

Physical mapping of the wheat genome: A coordinated effort to lay the foundation for genome sequencing and develop tools for breeders

CATHERINE FEUILLET^{a,*} AND KELLYE EVERSOLE^b

^aINRA-UBP UMR 1095, Amélioration et Santé des Plantes, Domaine de Crouelle, 2 234, Avenue du Brézet, 63100 Clermont-Ferrand, France

^bInternational Wheat Genome Sequencing Consortium, Eversole Associates, 5207 Wyoming Road, Bethesda, Maryland 20816, USA

(Received 10 December 2007; accepted in revised form 12 February 2008)

ABSTRACT

The International Wheat Genome Sequencing Consortium (IWGSC) was launched in 2005 with the aim of developing the tools and resources for sequencing the hexaploid wheat genome and providing breeders with state-of-the-art tools for improving wheat. The mid-term goals are to develop a physical map of the 21 chromosomes of bread wheat cv. Chinese Spring to accelerate map-based cloning and develop new markers, increase our knowledge about wheat genome organization, and assess sequencing technologies. In this framework, the feasibility of constructing the physical map using a chromosome-based approach has been evaluated through the development of the physical map of chromosome 3B. This article provides an update on the different projects developed within the IWGSC to reach these goals as well as a progress report on the construction of the 3B physical map.

Keywords: wheat, physical mapping, sequencing, chromosome 3B, international consortium

INTRODUCTION

Genome sequences hold the key to understanding how an organism works. In the past decade, sequencing of model plant genomes, such as those of *A. thaliana* and rice, has revolutionized our understanding of plant biology but it has not yet efficiently translated into the improvement of major crop species such as maize, wheat, or barley. Moreover, because comparative genomic studies have revealed the limits of conservation between rice and the other cereal genomes, genomic resources and programs have been developed for maize, sorghum, wheat, and barley to serve as the foundation for future genome sequencing and the acceleration of genomic-based improvement of these critically important crops.

In November 2003, a USDA–NSF funded international workshop of wheat geneticists and sequencing specialists set the basis for an international collaboration on wheat genome sequencing (Gill et al., 2004). The necessity of sequencing the wheat genome, the current understanding of its structure and sequence, the opportunity to sequence a wild diploid ancestor or the cultivated hexaploid bread wheat genome, and the different strategies for sequencing the genome were discussed and the first objectives defined. These objectives were (i) the construction of an accurate, sequence-ready physical (BAC contig) map of the reference hexaploid

*Author to whom correspondence should be addressed.
E-mail: catherine.feuillet@clermont.inra.fr

wheat genotype 'Chinese spring' for which large genetic stocks and aneuploid lines are available, (ii) the exploration of different strategies for gene enrichment, and (iii) assessing the feasibility of a chromosome-specific approach, i.e., to develop individual maps of each of the 21 chromosomes using chromosome-specific BAC libraries. A large consensus exists in the wheat community on the fact that sequencing the bread wheat genome will have a broad impact on education, new research technologies for large, repetitive, and polyploid genomes, and widespread application to the agricultural industry. Due to its recent history (Feldman, 2001), bread wheat is also one of the best models for understanding polyploidization, a common feature of plant genomes. Following these discussions, the International Wheat Genome Sequencing Consortium (IWGSC; www.wheatgenome.org) was launched in 2005 with the aim of "advancing agricultural research for wheat production and utilization by developing DNA-based tools and resources that result from the complete sequence of the common (hexaploid) wheat genome while ensuring that these tools and the sequence are available for all to use without restriction and without cost". The overall strategy of the consortium is to capture immediately significant outputs for wheat breeders and the wheat industry at large in parallel to continued advancements in basic research on the wheat genome.

In this strategy of providing the greatest resources to enhance wheat production while also advancing our basic understanding of the hexaploid wheat genome, the establishment of a physical map of the 21 wheat chromosomes is a priority to facilitate the map-based isolation of the hundreds of genes and quantitative trait loci (QTL) for traits of agronomic importance that have been identified over the past decades on the wheat genetic maps. This will accelerate immediately wheat improvement through the delivery of "perfect" markers for wheat breeding and the identification of new and favorable alleles from the huge reservoir of genetic resources that are present in seed banks all over the world. It will allow for a better understanding of the molecular basis of essential traits such as yield, quality, consistency, and stress (biotic and abiotic) resistance for tomorrow's agriculture, and permit the development of cisgenic wheat, i.e., wheat plants carrying wheat transgenes (Jacobsen and Schouten, 2007). Moreover, in the mid-term, the construction of the physical map is an important prerequisite for sequencing the wheat genome regardless of the ultimate sequencing strategy selected. Sequencing a 17-Gb polyploid genome remains expensive, even with the new emerging sequencing technologies (GSFLX, Illumina Solexa, SOLID, etc....) that offer a dramatic improvement of the cost

