# Functional genomics in Picea  abies

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# Scientific and socioeconomic interest

- Science of conifers
  - Evolution: The last major plant group without a sequenced genome
  - Ecology: Dominant members of boreal forests
  - Biology: Unique biological features
- Available genome sequence will aid and facilitate
  - Genomic improvement for biomass productivity, quality, health
  - Optimise cellulose and wood fibre qualities (new materials)
  - Optimised feedstock for bio-refineries
- Norway spruce is Sweden’s most economically important tree
  - 30% net exports
  - 3,000 spruce trees per citizen
  - Annual growth increment worth 2.2 billion €  or ~240 € per citizen per year

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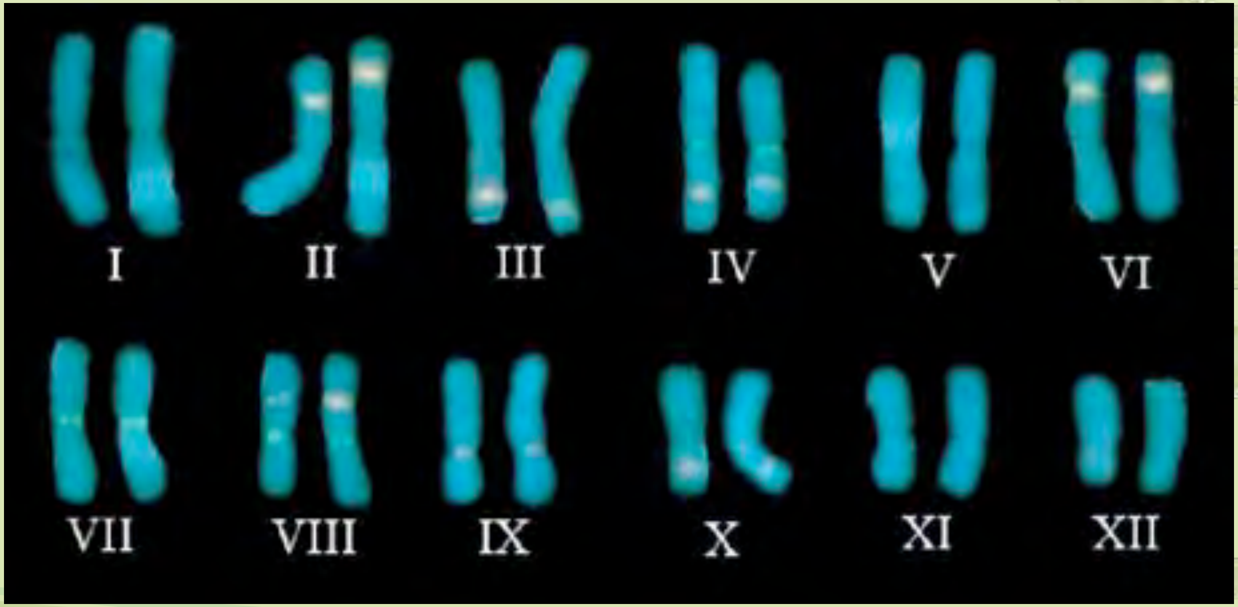
# Sequencing and assembly

- Challenges
  - 19.6 Gbp genome
  - 12 evenly sized chromosomes (Chromosome sorting not possible)
  - Fairly high heterozygosity
  - High repeat content



Vischi et al (2003)

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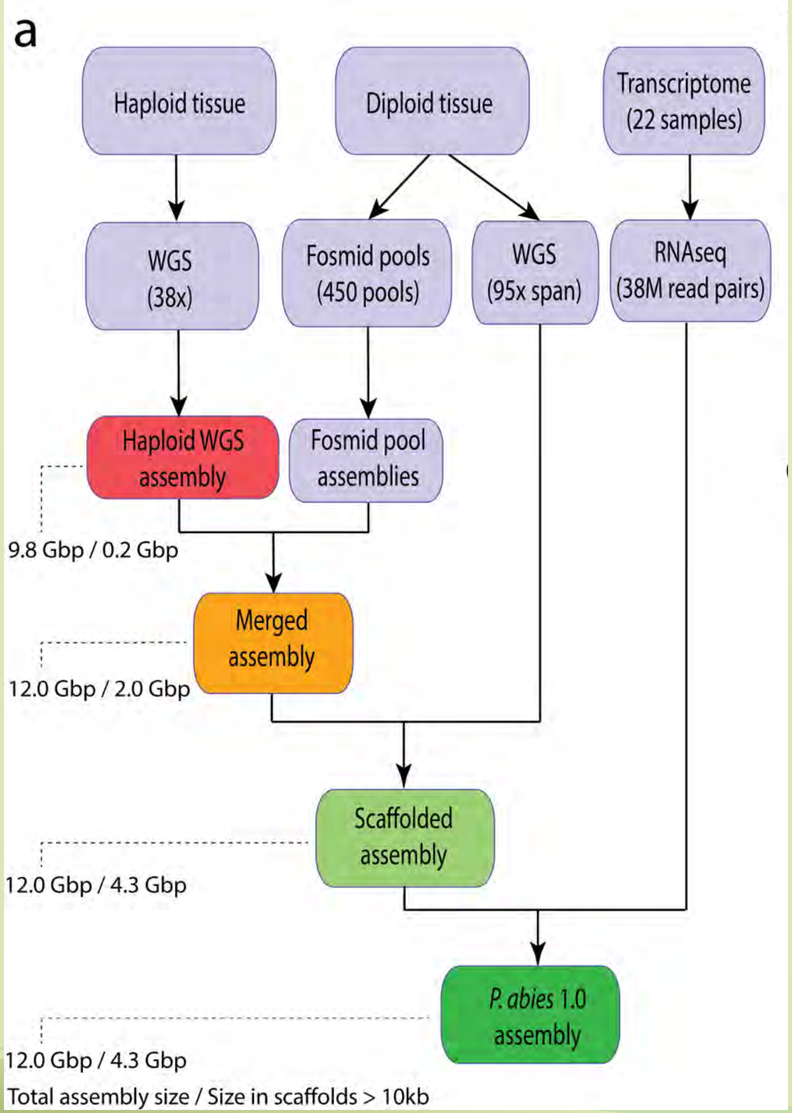
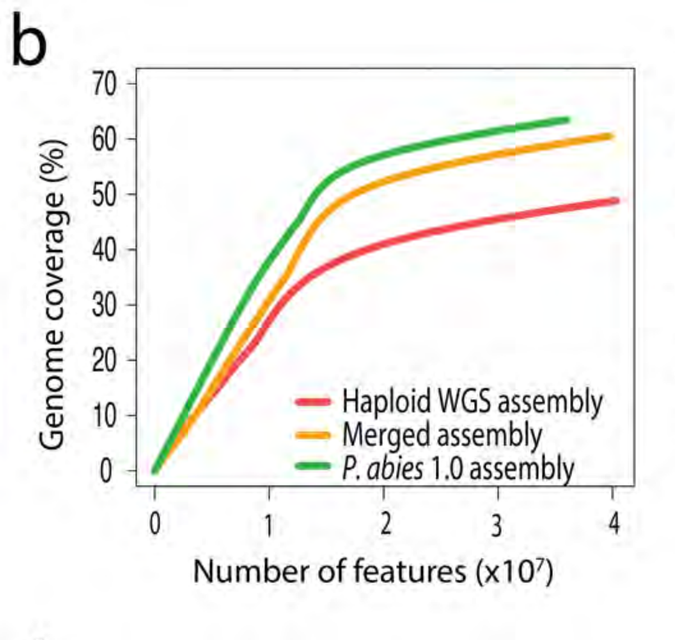
# Hierarchical assembly strategy





