A first look at the large and complex genome of Norway spruce (*Picea abies*)

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The Spruce Genome Project

Why sequence the Norway spruce genome?

The scientific argument:

- Evolutionary interesting group
  - conifers are evolutionary ancient and the last major plant group which have not at least one member with a complete genome sequence
- Ecological importance
  - conifers are dominant members of many ecosystems, primarily in boreal forests
- Unique biology?

Why sequence the Norway spruce genome?

The strategic argument:

- Norway spruce is the economically most important Swedish tree
- Genome sequence will spark research to generate:
  - New tools for breeding for tree productivity, quality, health
  - New knowledge and tools for cellulose and wood fibre modification (new materials)
  - New knowledge and tools for tree-based biorefineries

Challenges with sequencing a conifer genome

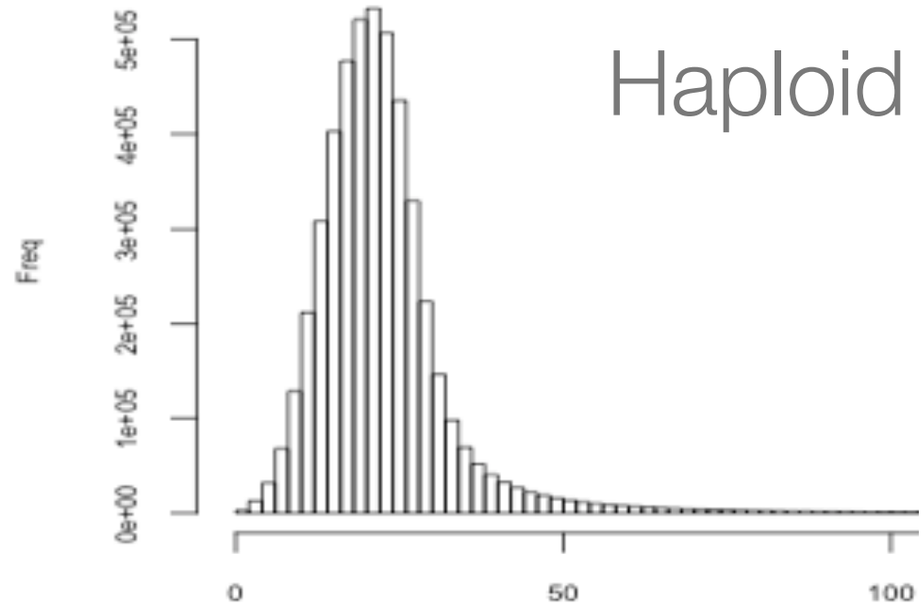
- Huge genome of approximately 20 Gb – seven to ten times the human genome
- The Norway spruce genome will be the largest genome sequenced so far
- >99% is likely moderately or highly repetitive DNA of unknown function
- Large gene families and abundant numbers of pseudo-genes
- <3% consists of sequences homologous to genes.

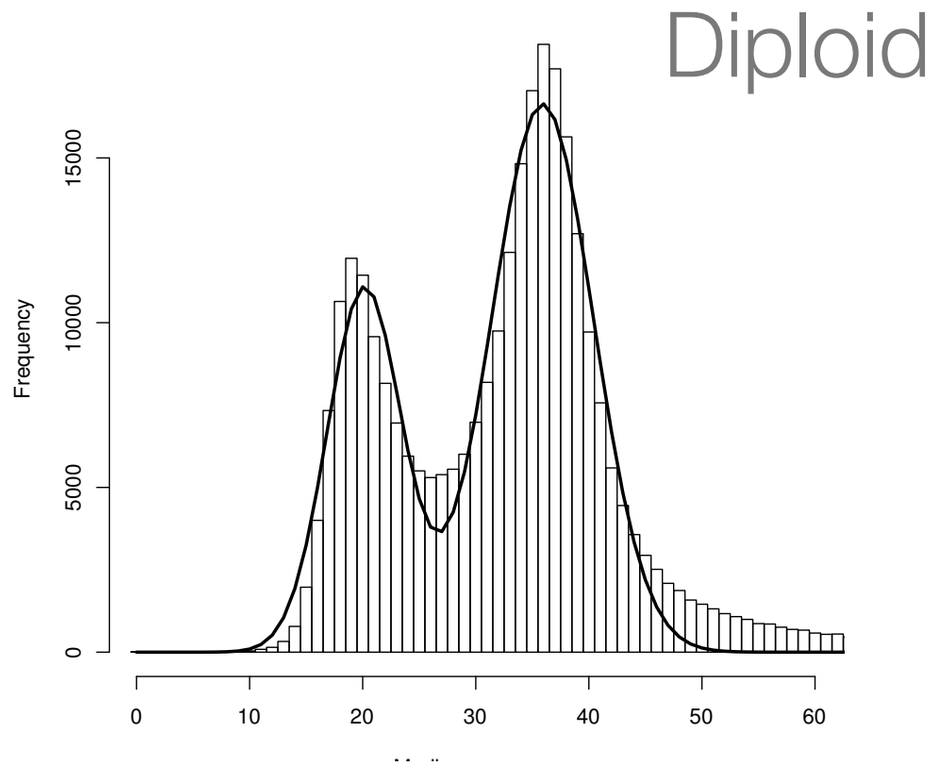
Sequencing status

Single-end Roche 454	1x read coverage ~450 bp 1x read coverage ~650 bp
Paired-end Illumina 2*100 bp	>50X read coverage 150, 300 and 650 bp fragments
Mate-pairs Hybrid Roche/Illumina	30X span cov 3 kb fragments 20X span cov 5 kb fragments
Fosmid ends	20X span cov
Fosmid pools	500 000 40 kb fosmids in pools of 1000
Silver standard Fosmids Roche 454	100 X
Mitochondria Roche 454 (long)	100 X

Current genome assemblies

	Haploid	Diploid
Coverage	20x (10x?)	50x + 1.5x 454
Contigs > 1 kbp	30%	44%
Contigs > 5 kbp	8%	12%
Contigs > 10 kbp	1%	3%
NG50:	204 bp	757bp





