Using LTC Software for Wheat Physical Mapping: Increasing Contig Lengths and MTP Quality

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See also poster P1130

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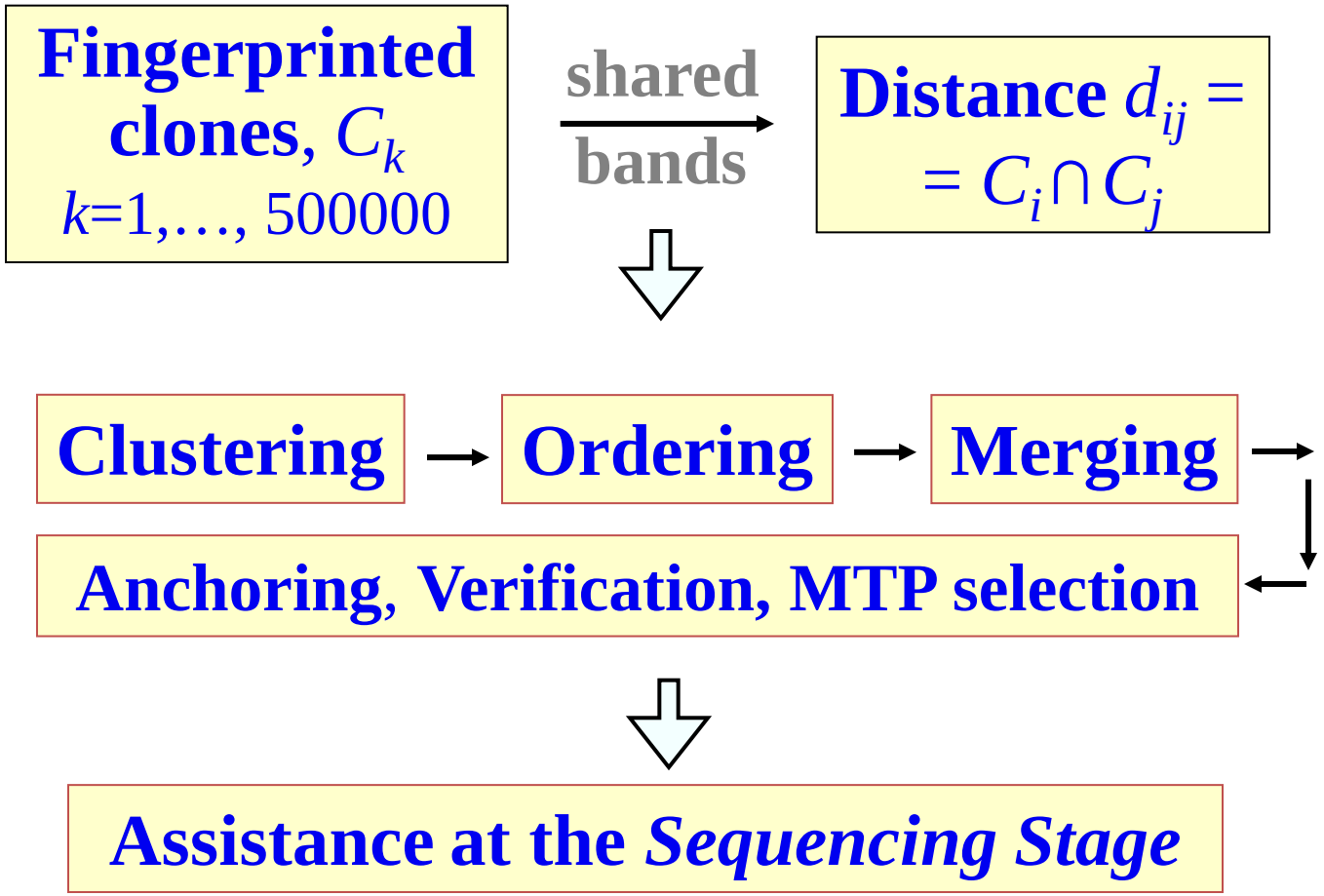
The major steps of physical mapping



Main difficulties in physical mapping

1. Chimerical clones
2. Low quality fingerprints
3. False clone overlaps due to repeats/duplications
4. 1-3 ➔ chimerical contigs
5. 1-4 ➔ problems in ordering
6. 1-5 ➔ problems in merging and anchoring
7. 3 & 5 ➔ gaps in MTP