efficiency in producing sequences. Moreover, the short read length offered currently by these techniques and the large amount of repetitive sequences present in the wheat genome make de novo assembly of nongenic low copy regions extremely difficult if not impossible (Wicker et al., 2006). Recent results of whole genome functional analyses such as the characterization of 1% of the human genome in the ENCODE project (Birney et al., 2007) clearly indicate that intergenic regions and regions formerly considered as "junk DNA" are likely as important as protein coding regions in the regulation of genome expression. Therefore, it is essential to define a strategy that will provide a high-quality wheat genome sequence which contains all information necessary to understand the molecular basis of wheat plant biology and allows its optimal exploitation for crop improvement. While waiting for the implementation of new technologies with increased read length and cost-effectiveness over the next few years, a targeted, systematic sequencing of the agronomically important regions in the genome could be initiated on a large scale as the physical maps become available and the regions carrying the traits of interest are mapped genetically at high resolution.

The roadmap of the IWGSC for the next four years was established and agreed to by the IWGSC coordinating committee and the broader wheat community at the ITMI meeting at Victor Harbour, Australia, in August 2006. The roadmap calls for the completion of the physical map of the D genome of the wild diploid *Ae. tauschii* so that it can be used to complete the construction of the physical maps for the 7 chromosomes of the D genome of hexaploid wheat. The IWGSC also aims to complete the maps of the remaining homoeologous A and B chromosome groups. The D genome project was initiated in 2001 (<http://wheat.pw.usda.gov/PhysicalMapping/>) and has established efficient protocols and software to perform BAC fingerprinting and contig assembly (Luo et al., 2003) that are used now for the hexaploid wheat genome project. For the hexaploid wheat genome, currently a whole-genome approach to develop a physical map is not seen to be practical, timely, or cost-effective as this would require fingerprinting and specific assembly of homoeologous BAC contigs comprising over 2 million BAC clones. Specifically, anchoring the homoeologous BAC contigs from such a large and complex set of contigs to their chromosomal location would be difficult if not impossible for all contigs and could result in significant cost increases. Therefore, after various discussions with experts from the large sequencing centers, the international community determined that the construction of physical maps in hexaploid wheat should be performed following a chromosome-specific strategy. This approach

relies on the recent improvement of chromosome sorting and BAC library construction technologies (Vrana et al., 2000; Kubalaková et al., 2002; Chalhoub et al., 2004) that permit the construction of chromosome-specific BAC libraries (Safar et al., 2004) that are used already or will be used in different IWGSC projects.

This article provides an overview of the IWGSC activities and projects as well as a progress report from the pilot project that aims at the construction of an anchored physical map of chromosome 3B from hexaploid wheat following the chromosome-based approach.

ACTIVITIES AND PROJECTS OF THE CONSORTIUM

As an international industry, academic, and governmental agency collaboration, the IWGSC is committed to providing wheat breeders and industry state-of-the-art tools and technologies that enable profitability throughout the wheat industry. The consortium is governed by a coordinating committee, comprised of scientific and financial contributors who support sequencing the bread wheat genome, and an executive director. General membership in the consortium is open to anyone, and all meetings are open to the public. Business meetings and workshops are held in conjunction with most major international plant genomics meetings.

To ensure the rapid delivery of tools to breeders, the IWGSC identifies short-term and long-term strategic goals, advocates for sequencing the wheat genome, coordinates international scientific efforts to build resources for wheat, and secures funding for collaborative efforts aimed at meeting identified goals. By implementing a milestone-based strategy, the consortium delivers products and tools while working towards the ultimate goal of a sequenced bread wheat genome. Projects coordinated and endorsed by the IWGSC fall within two broad categories: physical mapping (construction of physical maps for the D-genome and for hexaploid wheat) and sequencing (the development of the resources necessary for sequencing and the testing of technologies to determine the best method for sequencing). The following provides additional information on IWGSC coordinated and endorsed projects:

Physical mapping

Completed projects

- 3B—Led by C. Feuillet (INRA, France), this has served as the pilot project for developing a physical map of a flow-sorted chromosome and is discussed at length below. At this time, the project has yielded a 9.6X chromosome landing ready physical map of chromosome 3B of ‘Chinese spring’.

