Rye DArT arrays effectively anchor BACs onto genetic map

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Background

A physical map is essential for *de novo* sequencing and provides the necessary context for exploring genome structure and organization. A key point with respect to physical map construction and exploitation is the anchoring of BAC clones to high-resolution genetic maps - integration of physical and genetic maps. Many of the currently established anchoring techniques tend to be low-throughput or involve many processing steps. One of the proposed methods to speed up addressing of genetic markers to individual BAC clones involves the use of microarrays (Luo et al. 2009, Liu et al.2011).
In this study we applied Diversity Array Technology to anchor clones from the BAC library of the rye inbred line L318 on the high density, integrated genetic map of the rye genome and used the available sequence data (DArT marker and BAC clone sequences) to verify bioinformatically the reliability of the method.

Materials and Methods

- BAC library of the rye inbred line L318 consisting of 104 832 clones , with the average insert size 121 kb - genome coverage ca. 2x (Bakera et al. 2015)
- three dimensional BAC clone pooling strategy (AMPLICONexpress http://www.genomex.com/aexDownloads.php) – in this strategy individual clones from seven 384-well library plates are combined into 24 pools with every clone present in the superpool (containing all clones), two matrix plate pools, two matrix column pools, and two matrix row pools.
- BAC DNA isolation protocol involving the use of SLS (Sinnet et al. 1998, modified)
- the 11 520-clone rye DArT-genotyping array (Bolibok-Brągoszewska et al. 2010)
- compilated genetic map of the rye genome comprising 4048 DArT loci arranged in linear order (Bolibok-Brągoszewska et al. 2015), derived form the consensus rye genetic map of Milczarski et al. (2011)
- sequences of 6202 Dart Markers: 3355 sequences in 1117 bins and 2847 individual unique sequences (Bolibok-Brągoszewska et al. 2015),
- sequences of seven BAC clones (four clones included in the superpools analyzed)
- BLAST algorithms (Camacho al. 2009).

Results

- in total ca. 43 thousand BAC clones were screened (samples from 16 superpools arranged into four 96-well pates / 41% of the BAC library / 65% of the rye genome).
- 5897 DArT-BAC addresses were obtained with stringent selection criteria , this number comprised 3974 individual DArT markers (among them 1428 genetically mapped) and 2053 individual BAC clones
- over 724 DArTs were addressed to more than one BAC clone and more than one DArT was addressed to 1923 BAC clones. This should facilitate the future arrangement of BAC clones into contigs
- 748 BAC clones were anchored onto genetic map via at least one DArT marker (from 74 for 7R to 144 to for 6R. (Fig 1). The anchored BACs are dispersed throughout the genome (Fig 2).
- In most cases where multiple DArT markers were assigned to the same BAC clone, the DArT markers were found to be closely linked on the genetic map and/or were members of the same sequencing bin (Tab 1).
- in total 12 DArT markers (all sequenced) were addressed to two of the sequenced BAC clones that were included in the superpools analyzed; regions exhibiting a significant similarity (E-value < 10e-5) to the DArT markers sequences could be found in the sequences of the respective BAC clones (Tab 1).

