LTC software for physical mapping: contig assembly, MTP selection and verification of clone overlaps at sequence level

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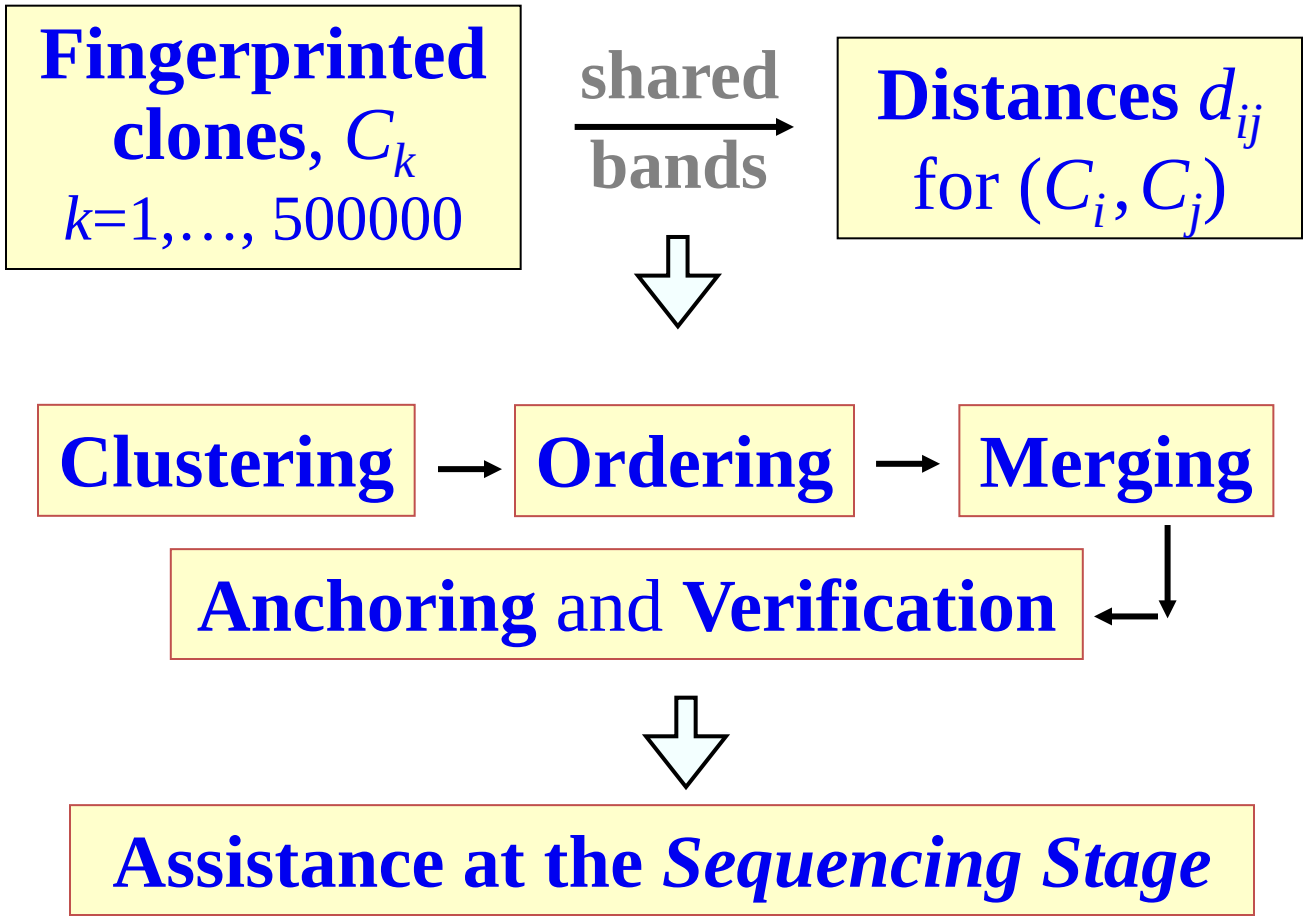
The major steps of physical mapping



