTCAGGTCATTTCATCGCTGACAGCTTC
CCTGAGGTTTGATGGTGCTCTGAATGTTGATGTCAATGAATTCCAGACCAACTTGGTGCC
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CTACCATGAGCAGCTGTCTGTTGCTGAGATCACCAACAGCGCCTTTGAGCCTTCATCTAT
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TGATGTTGTTCCCAAGGATGTCAACGCTGCTGTGGCCACCATCAAGACCAAGCGCACTAT
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TGTTGTCCCAGGCGGTGACCTTGCCAAGGTCCAGAGGGCTGTGTGCATGATCTCCAACTC
CACCAGTGTCGTCGAGGTCTTCTCCCGCATTGACCACAAGTTTGACCTCATGTACGCCAA
GCGTGCCTTCGTCCACTGGTACGTGGGTGAGGGCATGGAGGAGGGAGAGTTCTCTGAGGC
CCGTGAGGATCTTGCTGCCCTGGAGAAGGACTATGAAGAAGTTGGTGCTGAGTTCGACGA
GGGTGAGGACGGTGACGAGGGCGACGAGTACTAGAGCCTGCCTCCTGGTGCTTTCCCAAG
GCGTGCTGCTGCTATCCCATGATCTGCCCGAGTGGCTTTATCTGTTATCTGTCTGTTTGA
ATCTTTGCTTTGTGGTGTTTGTTTTACAACCTGTTGTGTTGTAAGACCCTTGTATCTTTG
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AAAAAAAAAAAAAAA
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>TUBA-2-2 (TUBA-2B)
AAGGCGTCTTCGTACTCGCCTCTCTCCGCGCAACCGAGCCTTCGCCCTCCTCCTTCCCCA
TTTCGCCAGCGGCGCAGCCCACCAACCACCCCCACCCGCCGCCATGAGGGAGTGCATCTC
GATCCACATCGGCCAGGCCGGCATCCAGGTCGGGAACGCGTGCTGGGAGCTCTATTGCCT
CGAGCATGGCATTCAGCCTGATGGCCAGATGCCCGGTGACAAGACCGTTGGGGGAGGTGA
TGATGCTTTCAACACCTTCTTCAGCGAGACTGGTGCTGGGAAGCACGTCCCCCGTGCTGT
CTTCGTAGATCTCGAGCCCACTGTGATTGATGAGGTGAGGACTGGCGCTTACCGCCAGCT
CTTCCACCCTGAGCAGCTTATCAGTGGCAAGGAGGATGCAGCCAACAACTTCGCCCGTGG
TCATTACACCATTGGCAAGGAGATTGTTGATCTCTGCCTAGATCGTATCAGGAAGCTTTC
AGACAACTGCACTGGTCTGCAGGGATTCCTTGTCTTCAACGCTGTTGGAGGTGGAACTGG
CTCTGGCCTTGGTTCGCTTCTCCTGGAGCGTCTCTCTGTTGACTATGGAAAGAAGTCCAA
GCTTGGGTTCACAGTTTACCCATCTCCCCAGGTCTCCACTTCTGTTGTTGAGCCATACAA
CAGTGTCCTGTCCACCCACTCACTCCTTGAGCACACCGATGTCTCTATCCTTCTTGACAA
TGAGGCCATCTATGACATCTGCCGCCGCTCCCTTGACATTGAGCGCCCAACATACACCAA
CCTCAACAGGCTTGTTTCTCAGGTCATTTCATCATTGACTGCTTCCCTGAGGTTTGATGG
TGCTCTGAATGTTGATGTCAACGAGTTCCAGACCAACCTGGTGCCCTACCCGAGGATCCA
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GTGCCCCACTGGCTTCAAGTGTGGTATCAACTACCAGCCACCTGGTGTCGTCCCAGGCGG
TGACCTTGCCAAGGTCCAGAGGGCTGTGTGCATGATCTCCAACTCCACCAGTGTTGTCGA
GGTCTTTTCCCGCATCGACCACAAGTTTGACCTGATGTACGCCAAGCGTGCCTTCGTCCA
CTGGTACGTGGGTGAGGGCATGGAGGAGGGAGAGTTCTCTGAGGCCCGTGAGGATCTTGC
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CGAGGGCGATGAGTACTAGAGCCTGCCTCCTGGTGCTTTCCCAAGGCATGCTGCTGCAAT
CCTATGATCTGCCCGAGTGGCTTTATCTGTTATCTGTCTGTTTGAACATTTGCTTTGTGG
TGTTTGTTTTACAACCTGTTGTGTTGTAAGAACCTTGTATGTTTGAACCTGTTATGCACC
TTGGTTAATATGCATGCTATCTGGTTATCTAAAAAAAAAAAAAAAAAAAA
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>TUBA-2-3 (TUBA-2D)
GAAAGGCGTCTTCGTACTCGCCTCTCTCCGCGCATCCTAGCCTTCGCCCTCCTCCTTCCC
CATTTCGCCAGCGGCGCAGCCCACCAACCACCCCCACCCGCCGCCATGAGGGAGTGCATC
