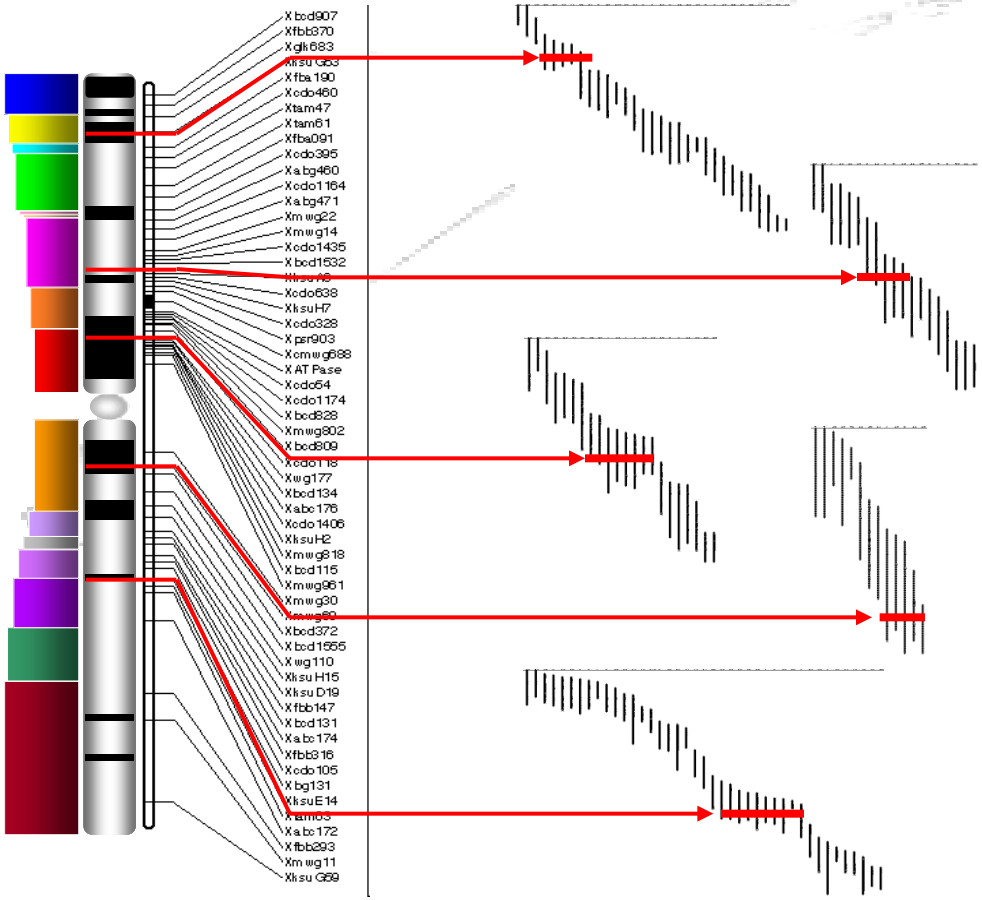
Plant & Animal Genome XVIII Conference
January 9-13, 2010
San Diego, California

IWGSC: Physical Mapping Standard Protocols Workshop

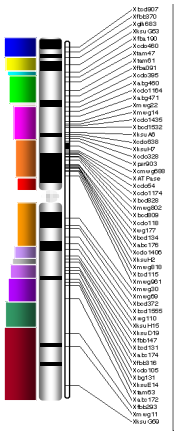
Physical map anchoring

First approach for physical contig anchoring

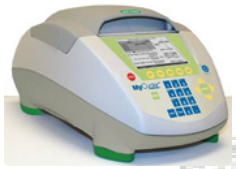
Forward anchoring: from genetic maps to contigs using genetically-mapped markers (SSRs, ESTs, RFLPs, DArTs, SNPs…)



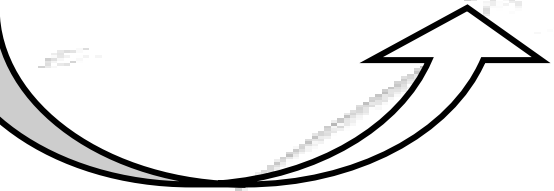
PCR screening of the 3-D MTP pools

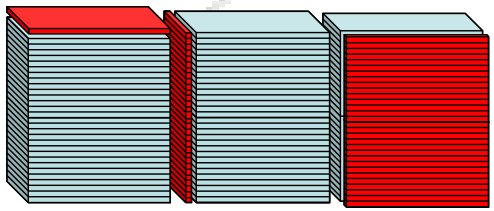


Genetically mapped markers (SSRs, ESTs, SNPs, ISBPs…)



PCR screening





3-D MTP pools

↓

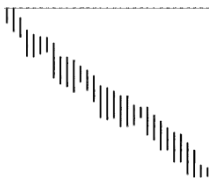
1 plate pool + 1 row pool + 1 column pool

↓

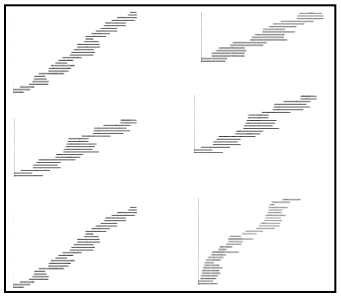
1 complete unambiguous BAC address

↓

1 physical contig



elephant: electronic physical map anchoring tool



Physical contigs
(FPC data)

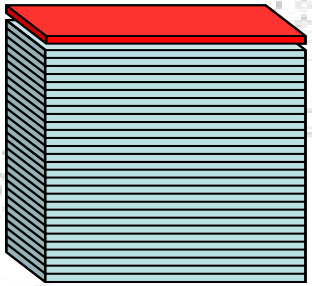
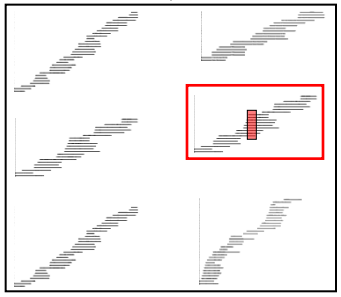


Plate pool PCR
screening results

(15 X coverage)