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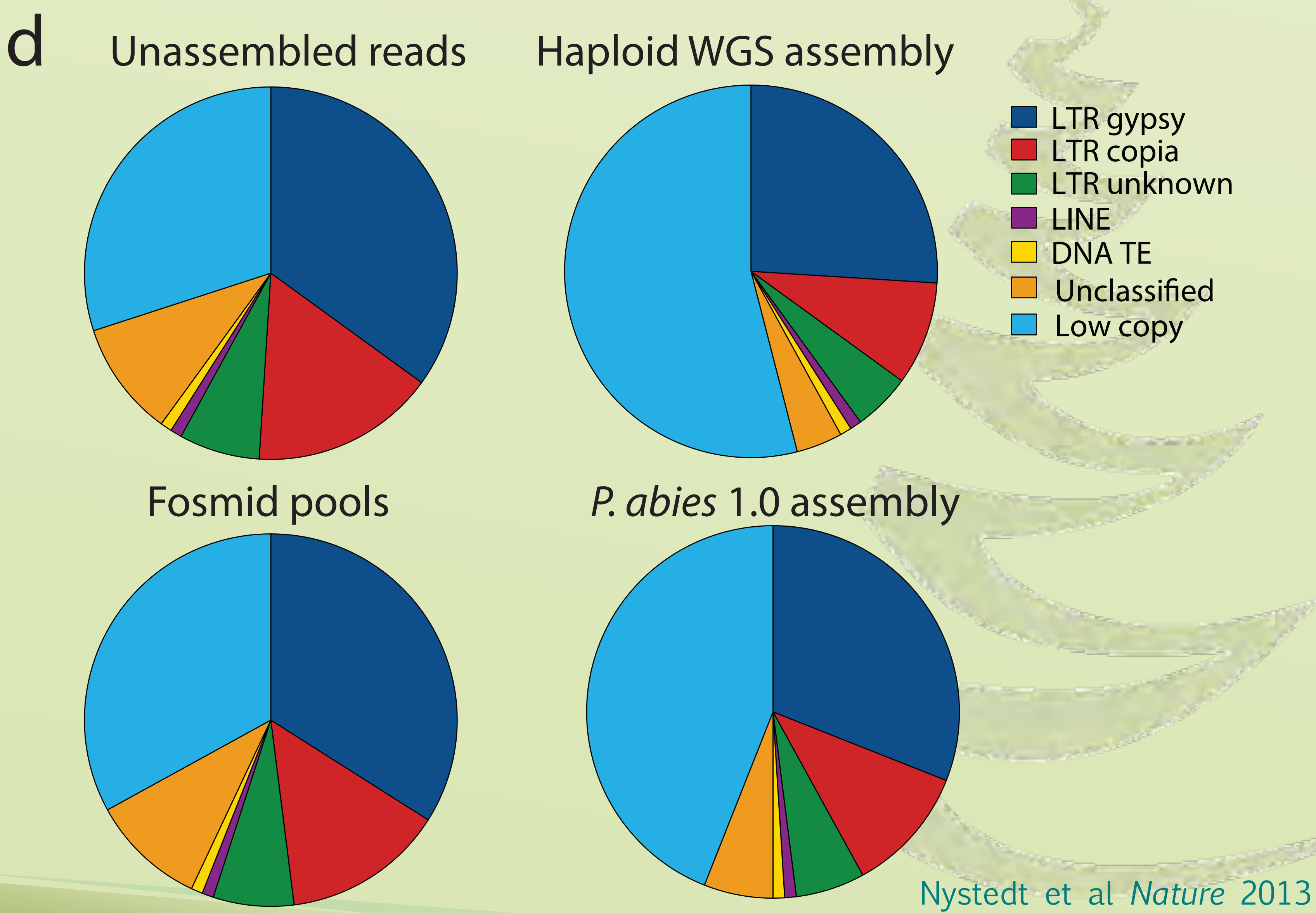
# Fosmid pool sequencing



Nystedt et al *Nature* 2013

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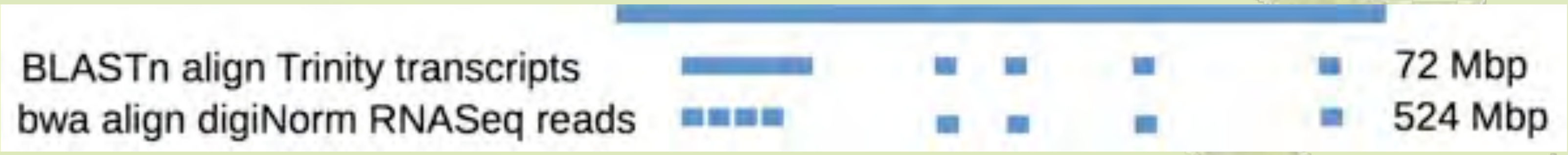
# Genome sub-selection for gene annotation

- Sub-selection of genomic scaffolds were done before gene prediction


BLASTn align Trinity transcripts
72 Mbp
bwa align digiNorm RNASeq reads
524 Mbp

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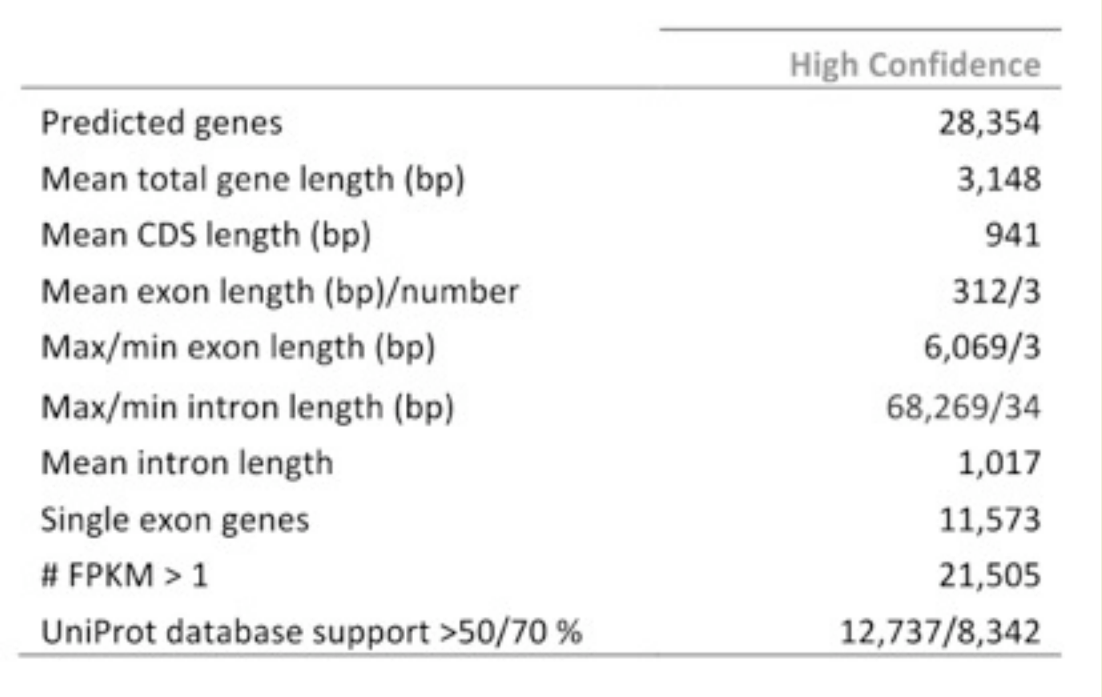
# Picea abies gene annotation

- Augustus+Eugene were run on scaffolds showing evidence for transcription (bwa mapping RNAseq reads) + all Scaffolds >10 Kbp
- 26,597 High Confidence gene predictions
  - >70 % supporting evidence coverage
- 32,263 Medium Confidence predictions
  - 30-70 % coverage
- 8,197 Low Confidence predictions
  - <30 % coverage



| | High Confidence |
|---|---|
| Predicted genes | 28,354 |
| Mean total gene length (bp) | 3,148 |
| Mean CDS length (bp) | 941 |
| Mean exon length (bp)/number | 312/3 |
| Max/min exon length (bp) | 6,069/3 |
| Max/min intron length (bp) | 68,269/34 |
| Mean intron length | 1,017 |
| Single exon genes | 11,573 |
| # FPKM > 1 | 21,505 |
| UniProt database support >50/70 % | 12,737/8,342 |

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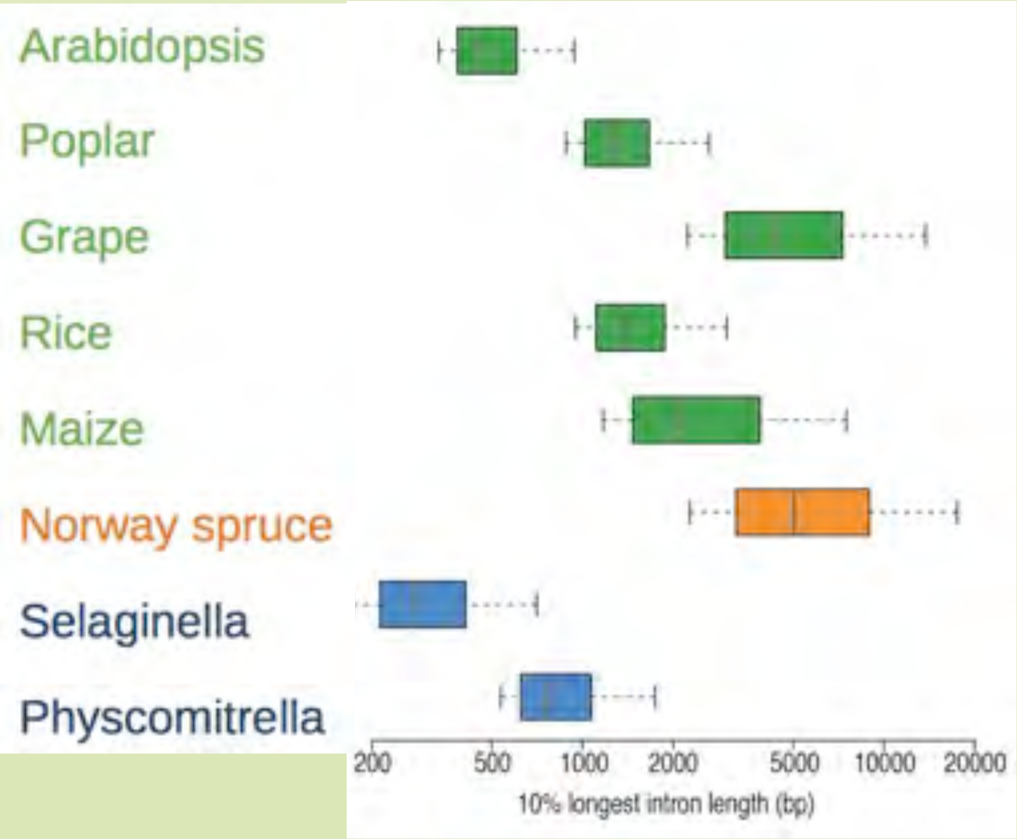
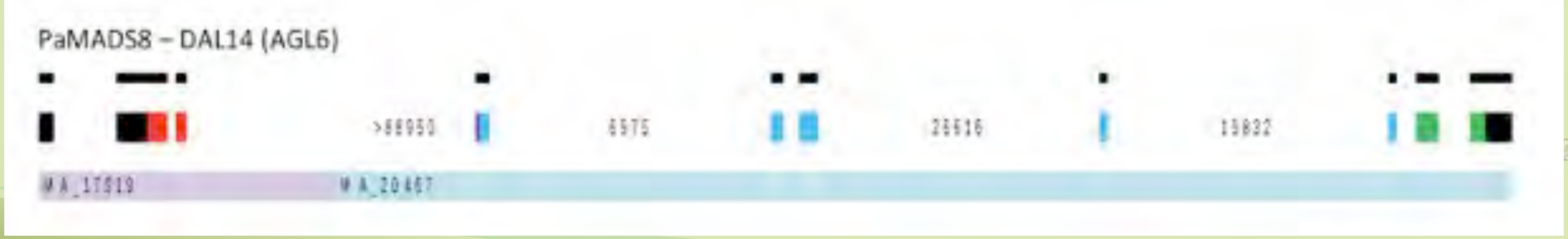
# Long introns