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**

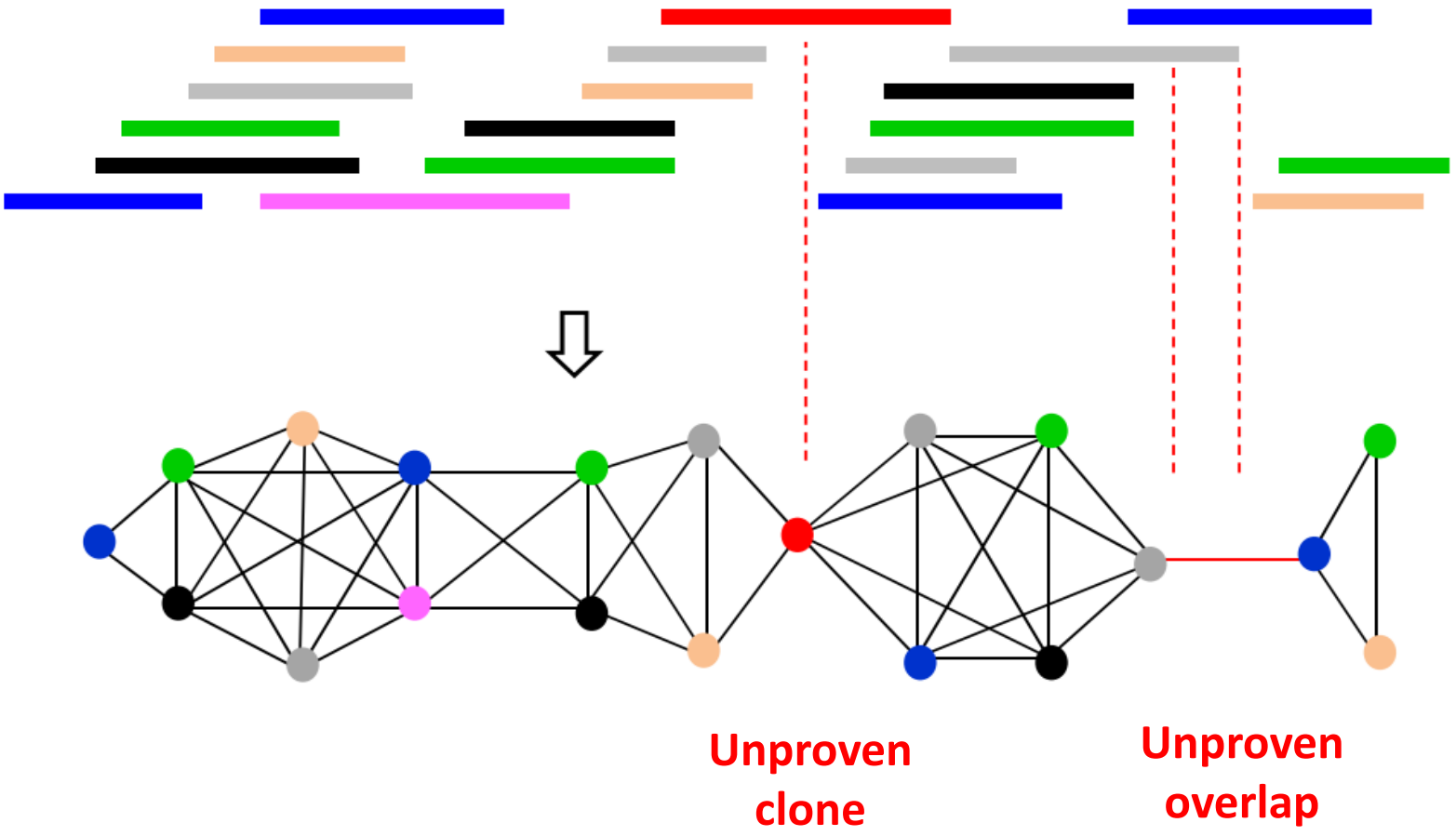
Contig assembly: LTC vs. FPC

- **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

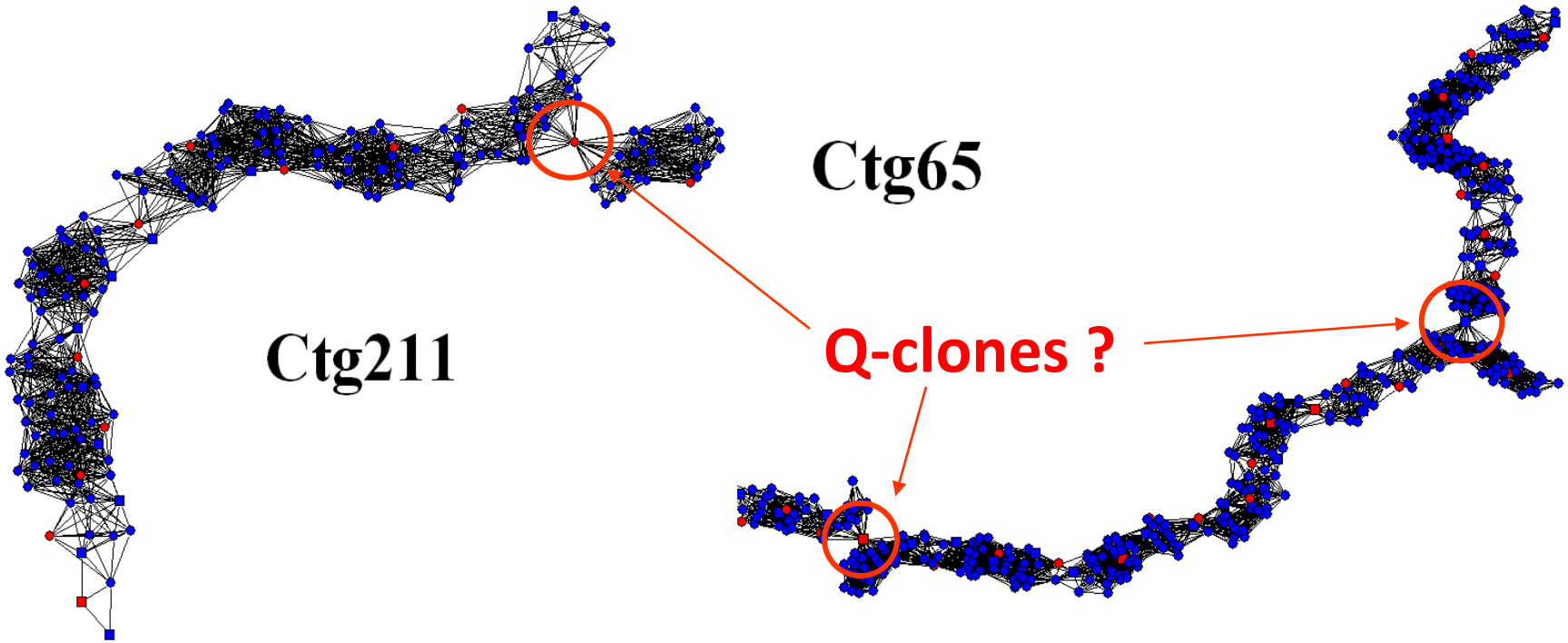
➔ Simpler anchoring

Net representation of clone overlaps



Testing FPC contig quality by using LTC