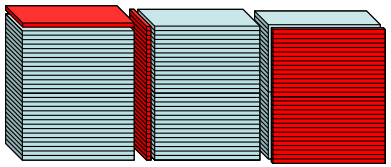
elephant



Positive contig

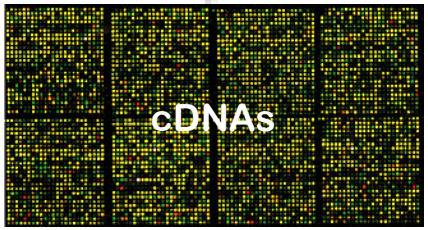
[Paux et al. (2007) Functional & Integrative Genomics]

Expression chip-based anchoring



3-D MTP pools

Hybridization
on microarrays

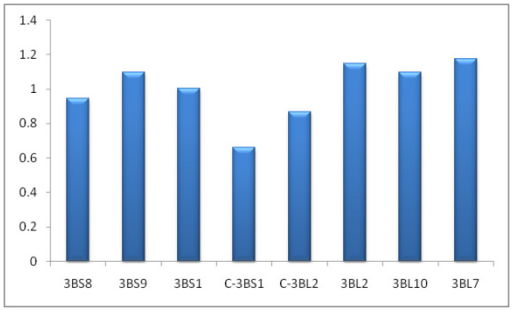


Barley Agilent 15K chip

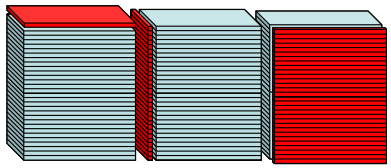
Coll. with R. Waugh & P. Hedley

743 ESTs anchored to 3B physical map

including 148 genetically mapped to barley chromosome 3H

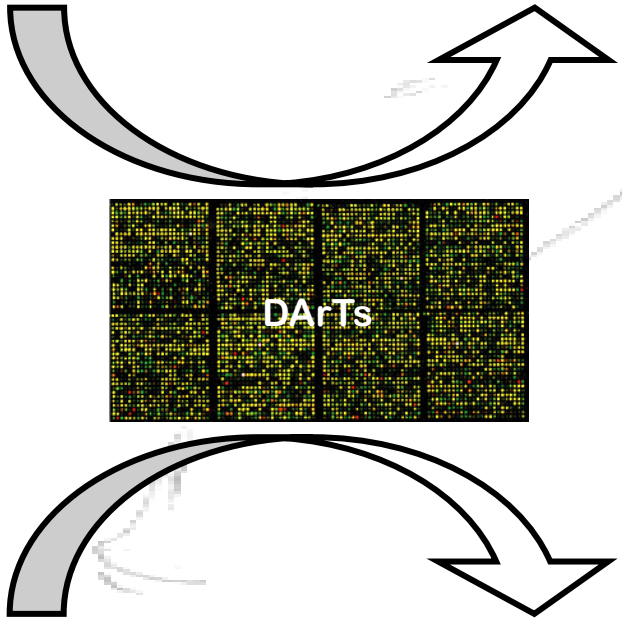


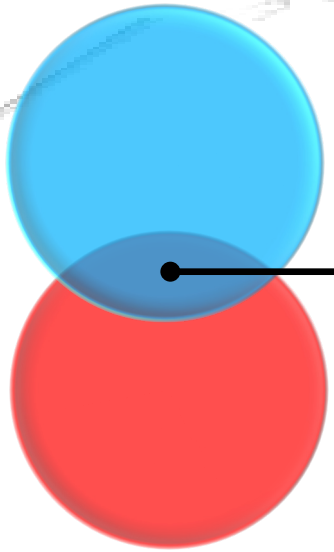
DArT-based anchoring



3-D MTP pools

600 DArTs anchored to 3B physical map



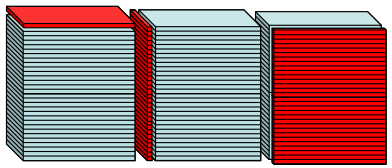


31 markers in common!

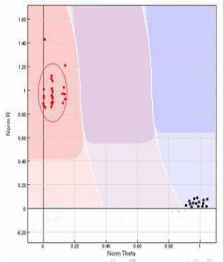
CsRe mapping population

233 DArTs mapped to chromosome 3B

Illumina-based anchoring



3-D MTP pools



SNPs anchored to BAC contigs

BMC Genomics

Methodology article

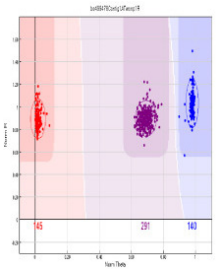
A high-throughput strategy for screening of bacterial artificial chromosome libraries and anchoring of clones on a genetic map constructed with single nucleotide polymorphisms

Ming-Cheng Luo[1], Kenong Xu[1], Yaqin Ma[1], Karin R Deal[1], Charles M Nicolet[2] and Jan Dvorak*[1]