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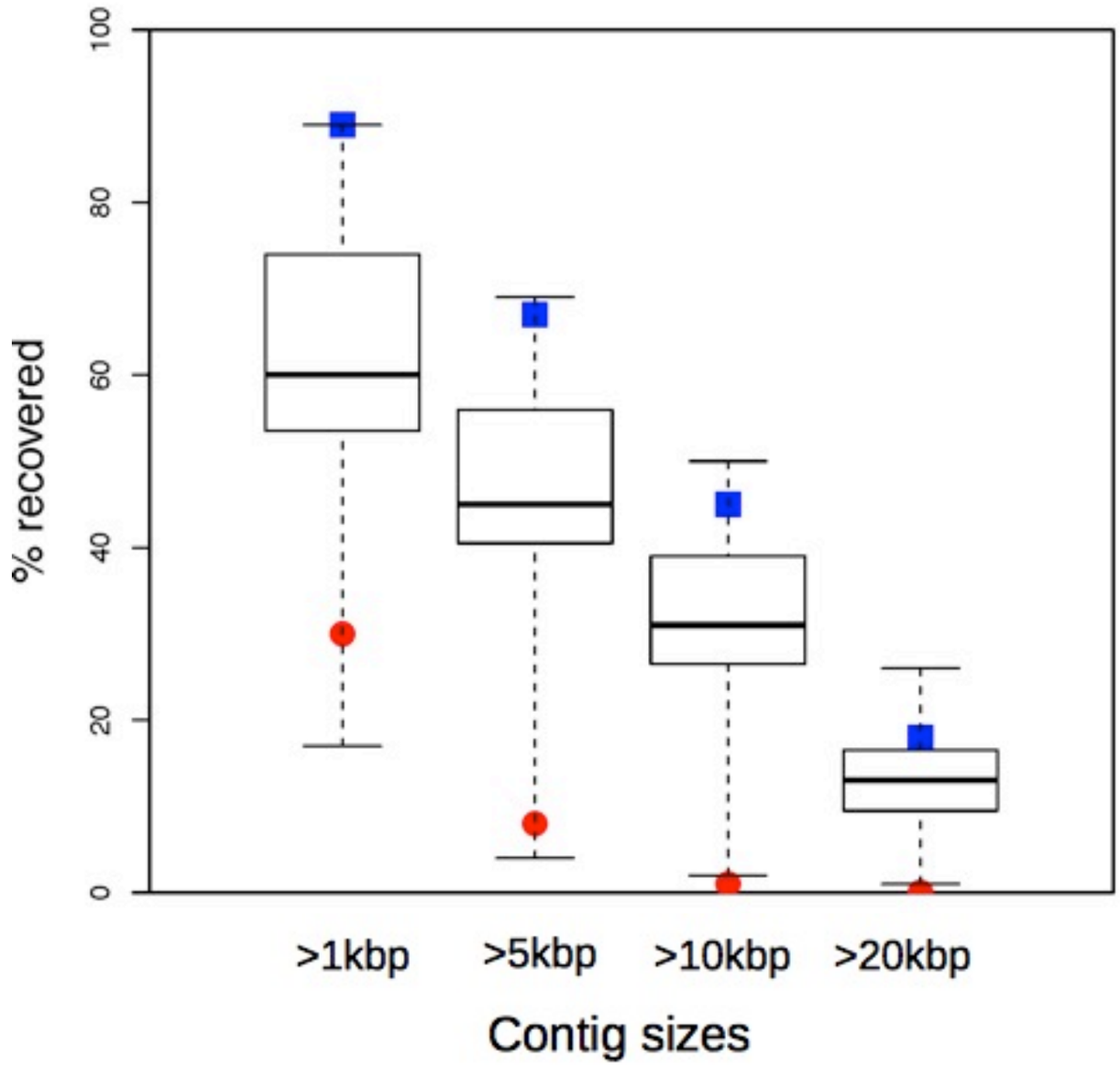
Fosmid pool strategy

Fosmid pools:

First round:
500 pools (1x)
300 libraries made
56 pools sequences and analyzed

Second round:
1500 pools (3x)
Production in progress

Even with relatively few pools analyzed results already “beat” WGS assemblies



More WGS information:

Genome assembly:

- *Sunday Jan 15, 4:10 pm:* Sequencing and assembly of the largest and most complex genome to date - the Norway spruce (*Picea abies*) - *Björn Nystedt*

Fosmid sequencing:

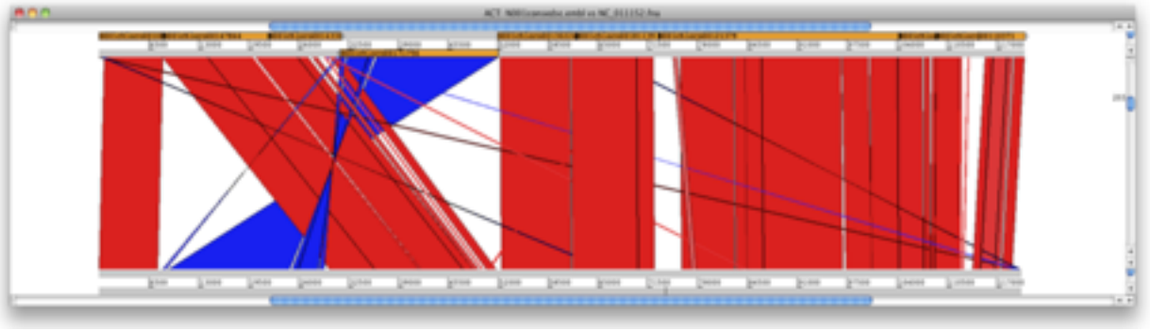
- *Tuesday Jan 17, 2:10 pm:* Fosmid pool sequencing of the 20 Gbp genome of Norway spruce (*Picea abies*) - *Björn Nystedt*

Sequencing the *Populus tremula* genome:

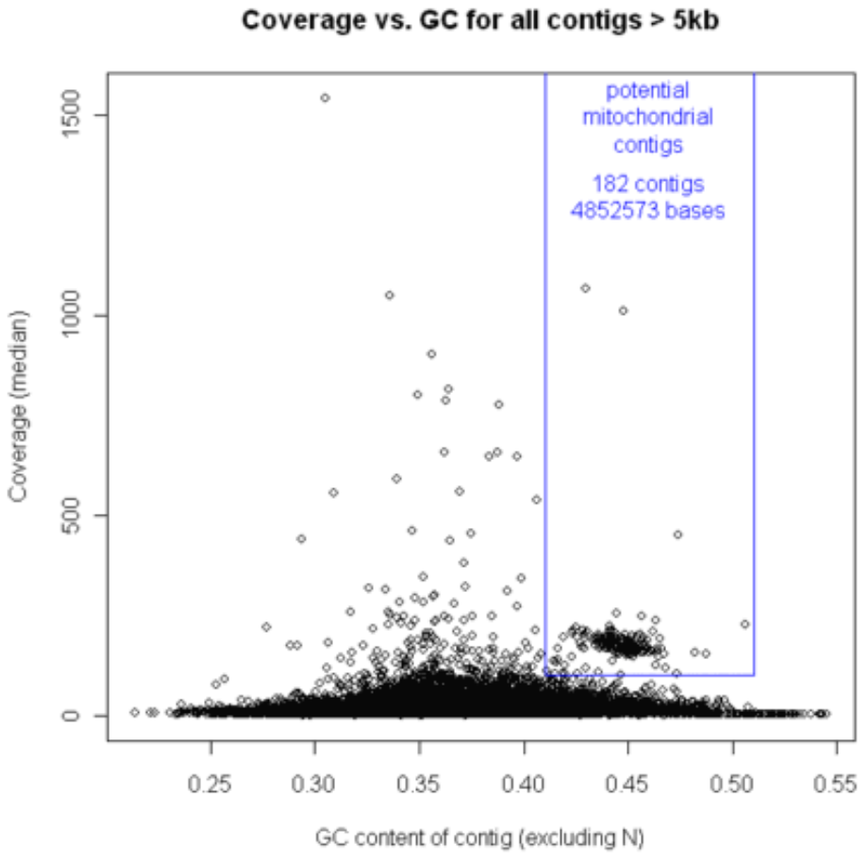
- *Sunday Jan 15, 11:30 am:* The genome of aspen (*Populus tremula*) - the most complex genome sequenced to date? - *Stefan Jansson*