Funded projects

- 1A, 1B, 3D, (3Bv2)—Triticeae Genome project funded by the EU Commission under FP7—Coordinated by C. Feuillet (INRA, France) with 17 EU partners, this European project will complete the physical maps of group 1 and 3 chromosomes in wheat (and barley). Map-based cloning of targeted QTL, molecular breeding and bioinformatics platforms will be developed within the framework of this project as well.
- 2AL—Led by K. Singh (Punjab Agricultural University, India), this project for the construction of the physical map of the long arm of chromosome 2A is funded by DBT (Department of Biotechnology), of the Indian Ministry of Science and Technology.
- 2D—Led by J. Jia (KL-CGB, CAAS, China), funded by the CAAS (Chinese Academy of Agricultural Sciences), this project will develop a physical map of chromosome 2D.
- 3AS/3AL—Led by B. Gill (Kansas State University, USA) and funded by the USDA (CSREES-NRI), these projects are developing anchored physical maps of the short and long arms of chromosome 3A. (Sequencing components of the 3AS project are discussed under “sequencing” below.)
- 4A—Led by J. Dolezel (Institute for Experimental Botany, Czech Rep.), this project resulted in the construction of a BAC library and funding is being sought for physical mapping.
- 5A—Led by L. Cattivelli (Experimental Institute for Cereal Research, Italy) and funded by the Agricultural Research Council of Italy, this project will develop a physical map of chromosome 5A.

Projects awaiting final review by funding agencies

- 1,4,6 D of hexaploid and all of *Ae. tauschii*—“D-Genome project”—Led by J. Dvorak, B. Gill, and O. Anderson (UC-Davis, KSU, and USDA-ARS, respectively), this project, submitted to the US National Science Foundation, will complete the *Ae. tauschii* physical map and, subsequently, be used as a framework for developing physical maps of chromosomes 1,4, and 6 of the D genome of ‘Chinese spring’ and all other D genome chromosomes of hexaploid wheat taken by other groups (see below).

Projects Planned to be submitted

- 2B—Led by M. Bevan (JIC, UK), this project will develop the physical map of chromosome 2B of ‘Chinese spring’ and will be submitted to funding agencies in late 2008 or 2009.
- 4B—Led by M. Nachit (ICARDA, Syria) & D. Habash (Rothamstead Research, UK) the development of the physical map of chromosome 4B will be

included in the proposed 4Phoenicia project that will build scientific capabilities for the Mediterranean region to underpin wheat genomics for sustainable agriculture.

- 5B—Led by E. Salina (Institute of Cytology & Genetics, Russia), this project will develop, in collaboration with European and US partners, the physical map of chromosome 5B of ‘Chinese spring’ and will be submitted to funding agencies in 2008 or 2009.
- 5D—Led by H. Budak (Sabanci University, Turkey), this project, to establish a physical map of chromosome 5D of ‘Chinese spring’, is under development and will be submitted to funding agencies in 2008.
- 7D (A,B)—Led by R. Appels (Murdoch University, Australia), this project to establish a physical map of chromosome 7D of ‘Chinese spring’ is under development.. Projects to develop the physical maps of chromosomes 7A and 7B will be prepared subsequently in Australia.

Chromosomes for which leaders have yet to be identified

Scientific leadership and funding is being sought for 2 remaining chromosomes: 6A and 6B. A summary of the current status of the physical mapping projects is

provided in Fig. 1 and updates are available at the IWGSC website www.wheatgenome.org.

Sequencing

1. Sequencing of Megabase sized contigs on chromosome 3B.
 - a. C. Feuillet, (INRA France): Two projects (supported by the ANR-Genoplante and the Genoscope) are underway currently to sequence more than 20 Mb of BAC contigs distributed in different regions of chromosome 3B.
 - b. R. Appels (Murdoch University, Australia): Sequencing of two Mb-sized contigs located on chromosome 3BS (supported by GRDC) is currently underway.
2. 3AS Sample Sequencing project - Led by B. Gill (KSU, USA) and funded by the USDA (CSREES-NRI), this project will generate 18.4 Mb of sequence from the chromosome 3AS BAC libraries, sequence 48 targeted BAC clones, and BAC end sequence 10,000 random clones. The second sequencing component of this project is to compare the 3AS BAC sequences with sequences from homoeologous chromosome arm 3BS.