Contigs anchored to genetic maps

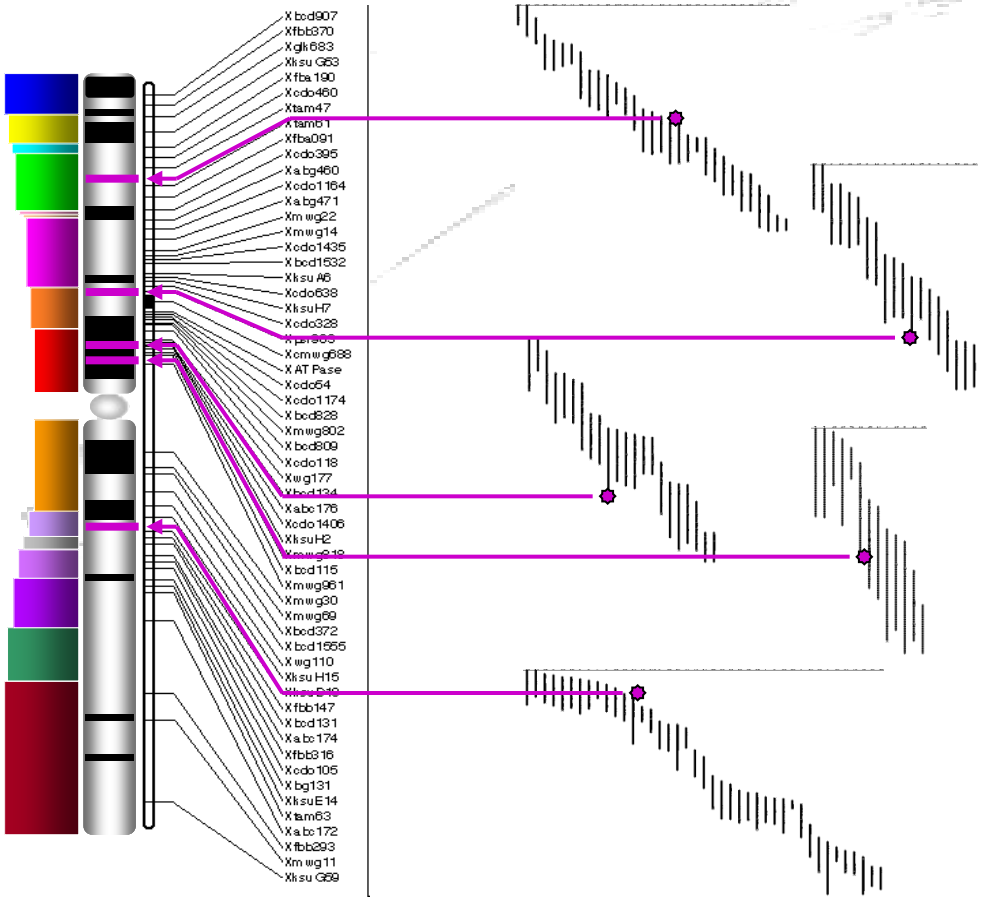
Mapping populations

SNPs genetically mapped



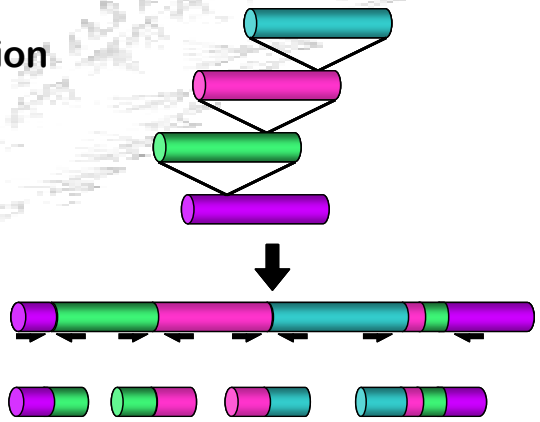
Second approach for physical contig anchoring

Reverse anchoring: from contigs to genetic maps using BAC or BAC-end sequence-derived markers (SSRs, ISBPs, SNPs…)



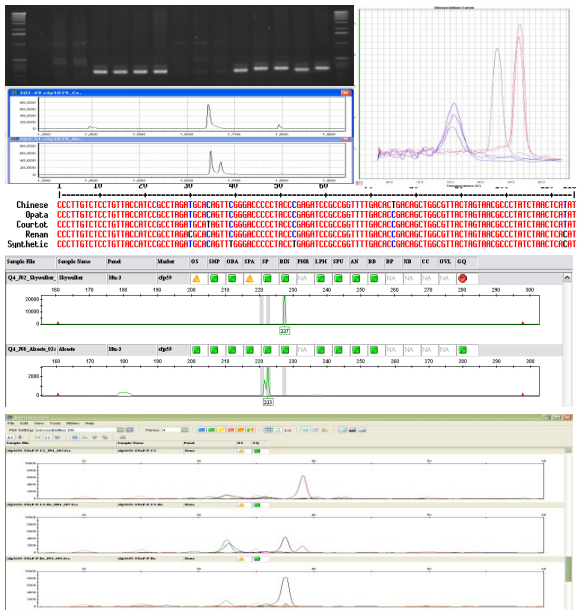
Insertion-Site Based Polymorphism

- Polymorphism rate
  - ✓ Melting curve analysis: 35% in elite pool *vs.* 70% in core collection
  - ✓ Sequencing: 60% in elite pool *vs.* 80% in core collection



- Infinite source for molecular markers
  - ✓ Average density: 1 ISBP / 5.4 kb
  - ✓ More than 3 millions ISBP in the whole wheat genome
  - ✓ One SNP / 99 bp

- Wide range of detection techniques
  - ✓ agarose or acrylamide gel electrophoresis
  - ✓ melting curve analysis
  - ✓ temperature gradient electrophoresis
  - ✓ fluorescent capillary electrophoresis
  - ✓ sequencing
  - ✓ SNaPshot
  - ✓ Illumina platform
  - ✓ microarray-based genotyping



[Paux et al. (2010) Plant Biotechnology Journal]

Third approach for physical contig anchoring

Hybrid anchoring: from random sorted chromosome shotgun sequences to genetic maps and contigs using sequence-derived markers (ISBPs, SSRs, ESTs, SNPs…)