Picea abies organelle genomes

- cpDNA:
  - Chloroplast genome of expected size ~120 kbp
  - 1 run 454 assembled into 9 chloroplast contigs
  - Scaffolding with 1% of 1 lane of Illumina MP data => 1 circular scaffold
  - Detected 1 translocated inversion compared to *Picea sitchensis*

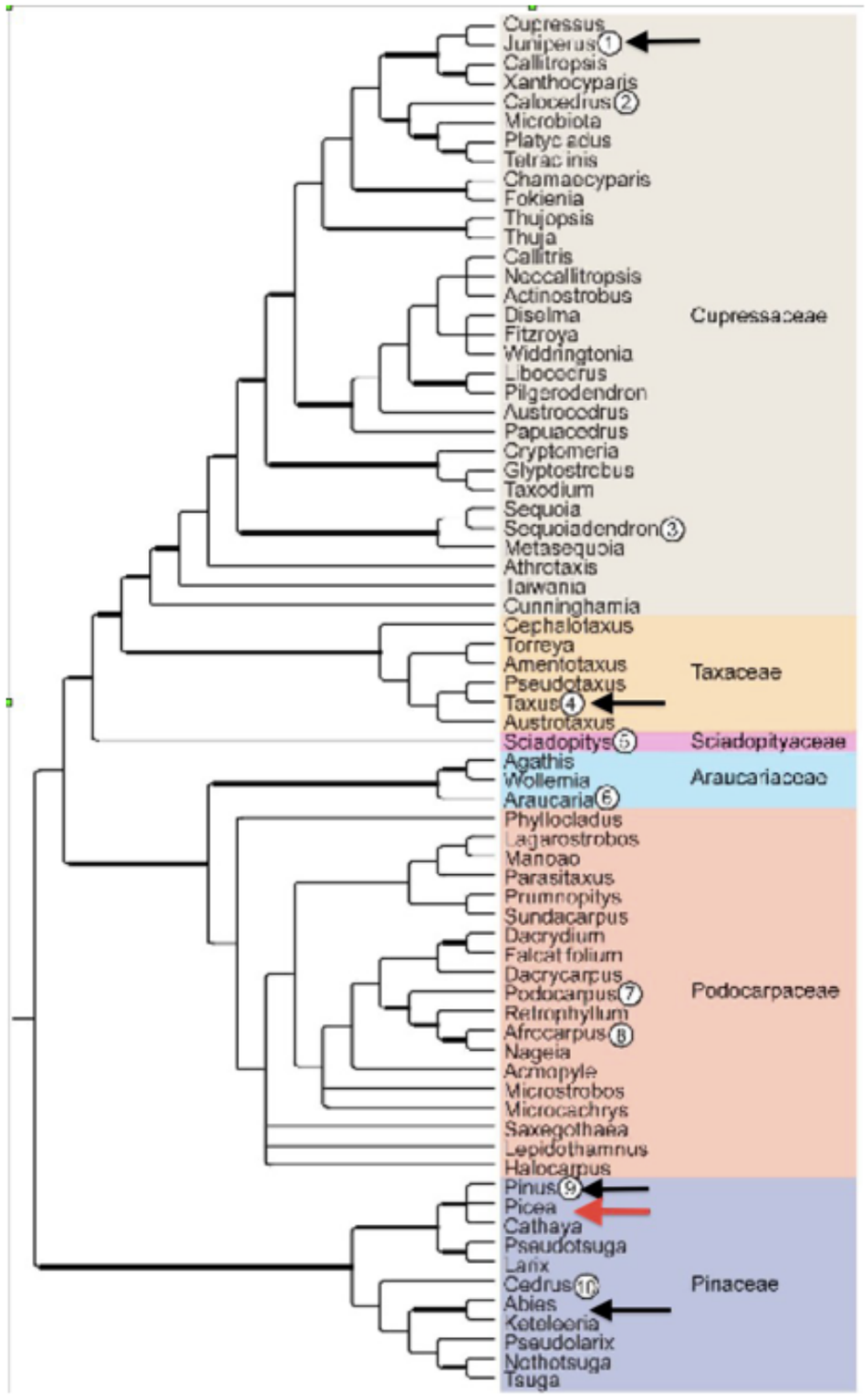


- **mtDNA:**
  - Mitochondrial genome possibly large - estimated size ~4.8 Mb
  - N50 contigs: 50 kbp
    N50 scaffolds: 289 kbp
  - Much longer contigs than nuclear DNA - less repeats?



Comparative genomics of conifer repetitive elements

- 5x Illumina sequencing and low-coverage 454 sequencing of five additional conifer species:
  - *Pinus sylvestris* (20x)
  - *Abies siberica*
  - *Taxus baccata*
  - *Juniper communis*
  - *Gnetum*
- Main objective is to analyze major repat content in the different genomes
- *Pinus sylvestris* sequencing will be extended in EU FP7 project “ProCoGen” starting early 2012



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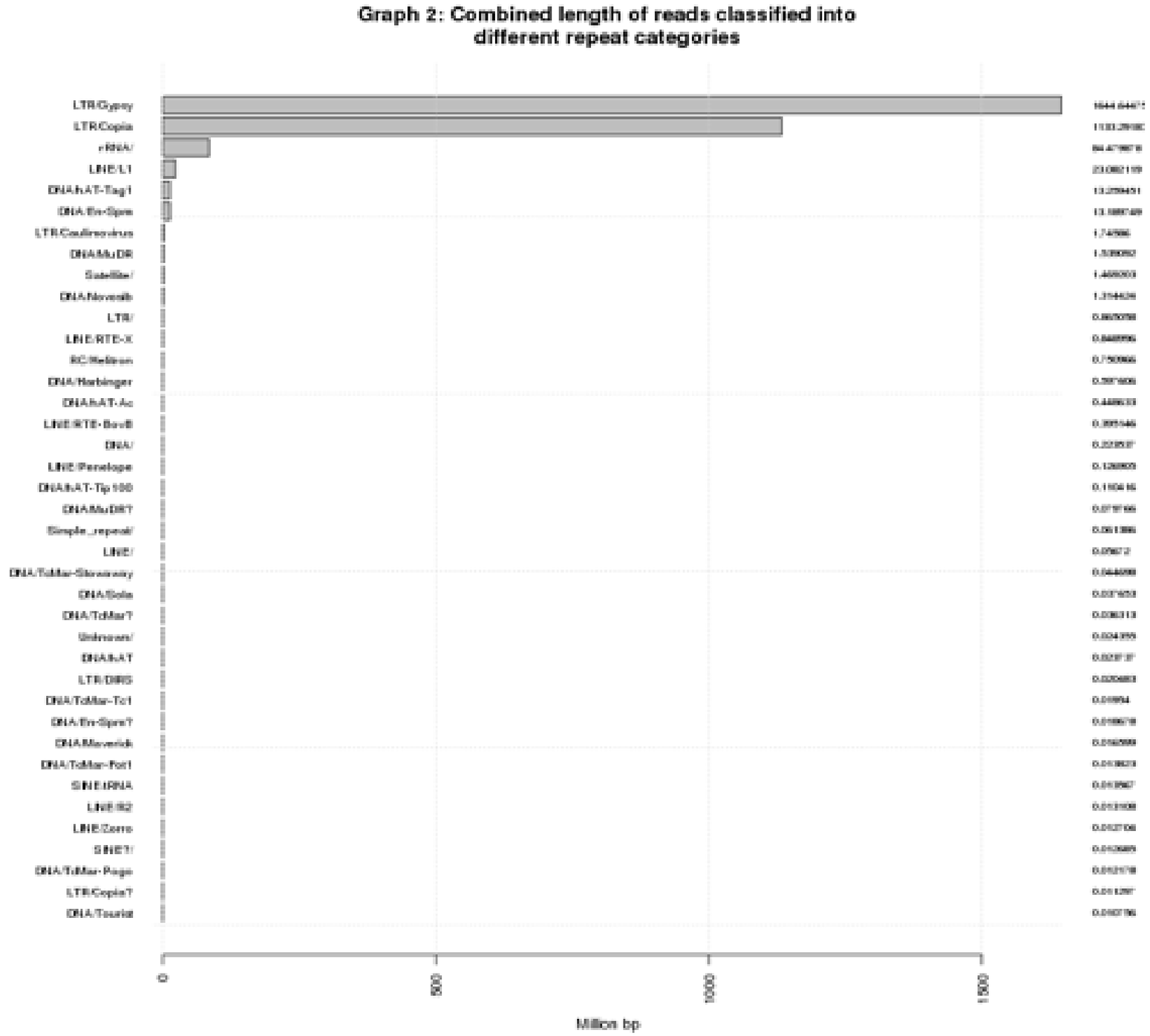
Analyzing the repetitive part of the genome