⇨ **LTC**

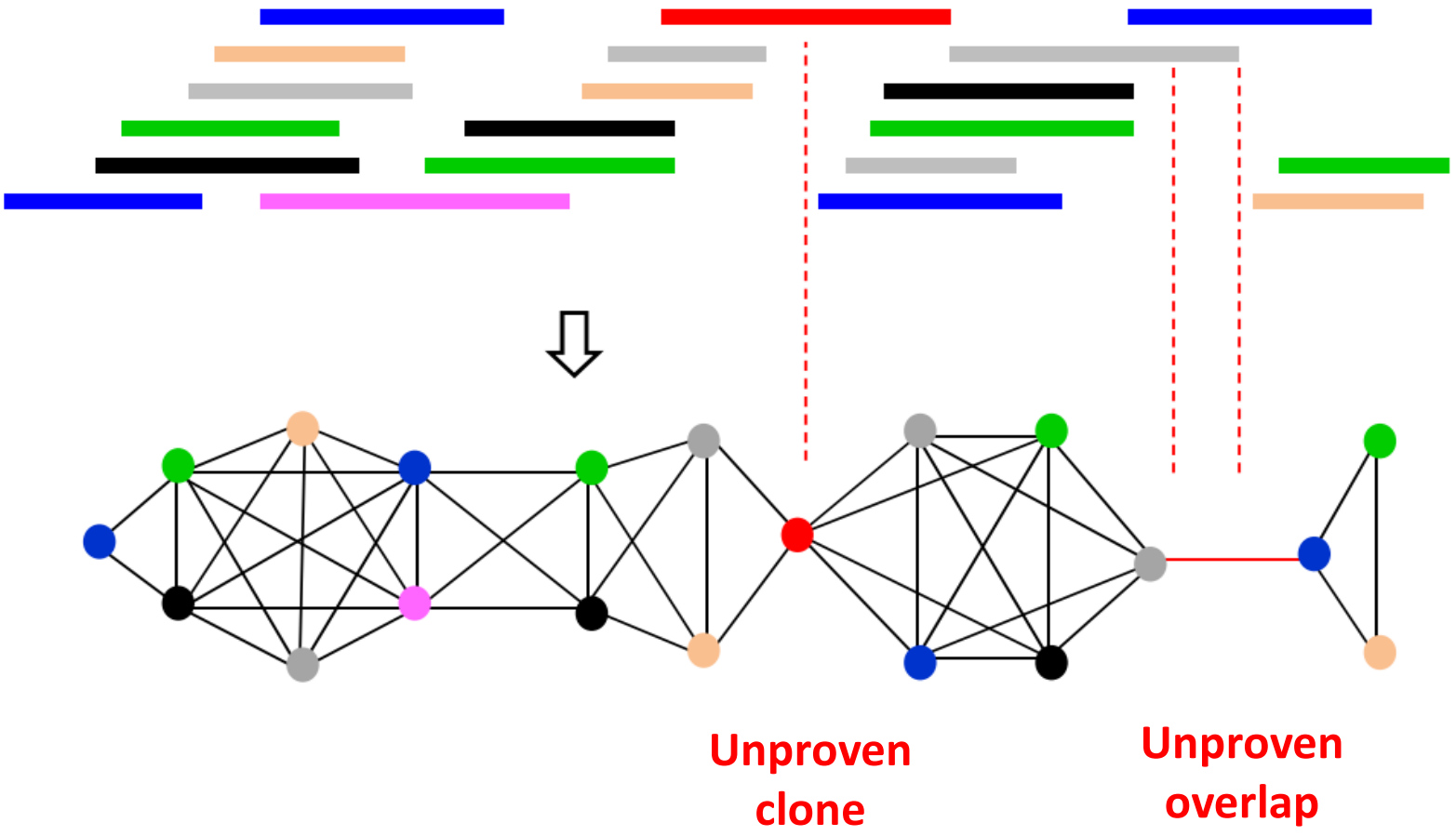
Contig assembly: LTC vs. FPC

- **Parallel clone overlaps** instead of consensus band/tag maps → more powerful detection of problematic clones and clone overlaps
- **Linear structure** of the net of significant clone overlaps → No contradictions of the contig topology with chromosome linear structure