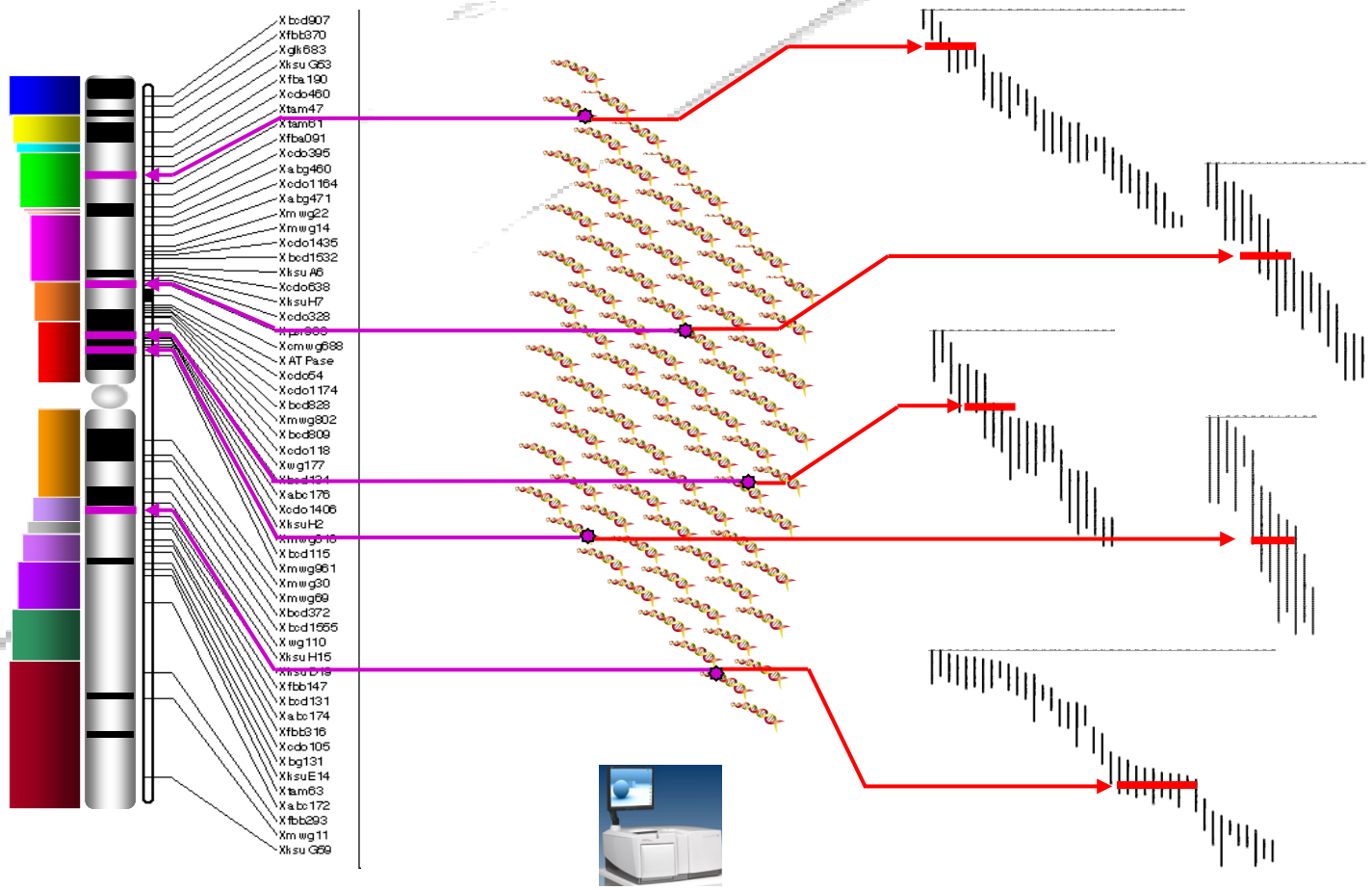


IMaGe: ISBP Microarray-based Genotyping

- ✓ Aneuploid lines
- ✓ Mapping populations
- ✓ RH panel…

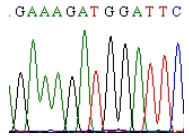
Hybridization on microarrays

Mapping positions

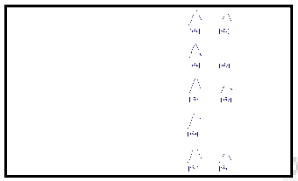
Technological developments underway

Patent pending

Recombination-based mapping



New markers



Individual scoring

2000-3000 Chinese Spring x Renan F8 RILs

Genetic mapping of markers
& contigs

Recombination-based mapping

Advantages:

- ✓ Relative order of markers
- ✓ Links to QTLs

Drawbacks:

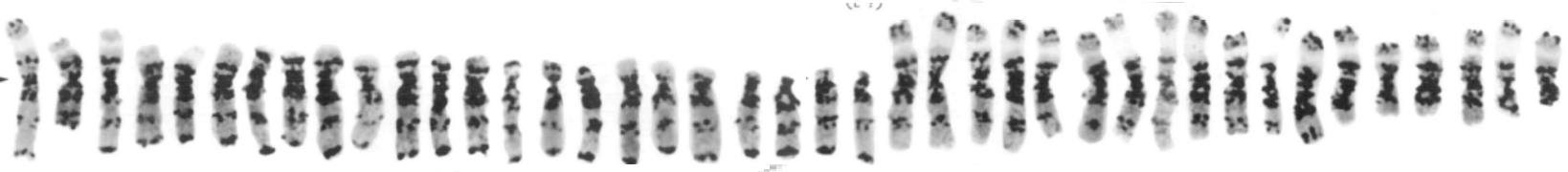
- ✓ Dependent on recombination
- ✓ Polymorphic markers

Deletion stocks of hexaploid wheat

1A

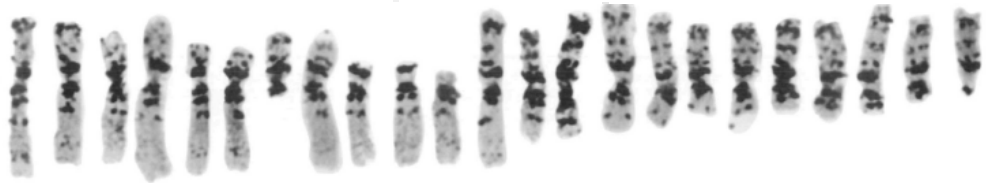
11 deletion breakpoints + 1 nullitetrasomic + 2 ditelosomic lines

1B



40 deletion breakpoints + 1 nullitetrasomic + 2 ditelosomic lines

3B



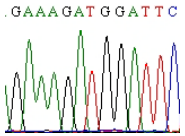
22 deletion breakpoints + 1 nullitetrasomic + 2 ditelosomic lines

3D

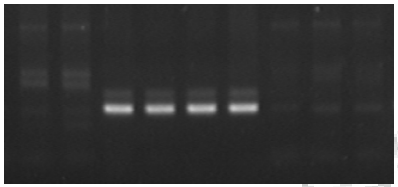
12 deletion breakpoints + 1 nullitetrasomic + 2 ditelosomic lines

[adapted from Endo and Gill (1996) Journal of Heredity]