TCGATCCACATCGGCCAGGCCGGCATCCAGGTCGGGAACGCGTGCTGGGAGCTCTACTGC
CTCGAGCATGGCATTCAGCCTGATGGCCAGATGCCCGGTGACAAGACCGTTGGGGGAGGT
GATGATGCTTTCAACACCTTCTTCAGCGAGACTGGTGCTGGGAAGCATGTCCCCCGTGCT
GTCTTTGTAGATCTCGAGCCCACTGTGATTGATGAGGTGAGGACTGGTGCTTACCGCCAG
CTCTTCCACCCTGAGCAGCTTATCAGTGGCAAGGAGGATGCAGCCAACAACTTCGCCCGT
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TCAGACAACTGCACTGGTCTCCAGGGATTCCTTGTATTCAACGCTGTTGGAGGTGGAACT
GGCTCTGGCCTTGGCTCGCTTCTCCTGGAGCGCCTCTCTGTTGACTATGGAAAGAAGTCC
AAGCTTGGGTTCACGGTGTACCCATCTCCCCAGGTCTCCACCTCTGTTGTTGAGCCATAC
AACAGTGTCCTGTCCACCCACTCACTCCTTGAGCACACTGATGTCTCTATCCTTCTTGAC
AATGAGGCCATCTATGACATCTGCCGCCGCTCCCTTGACATTGAGCGCCCAACATACACC
AACCTCAACAGGCTTGTTTCTCAGGTCATTTCATCACTGACAGCTTCCCTGAGGTTTGAT
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CACTTCATGCTTTCCTCCTATGCCCCAGTGATCTCAGCTGAGAAGGCTTACCATGAGCAG
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TGGTGCCCCACTGGCTTCAAGTGTGGTATCAACTACCAGCCACCAGGTGTCGTCCCAGGC
GGTGACCTTGCCAAGGTCCAGAGGGCTGTGTGCATGATCTCCAACTCCACCAGTGTCGTC
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AA
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>TUBA-3-1 (TUBA-3A)
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CCGCCGCTCCGACCCGCCGCCATGAGGGAGTGCATCTCGATCCACATCGGCCAGGCCGGT
ATCCAGGTCGGGAACGCGTGCTGGGAGCTCTACTGCCTCGAGCATGGCATTCAGCCTGAT
GGACAGATGCCCGGTGACAAGACCGTTGGGGGAGGTGATGATGCTTTCAACACCTTCTTC
AGTGAGACTGGTGCTGGGAAGCATGTCCCCCGCGCGGTCTTTGTTGATCTTGAGCCCACT
GTGATTGATGAGGTGAGGACTGGCACTTACCGCCAGCTCTTCCACCCTGAGCAGCTTATC
AGTGGCAAGGAGGATGCAGCCAACAACTTTGCCCGTGGTCACTACACCATTGGCAAGGAG
ATTGTTGACCTATGCCTTGACCGTATCAGGAAGCTTGCAGACAACTGCACTGGTCTCCAG
GGGTTCCTTGTCTTCAACGCTGTTGGAGGTGGAACTGGCTCTGGCCTTGGTTCTCTTCTC
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TCCCCTCAGGTCTCCACCTCTGTTGTTGAGCCATACAACAGTGTCCTGTCCACCCACTCT
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CGCCGCTCCCTTGACATTGAGCGCCCAACATACACCAACCTCAATAGGCTCGTTTCTCAG
GTCATCTCATCCCTGACTGCTTCCCTGAGGTTTGATGGTGCTCTGAATGTTGATGTGAAC
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CCAGTGATCTCAGCTGAGAAGGCTTACCATGAGCAGCTCTCTGTCGCTGAGATCACCAAC
AGCGCCTTCGAGCCTTCCTCCATGATGGCCAAGTGTGACCCCCGCCACGGCAAGTACATG
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GCCGTGTGCATGATCTCCAACTCCACCAGCGTTGTCGAGGTCTTCTCCCGCATCGACCAC
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GAGGAGGGTGAGTTCTCTGAGGCCCGTGAGGATCTTGCTGCCCTGGAGAAGGACTATGAA
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AGATGAGTTTGCGACCTGATGTACGTCAAGCGTCCCTTCGTCTACTACTATCCTGTGATC
TGCCCAAGCGGCTTTATCTGTTGTCTGTCTGTTTGAATGTTTGCTGTGTGGTGTTTGGTT