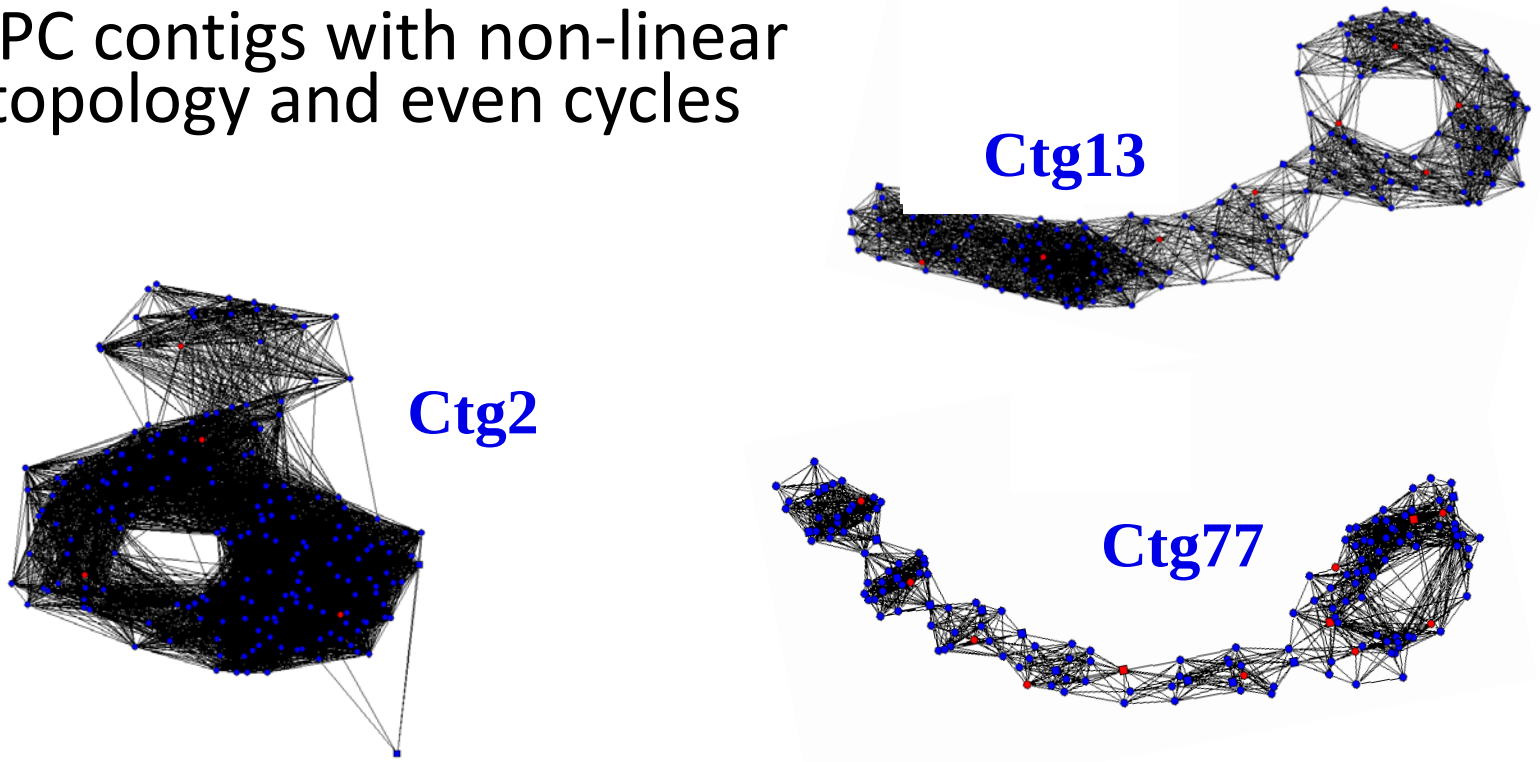
Some FPC contigs have non-linear topological structure inconsistent with chromosome linear structure:



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

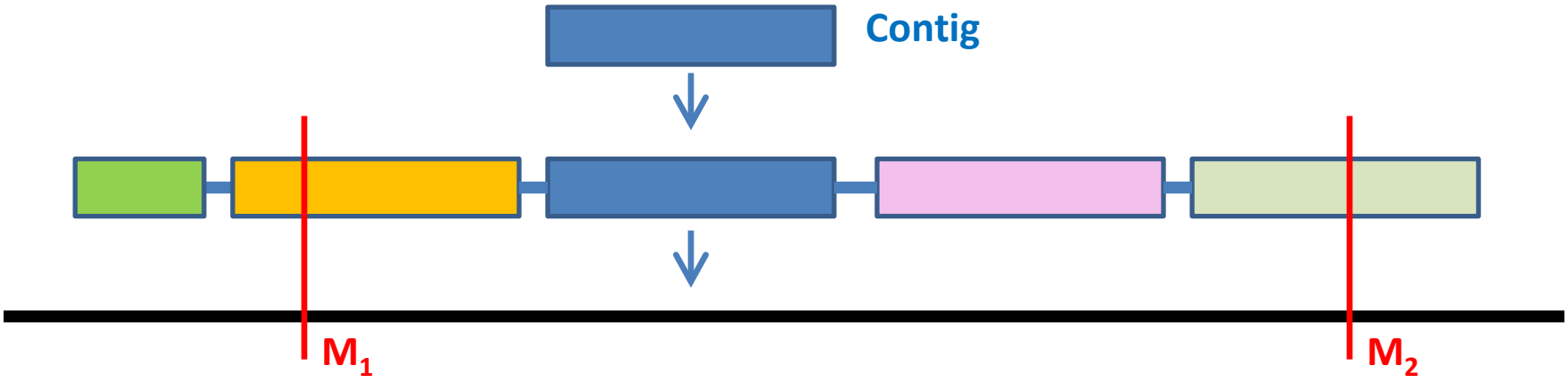
Example: wheat 1BS (314 Mb, HICF, x15, ~50,000 BACs)