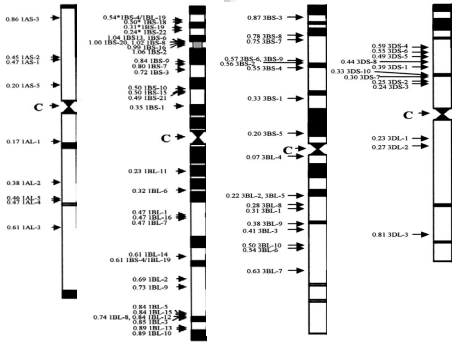
Deletion bin mapping



New markers



Individual scoring

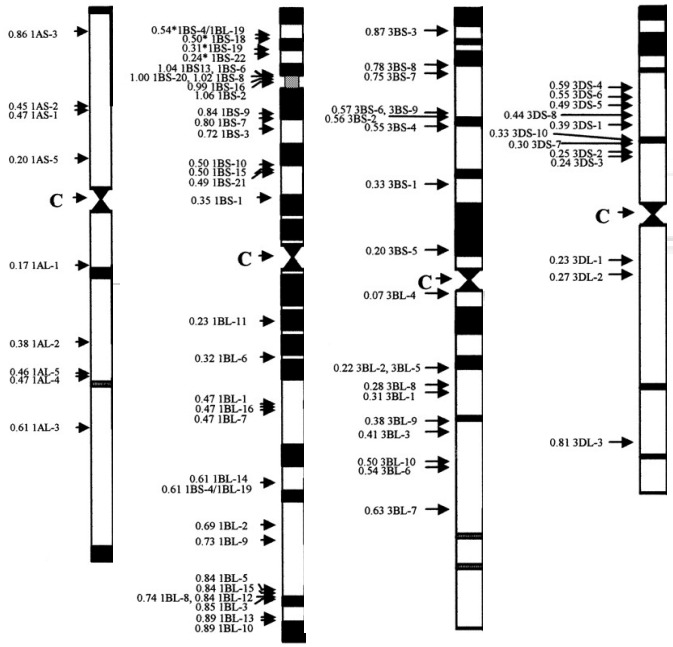


Ditelosomic, nullitetrasomic and deletion lines

Deletion bin mapping of markers
& contigs

[adapted from Dilbirligi et al. (2004) Genetics]

Deletion bin mapping



↳ Advantages:

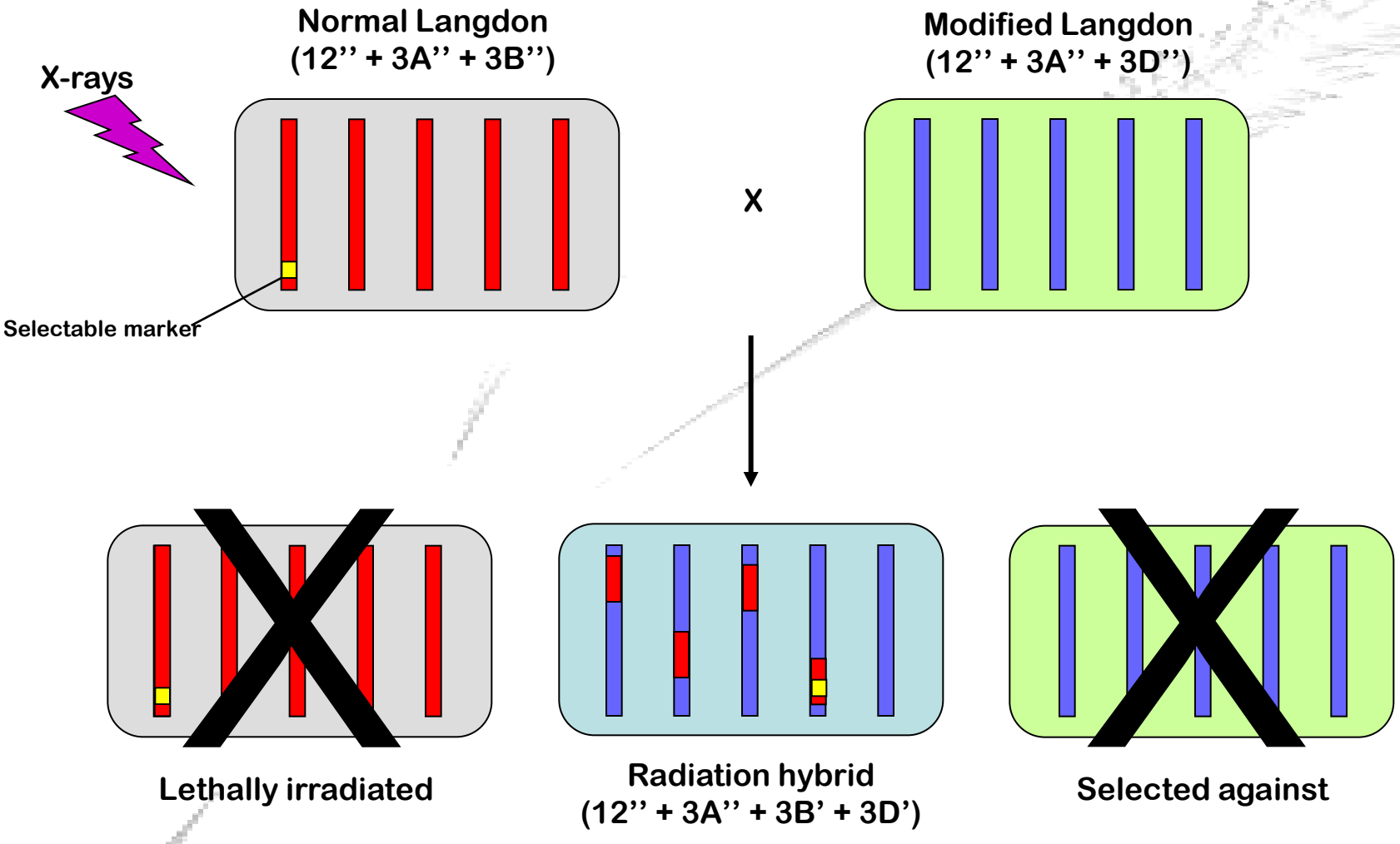
- ✓ Independent on recombination
- ✓ No need for polymorphic markers