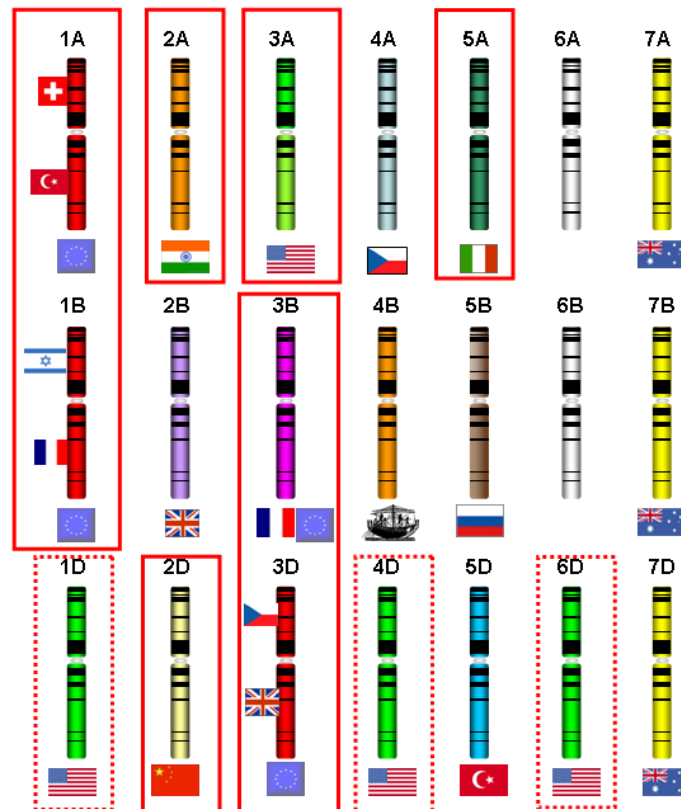


Fig. 1. Project leadership status for chromosome-based physical maps of bread wheat. Chromosomes boxed in red represent funded (solid line) or pending (interrupted line) projects in August 2008 (for an update visit the IWGSC website at www.wheatgenome.org).

3. New generation sequencing technologies. A number of small projects are underway currently to evaluate the feasibility of using new sequencing technologies to accelerate marker development and reduce MTP sequencing cost while maintaining quality and without losing access to the nongenic but yet relevant sequences.

Comparative genomics studies

1. Wheat–Brachypodium–Rice Comparative Analysis—Led by K. Devos (USA-UG) funded by NSF Plant Genome Research Program, this project will compare 22 Mb of *Ae. tauschii* with the *B. distachyon* sequence, extend the comparative analysis to the A&B genomes of ‘Chinese spring’, and quantify the degree of colinearity between *Ae. tauschii*, *B. distachyon*, wheat, and rice.
2. ANR EXEGESE project (C. Feuillet, INRA) for comparative analysis at the Rph7 locus on chromosome 3BS between rice, wheat, barley, sorghum, maize, and brachypodium
3. 3AS Physical Map and Sample Sequencing project - Led by B. Gill (KSU, USA) and funded by the USDA (CSREES-NRI), this project will compare the 3AS BAC sequences (discussed above) with sequences from homoeologous chromosome arm 3BS.

ESTABLISHING A PHYSICAL MAP OF THE WHEAT GENOME USING A CHROMOSOME-BASED APPROACH: PROGRESS REPORT OF A PILOT PROJECT ON CHROMOSOME 3B

The availability of a BAC library specific for chromosome 3B (Safar et al., 2004) provided an opportunity to test the feasibility of constructing a physical map of the hexaploid wheat genome with a chromosome-based approach (Gill et al., 2004). To establish the physical map of chromosome 3B, high information content fingerprints (HICF) were generated from the 67,968 BAC clones of the 3B BAC library using a modified SNaP-shot protocol (Luo et al., 2003) and were assembled into contigs using the FPC (FingerprintPrinted Contigs) software (Soderlund et al., 2000). Using a single ABI 3730 XL capillary sequencer, it was possible to fingerprint all BAC clones of the 3B BAC library within 10 weeks. To date, the physical map consists of about 1000 contigs that cover nearly 80% of the chromosome (Paux et al., unpublished data).