2,384 HC genes contain 2,697 introns >5 Kbp

2,679 contain TE



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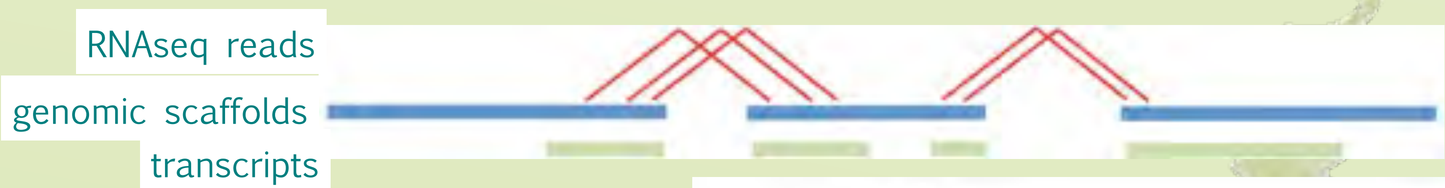
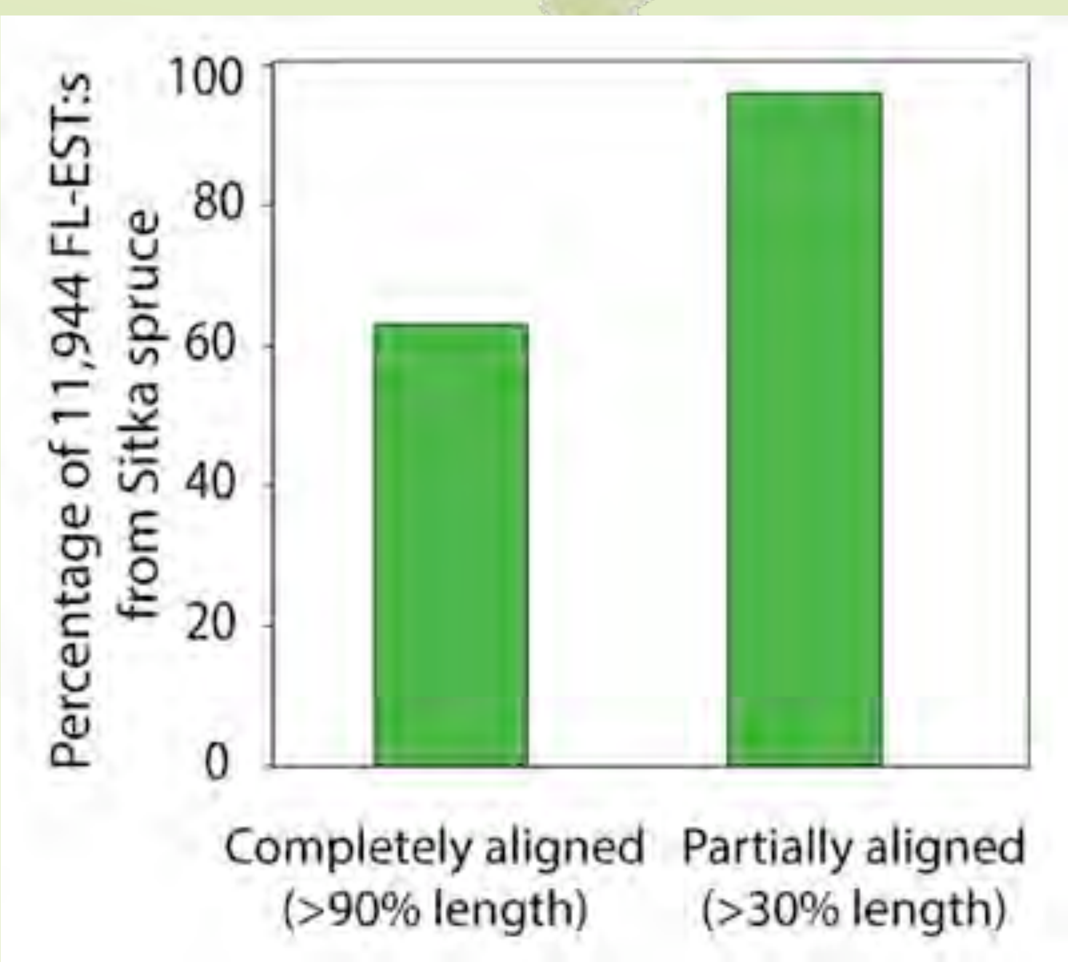
# RNAseq scaffolding



- For a relatively large fraction of genes, assembly breaks in long (>10kb) introns
- Scaffolding using RNAseq data alleviates this to some extent (59% → 64%)

- Transcript, PacBio and gene fusion based scaffolding
- expected to complete ~5000 additional genes



Nystedt et al *Nature* 2013

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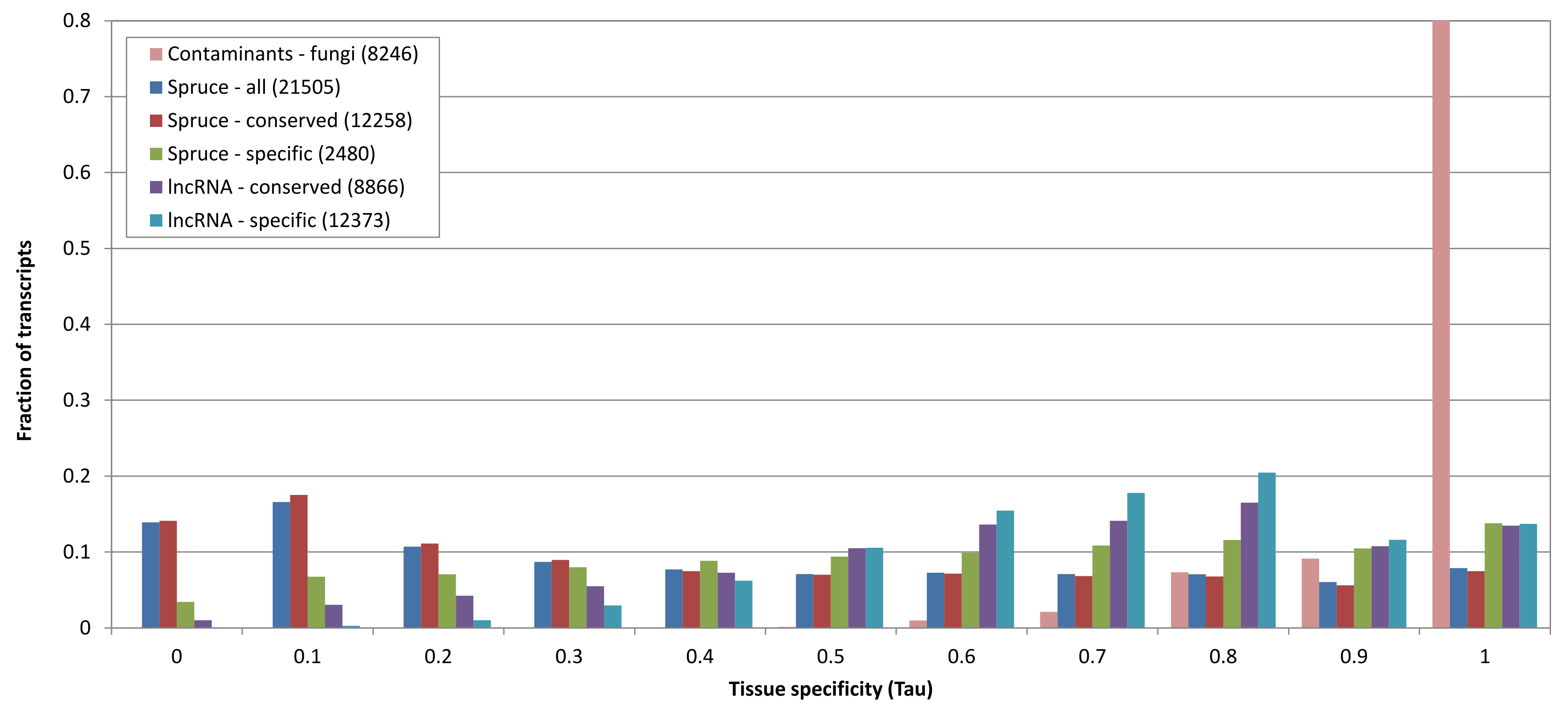
# Expression across tissues