↳ Drawbacks:

- ✓ No relative order in bins
- ✓ Large genomic segments

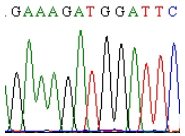
[adapted from Dilbirligi et al. (2004) Genetics]

Radiation hybrid mapping

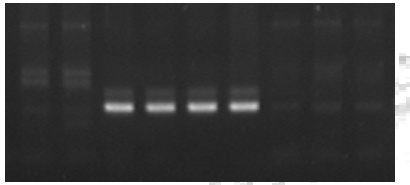


[kindly of S. Kianian]

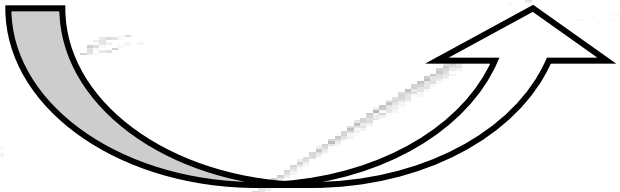
Radiation hybrid mapping

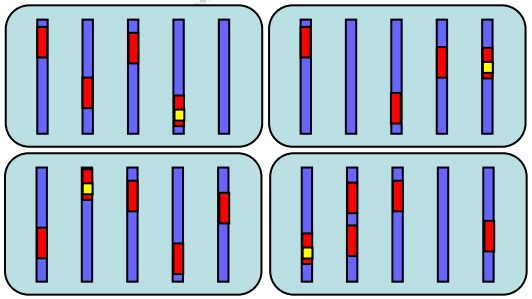


New markers



Individual scoring

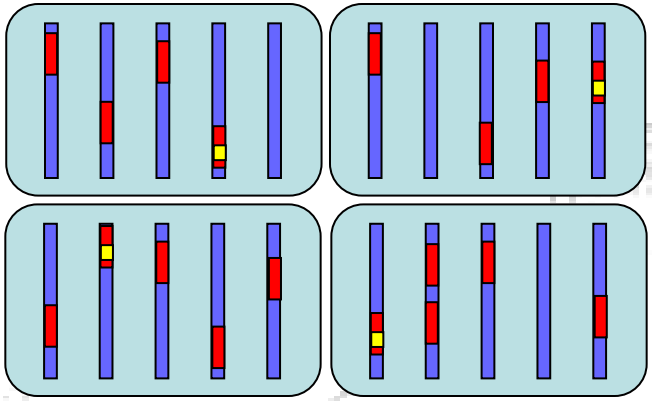




RH panel

RH mapping of markers
& contigs

Radiation hybrid mapping



Advantages:

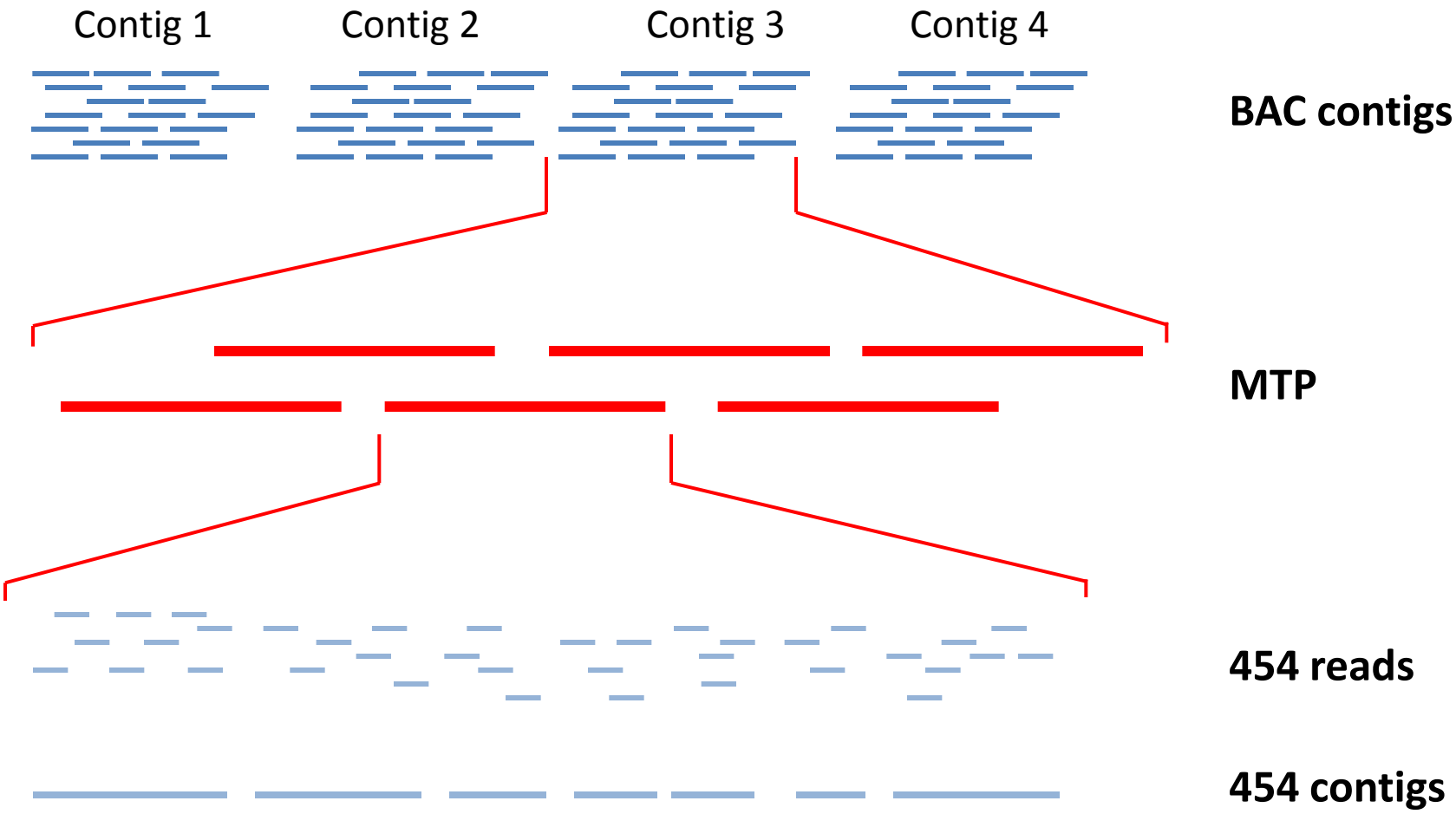
- ✓ Independent on recombination
- ✓ No need for polymorphic markers
- ✓ Resolution compatible with marker ordering (300 kb)
- ✓ No need for large population