➔ Longer and more reliable contigs

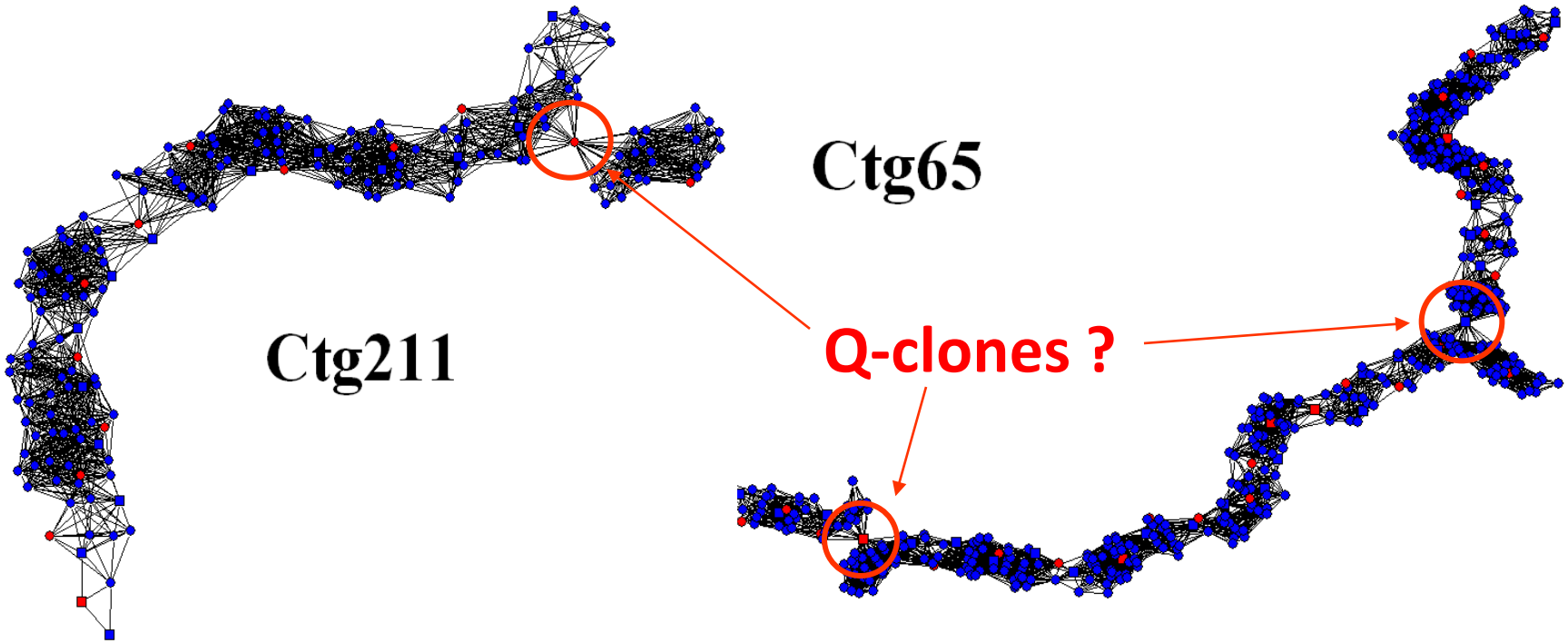
➔ Simpler anchoring

Net representation of clone overlaps



Testing FPC contig quality by using LTC

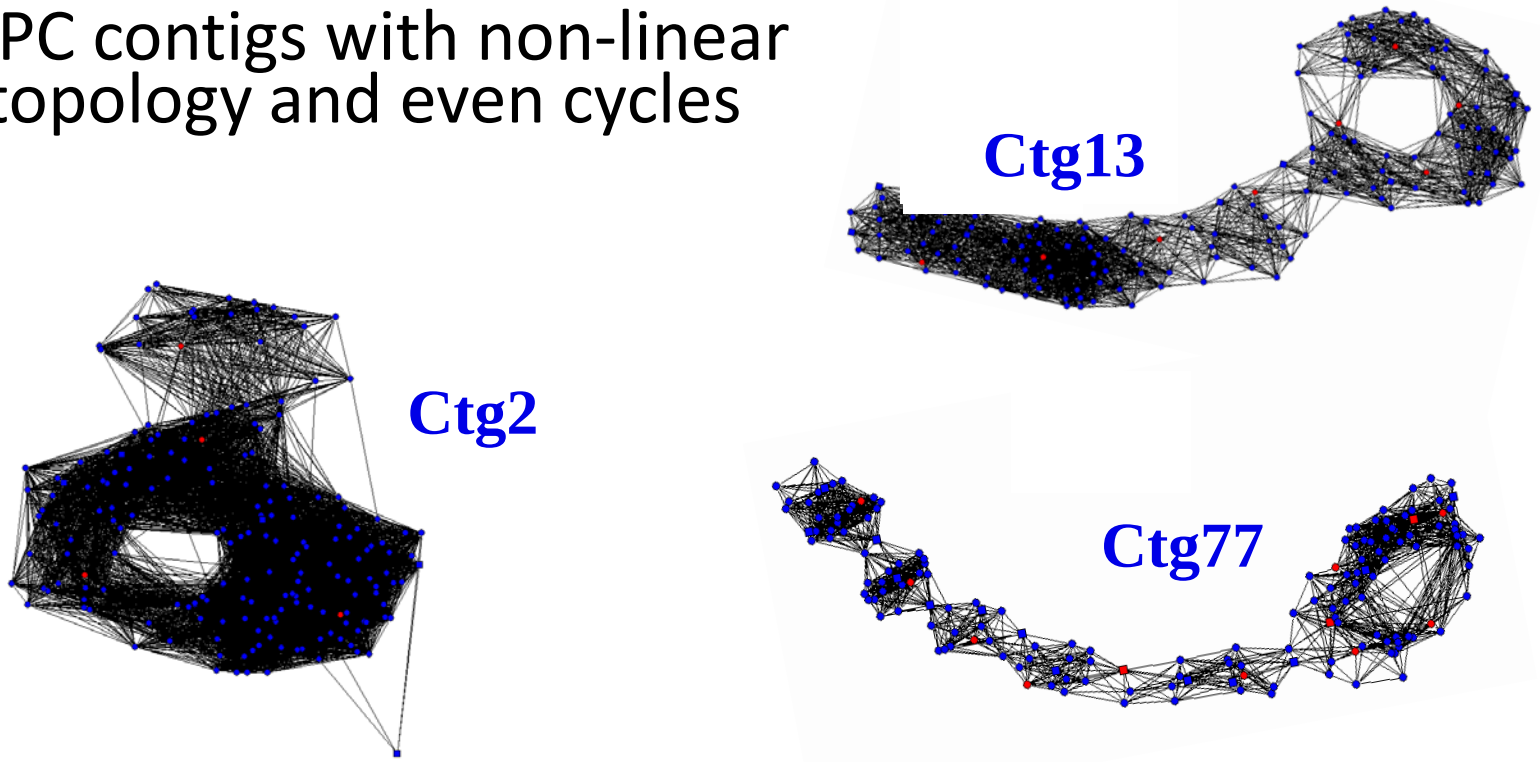
Some FPC contigs have non-linear topological structure inconsistent with chromosome linear structure:



Vertices represent the clones; **edges** represent the significant overlaps (with cutoff 1e-25 Sulston score)

Testing FPC contig quality by using LTC

FPC contigs with non-linear topology and even cycles



Edges represent significant overlaps (with cutoff 1e-25 Sulston score). Increasing the stringency up to e-75

does not help in non-trivial linearization!

Scaffolding of physical contigs

- Visual and analytical control of the net of significant clone overlaps
- Coordinating of scaffolding with anchoring

➔ Long well anchored physical scaffolds

Example: wheat 1BS (314 Mb, HICF, x15, ~50,000 BACs)

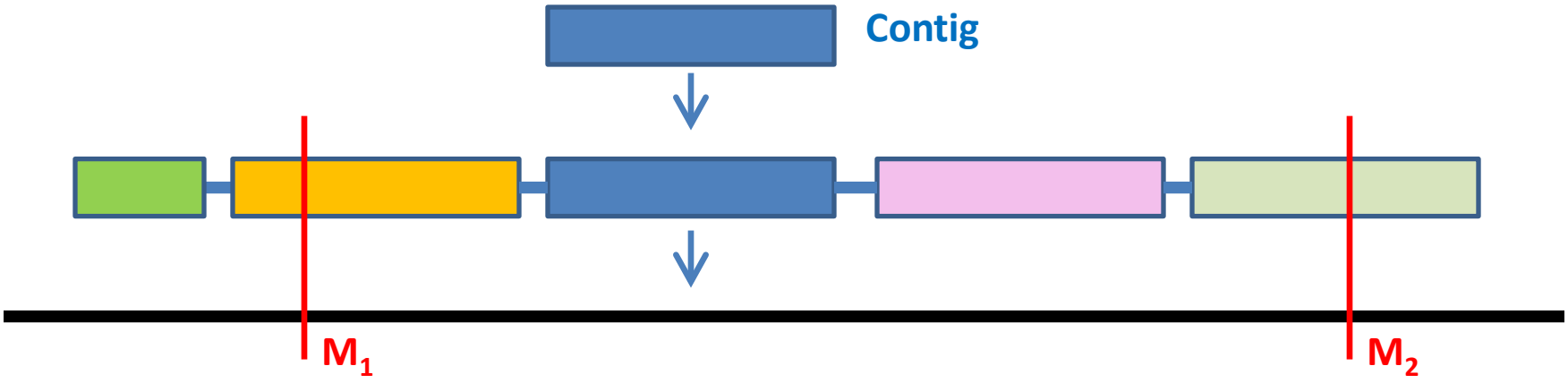
	FPC	LTC contigs	LTC scaffolds
Clones in contigs (≥6)	34,104	33,846	34,027
Longest contig (Mb)	4.7	**7.0**	**20.9**
N50 (Mb)	1.0	**2.4**	**8.5**
L50 (contigs)	81	**35**	**11**