- Using low coverage 454 data to identify and classify repeats
- 1838 repeats identified, 1404 of these can be assigned to known classes
- These repeats mask 69% of the 454 read set



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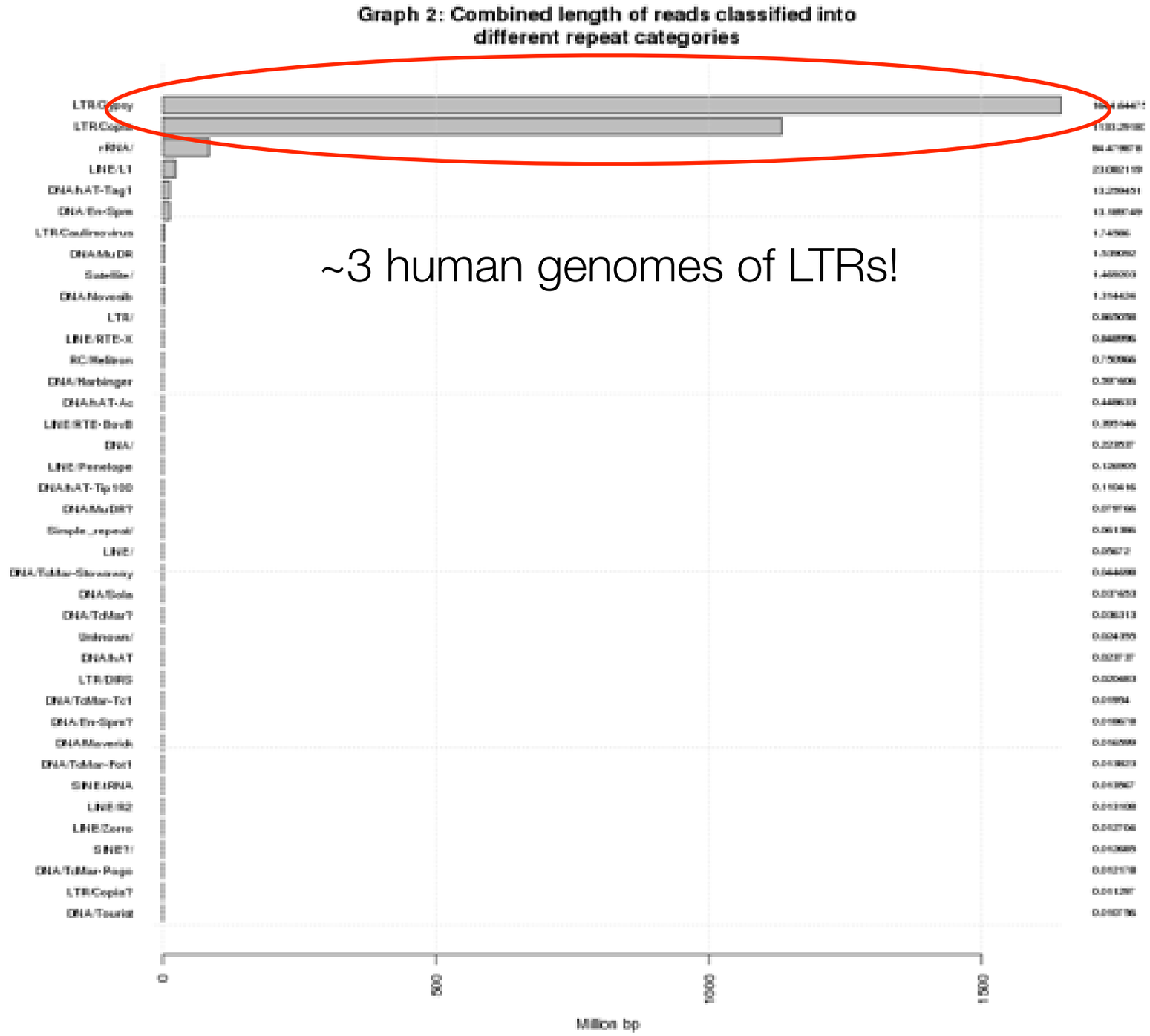


More information on repeats:

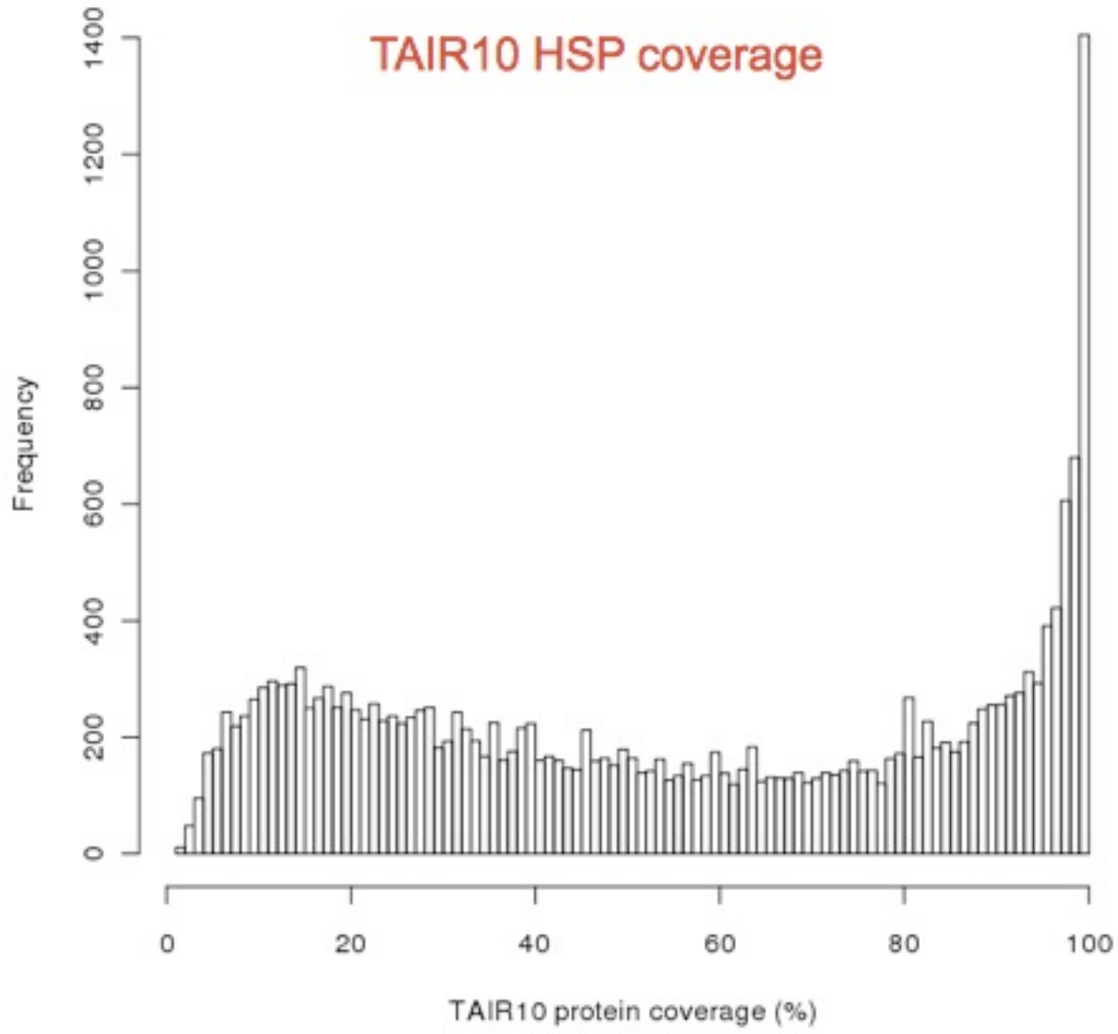
- *Sunday Jan 15, 8:30 am:* The repetitive DNA of conifers - lessons from the Norway spruce (*Picea abies*) genome and resequencing of 5 other conifers - *Anna L Wetterbom*

Transcriptome analyses

- **Phase 1 - gene calling and discovery**
  - 24 tissue / seasonal samples from Z4006
  - Normalised mRNA and total RNA pools
    - polyA selected and random hexamer
    - 454 + Newbler assembly (complete)
- RNA-Seq of all individual samples
  - Illumina strand-specific RNA-Seq

Transcriptome analyses

- Assembly of 454 data
  - 26367 Isogroups / 35992 Isotigs
  - 12,726 in common to Candian white spruce EST set
  - Comparison to TAIR10 shows our 454 EST set is comprehensive and covers as many proteins full-length
  - ~33% of white spruce and 454 Isotigs align within a single shotgun contig and ~66% are well covered but fragmented

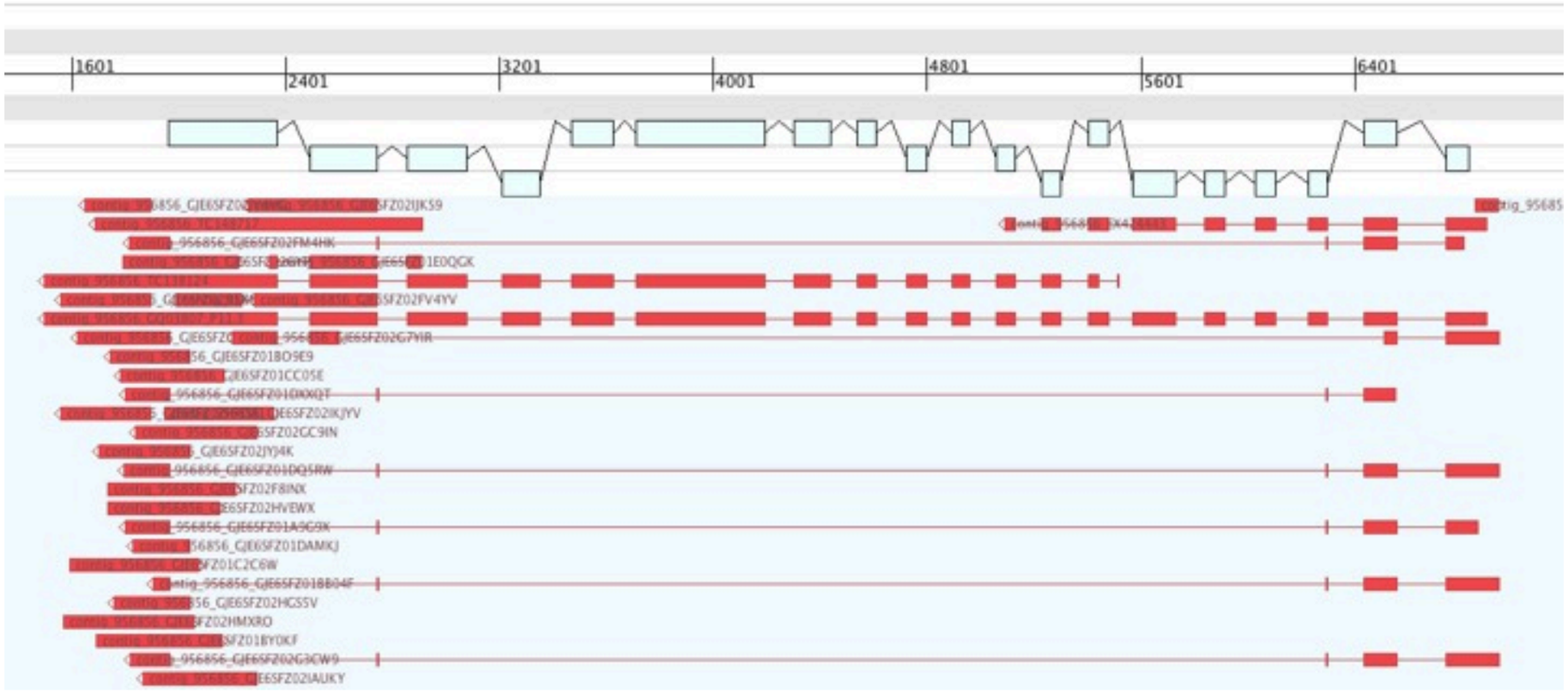


Transcriptome analyses

- Compact genes, with short introns (similar to angiosperm genes)