Drawbacks:

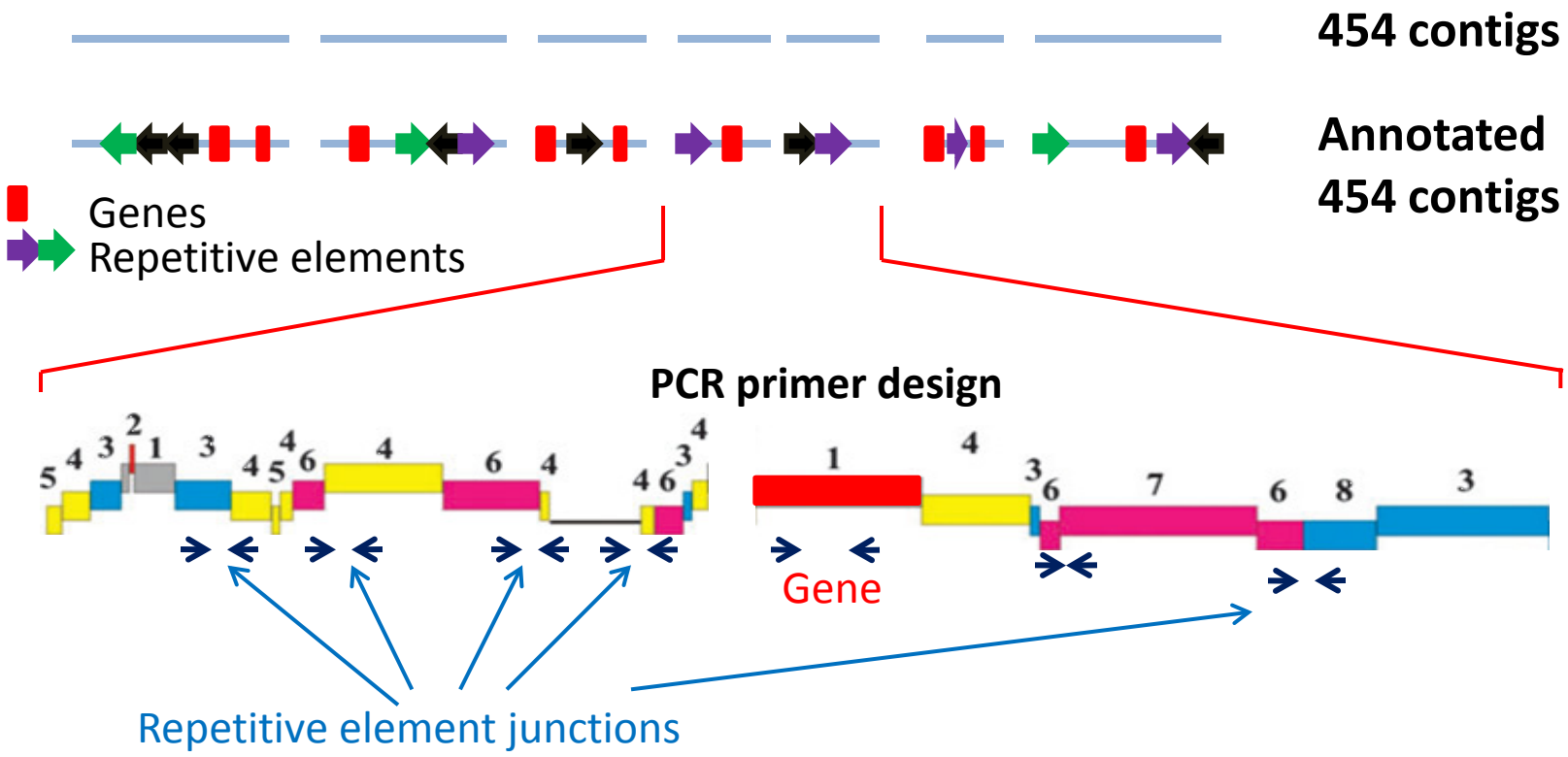
- ✓ Few results on wheat
- ✓ Tricky to develop RH panel

Eduard Akhunov, KSU

Anchoring strategy for the wheat chromosome 3A



Anchoring strategy for the wheat chromosome 3A

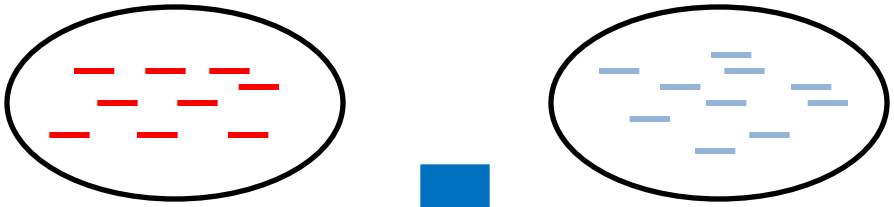


Generate amplicons for parental lines of mapping populations:

1. T. monococcum
2. T. dicoccoides x T. durum

Anchoring strategy for the wheat chromosome 3A

Amplicon pools

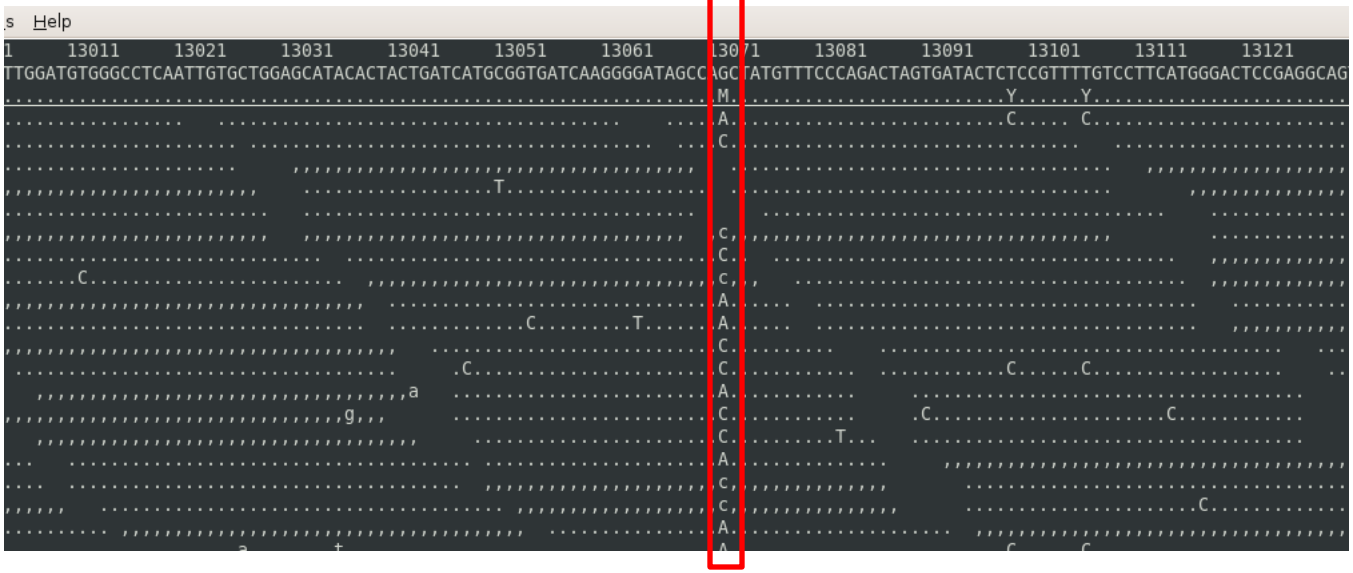


P1

P2

454 sequencing

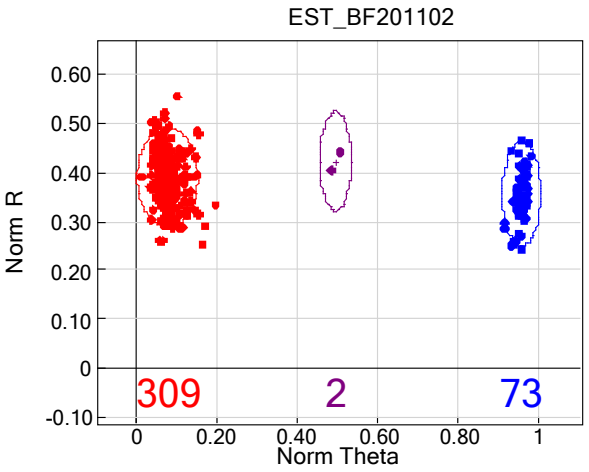
Map 454 reads to the reference sequence

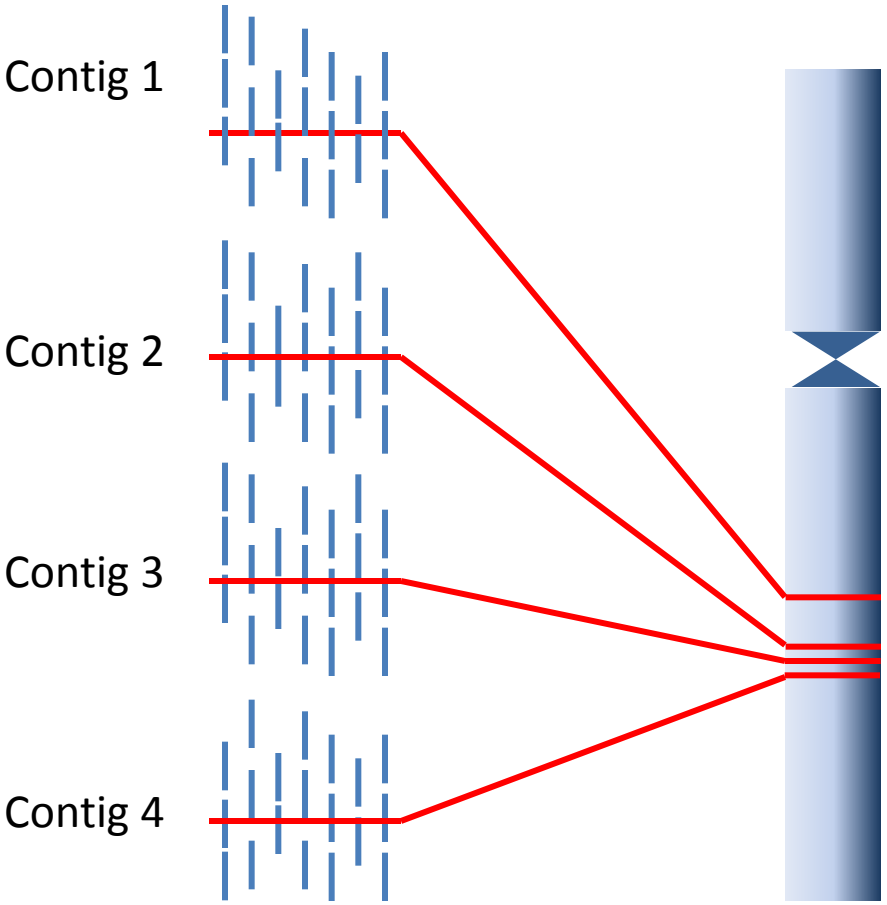


SNP

Anchoring strategy for the wheat chromosome 3A

Illumina Oligo Pool Assay (OPA) + Golden Gate genotyping assay:
- 1536-plex SNP assay
- 480 plants could be genotyped for $50,000





Next generation sequencing platforms changed the way how anchoring can be accomplished:

a. BAC contigs

b. Minimum Tiling Path sequencing

c. Development of anchoring markers and mapping

d. Integrated physical map