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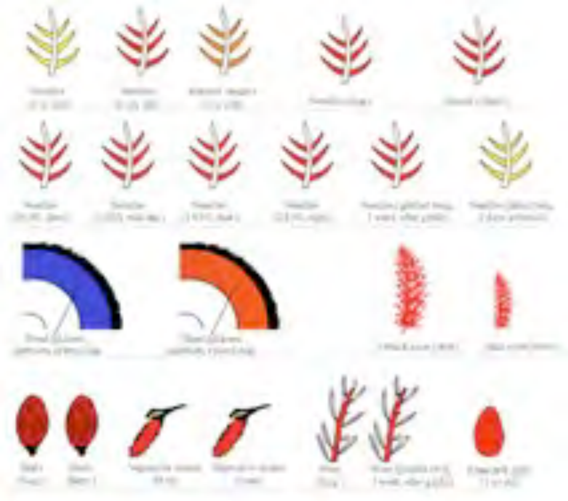
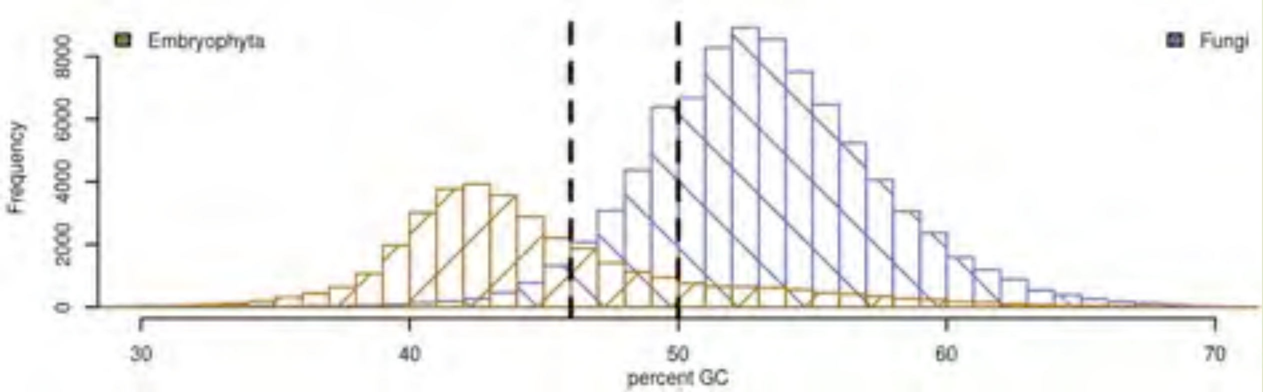
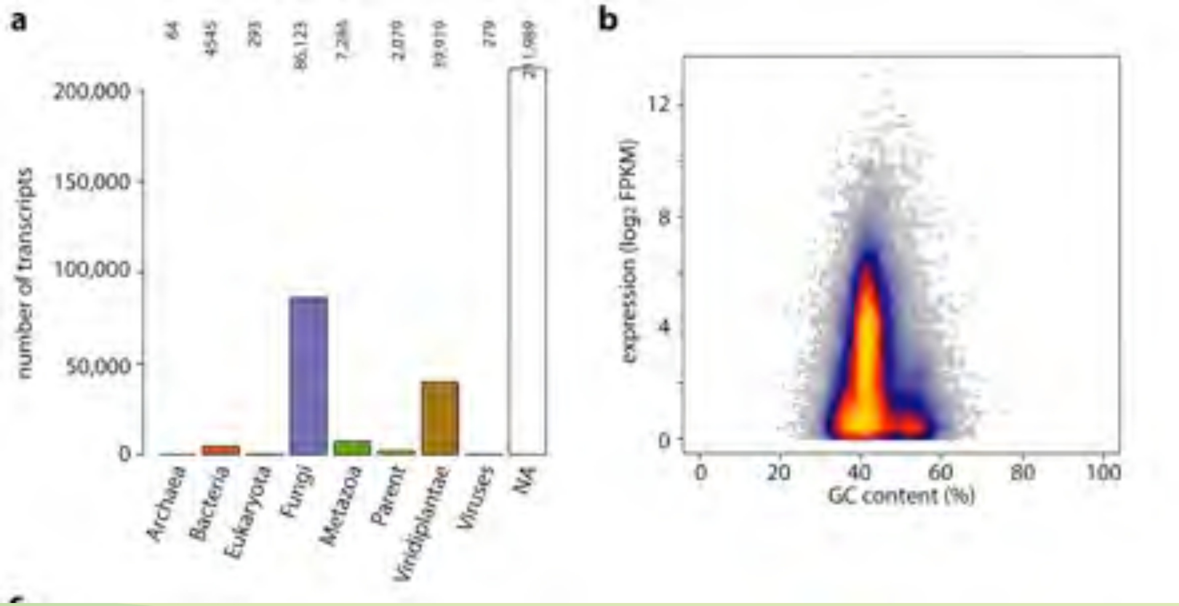
# An expression catalogue



22 libraries, 50 M PE reads per library





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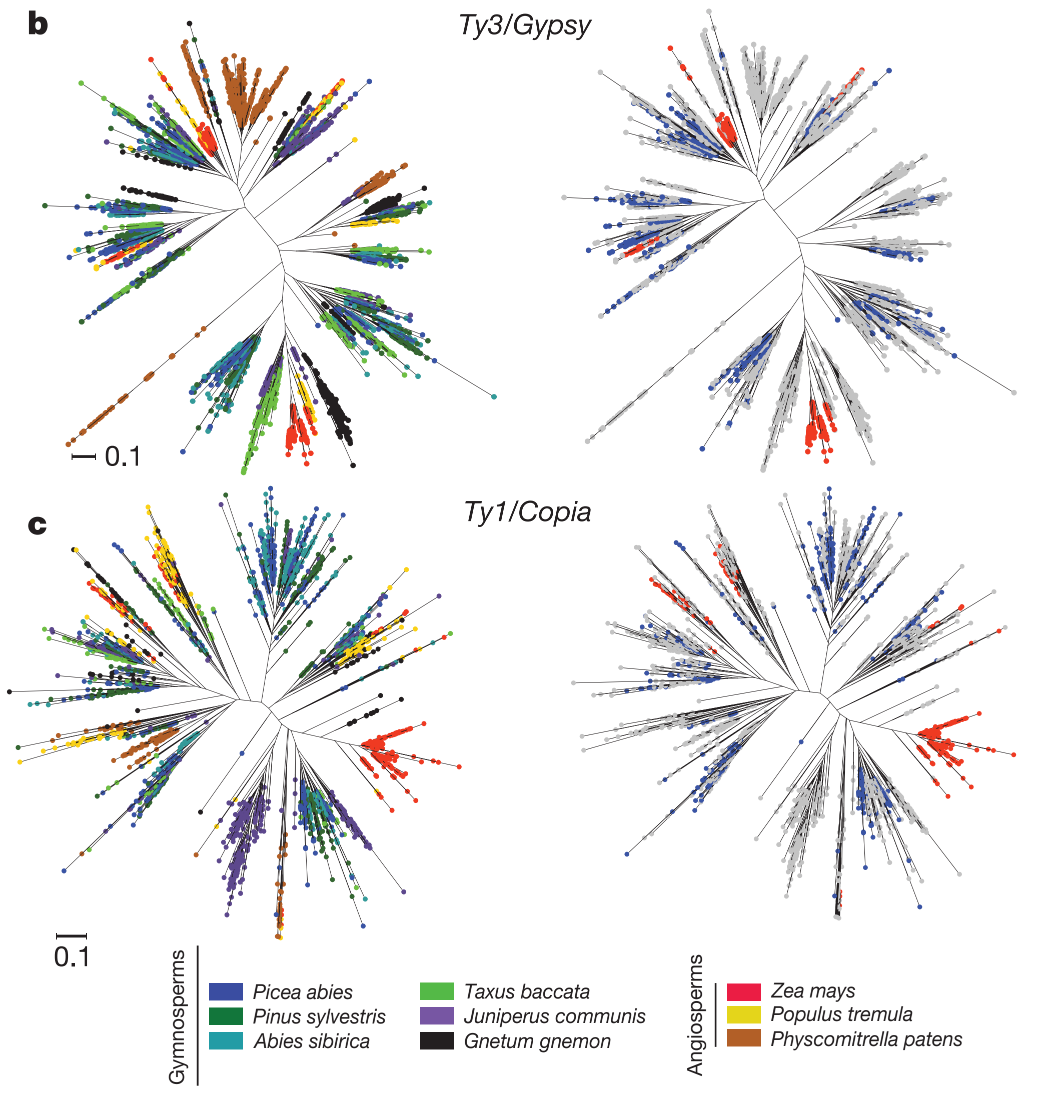
# Identification of repetitive sequences