Gene structure

454 EST

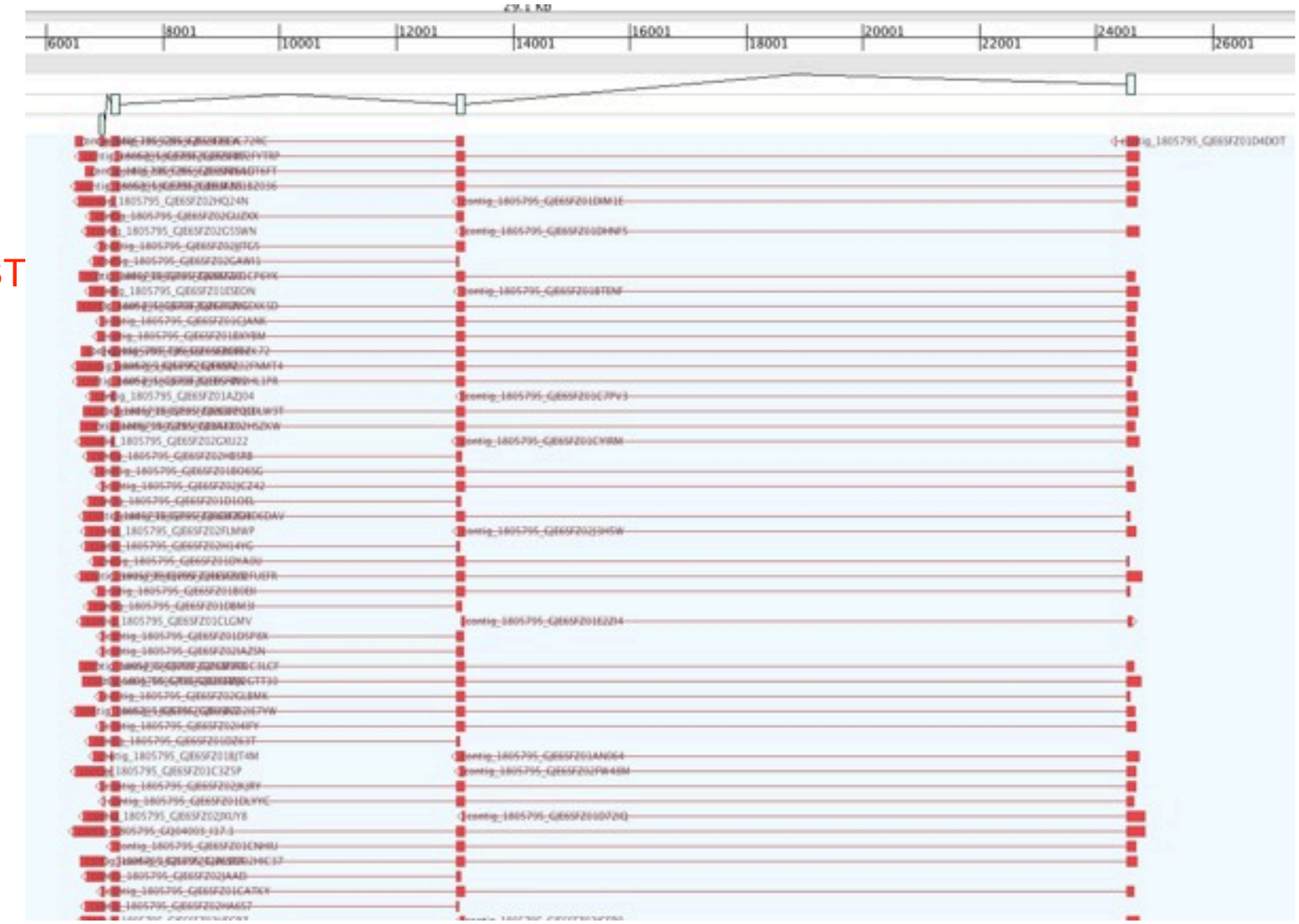


Transcriptome analyses

- Genes with a mixture of long and short introns:

Gene structure

Intron size 123 bp 5,782 bp 11,366 bp



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Transcriptome analyses

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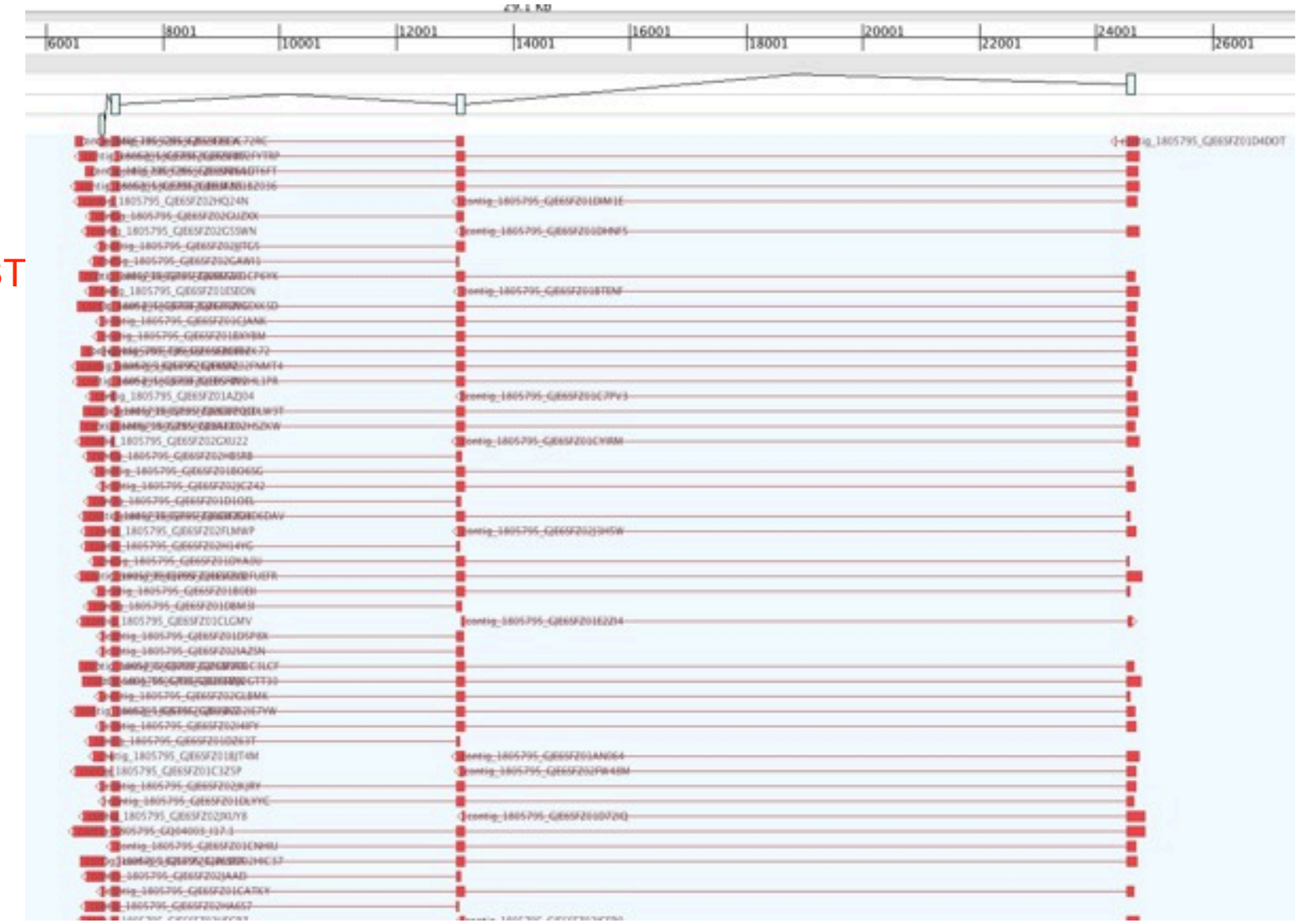
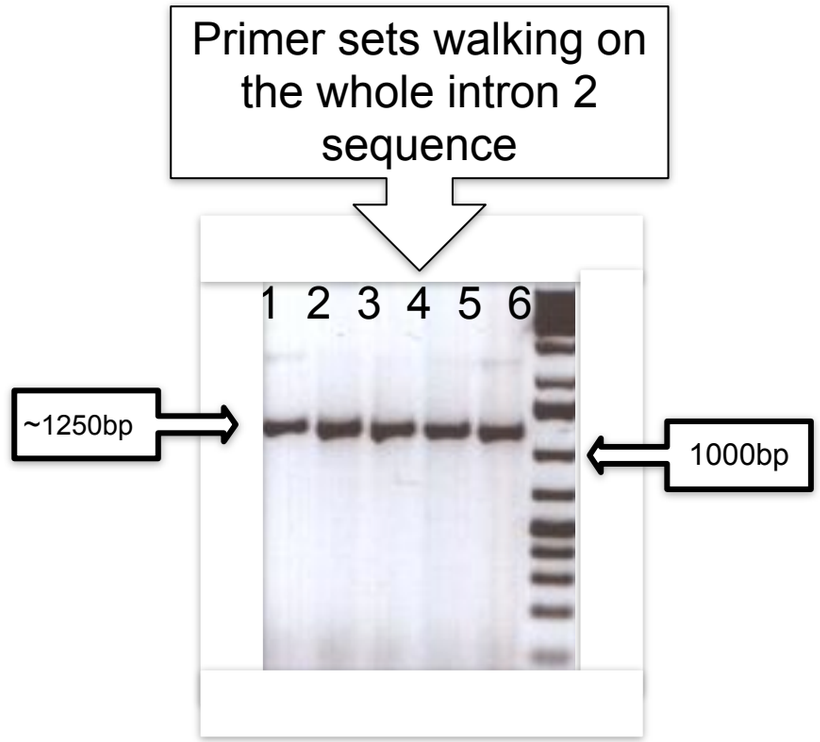