	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

Anchoring of long contigs

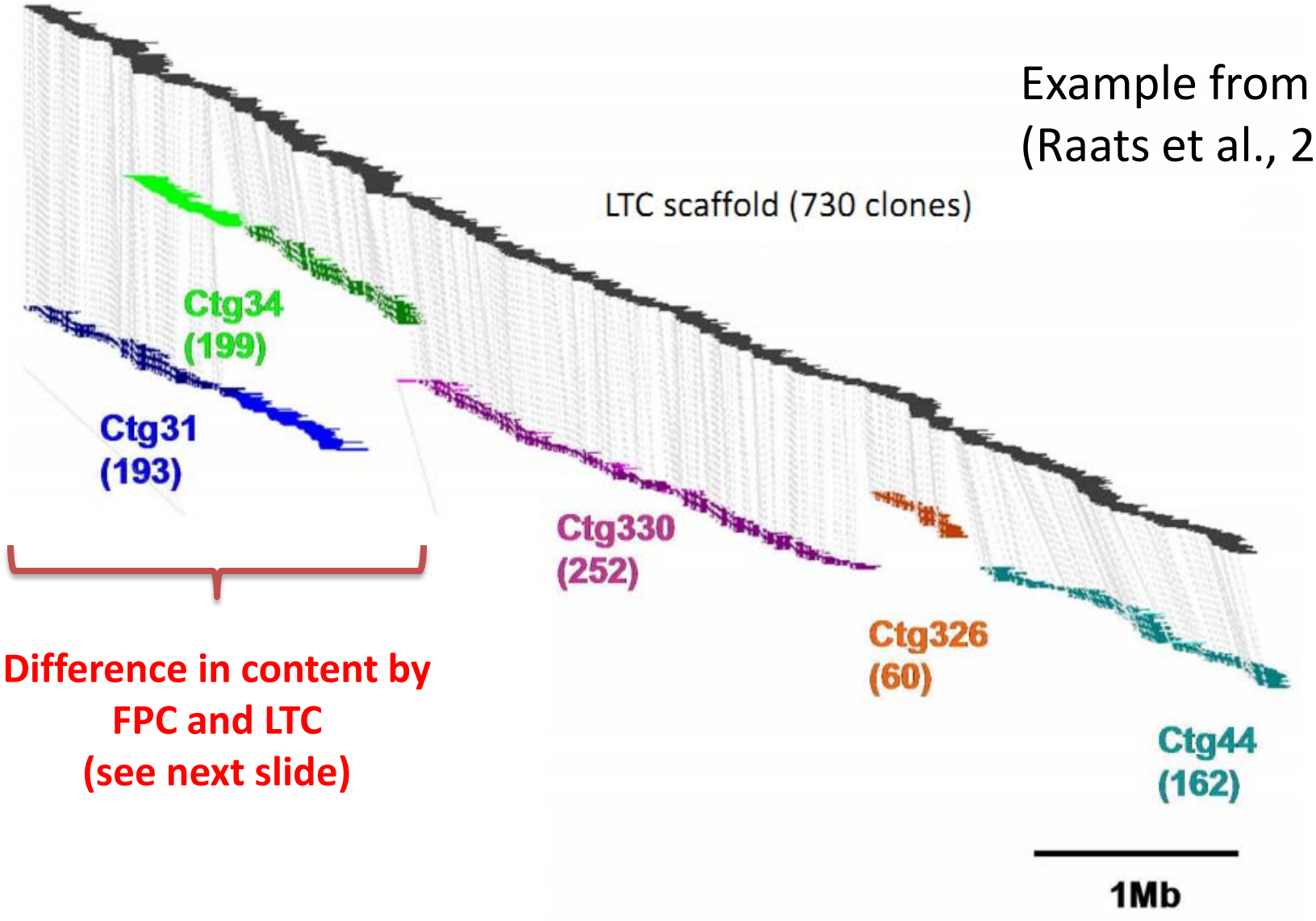
- Much less markers are needed
- Especially useful for regions with suppressed recombination, e.g., “near” the centromeres
- More effective contig orientation in chromosomes