TACAACCTGTTGTGTTGTATGAACCTGTGGTATGTTTGAACCTGCTTCGCACCTTGGTCA
ATATGCATGTTATCTGGTTTGCCTAAAAAAAAAAAAAAA
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>TUBA-3-2 (TUBA-3B)
CGGCGTCTTCGTACTCGCCTCTCTCGCGCGCTTCCGAGCTTTGTCCTTCGTCCTCCTCCC
CCGACCTCTCCAGCAGCGTAGCGTAGCGCCCGCTTCCGCCGTTCCGACCCGCCGCCATGA
GGGAGTGCATCTCGATCCACATCGGCCAGGCCGGTATCCAGGTCGGGAACGCGTGCTGGG
AGCTCTACTGCCTCGAGCATGGCATTCAGCCTGATGGACAGATGCCTGGTGACAAGACTG
TTGGGGGAGGTGATGATGCTTTCAACACCTTCTTCAGTGAGACTGGTGCTGGGAAGCATG
TCCCCCGTGCTGTCTTTGTTGATCTTGAGCCCACTGTGATTGATGAGGTGAGGACTGGCA
CTTATCGCCAGCTCTTCCACCCTGAGCAGCTTATCAGTGGCAAGGAGGATGCAGCCAACA
ACTTTGCCCGTGGTCACTACACCATTGGCAAGGAGATTGTTGACCTATGCCTGGACCGTA
TCAGGAAGCTTGCAGACAACTGCACTGGTCTTCAGGGATTCCTCGTCTTCAACGCTGTTG
GAGGTGGAACTGGCTCTGGCCTTGGTTCTCTTCTCCTGGAGCGGCTCTCTGTTGACTATG
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CAACATACACCAACCTCAACAGGCTCGTTTCTCAGGTCATCTCATCCCTGACTGCTTCCC
TGAGGTTTGATGGTGCTCTGAATGTTGATGTCAACGAGTTCCAGACCAACCTGGTGCCCT
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ACCATGAGCAGCTCTCTGTTGCCGAGATCACCAACAGCGCCTTCGAGCCTTCCTCCATGA
TGGCAAAGTGTGACCCCCGCCACGGCAAGTACATGGCCTGCTGCCTCATGTACCGTGGTG
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CCAGCGTTGTCGAGGTCTTCTCCCGCATCGACCACAAGTTCGACCTGATGTACGCCAAGC
GTGCCTTCGTCCACTGGTACGTGGGTGAGGGCATGGAGGAGGGAGAGTTCTCTGAGGCCC
GTGAGGATCTCGCTGCCCTGGAGAAGGACTATGAAGAAGTTGGTGCTGAGTTCGACGAGG
GTGAGGATGGTGATGAGGGTGATGAGTACTAGAGCAGCTGAGATTGCGACCTGATGATCT
GCCCGAGTGGCTTTATCTGTTTTCTGTCTGTTTGAATTGAATGTTTGCTGTGGGGTGTTT
GGTTTACAACCTGTTGTGTTGTATGAACCTGCTTCGCACCTTGGTCAATATGCATGTTAT
CCGGTTTGCCTACACCATAAAAAAAAAAAAAAAAAAAAA
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>TUBA-3-3 (TUBA-3D)
CGGCGTCTTCGTACTCGCCTCTCTCTCGCGCTTCCGAGCTTTGGCCTCCCTCCCCTCCTC
CCCGCAATCTCCCCACCAGCGTAGCGCAGCGCGCGCCTCCGCCGCTCCGACCCGCAGCCA
TGAGGGAGTGCATCTCGATCCACATCGGCCAGGCCGGCATCCAGGTCGGGAACGCGTGCT
GGGAGCTCTACTGCCTCGAGCATGGCATTCAGCCTGATGGACAGATGCCTGGTGACAAGA
CCGTTGGGGGAGGTGATGATGCTTTCAACACCTTCTTCAGTGAGACTGGTGCTGGGAAGC
ATGTTCCCCGTGCTGTCTTTGTTGATCTTGAGCCCACTGTGATTGATGAGGTGAGGACTG
GCACCTATCGCCAGCTCTTCCACCCTGAGCAGCTTATCAGTGGCAAGGAGGATGCAGCCA
ACAACTTTGCCCGTGGTCACTACACCATTGGCAAGGAGATTGTTGACCTATGCCTTGACC
GTATCAGGAAGCTTGCAGACAACTGCACTGGTCTCCAGGGATTCCTTGTCTTCAACGCTG
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AGGGTGAGGATGGTGATGAGGGTGATGAGTACTAGAGCTGCTGAGTTCAACGACCTGATG
TACGCCAAGCGTTCCTTCGTGTGATCTGCCCGAGTGGCTTTATCTGCTGTCTGTCTGTTT
GAATGTTTGCTGTGTGGTGTTTGGTTTACAACCTGTGGTATGTTTGAACCTGCTTCGCAC
CTTGGTCAATATGCATGTTATCTGGTTTGCCTTAAAAAAAAAAAAAAA
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>TUBA-4-1 (TUBA-4A)
ACTACACATCCGGCGTCTTCACAAACGCCGGCTCGCTCTCGCTCCGTCTCCCTCCCTCCT