- Identitification
  - For each species: random samples of 100000 454 reads longer than 500 bp
  - RepeatScout + cap3 + cd-hit-est clustering

- Characterization
  - Similarity (blastX and tblastx) searches against other TEs database and nr GenBank.
  - removal of plastid contaminants and candidates having hits with known gene families

# LTRs are widespread ad shared between species



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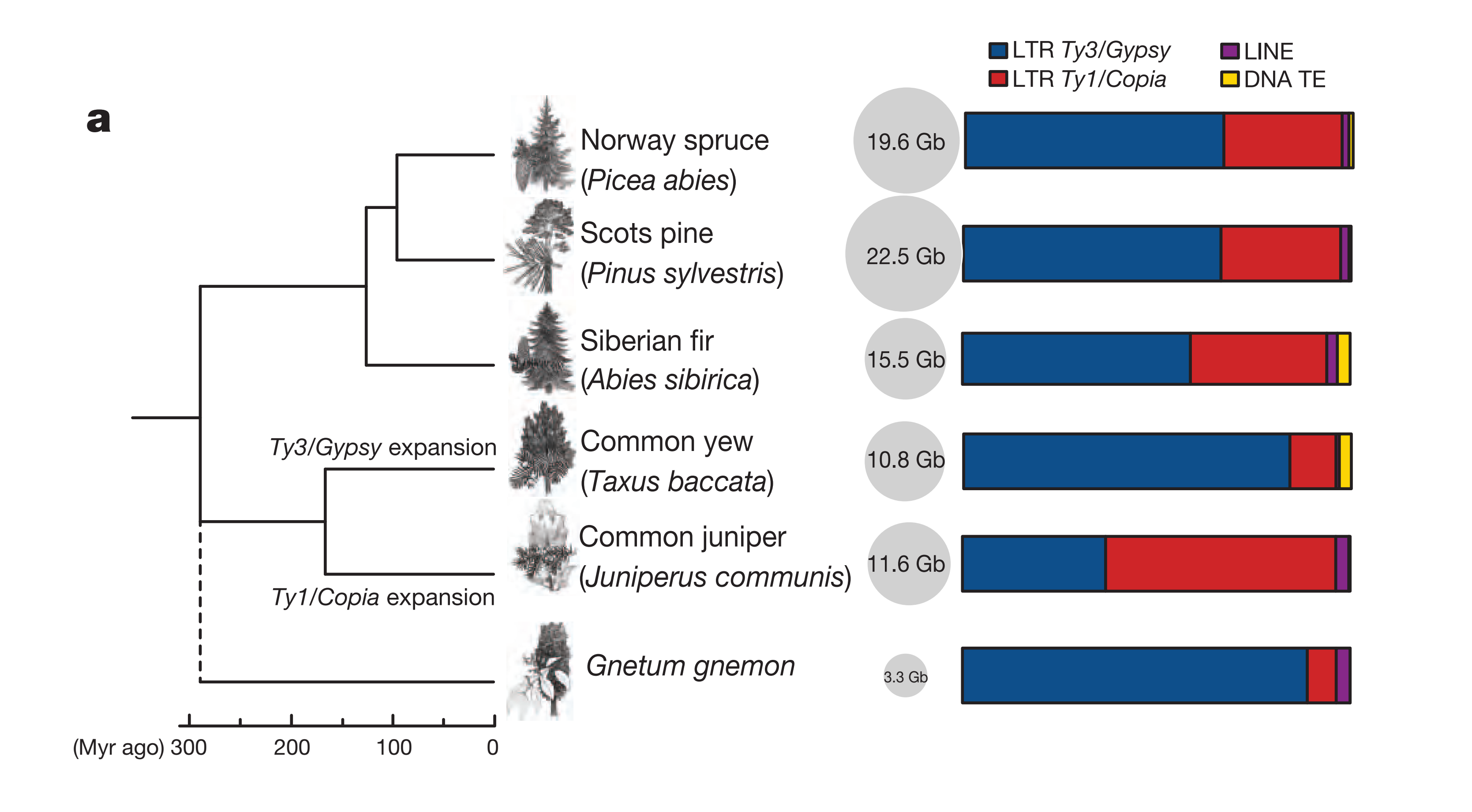
# Repetitive structure of the Norway spruce genome



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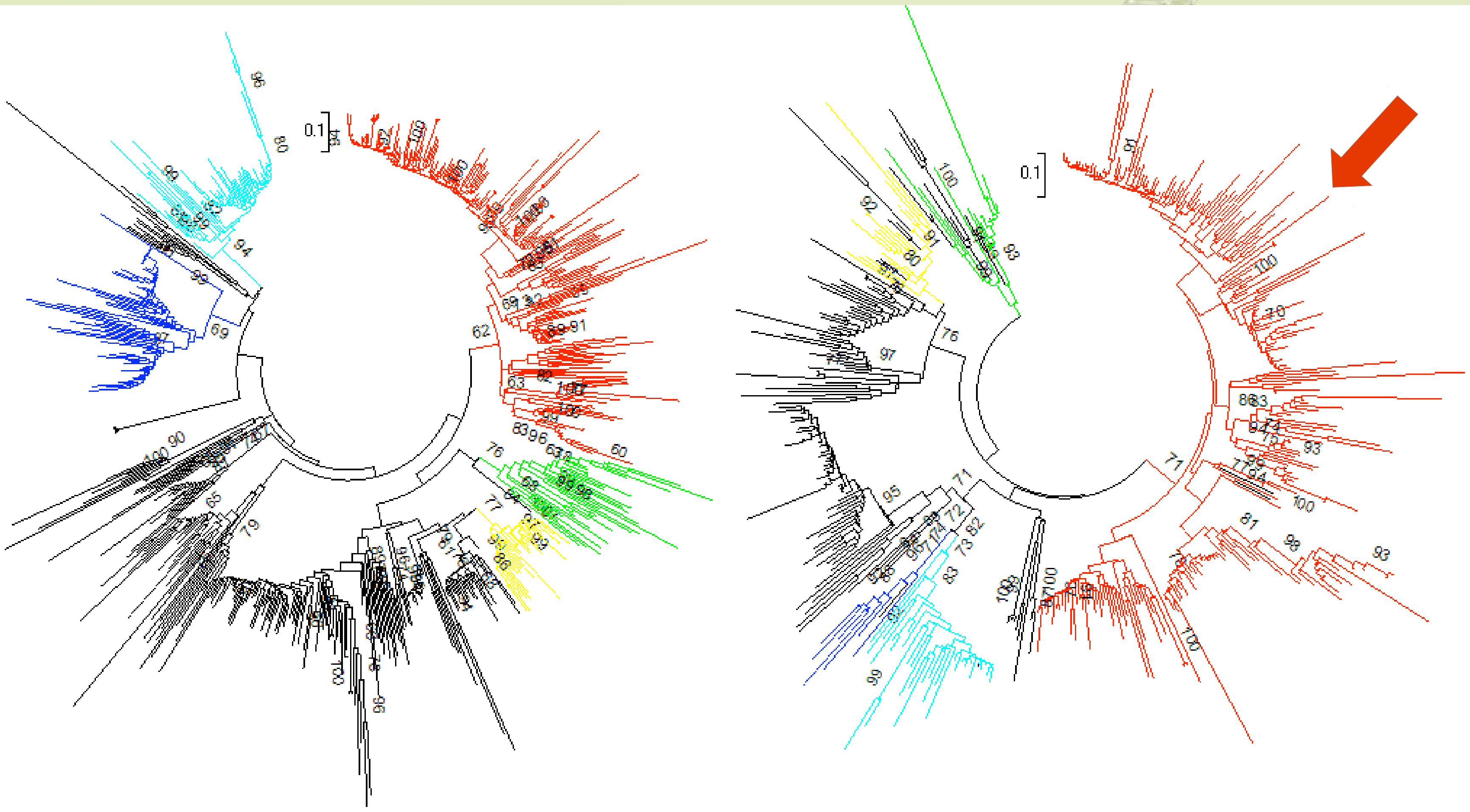
# The same LTR group have different fates in different conifer species