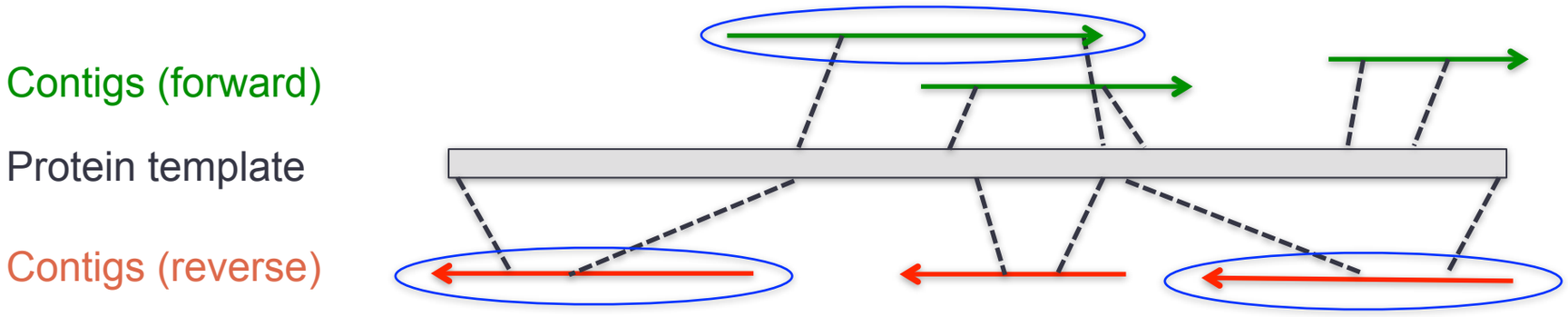
454 EST

Verification:
PCR products (1250 bp) amplified with overlapping primer sets across intron 2 (predicted size 5782bp).