CCCCCGCTCCGCGCCCTCCGCCGCCAGATCCCGTCGAGCCCCCGCGCCCCCGCGTCGAA
GATGAGGGAGTGCATCTCGATCCACATCGGGCAGGCCGGCATCCAGGTCGGCAACGCGTG
CTGGGAACTTTACTGCCTCGAGCACGGCATCCAGCCTGATGGCCAGACGAACGGCGACAA
GACCATCGGAGGTGGTGATGACGCCTTCAACACCTTCTTCAGCGAGACCGGAGCCGGCAA
GTACGTGCCCCGTGCGGTCTTCGTCGATCTTGAGCCCACCGTGATTGACGAGGTCCGCAC
CAGCGCCTACCGCCAGCTCTTCCACCCCGAGCAGCTCATCAGCGGCAAGGAGGACGCCGC
CAACAACTTCGCCCGTGGTCACTACACAATTGGCAAGGAGATTGTGGATCTCTGCCTCGA
CCGCATCCGCAAGCTGGCCGACAACTGCACTGGCCTGCAGGGCTTCCTGGTCTTCAACGC
CGTTGGTGGTGGAACCGGGTCTGGGCTTGGGTCGCTCCTCCTTGAGCGCCTGTCCGTGGA
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TGTGGTCGAGCCCTACAACAGTGTGCTGTCCACCCACTCCCTGCTGGAGCACACCGATGT
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GAGGCCCACCTACACCAACCTGAACCGCCTCGTCTCTCAGGTGATCTCATCCCTGACCAC
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GGGCGACGTGGTGCCCAAGGACGTGAACGCGGCGGTGGCCACCATCAAGACCAAGCGCAC
CATCCAGTTCGTGGACTGGTGCCCCACGGGGTTCAAGTGCGGCATCAACTACCAGCCGCC
CACCGTGGTGCCTGGCGGCGACCTGGCCAAGGTGCAGAGGGCCGTCTGCATGATCTCCAA
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CAAGCGCGCCTTCGTGCACTGGTATGTGGGCGAGGGCATGGAGGAGGGCGAGTTCTCCGA
GGCCCGTGAGGACCTGGCTGCCCTGGAGAAGGACTACGAGGAGGTCGGCGCTGAGGGTGG
CGACGATGAGGATGGCGAGGAGGACGACGACTACTGATCTGCTCGTTCGCTCGACGGGGG
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>TUBA-4-2 (TUBA-4B)
TCTTCACAAACGCCGGCTCTCCCCCGCTCCGCCTCCCTCCCTCATCCTCCCCCGCTCCGC
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CATCTCGATCCACATCGGGCAGGCCGGAATCCAGGTCGGCAACGCGTGCTGGGAACTTTA
CTGCCTCGAGCACGGCATCCAGCCTGACGGCCAGACGAACGGTGACAAGACCATCGGAGG
TGGTGATGACGCCTTCAACACCTTCTTCAGCGAGACCGGAGCCGGCAAGTACGTGCCCCG
CGCGGTCTTCGTCGACCTCGAGCCCACCGTGATCGACGAGGTCCGCACCAGCGCCTACCG
CCAGCTCTTCCACCCCGAGCAGCTCATCAGCGGCAAGGAGGACGCGGCCAATAACTTCGC
CCGTGGTCACTACACAATCGGCAAGGAGATTGTGGATCTCTGCCTGGACCGCATCCGCAA
GCTGGCCGACAACTGCACTGGCCTGCAGGGCTTCCTGGTCTTCAACGCCGTCGGCGGTGG
AACCGGGTCTGGGCTTGGGTCGCTCCTCCTCGAGCGCCTGTCCGTGGACTATGGAAAGAA
GTCCAAGCTCGGGTTCACCGTGTACCCATCTCCTCAGGTGTCGACCTCTGTGGTTGAGCC
CTACAACAGTGTGCTGTCCACCCACTCCCTGCTGGAGCACACCGATGTCTCCATCCTGCT
CGACAACGAGGCCATCTACGACATCTGCAGGCGCTCCCTGGACATCGAGAGGCCCACCTA
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CGACGGTGCCCTGAACGTGGACGTGACCGAGTTCCAGACCAACCTGGTCCCGTACCCTCG
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GCAGCTGTCGGTGTCGGAGATCACCAACAGCGCGTTCGAGCCGTCGTCCATGATGGCCAA
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GCCCAAGGACGTGAACGCGGCGGTGGCCACCATCAAGACCAAGCGCACCATCCAGTTCGT
GGACTGGTGCCCCACGGGGTTCAAGTGCGGCATCAACTACCAGCCGCCCACCGTGGTGCC
CGGCGGCGACCTGGCCAAGGTGCAGAGGGCCGTCTGCATGATCTCCAACTCCACCAGCGT