Raats et al. *Genome Biology 2013,* ***14:R138***

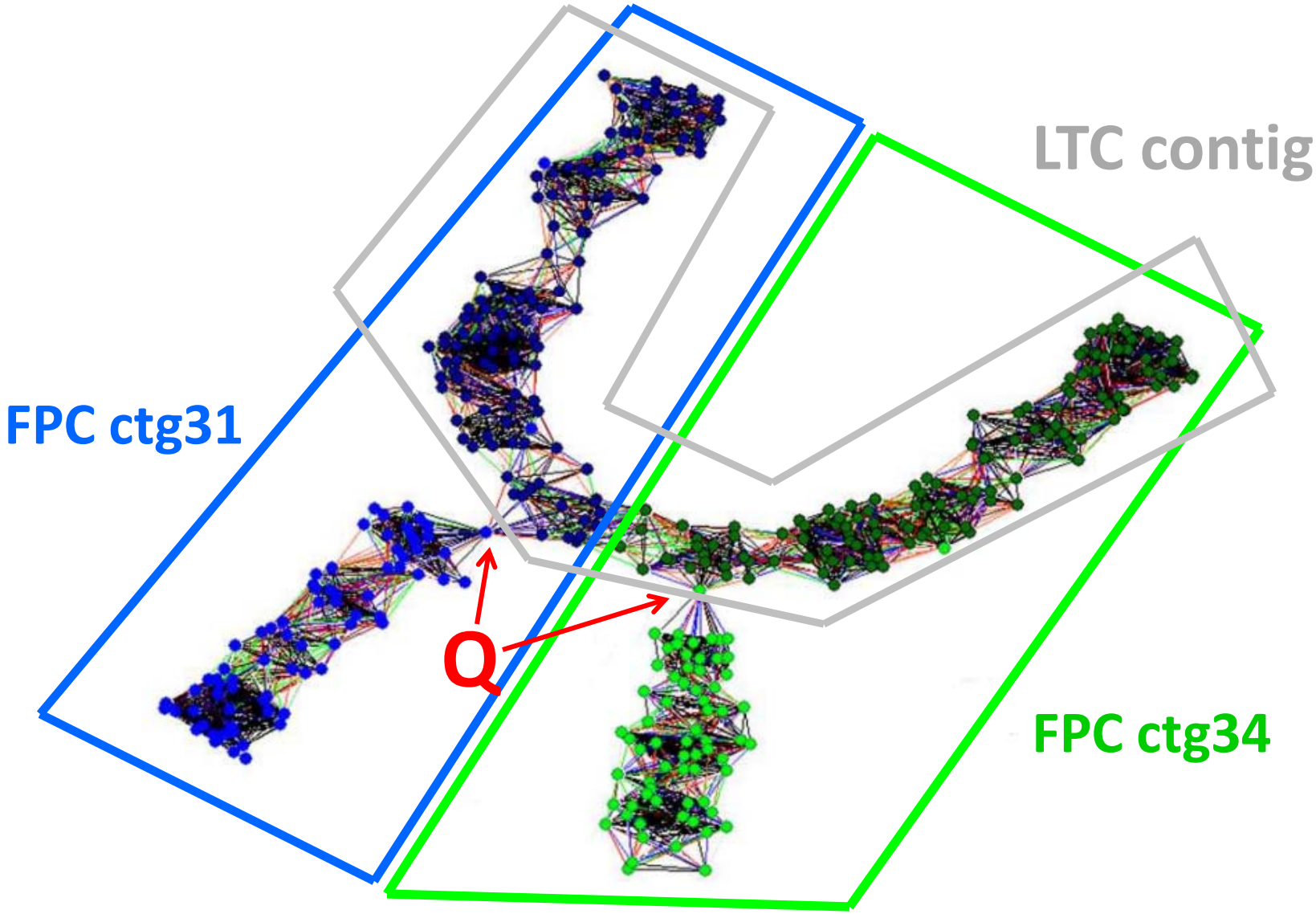
Anchoring of long contigs

- Much less markers are needed
- Especially useful for regions with suppressed recombination, e.g., “near” the centromeres
- More effective contig orientation in chromosomes

Scaffolds → possible anchoring and orientation even for contigs having no markers