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



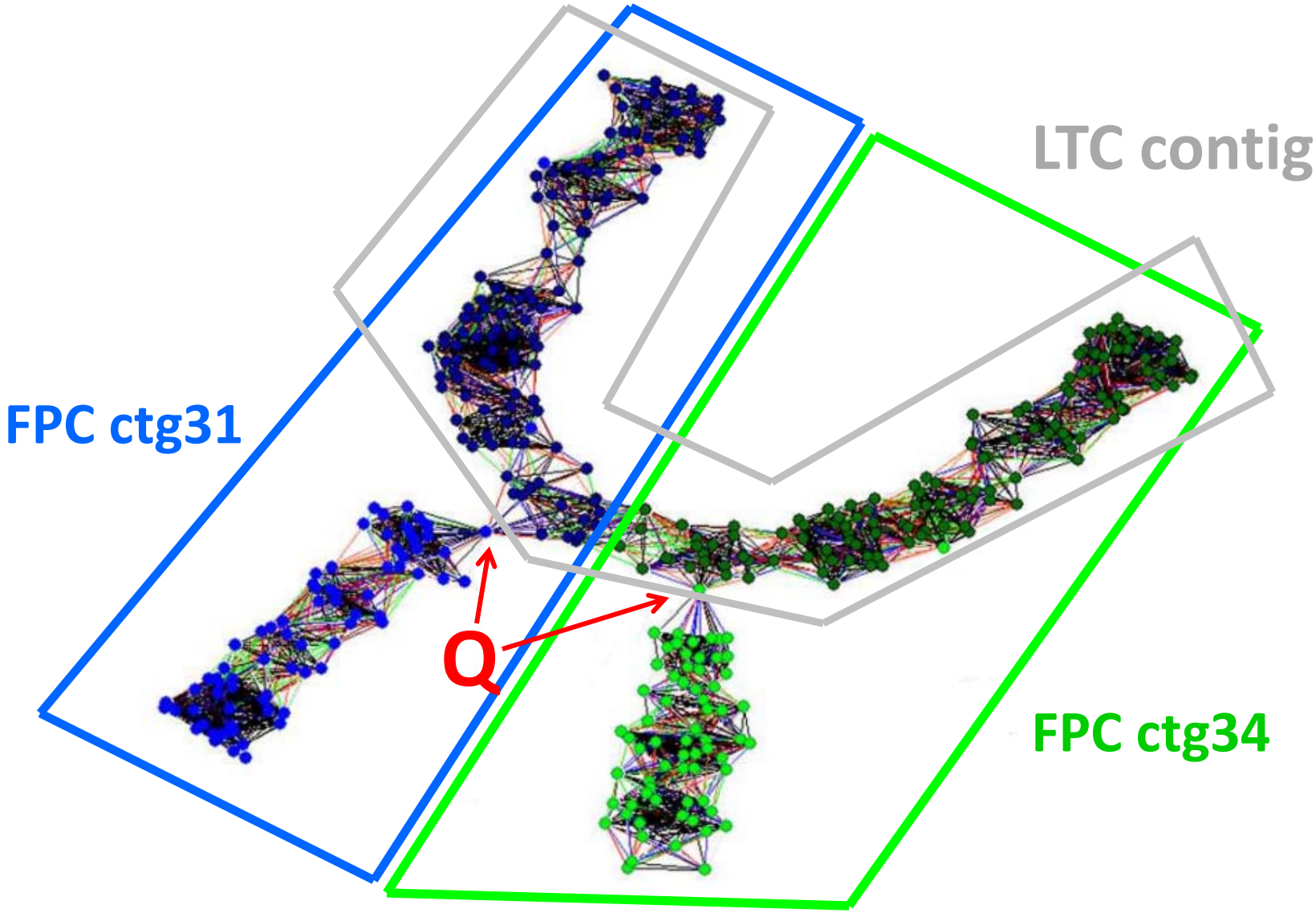
LTC scaffolds vs. FPC contigs

Example from 1BS
(Raats et al., 2013)



Difference in content by FPC and LTC (see next slide)