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CGTGCACTGGTACGTGGGCGAGGGCATGGAGGAGGGCGAGTTCTCCGAGGCCCGTGAGGA
CCTGGCCGCCCTGGAGAAGGACTACGAGGAGGTCGGCGCCGAGGGCGGCGATGATGAGGA
TGGCGAGGAGGACGACGACTACTGATCTGCTCGTCCGCTCGACGGAGGATCTGTCTCCTT
TGCTGCCTATCTTTAACTACATGTTGCTGTGCTGTCCTGTTTTGGAAACTTGTGTCTGGG
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AAAAAAAAAAAA
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>TUBA-4-3 (TUBA-4D)
CACAAACGCCGGCTCGCTCCCCATCCTTTTCCGTGCTCCGCCTCCCCGCCCCGCGCCCTC
CGCCGCCAGATCCCGTCGAGCCCCCCGCGCCCCCGCGTCGAAGATGAGGGAGTGCATCTC
GATCCACATCGGGCAGGCCGGCATCCAGGTCGGCAACGCGTGCTGGGAACTTTACTGCCT
CGAGCACGGCATCCAGCCTGATGGCCAGACGAACGGTGACAAGACCATCGGAGGTGGTGA
TGACGCCTTCAACACCTTCTTCAGCGAGACCGGAGCCGGCAAGTACGTGCCCCGTGCGGT
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CTTCCACCCCGAGCAGCTCATCAGCGGCAAGGAGGACGCAGCCAACAACTTCGCCCGTGG
TCACTACACAATCGGCAAGGAGATTGTGGATCTCTGCCTCGACCGCATCCGCAAGCTGGC
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GTCTGGGCTTGGGTCGCTCCTCCTCGAGCGCCTGTCCGTGGACTATGGAAAGAAGTCCAA
GCTCGGGTTCACCGTGTACCCATCTCCTCAGGTGTCGACCTCTGTGGTCGAGCCCTACAA
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CGAGGCCATCTACGACATCTGCAGGCGCTCCCTGGACATCGAGAGGCCCACCTACACCAA
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TGCCCTGAACGTGGACGTGACCGAGTTCCAGACCAACCTGGTCCCGTACCCTCGGATCCA
CTTCATGCTCTCGTCCTACGCGCCGGTCATCTCGGCGGAGAAGGCGTACCACGAGCAGCT
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GGACGTGAACGCGGCGGTGGCCACCATCAAGACCAAGCGCACCATCCAGTTCGTGGACTG
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CGACCTGGCCAAGGTGCAGAGGGCCGTCTGCATGATCTCCAACTCCACCAGCGTCGTCGA
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GGAGGACGACGACTACTGATCTGCTCGTCCGCTCGACGGAGGATCCGTCTCCTCTGCCGC
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>TUBA-5-1 (TUBA-5A)
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GAGGGAGATGAGGGAGATCATAAGCATCCACATCGGGCAGGCGGGCATCCAGGTGGGGAA
TTCCTGCTGGGAGCTCTACTGCCTCGAGCATGGCATCCAGCCCGACGGCCTCATGCCCAG
TGATACCTCGGTTGGGGTTGCAAAGGATGCATTCAACACATTCTTCAGCGAAACGGGTTC
AGGGAAGCATGTTCCGAGGGCCCTGTTCGTCGATCTGGAGCCCACGGTCATCGACGAGGT
GAGGACGGGGGCATACCGCCAGCTCTTCCATCCTGAGCAGCTCATCTCCCACAATGAAGA
TGCTGCTAACAACTTTGCCCGCGGACACTACACAGTTGGAAGAGAAGTGGTGGACCTTTG
CCTTGACCGGATCAGAAAATTGGCGGACAACTGCACTGGCCTCCAAGGTTTCTTGGTTTT
CAATGCTGTCGGTGGCGGAACTGGCTCAGGACTTGGTTCATTGCTTCTGGAGCGCCTATC
GGTCGACTATGGCAGGAAATCCAAGCTCGGTTTCACCATCTATCCTTCACCACAGATTTC
GACGGCCGTTGTGGAGCCATACAACAGCGTGCTCTCGACCCACTCGCTGATCGAGCACAC
CGACGTGGTGGTGCTCCTGGACAACGAGGCCATCTACGACATCTGCAAGAGGTCCCTGGA
CATCGAGCGCCCGACCTACACCAACCTGAACCGGCTGATATCCCAGGTGATATCGTCCCT
GACCACGTCGCTGCGGTTCGACGGCGCCATCAACGTGGACATAACCGAGTTCCAGACCAA