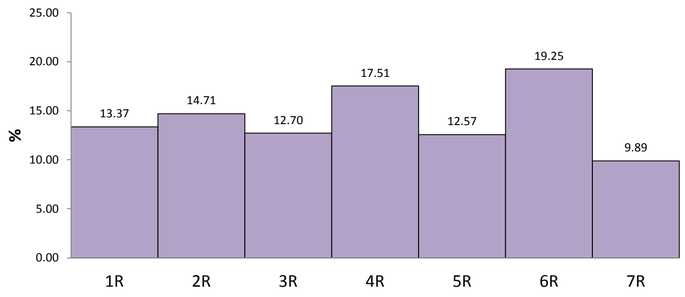

Fig 1. Percentage of BAC clones anchored on individual rye chromosomes

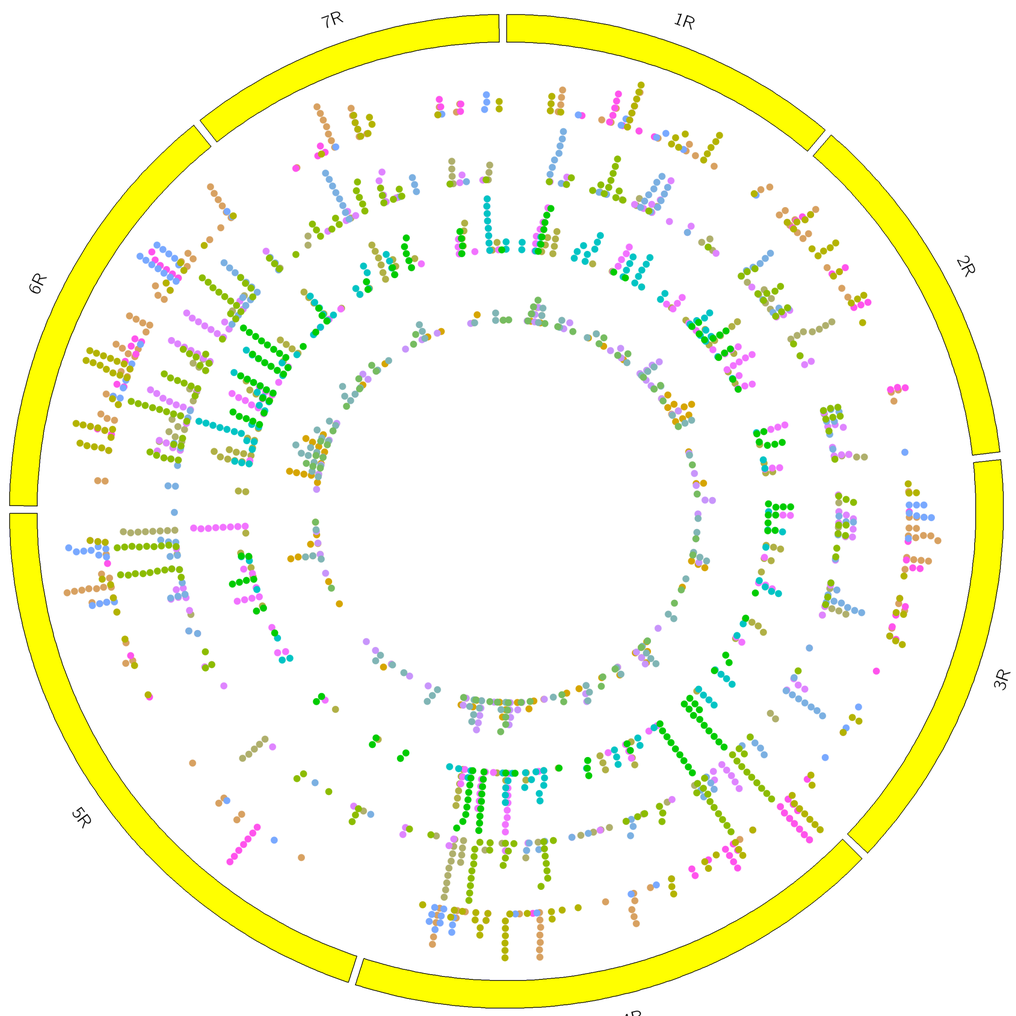

Fig2. Distribution of the anchored BAC clones (small circles) across the rye genetic map. Different colors indicate BAC clones form different superpools. Four inner tracks correspond to four 96-well plates with BAC pool samples, that were analyzed separately. The figure was generated using Circos software (Krzywinski et al. 2009)

Tab 1. Verification of the BAC to Dart addressing based on genetic map positions, membership in sequence bins, and BAC clone sequences

BAC clone	addressed DArT marker	the number of BAC to DArT addresses involving the DArT marker	the number of BAC to DArT addresses involving the BAC clone	genetic map position of the DArT marker (chromosome)	genetic map position of the DArT marker (cM)	sequencing bin membership of the DArT marker (number of DArT marker sequences in the bin)	sequenced BAC clones in which regions exhibiting similarity to the DArT marker sequence were found
S2p1H1	390135	1	10	na	na	260 (2)	S8p6I18;S2p1H1
S2p1H1	400857	1	10	4R	279.4	260 (2)	S8p6I18;S2p1H1
S2p1H1	399265	1	10	4R	278.5	410 (5)	S8p6I18;S2p1H1
S2p1H1	505672	1	10	4R	278.9	410 (5)	S8p6I18;S2p1H1
S2p1H1	401848	1	10	4R	278.5	410 (5)	S8p6I18;S2p1H1
S2p1H1	401336	1	10	4R	278.3	410 (5)	S8p6I18;S2p1H1
S2p1H1	508878	1	10	4R	278.8	410 (5)	S8p6I18;S2p1H1
S2p1H1	401713	1	10	4R	278.5	unique sequence	S8p6I18;S2p1H1
S2p1H1	402360	1	10	na	na	unique sequence	S8p6I18
S2p1H1	505181	1	10	na	na	unique sequence	S8p6I18;S2p1H1
S4p6F8	389569	3	2	4R	205.7	unique sequence	-
S4p6F8	402162	1	2	na	na	unique sequence	S32p4E22;S33p1K4;S4p6F8;S15p4I13

Conclusion

Obtained results indicate that Diversity Array Technology enables high throughput and reliable rye DArT to BAC addressing and, in consequence, BAC clone anchoring onto genetic map .

The presented approach could thus greatly facilitate physical map construction in rye

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