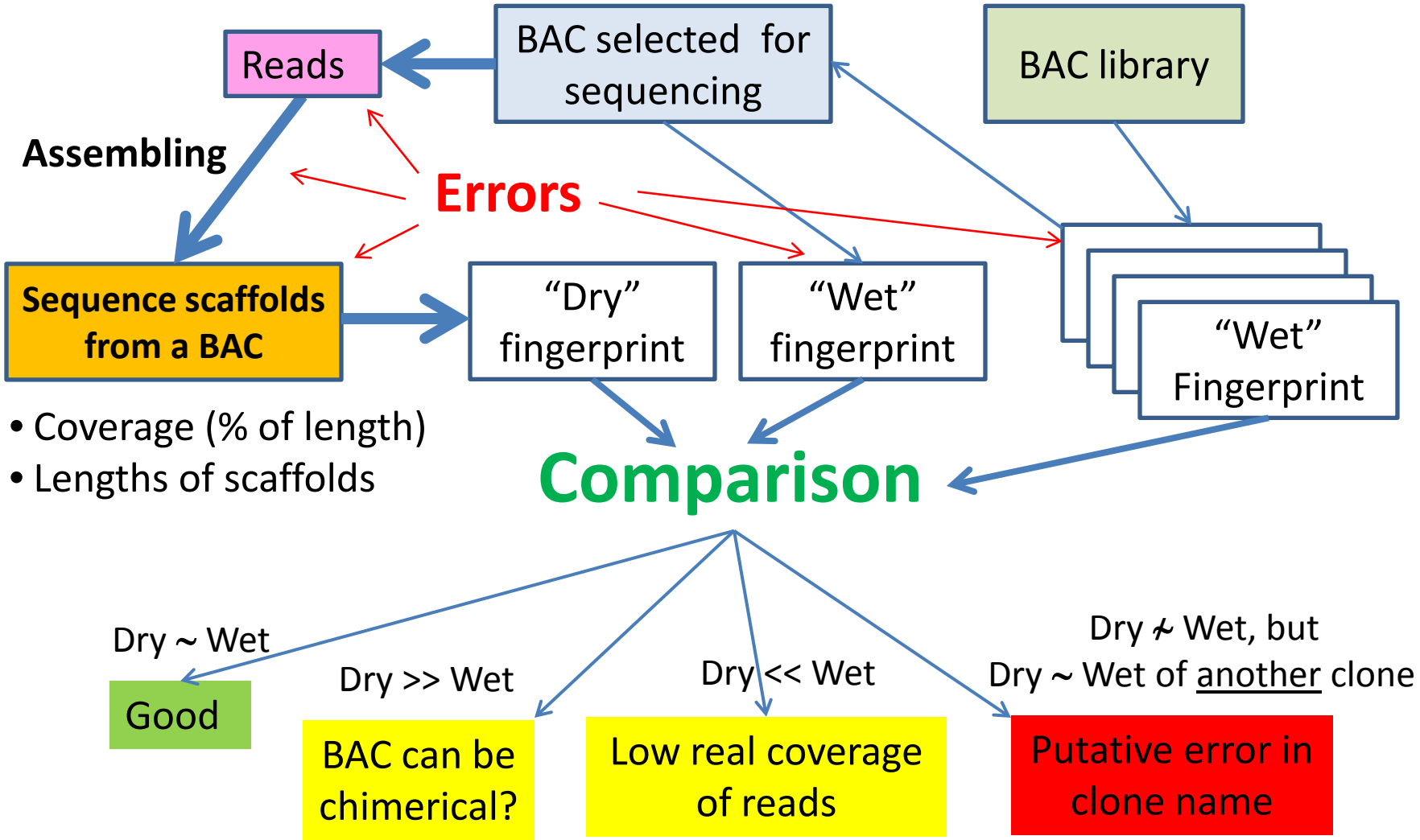
LTC scaffolds vs. FPC contigs



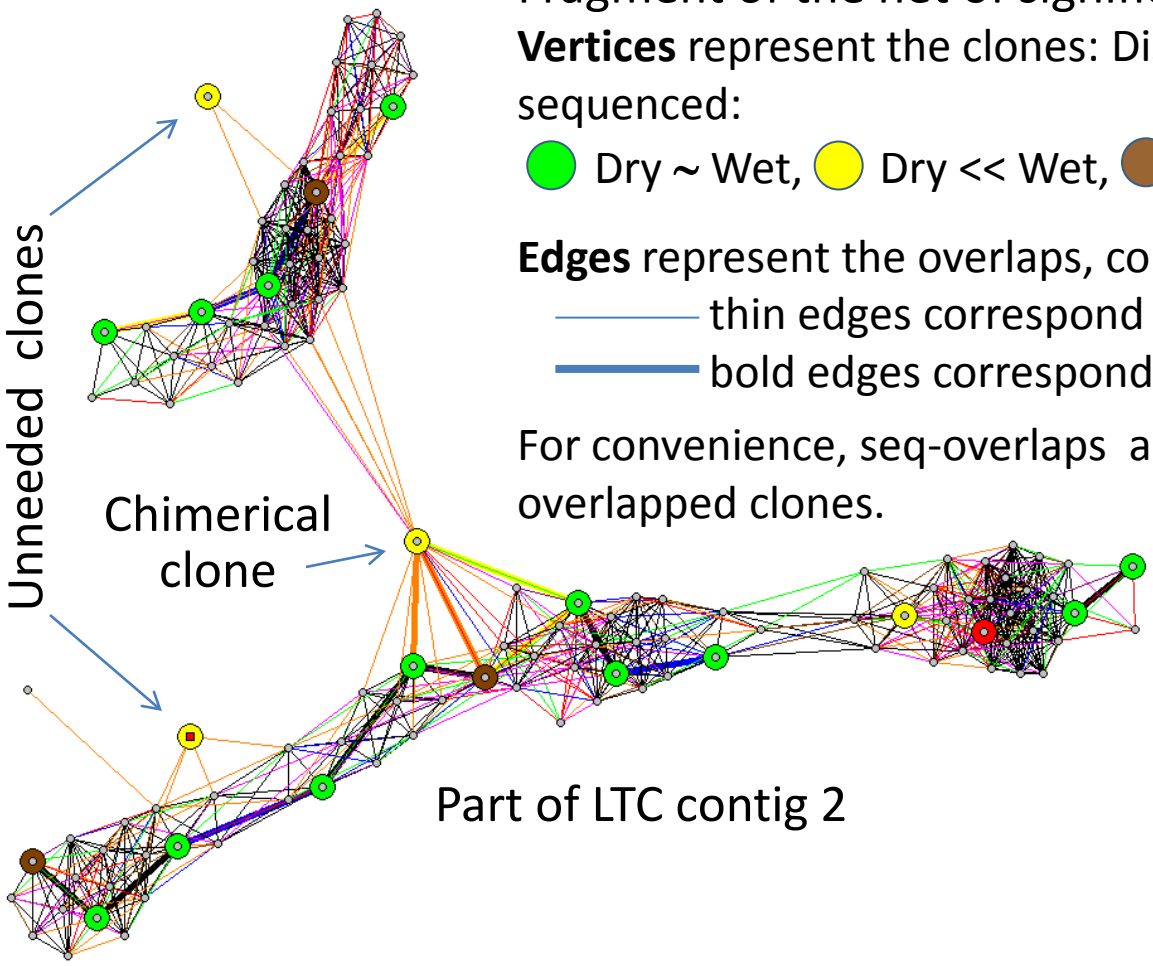
Selecting clones for sequencing by LTC

- 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 gaps

- **Check the physical contig** (a gap can be a result of error in physical contig assembly)
