THE POTENCIAL OF LOW-COPY FISH IN PHYSICAL MAPPING

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Background

The process of creating high resolution physical maps relies on ordering contigs of overlapping BAC clones and integration with genetic maps. Unfortunately, the ordering contigs is very difficult in the region with highly reduced frequency of recombination, where genetic maps suffer from the poor resolution. The existence of non-recombining regions underline the necessity of additional approach, which could help to overcome this imperfection. FISH (fluorescence *in situ* hybridization) is one of the possible techniques, which is not limited by the presence of crossing overs and could increase the number of the markers in these problematic areas. In this work, we concentrated on mapping of short sequences and our results indicate, that single-copy FISH in barley is feasible. Furthermore, this progress opens the possibilities also for other application. Recently, genomes or their portions are massively sequenced and FISH with short, repeat-free probes, is an attractive alternative for anchoring contigs and could facilitates the genome sequence assembling.

Plant material

- *Hordeum vulgare* L. (2n=14), cv. Morex

Target DNA:
- Mitotic metaphase and prometaphase chromosome plates prepared from synchronized root tip meristem cells

Methods

Probe preparation

Plasmid isolation
↓
cDNA amplification
↓
Nick translation
↓
Probe purification

Chromosome preparation

Cell cycle synchronization
↓
Root tip fixation
↓
Squashing
↓
Postfixation

⇩

Fluorescence *in situ* hybridization (FISH)

⇩

Image processing

Final adjustment (Adobe Photoshop 6.0.)
↓
Chromosome straightening (image processing program ImageJ v1.43)

Conclusions

- We focused on ordering single copy sequences in non-recombining region of barley chromosome 7H

- Using FISH, we ordered 13 out of 15 cDNA clones. Signals of the clones FLbaf129g09 and FLbaf25l12 co-localized to the same positions and clones FLbaf24d09 and FLbaf25l12 provided ambiguous results.

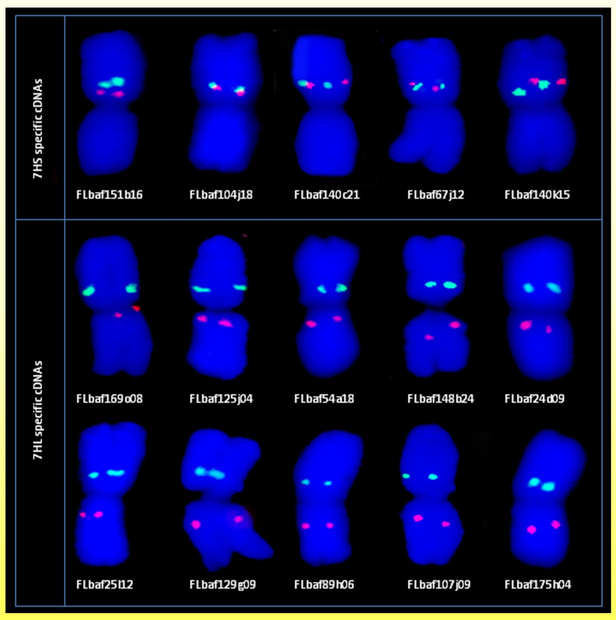
- In this study, we underline the importance of cytogenetics in physical mapping. We proved that cytogenetic tools could significantly increase the resolution of the physical map in non-recombining regions.