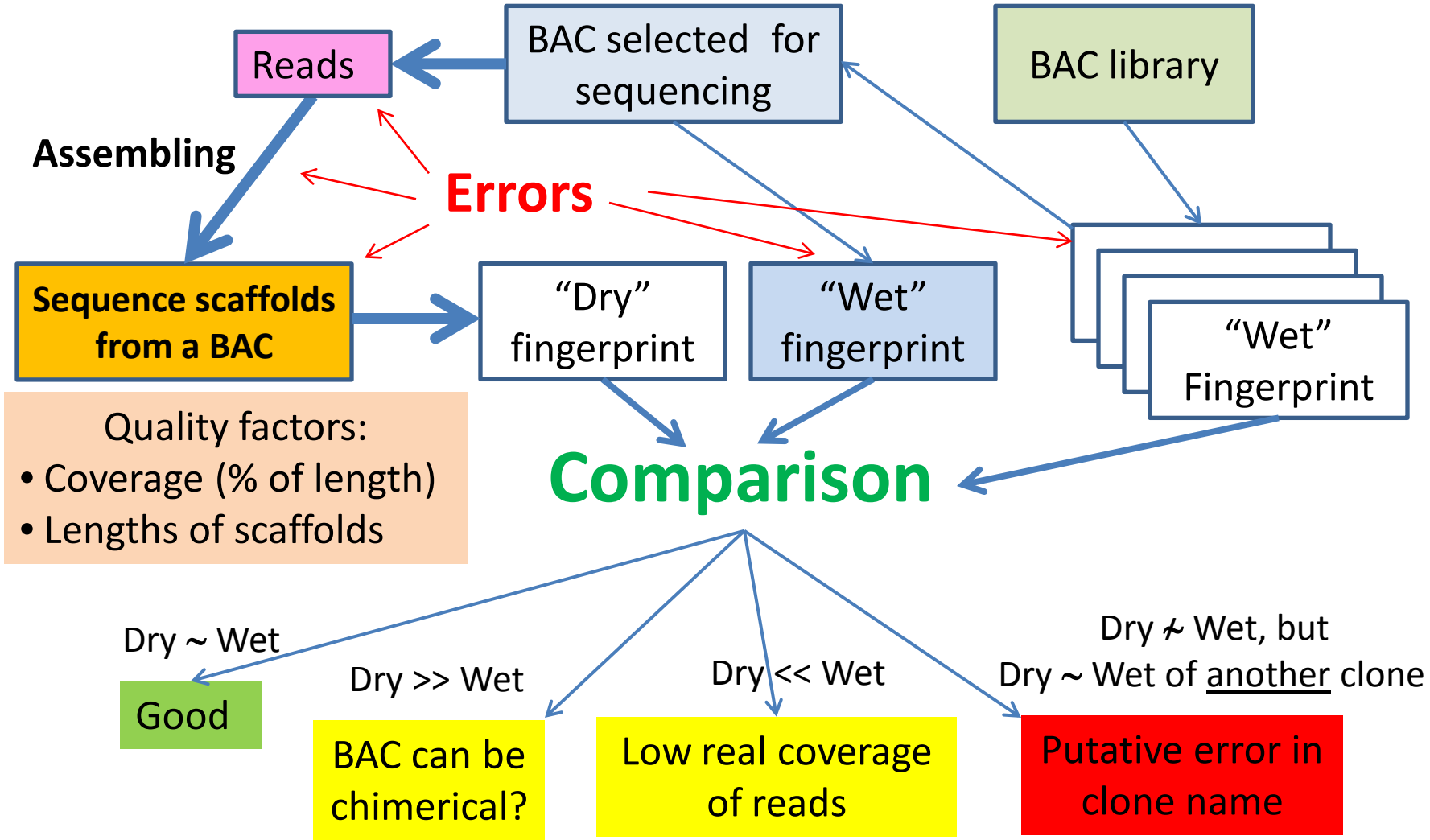
LTC scaffolds vs. FPC contigs



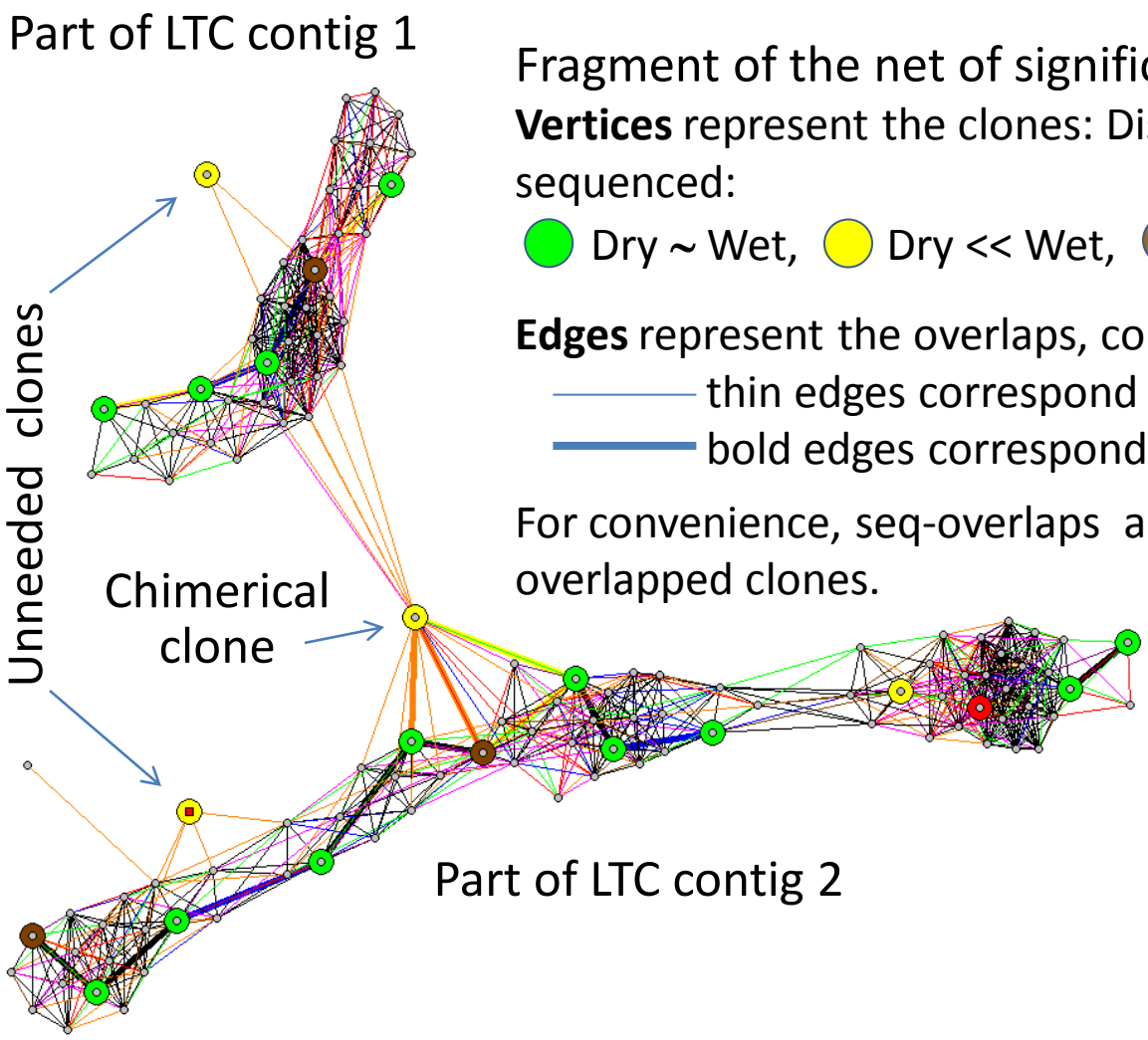
Selecting clones for sequencing by LTC

- Possibility to give priority to previously selected MTP clones (for anchoring or for BAC-end sequencing)
- Larger (more sure) overlaps of neighbor clones to avoid non-significant overlaps at sequence level in highly repeated genomes → **less gaps**
- Reducing the risk of errors caused by Q-clones and false clone overlaps → **more reliable MTP**
- Supplementing the list of MTP clones by potential "bridges" for end-to-end merging → **longer contigs**