- **Check overlaps** based on fingerprint and sequencing quality
- **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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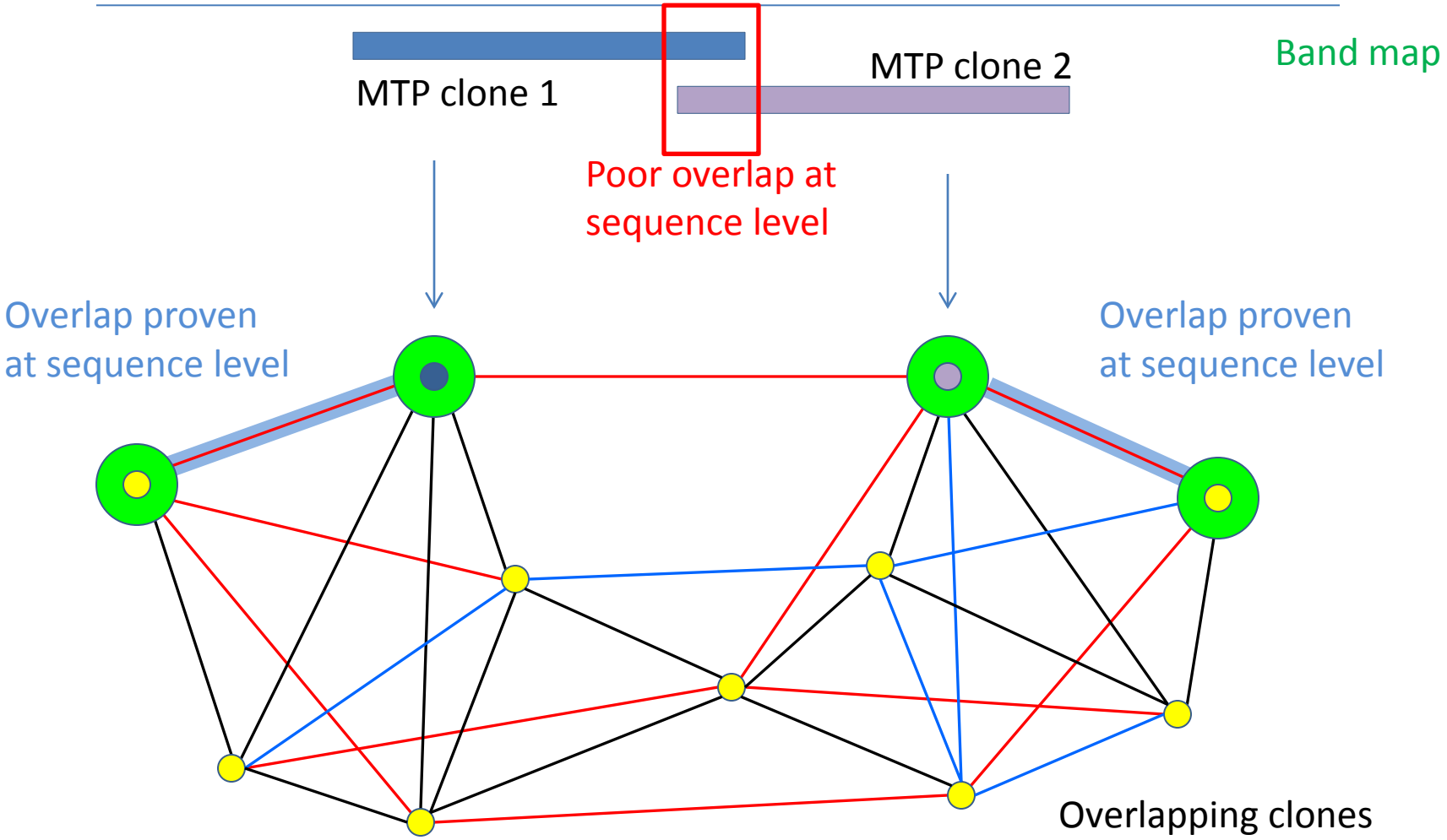
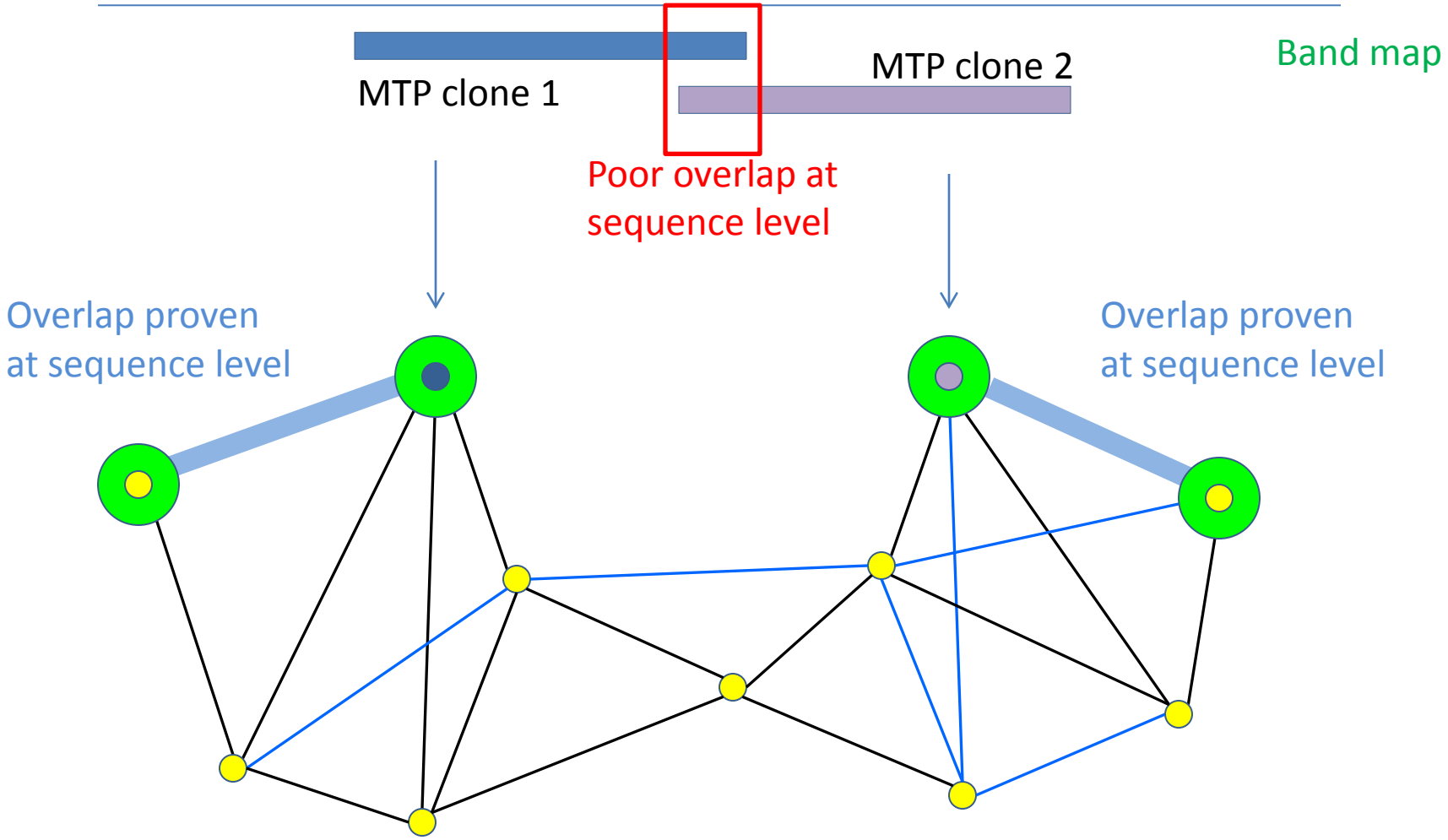
Example of gap repairing