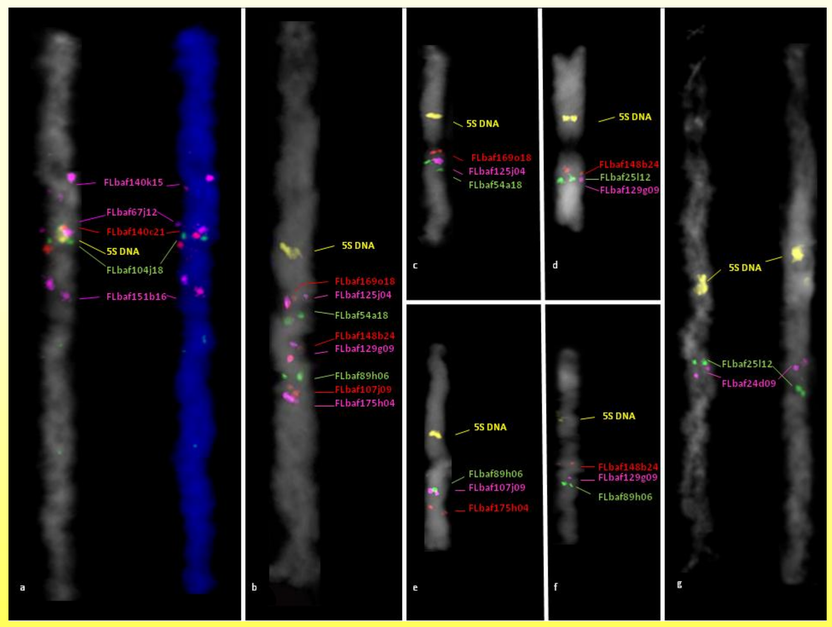
Results

1) Blast results

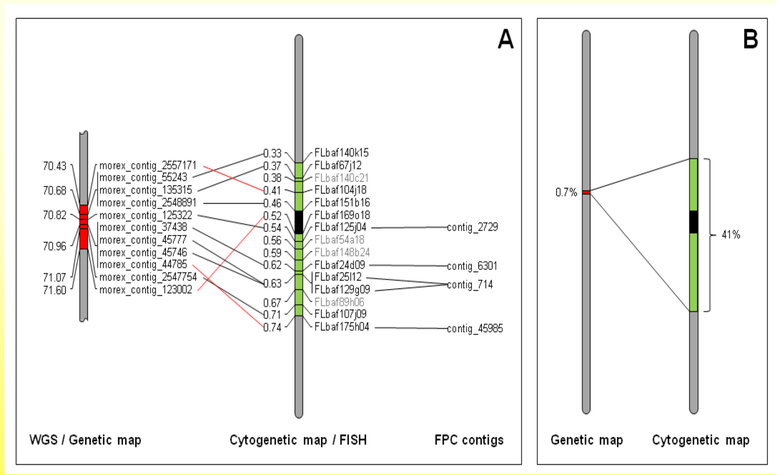
Full-length cDNA	Accession number	Length (bp)	Signal location	Relative position*	Hit name	Chromosome ID	Genetic position (cM)	FPC_contig
FLbaf140k15	AK248620	2,127	7HS	0.33	morex_contig_55243	7H	70.68	---
FLbaf67j12	AK250219	2,3	7HS	0.37	morex_contig_135315	7H	70.68	---
FLbaf140c21	AK252013	3,4	7HS	0.38	morex_contig_160232	---	---	---
FLbaf104j18	AK251038	2,475	7HS	0.41	morex_contig_2557171	7H	70.43	---
FLbaf151b16	AK252317	2,328	7HS	0.46	morex_contig_2548891	7H	70.68	---
FLbaf169o18	AK248228	2,488	7HL	0.52	morex_contig_123002	7H	71.60	---
FLbaf125j04	AK251498	3,382	7HL	0.54	morex_contig_125322	7H	70.82	contig_2729
FLbaf54a18	AK249749	2,661	7HL	0.56	morex_contig_359593	---	---	---
FLbaf148b24	AK252034	2,568	7HL	0.59	morex_contig_1564111	---	---	---
FLbaf24d09	AK249246	3,499	7HL	0.62	morex_contig_37438	7H	70.96	contig_6301
FLbaf25l12	AK249387	2,447	7HL	0.63	morex_contig_45777	7H	70.96	contig_714
FLbaf129g09	AK251673	2,295	7HL	0.63	morex_contig_45746	7H	70.96	contig_714
FLbaf89h06	AK250597	3,083	7HL	0.67	morex_contig_6028	---	---	---
FLbaf107j09	AK248217	2,038	7HL	0.71	morex_contig_2547754	7H	71.07	---
FLbaf175h04	AK252946	3,101	7HL	0.74	morex_contig_44785	7H	70.96	contig_45985

2) FISH mapping





3) Barley chromosome 7H comparative map



A: Comparison of the cytogenetic map and genetic order of fl-cDNA (TIBGSC, 2012). We corrected the position of three cDNAs using FISH.

B: According to our results, the locus which was considered to represent 0,7 % of the genetic map, actually make up more than 40 % of the chromosome length .

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References: TIBGSC (2012) A physical, genetical and functional sequence assembly of the barley genome.Nature 491, 711-716