*P. abies*  Ty1-copia  *Abies alba*

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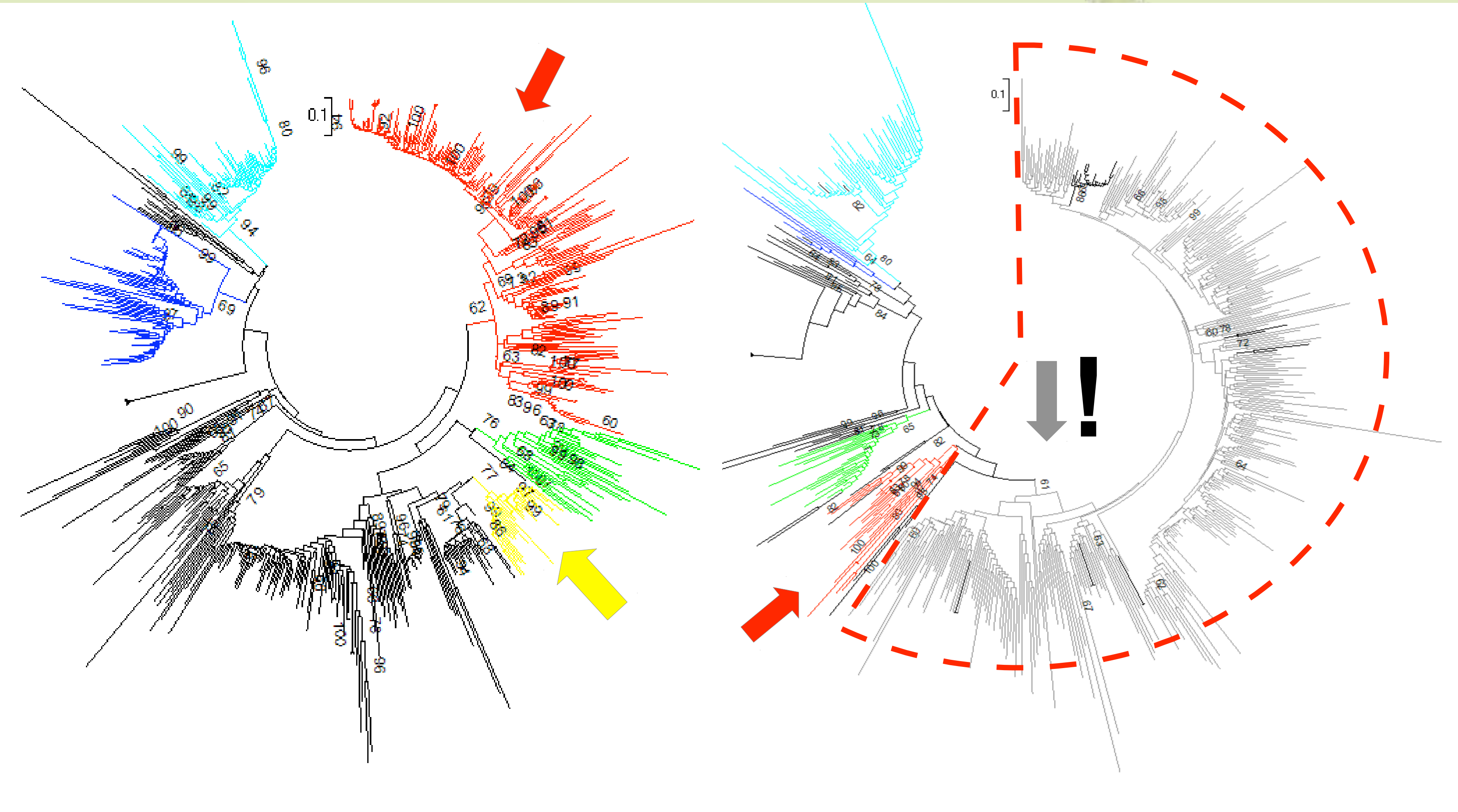
# The same LTR group have different fates in different conifer species - an extreme case!



*P. abies*

Ty1-copia

*Juniperus communis*

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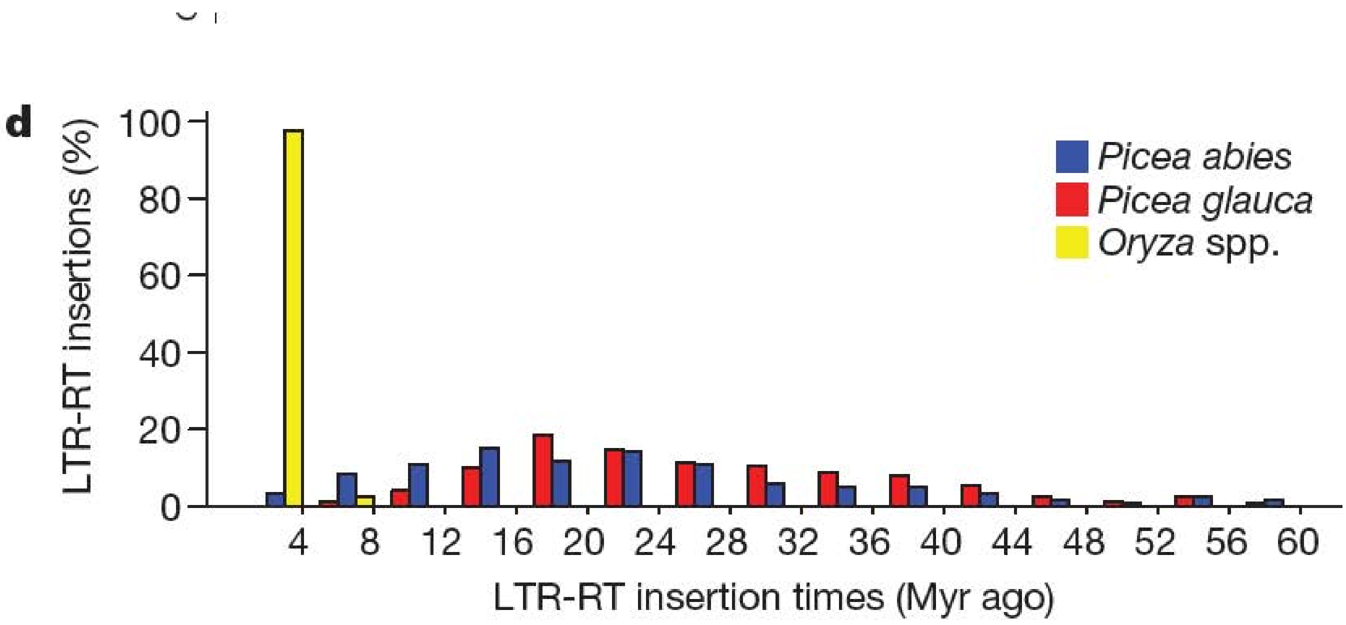
# Summary of the repetitive fraction

- Conifer large genomes are mostly repetitive
- LTR-RTs are the most represented TE class and are shared across different genera;
- LTR-RTs from the same group had different fates in different species...
- ...nothing unexpected and nothing that different from what was already described in several angiosperms!

# Amplification of conifer LTRs occurred in ancient times



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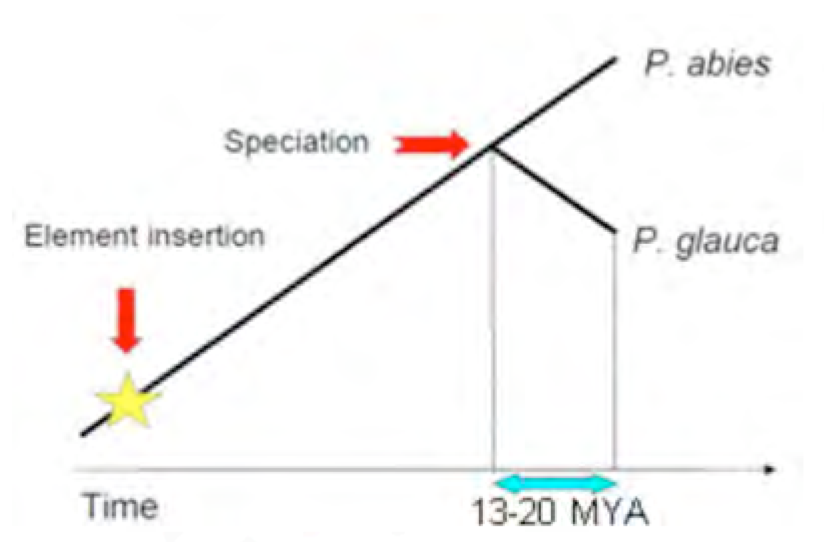
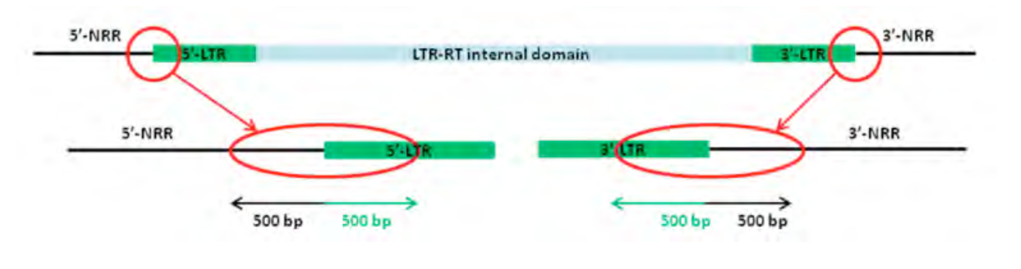
# LTR amplification in Picea mostly pre-dates speciation