The most laborious task in the construction of a physical map is the anchoring of the physical contigs to the genetic map. In most of the plant genome physical mapping projects published so far, anchoring has been performed through hybridization with RFLP (Restriction

Fragment Length Polymorphism) and overgo probes that correspond to gene sequences and/or by PCR using microsatellites, CAPS (Cleaved Amplified Polymorphic Sequence) or STS (Sequence Tagged Site) markers. In order to anchor the 3B physical contigs to the genetic map, PCR screening was performed with more than 2,000 molecular markers following two approaches. The first strategy used markers (SSR, EST) that are located on chromosome 3B genetic or cytogenetic maps (Munkvold et al., 2004; Sourdille et al., 2004) to screen the BAC library and anchor the corresponding FPC contigs. Under the second approach, markers were derived from the BAC contigs themselves and mapped onto the genetic and/or cytogenetic maps. To do that, we generated 19,400 BAC-end sequences (BES) representing a cumulative length of nearly 11 Mb (1.1% of the chromosome length) distributed among the contigs of chromosome 3B (Paux et al., 2006). The systematic identification of junctions between transposable elements (TEs) allowed the development of RBIP (Retrotransposon-Based Insertion Polymorphism)-type markers (Flavell et al., 1998) that consist of PCR amplicons spanning the TE junctions. The main advantage of this type of markers is that they are (i) very abundant, as more than 80% of the wheat genome consists of transposable elements that are mostly nested into each other; (ii) mostly unique in the genome since there is a low chance that the same insertion event occurred at another locus; and (iii) genome specific, as they originate from a specific chromosome sequence (Paux et al., 2006) More than 700 ISBP (Insertion Site Based Polymorphism) markers have been developed across chromosome 3B and have been used for anchoring the contigs on the genetic and cytogenetic maps (Paux et al., unpublished data). Finally, a minimal tiling path (MTP) has been established for chromosome 3B that can be used now for structural and functional studies on chromosome 3B.

PCR-based methods are very cost effective for anchoring of physical maps to genetic maps. However, with large genomes such as wheat, the number of PCR required to identify the BAC clones bearing a single target marker sequence can become very high. To reduce significantly the number of reactions, several pooling strategies have been proposed, all of which rely on concentrating the BAC library into pools that represent overlapping groups of clones. The most powerful is six-dimensional pooling (Klein et al., 2000), which reduces the number of PCR needed to get the complete unambiguous address of a BAC clone in a single round, by a factor of 384 compared to non-pooling strategies (Yim et al., 2007). Six-dimensional pools, though, are expensive to produce and require specific automated platforms that are not available in every laboratory. Be-

cause of these limitations, lower dimensional pools such as three-dimensional (3D) pools that consist of pools of 384-well plates, rows, and columns are used often. One of the drawbacks of 3D-pools is an increased number of ambiguous BAC addresses after screening. Additional PCR reactions must then be performed to eliminate the ambiguity and this, therefore, reduces the efficiency of this pooling strategy. To increase the efficiency of anchoring and reduce the cost of anchoring during the construction of the 3B chromosome physical map, a new software called “*elephant*” (*electronic physical map anchoring tool*) has been developed (Paux et al., 2007). This freely available Perl script combines BAC contig information generated by FPC with results from BAC library pools screened to identify BAC addresses with a minimal amount of PCR reactions. Using *elephant* during the construction of the 3B physical map, we have shown that a one-dimensional pool screening can be sufficient to anchor a BAC contig, while reducing the number of PCR by 384 fold.

Thus, these preliminary data on the construction of the first chromosome-based physical map of a hexaploid wheat chromosome show that:

- The chromosome-based strategy is feasible and that even with a single enzyme (*HindIII*) BAC library covering 8× of the chromosome, it is possible to generate contigs ranging from 300 kb to 4 Mb that are suitable for map-based cloning and cover about 80% of the chromosome;
- Systematic BAC-end sequencing of the MTP is a powerful approach to develop genome-specific markers from each of the wheat chromosomes and chromosome arms for contig anchoring and for breeding;
- The 10% of contaminating clones that are inherent to the construction of BAC libraries from sorted chromosomes do not interfere with the establishment of the physical contigs; and
- A single lab equipped with a capillary sequencer and basic robotic equipment can establish a physical map of a wheat chromosome.

CONCLUSION

The successful pilot project to develop a physical map on chromosome 3B of ‘Chinese spring’ by a single laboratory has opened up the route for the international collaborative effort on the 20 remaining chromosomes of hexaploid wheat. As indicated above, the effort is underway to construct the physical maps for the remaining chromosomes of hexaploid wheat. Project leaders have been identified for most of the chromosomes and several projects have been funded. The IWGSC will continue to seek sponsorship and funding for the development of physical maps

of all of the chromosomes of hexaploid wheat to ensure that the entire wheat industry can begin to rapidly exploit genomic information while efforts are underway to obtain a complete sequence of bread wheat.