Controlling the sequencing quality



LTC control of MTP clone-overlaps at sequence level



Fragment of the net of significant clone overlaps (7BS data)

Vertices represent the clones: Disk indicate that the clone was sequenced:

Dry ~ Wet, Dry << Wet, Dry >> Wet, Dry ≁ Wet

Edges represent the overlaps, color reflects significance:

thin edges correspond to HICF-based overlaps

bold edges correspond to seq-based overlaps

For convenience, seq-overlaps are shown only for HICF-overlapped clones.

LTC candidate solutions to cure the detected **sequence** gaps

- **Check the physical contig:** a gap can be a result of error(s) in physical contig assembly
- **Check overlaps** in fingerprints
- **Check sequence quality**: coverage, length and correspondence of wet and dry fingerprints
- **Add clones** to connect the sides of the gap via significant fingerprint-based overlaps
- If well sequenced clones appeared to overlap on fingerprint but not sequence level, try to **increase cutoff at the fingerprint level**

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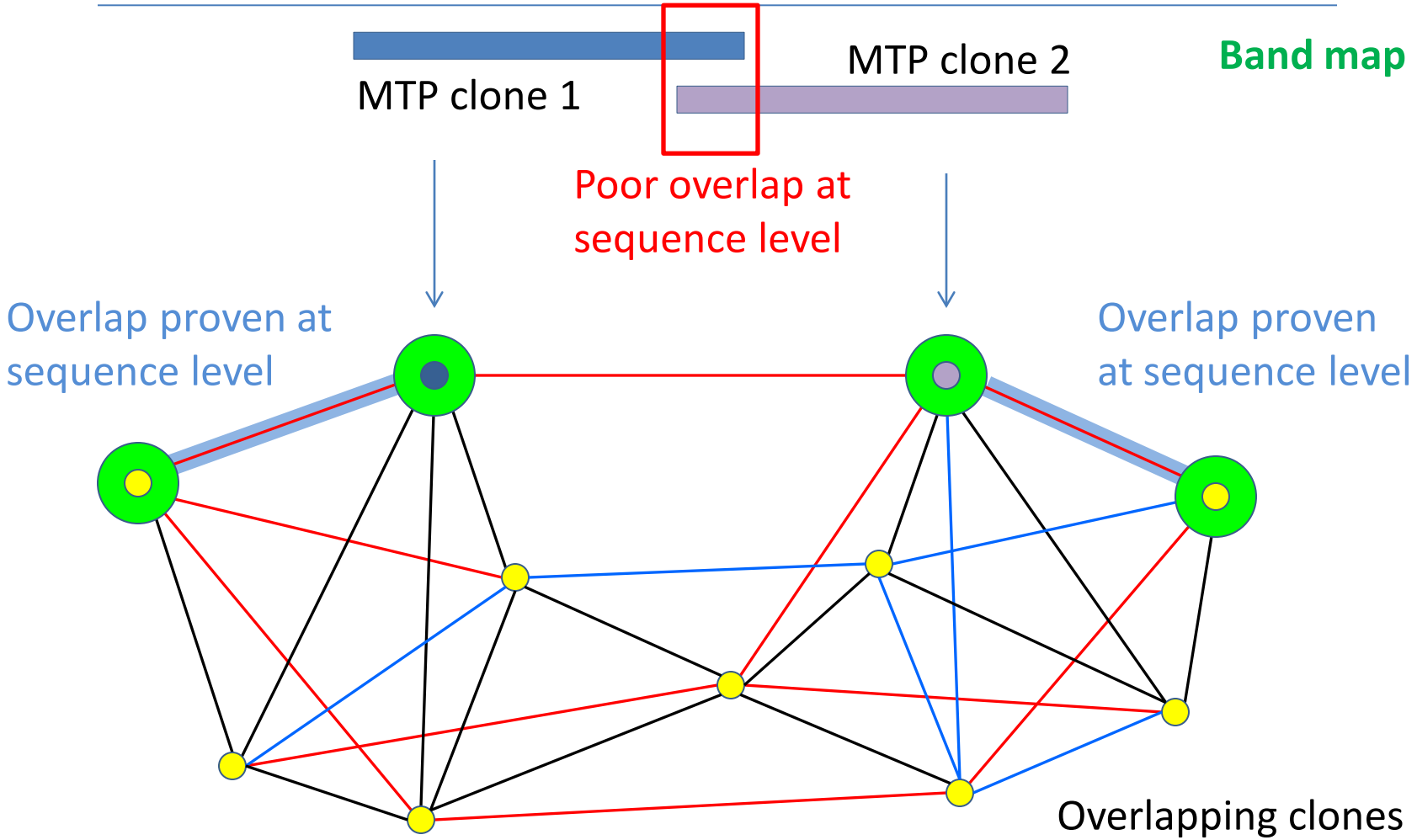
Example of gap repairing