- 113 LTR-RTs complete elements were identified in 25 *P. glauca* BACs
- The tracts spanning for 1000 bp all the boundaries genome-LTR-RT-genome were mapped onto *P. abies* assembly
- 45 elements: not mappable
- 5 inserted in *P. glauca* after speciation (empty spot in *P. abies*); younger than 13 MY
- 63 inserted before speciation: older than 13 MY





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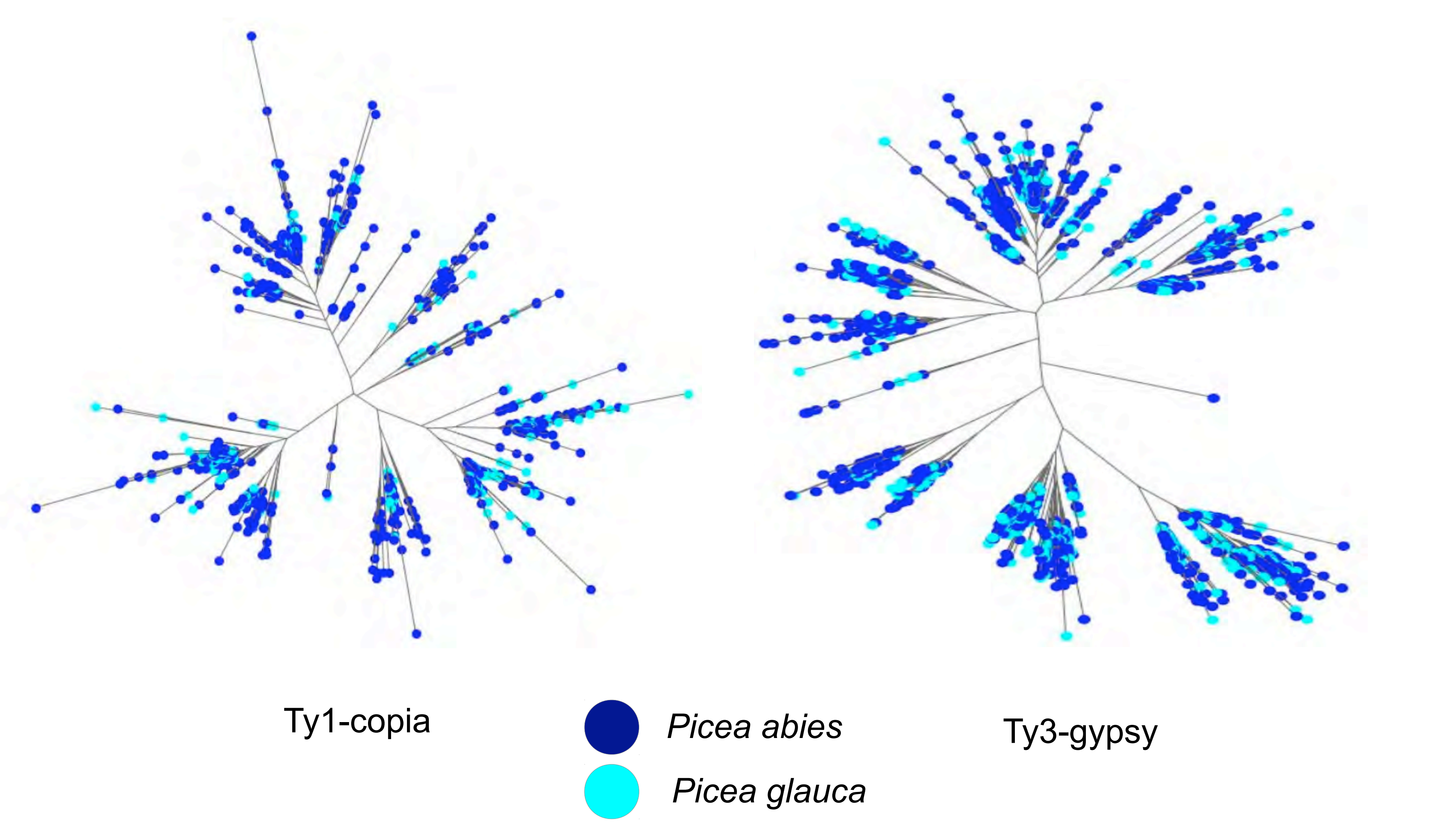
# LTR amplification in Picea mostly pre-dates speciation



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# Summary of LTR insertion dynamics

- LTR-RTs massively amplified in ancient times;
- There is no (or limited) evidence of recent LTR-RTs insertional activity;
- A huge amount of ancient inserted LTR-RTs escaped removal
- The emerging scenario is totally different from the one in angiosperms and leads to a crucial question:
- Why (How) these elements have been retained?

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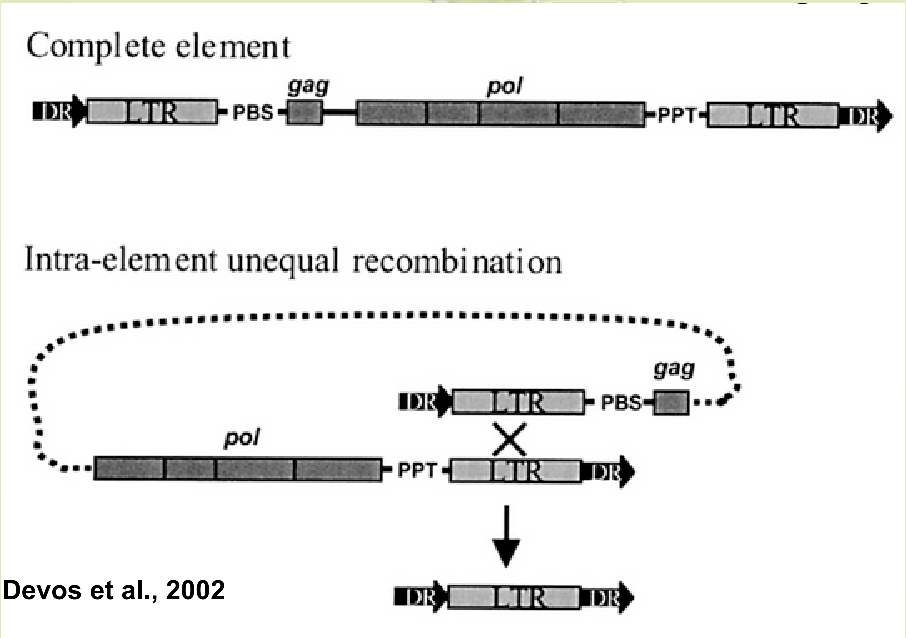
# LRT removal through unequal recombination

- We searched for evidence of UR for 3 LTR-RT families in *P. abies*
- We used 4 BACs, 20 fosmids and the assembly contigs longer than 50 kbp



Devos et al., 2002

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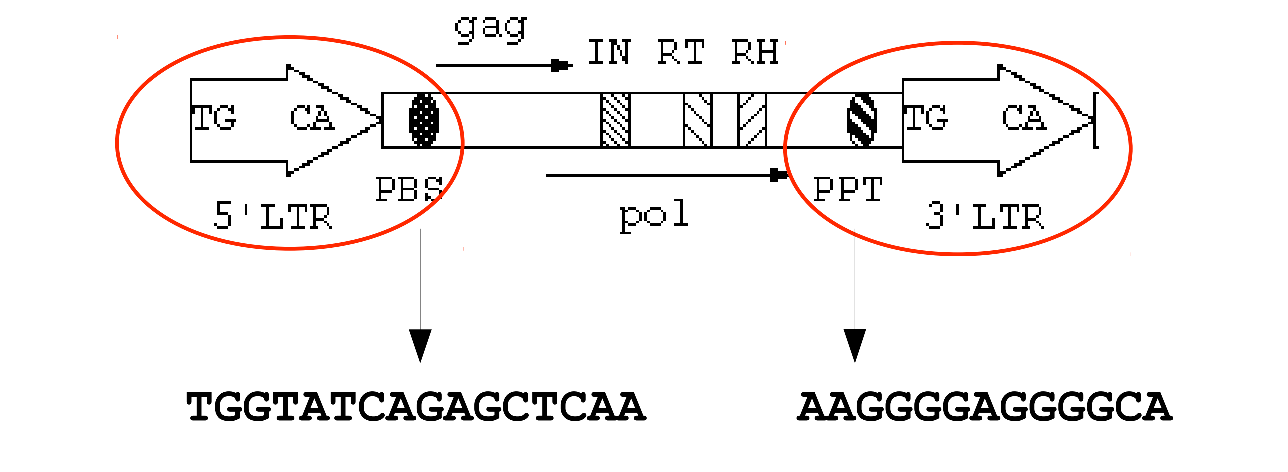
# Estimating LTR removal through unequal recombintion



- LTRs can be assigned to complete elements or identified as a solo-LTR
  - If it is followed by PBS...it's the 5' LTR of a complete element;
  - If it is preceded by PPT...it's the 3' LTR of a complete element;
- If there is no evidence of PPS or PPT and there are TSD at end → solo LTR
- Any other case → UNCLEAR (amount should be negligible)
- Ratio complete_elements: sLTRs ---> (5'LTR +3'LTR)/2 : sLTR

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# Estimating LTR removal through unequal recombintion

| LTR Family | Complete elements | Solo LTRs | Ratio |
|---|---|---|---|
| ALISEI | 27 | 5 | 0.185 |
| 3K05 | 26 | 5 | 0.192 |
| 4D08_5 | 43 | 0 | 0.0 |
| ***Total*** | ***96*** | ***10*** | ***0.104*** |

- In *P. abies* the ratio of solo-LTRs to complete elements is ~1:9
- In *A. thaliana*, rice and barley the corresponding ratios are 1:1, 0.6:1 and 16:1.