Concurrently with the development of the physical maps, the IWGSC is exploring the feasibility of using the new sequencing technologies for the construction of physical maps and for sequencing the bread wheat genome.

REFERENCES

- Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigo, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., Dermitzakis, E.T., et al. 2007. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 447: 799–816.
- Chalhoub, B., Belcram, H., Caboche, M. 2004. Efficient cloning of plant genomes into bacterial artificial chromosome (BAC) libraries with larger and more uniform insert size. *Plant Biotechnol. J.* 2: 181–188.
- Feldman, M. 2001. In: Bonjean, A.P., Angus, W.J., eds. *The world wheat book: a history of wheat breeding*. Lavoisier Publishing, Paris, pp. 3–56.
- Flavell, A.J., Knox, M.R., Pearce, S.R., Ellis, T.H. 1998. Retrotransposon-based insertion polymorphisms (RBIP) for high throughput marker analysis. *Plant J.* 16: 643–650.
- Gill, B.S., Appels, R., Botha-Oberholster, A.-M., Buell, C.R., Bennetzen, J.L., Chalhoub, B., Chumley, F., Dvorak, J., Iwanaga, M., et al. 2004. A workshop report on wheat genome sequencing: international genome research on wheat consortium. *Genetics* 168: 1087–1096.
- Jacobsen, E., Schouten, H.J. 2007. Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. *Trends Biotechnol.* 25: 219–223.
- Klein, P.E., Klein, R.R., Cartinhour, S.W., Ulanich, P.E., Dong, J., Obert, J.A., Morishige, D.T., Schlueter, S.D., Childs, K.L., et al. 2000. A high-throughput AFLP-based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. *Genome Res.* 10: 789–807.
- Kubalakova, M., Vrana, J., Cihalikova, J., Simkova, H. 2002. Flow karyotyping and chromosome sorting in bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 104: 1362–1372.
- Luo, M.C., Thomas, C., You, F.M., Hsiao, J., Ouyang, S., Buell, C.R., Malandro, M., McGuire, P.E., Anderson, O.D., et al. 2003. High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis. *Genomics* 82: 378–389.
- Munkvold, J.D., Greene, R.A., Bermudez-Kandianis, C.E., La Rota, C.M., Edwards, H., Sorrells, S.F., Dake, T., Benschler, D., Kantety, R., et al. 2004. Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. *Genetics* 168: 639–650.
- Paux, E., Roger, D., Badaeva, E., Gay, G., Bernard, M., Sourdille, P., Feuillet, C. 2006. Characterizing the composition

- and evolution of homoeologous genomes in hexaploid wheat through BAC-end sequencing on chromosome 3B. *Plant J.* 48: 463–474.
- Paux, E., Legeai, F., Guilhot, N., Adam-Blondon, A.F., Alaux, M., Salse, J., Sourdille, P., Leroy, P., Feuillet, C. 2007. Physical mapping in large genomes: accelerating anchoring of BAC contigs to genetic maps through in silico analysis. *Funct. Integrative Genomics* 8: 29.
- Safar, J., Bartos, J., Janda, J., Bellec, A., Kubalaková, M., Valarik, M., Pateyron, S., Weiserová, J., Tusková, R., et al. 2004. Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosomes from bread wheat. *Plant J.* 39: 960–968.
- Soderlund, C., Humphray, S., Dunham, A., French, L. 2000. Contigs built with fingerprints, markers, and FPC V4.7. *Genome Res.* 10: 1772–1787.
- Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G.L., Gay, G., Qi, L., Gill, B.S., Dufour, P., Murigneux, A., et al. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Funct. Integrative Genomics* 4: 12–25.
- Vrana, J., Kubalaková, M., Simkova, H., Cihalikova, J., A. Lysak, M., Dolezel, J. 2000. Flow sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). *Genetics* 156: 2033–2041.
- Wicker, T., Schlagenhauf, E., Graner, A., Close, T.J., Keller, B., Stein, N. 2006. 454 sequencing put to the test using the complex genome of barley. *BMC Genomics* 7: 275.
- Yim, Y.S., Moak, P., Sanchez-Villeda, H., Musket, T.A., Close, P., Klein, P.E., Mullet, J.E., McMullen, M.D., Fang, Z., et al. 2007. A BAC pooling strategy combined with PCR-based screenings in a large, highly repetitive genome enables integration of the maize genetic and physical maps. *BMC Genomics* 8: 47.