CCTGGTGCCGTACCCGAGGATCCACTTCATGCTCTCCTCCTACGCGCCCATCATCTCCGC
GGAGAAGGCCTTCCACGAGCAGCACTCCGTCCCCGAGATCACCAACTCCGTGTTCGAGCC
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CAGCAACAACACGGCCGTGGCCGAGGTCTTCTCGCGCATCGACCGCAAGTTCGACCTCAT
GTACGCCAAGCGCGCGTTCGTGCACTGGTACGTCGGGGAAGGGATGGAGGAGGGGGAATT
CTCGGAGGCCAGGGAGGATCTGGCCGCGCTGGAGAAAGACTACGAGGAGGTCGGAGCCGA
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>TUBA-5-2  (TUBA-5B)
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AGGGAGATGAGGGAGATCATAAGCATCCACATCGGGCAGGCGGGCATCCAGGTGGGGAAT
TCCTGCTGGGAGCTCTACTGCCTCGAGCATGGCATCCAGCCCGACGGCCTCATGCCCAGT
GATACCTCGGTTGGGGTTGCAAAGGATGCATTCAACACATTCTTCAGCGAAACGGGTTCA
GGGAAGCATGTTCCGAGGGCCCTGTTCGTCGATCTGGAGCCCACGGTCATCGACGAGGTG
AGGACGGGGGCATACCGCCAGCTCTTCCATCCTGAGCAGCTCATCTCCCACAATGAAGAT
GCTGCTAACAACTTTGCCCGCGGACACTACACAGTTGGAAGAGAAGTGGTGGACCTTTGC
CTTGACCGGATCAGAAAATTGGCGGACAACTGCACTGGCCTCCAAGGTTTCTTGGTTTTC
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GACGTCGTCGTTCTGCTGGACAACGAGGCCATCTACGACATCTGCAAGAGGTCCCTGGAC
ATCGAGCGCCCGACCTACACCAACCTGAACAGGCTGATATCCCAGGTGATATCGTCCCTG
ACCACCTCGCTGCGGTTCGACGGCGCCATCAACGTGGACATAACCGAGTTCCAGACCAAC
CTGGTGCCGTACCCGAGGATCCACTTCATGCTCTCTTCCTACGCGCCCATCATCTCCGCG
GAGAAGGCCTTCCATGAGCAGCACTCCGTCCCCGAGATCACCAACTCTGTGTTCGAGCCG
TCGAGCGTCATGGCCAAGTGCGACCCCCGGCACGGCAAGTACATGGCCTGCTGCCTCATG
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TACGCCAAGCGCGCGTTCGTGCACTGGTACGTCGGGGAAGGGATGGAGGAGGGGGAGTTC
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GGTGAAGACGACGAGGACGAAGGCGATGAGTACTGAGTTGATCAAGCATGGGTACGTGCT
TAATTGGGTGGCAGTGTTGTGCACTTCATGCCGTATGTTGTTCTTCTCTGTCCCTGTTTG
CTTGCCGTTGCATCTGTGATGTTCGGTGGTTAGCAGAGTACAAAAATTGTCAGATTGGTT
CGGGTGCACTGACGGCTCGGTGCCACATAGTCTGTGAGGAGGTGGTATCGATACATCATA
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>TUBA-5-3   (TUBA-5D)
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CTTCCGAGGCGACAGAGCTGCACAAGAGCAGAGCAGAGCATAGGGAGAGAGAGAGAGAGA
GAGAGAGAGATGAGGGAGATCATAAGCATCCACATCGGGCAGGCGGGCATCCAGGTGGGG
AATTCCTGCTGGGAGCTCTACTGCCTCGAGCATGGCATCCAGCCCGACGGCCTCATGCCC
AGTGACACCTCGGTTGGGGTTGCCAAGGATGCATTCAACACGTTCTTCAGCGAAACGGGT
TCAGGGAAGCACGTTCCGAGGGCCTTGTTCGTTGATCTGGAGCCCACGGTCATCGACGAG
GTGAGGACGGGGGCATATCGCCAGCTCTTCCATCCAGAGCAGCTCATCTCCCACAATGAA
GATGCTGCTAACAACTTTGCCCGCGGACACTACACAGTTGGAAGAGAAGTAGTGGACCTT
TGCCTTGACCGGATCAGAAAATTGGCGGACAACTGCACTGGTCTCCAAGGCTTCTTGGTT
TTCAATGCTGTCGGTGGTGGAACTGGCTCAGGGCTTGGTTCATTGCTTCTGGAGCGCCTA
TCGGTCGACTATGGCAGGAAATCGAAGCTCGGTTTCACCATCTATCCTTCACCACAGATT
TCGACGGCCGTTGTGGAGCCTTACAACAGCGTGCTGTCGACCCACTCGCTGATCGAGCAC
ACCGACGTGGTGGTGCTCCTGGACAACGAGGCCATCTACGACATCTGCAAGAGGTCCCTG