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# The emerging picture: how did conifer genomes become so large?

Massive LTR-RTs amplification
occurred for a long period in ancient
evolutionary times

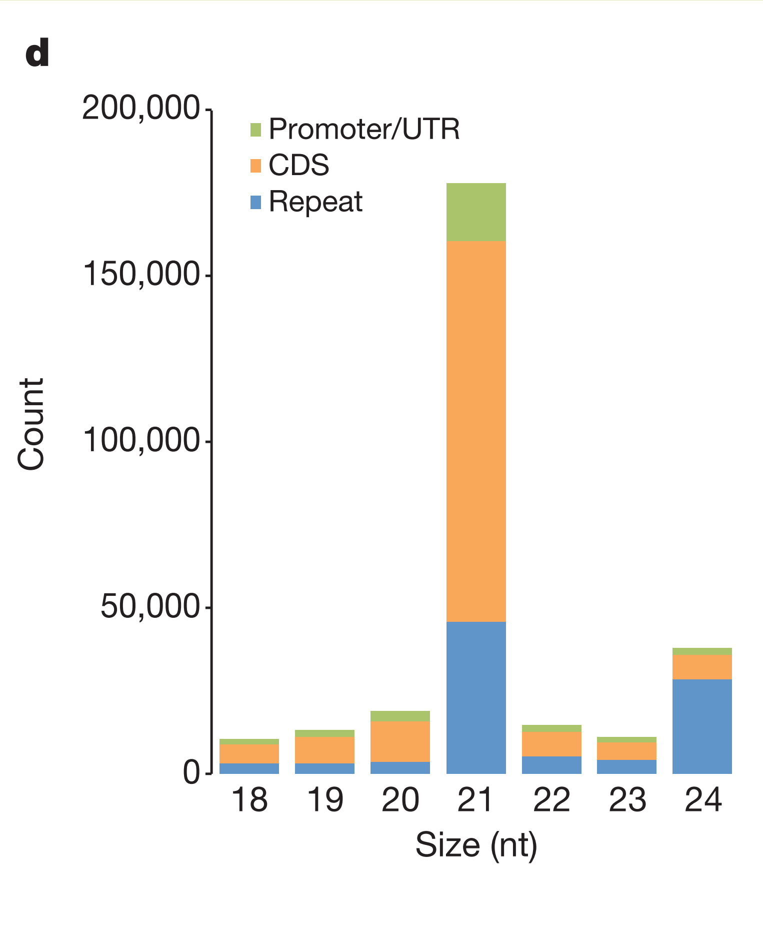
+

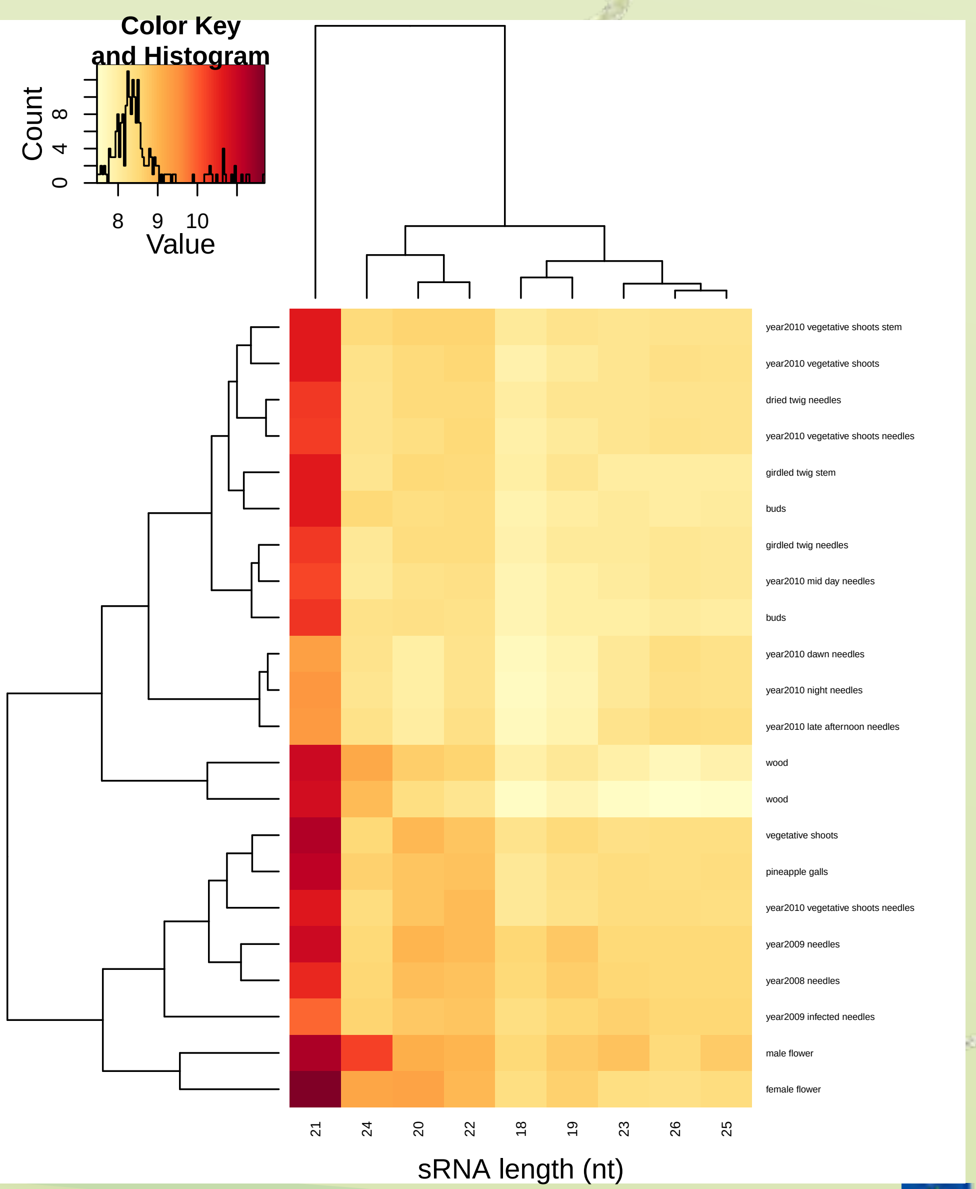
(very) limited homologous recombination
targeting these elements
– No LTR-RTs removal;
– No LTR-RT mediated damage to the
host genome

=

LTR-RT accumulation → Large genomes

# sRNA distribution





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# In-progress RNAseq and sRNA studies

- Root, needle, cambium and soil seasonal profiling: control and long-term fertilized + metagenomics and metatranscriptomics
- Needle developmental profiling
- Phloem and xylem seasonal profiling
- Developing wood cross section
- Somatic embryogenesis
- Reproductive organ development
- Embryo and seedling development
- Suspensor cell
- Diurnal expression in needles and cambium

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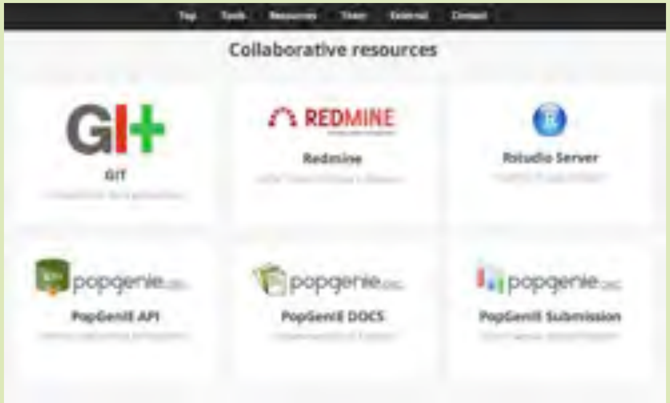
# Bioinformatic resources

PlantGenIE.org



popgenie
Welcome to v3 PopGenIE: The Populus Genome Integrative Explorer

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# The Norway Spruce Genome Team

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Susanne Larsson
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Detlef Weigel

**Skogforsk**
Bengt Andersson
Bo Karlsson

**SNIC Supercomputers**
Uppmax/PDC/NSC/HPC2N

**SNISS national infrastructure**

**CLCbio**

**Lucigen**

Knut och Alice Wallenbergs Stiftelse

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