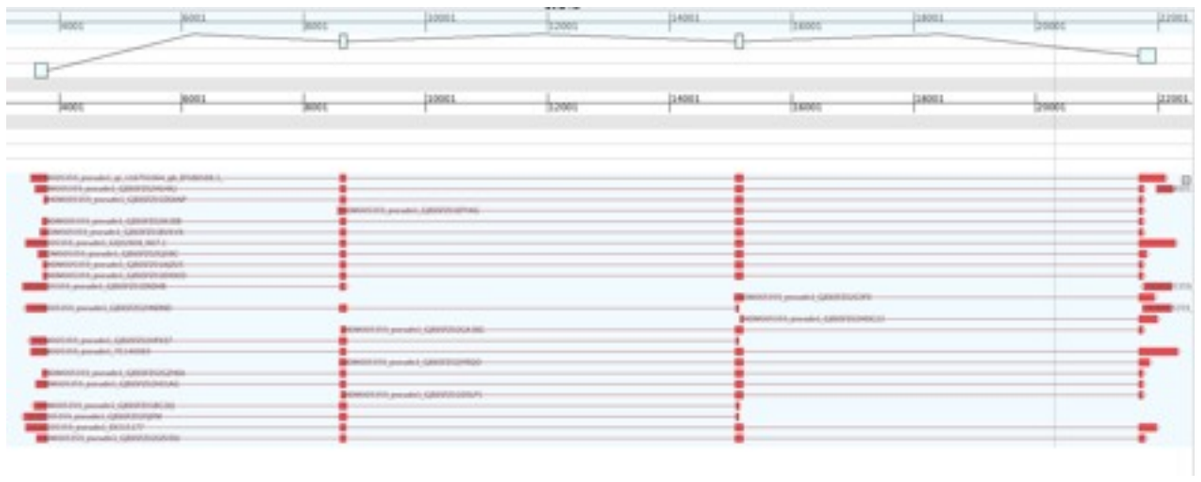


Transcriptome analyses

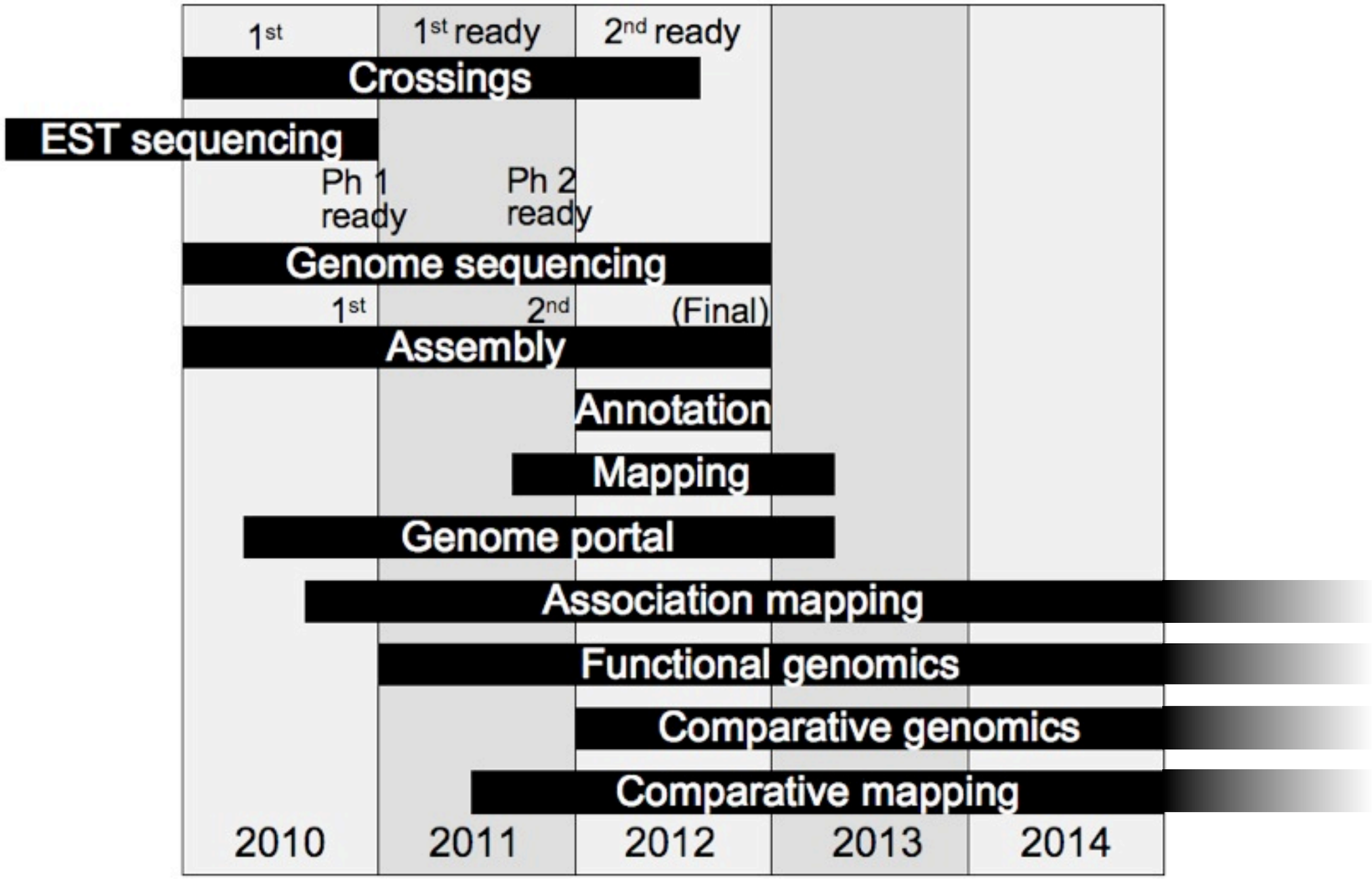
Potential long intron gene scattering in three contigs:



Joining three contigs into one (pseudo)scaffold
then 454 ESTs can properly align to the scaffold



Downstream proof-of-principle projects



Next phase of transcriptome analyses

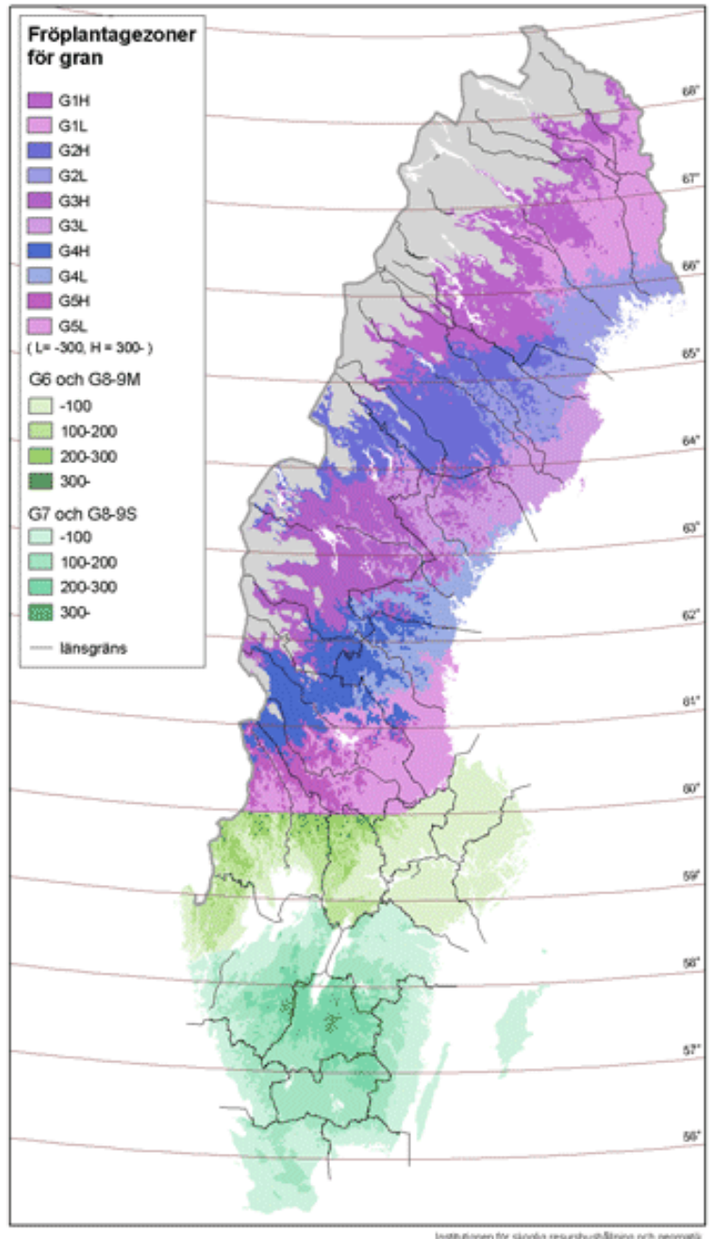
- **Phase 2 - biological discovery**
  - 18 projects, > 800 samples
  - Emphasis on wood development
  - Projects led by a UPSC PI
  - All data publicly available at http://congenie.org
  - Combined systems biology analysis of all data

Related talk:

Sunday Jan 15, 2:40 pm: Large-scale RNA-seq transcriptomics studies Exploring wood development and natural variation in aspen (*P. tremula*): Projects and Resource Development - *Nathaniel Street*

Downstream proof-of-principle projects

- Association mapping
  - Open-pollinated families from SkogForsk's breeding trials, two replicated populations of roughly equal size (1200 families per replicate)
- Functional genomics
  - Spruce somatic embryogenesis and transformation.
    - Analysis of selected transcription factors (in collaboration with STT)
- Comparative genomics
  - Spruce – Pine comparative mapping
  - Spruce – *Populus* - *Arabidopsis* comparisons



Making the data available:

ConGenIE - the Conifer Genome Integrative Explorer

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SNISS national infrastructure

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SciLifeLab
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