GACATCGAGCGCCCGACCTACACCAACCTGAACCGGCTGATATCCCAGGTGATATCGTCG
CTGACCACCTCGCTGCGGTTCGACGGCGCCATCAACGTGGACATAACCGAGTTCCAGACC
AACCTGGTGCCGTACCCGCGGATCCACTTCATGCTCTCCTCCTACGCGCCCATCATCTCC
GCGGAGAAGGCCTTCCACGAGCAGCACTCCGTCCCCGAGATCACCAACTCCGTGTTCGAG
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AAGAGGACCGTGCAGTTCGTCGACTGGTGTCCGACTGGGTTCAAGTGCGGGATAAACTAC
CAGCCGCCGACGGTGGTTCCGGGAGGGGACCTGGCCAAGGTGCGGCGGGCGGTGTGCATG
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TGCATCTGTGATGTTCGGTGGTTAGCAGAGTACAAAAATTGTCAAATTGGTTCGTGTGCT
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>Ta_TUBA-1-1
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RVRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTIYPSPQVSTA
VVEPYNSVLSTHSLLEHTDVAVLLDNEAIYDICRRSLDIERPTYTNLNRLISQIISSLTT
SLRFDGAINVDVTEFQTNLVPYPRIHFMLSSYAPVISAEKAYHEQLSVPEITNAVFEPSS
MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTVQFVDWCPTGFKCGINYQPP
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RVRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTIYPSPQVSTA
VVEPYNSVLSTHSLLEHTDVAVLLDNEAIYDICRRSLDIERPTYTNLNRLISQIISSLTT
SLRFDGAINVDVTEFQTNLVPYPRIHFMLSSYAPVISAEKAYHEQLSVPEITNAVFEPSS
MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTVQFVDWCPTGFKCGIKYQPP
SVVPGGDLAKVQRAVCMISNNTAVAEVFLRIDHKFDLMYAKRAFVHWYVGEGMEEGEFSE
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>Ta_TUBA-1-3
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RVRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTIYPSPQVSTA
VVEPYNSVLSTHSLLEHTDVAVLLDNEAIYDICRRSLDIERPTYTNLNRLISQIISSLTT
SLRFDGAINVDVTEFQTNLVPYPRIHFMLSSYAPVISAEKAYHEQLSVPEITNAVFEPSS
MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTVQFVDWCPTGFKCGINYQPP
SVVPGGDLAKVQRAVCMISNNTAVAEVFSRIDHKFDLMYAKRAFVHWYVGEGMEEGEFSE
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>Ta_TUBA-2-1
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RIRKLSDNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
VVEPYNSVLSTHSLLEHTDVSILLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTA
SLRFDGALNVDVNEFQTNLVPYPRIHFMLSSYAPVISAEKAYHEQLSVAEITNSAFEPSS
MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTIQFVDWCPTGFKCGINYQPP
GVVPGGDLAKVQRAVCMISNSTSVVEVFSRIDHKFDLMYAKRAFVHWYVGEGMEEGEFSE
AREDLAALEKDYEEVGAEFDEGEDGDEGDEY-
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>Ta_TUBA-2-2
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RIRKLSDNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
VVEPYNSVLSTHSLLEHTDVSILLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTA
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MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTIQFVDWCPTGFKCGINYQPP
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AREDLAALEKDYEEVGAEFDEGEDGDEGDEY-
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>Ta_TUBA-2-3
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RIRKLSDNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
VVEPYNSVLSTHSLLEHTDVSILLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTA
SLRFDGALNVDVNEFQTNLVPYPRIHFMLSSYAPVISAEKAYHEQLSVAEITNSAFEPSS
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>Ta_TUBA-3-1
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RIRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
VVEPYNSVLSTHSLLEHTDVAVLLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTA
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MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTIQFVDWCPTGFKCGINYQPP
SVVPGGDLAKVQRAVCMISNSTSVVEVFSRIDHKFDLMYAKRAFVHWYVGEGMEEGEFSE
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>Ta_TUBA-3-2
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RIRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
VVEPYNSVLSTHSLLEHTDVAVLLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTA
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AREDLAALEKDYEEVGAEFDEGEDGDEGDEY-
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>Ta_TUBA-3-3
MRECISIHIGQAGIQVGNACWELYCLEHGIQPDGQMPGDKTVGGGDDAFNTFFSETGAGK
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VVEPYNSVLSTHSLLEHTDVAVLLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTA
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RIRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
VVEPYNSVLSTHSLLEHTDVSILLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTT
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>Ta_TUBA-4-2
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VVEPYNSVLSTHSLLEHTDVSILLDNEAIYDICRRSLDIERPTYTNLNRLVSQVISSLTT
SLRFDGALNVDVTEFQTNLVPYPRIHFMLSSYAPVISAEKAYHEQLSVSEITNSAFEPSS
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>Ta_TUBA-4-3
MRECISIHIGQAGIQVGNACWELYCLEHGIQPDGQTNGDKTIGGGDDAFNTFFSETGAGK
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MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTIQFVDWCPTGFKCGINYQPP
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>Ta_TUBA-5-1
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SLRFDGAINVDITEFQTNLVPYPRIHFMLSSYAPIISAEKAFHEQHSVPEITNSVFEPSS
VMAKCDPRHGKYMACCLMYRGDVVPKDVNSAVHSIKTKRTVQFVDWCPTGFKCGINYQPP
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>Ta_TUBA-5-2
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>Ta_TUBA-5-3
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