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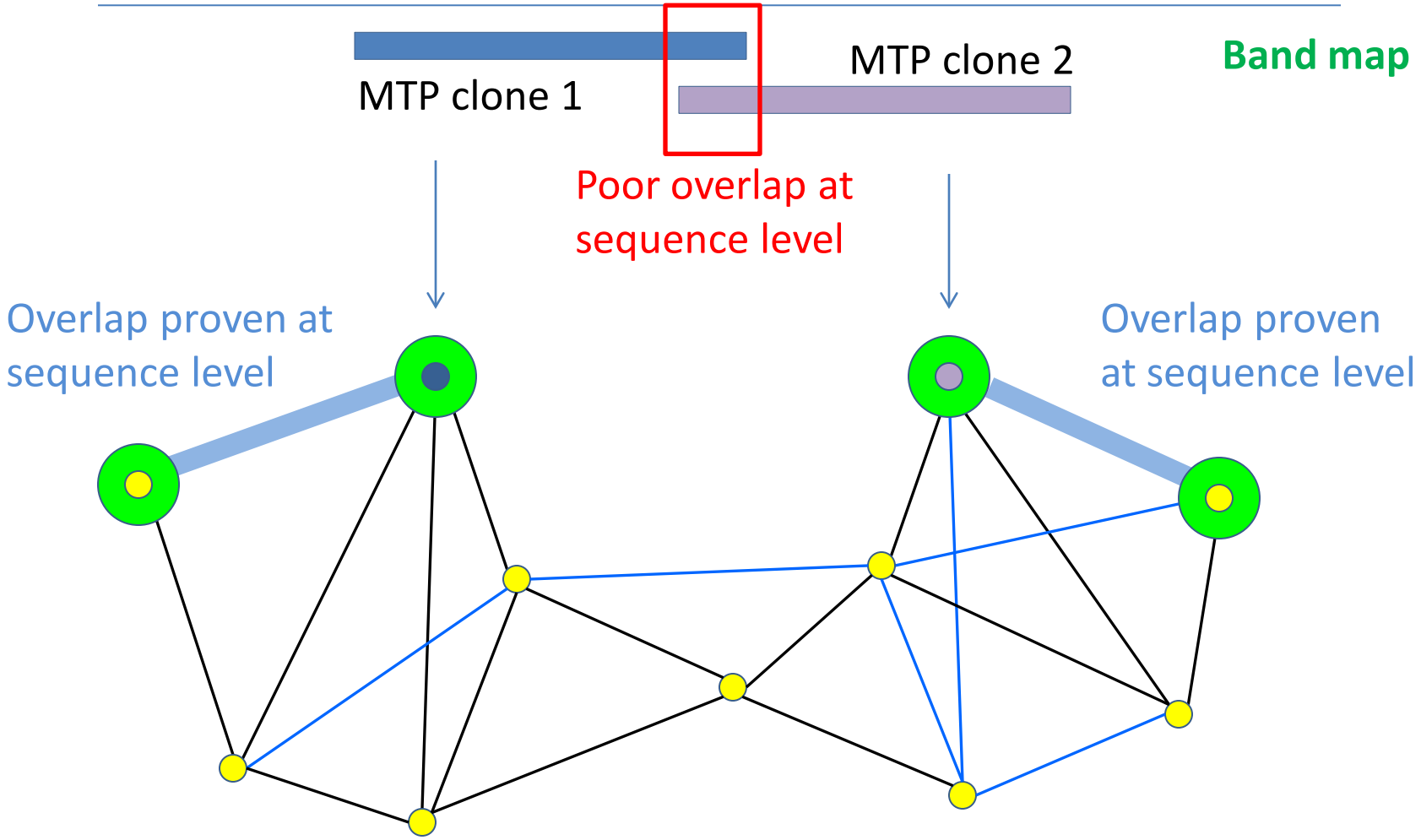
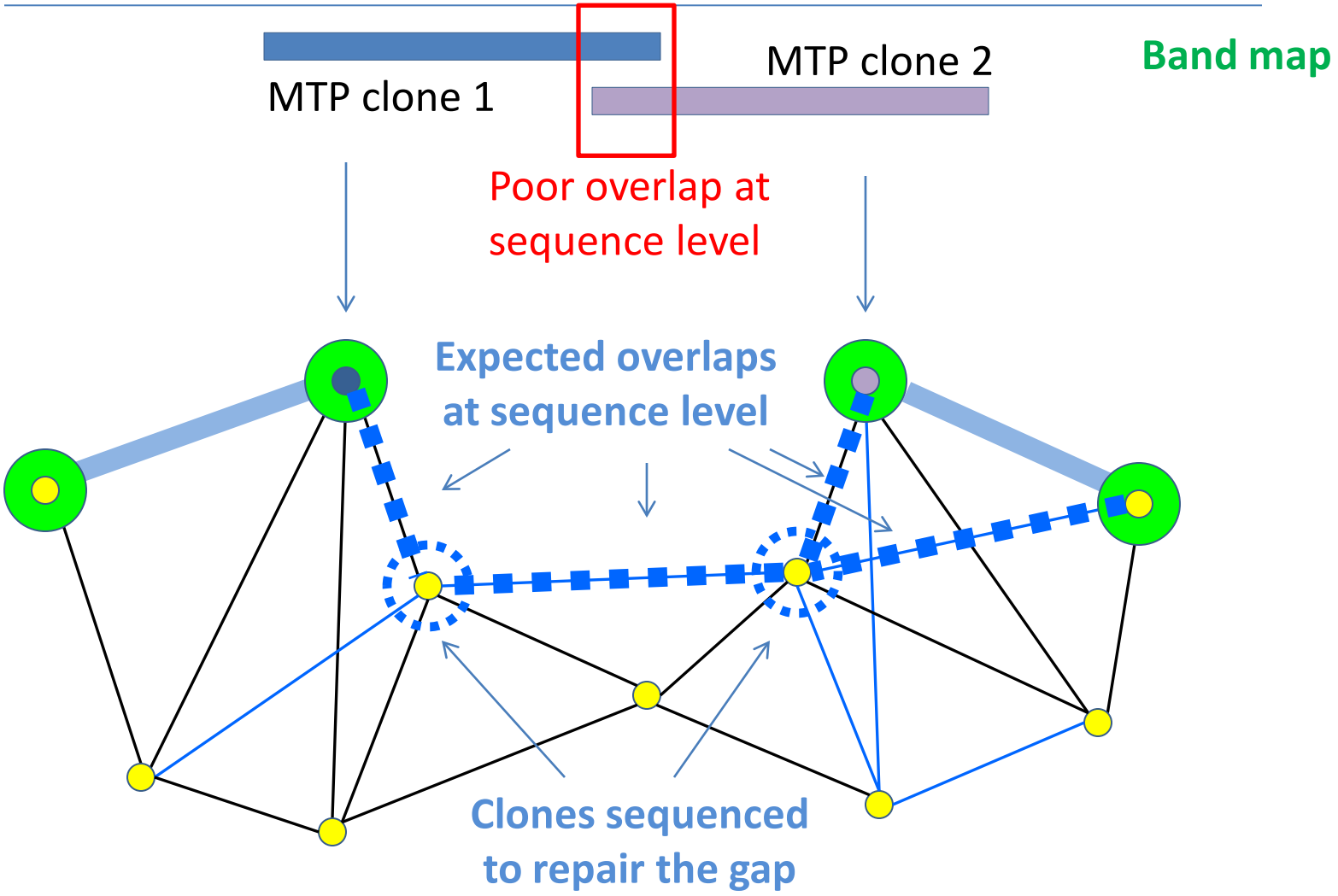