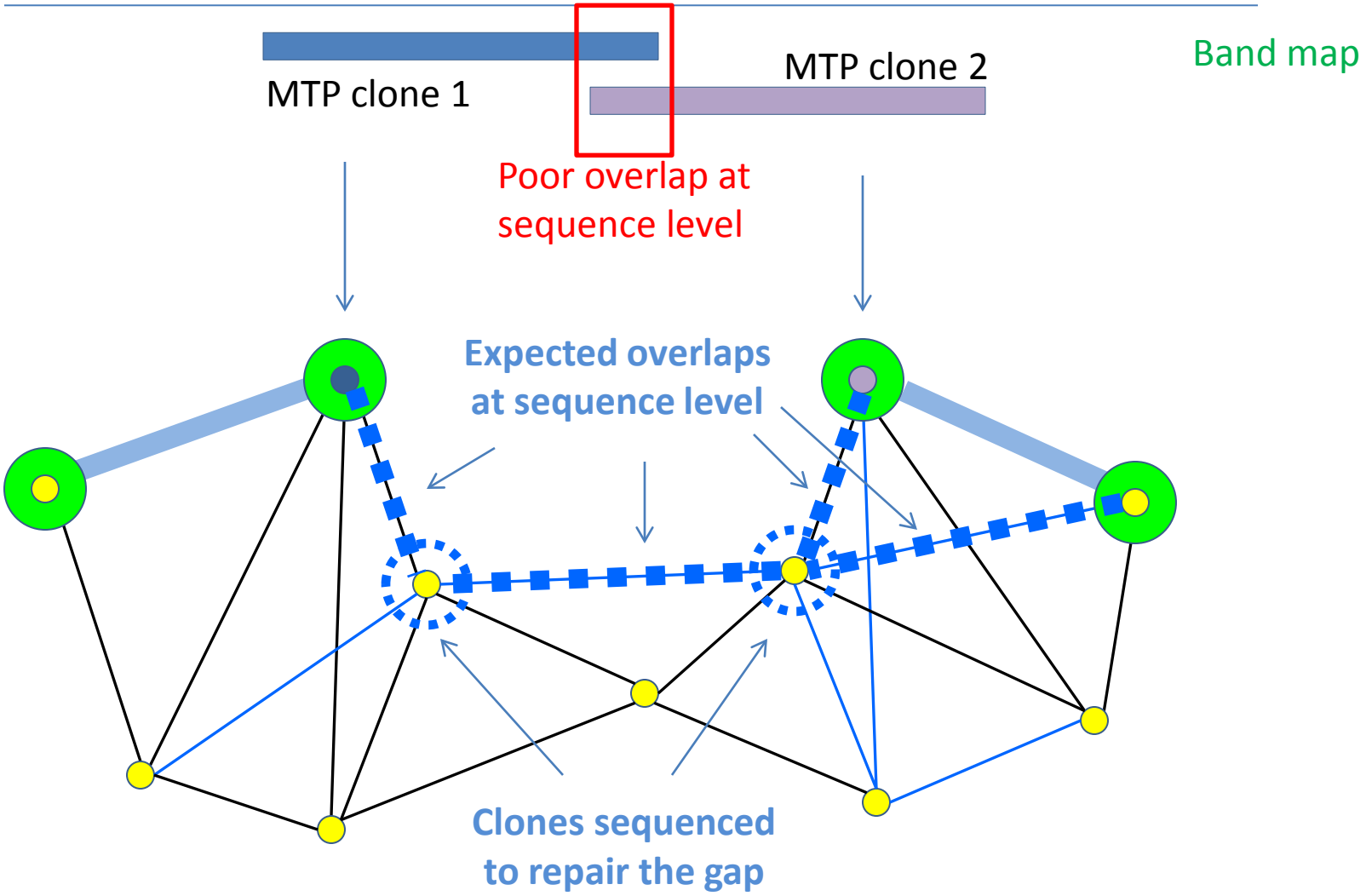


Example of gap repairing



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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Thank you for your attention

Phasing of wheat sequencing activities

- Selection of clones for sequencing: providing high quality physical contigs and selection of MTP clones, enabling to start the sequencing.
- Quality control of assembled sequence contigs based on cross-talks with fingerprints (a proof of principle: our ongoing collaboration with the Norwegian group on 7B).
- Curing of gaps at sequence level: by revising the physical scaffolds; will be assisted by anchoring of the physical contigs to existing maps and to sequences of orthologous genomic regions of related species.
- Improving within-clone sequence assemblies using fingerprinting information of overlapping BAC clones.