Clone overlaps detected at increased cutoff stringency

Example of gap repairing



Some prospects

- Simplification of scaffolding of physical contigs coordinated with anchoring
- Optimization of MTP selection by taking into account clone length, clone overlaps and putative (calculated) local coverage and repetitiveness
- Orientation, ordering and merging of sequence scaffolds assisted by fingerprinting information from overlapped fingerprinted clones (even not yet sequenced)

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Example: sequencing of YrH52 and Yr15 region (1BS)

Candidate region:

Length ~6Mb

Covered by 104 overlapping MTP clones

Pooling of neighbor MTP clones :

23 pools instead 104

→ lower cost of sequencing stage

Sequencing by MiSeq (x450 coverage)

Orientation, ordering and merging of sequence scaffolds

Sequence contig assembly (using EDENA):

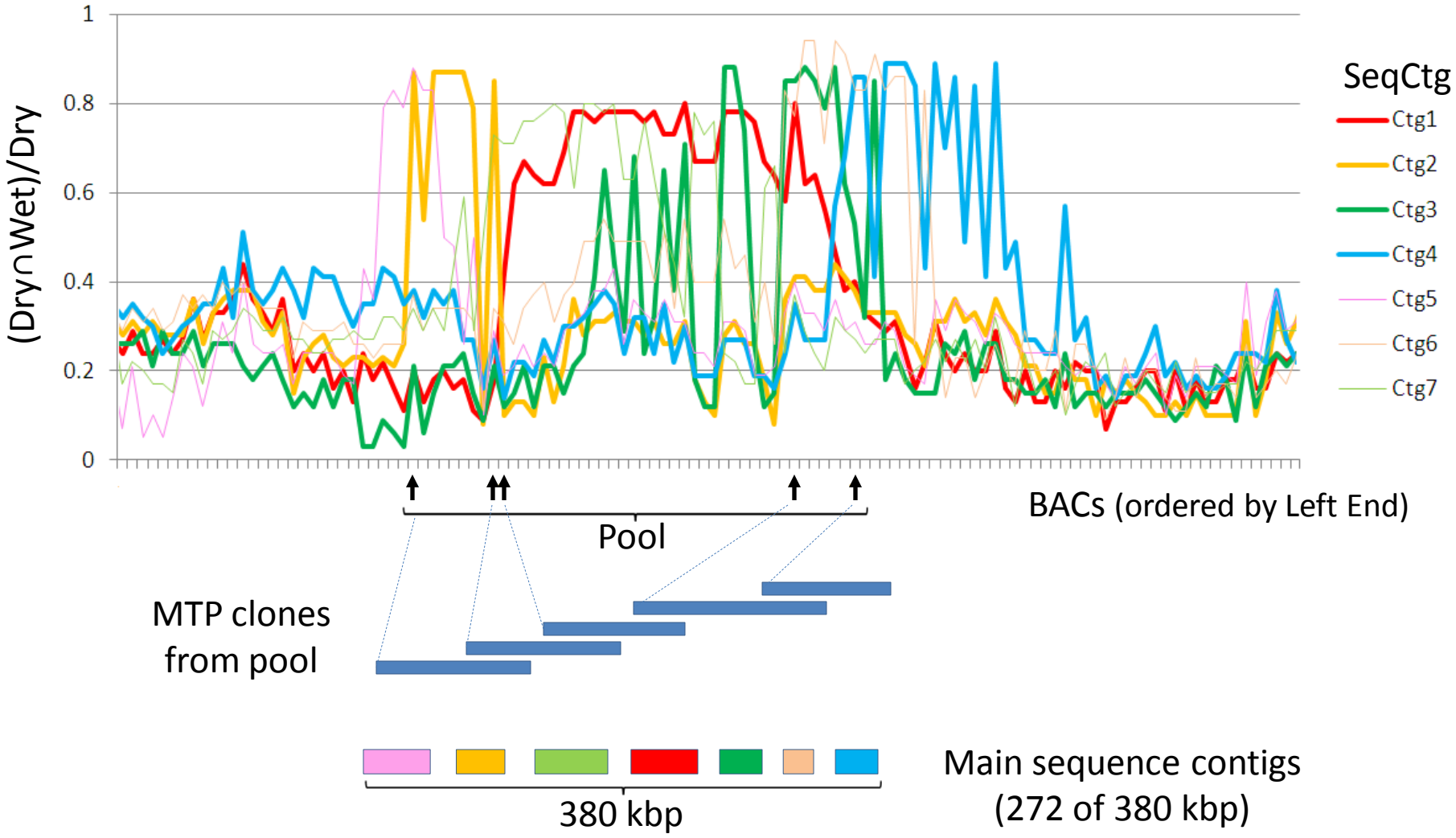
- 9-56 sequence contigs per pool
- Average total length of contigs per pool ~ 333 kbp
- **Only few "main" contigs** (longer than 15 kbp)

Sequence contigs → *in silico* fingerprinting

↓

Comparison with clones from physical scaffold (not MTP only)

Ordering and orientation of sequence contigs within pool



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