Looking for auxin transport genes in tobacco BY-2

Part II (June to November)

looking for the fragments, assembling contigs, confirming with ESTs/aa sequences

- standard approach - cDNA library (still dismissed)

- sequence similarity search (DNA or protein) in
  - GenBank (ncbi....National Center for Biotechnology Information)
  - TGI (tobacco genome initiative) searches –assemble your own gene
  - plantgbd.org, SGN.cornell.edu

- confirmation of ESTs/aa sequences in either DB

- Od: sgraham@pngg.org
- Odesláno: 28. února 2008 16:19
- Komu: Perry Lucie UEB
- Předmět: NCSU TGI Blast Results for query:

- Filtering On
- BLASTN 2.0MP-WashU [10-Aug-2004] [linux24-i686-ILP32F64 2004-08-10T15:48:49]

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- Reference: Gish, W. (1996-2004) http://blast.wustl.edu

- Notice: this program and its default parameter settings are optimized to find nearly identical sequences rapidly. To identify weak protein similarities encoded in nucleic acid, use BLASTX, TBLASTN or TBLASTX.

- WARNING: the gapsepqmax and gapsepsmax parameters are deprecated and replaced
- by hspsepqmax and hspsepsmax, respectively, which are now for use
- with both gapped and ungapped alignments.

- Query= (Length: 77)
- (77 letters)

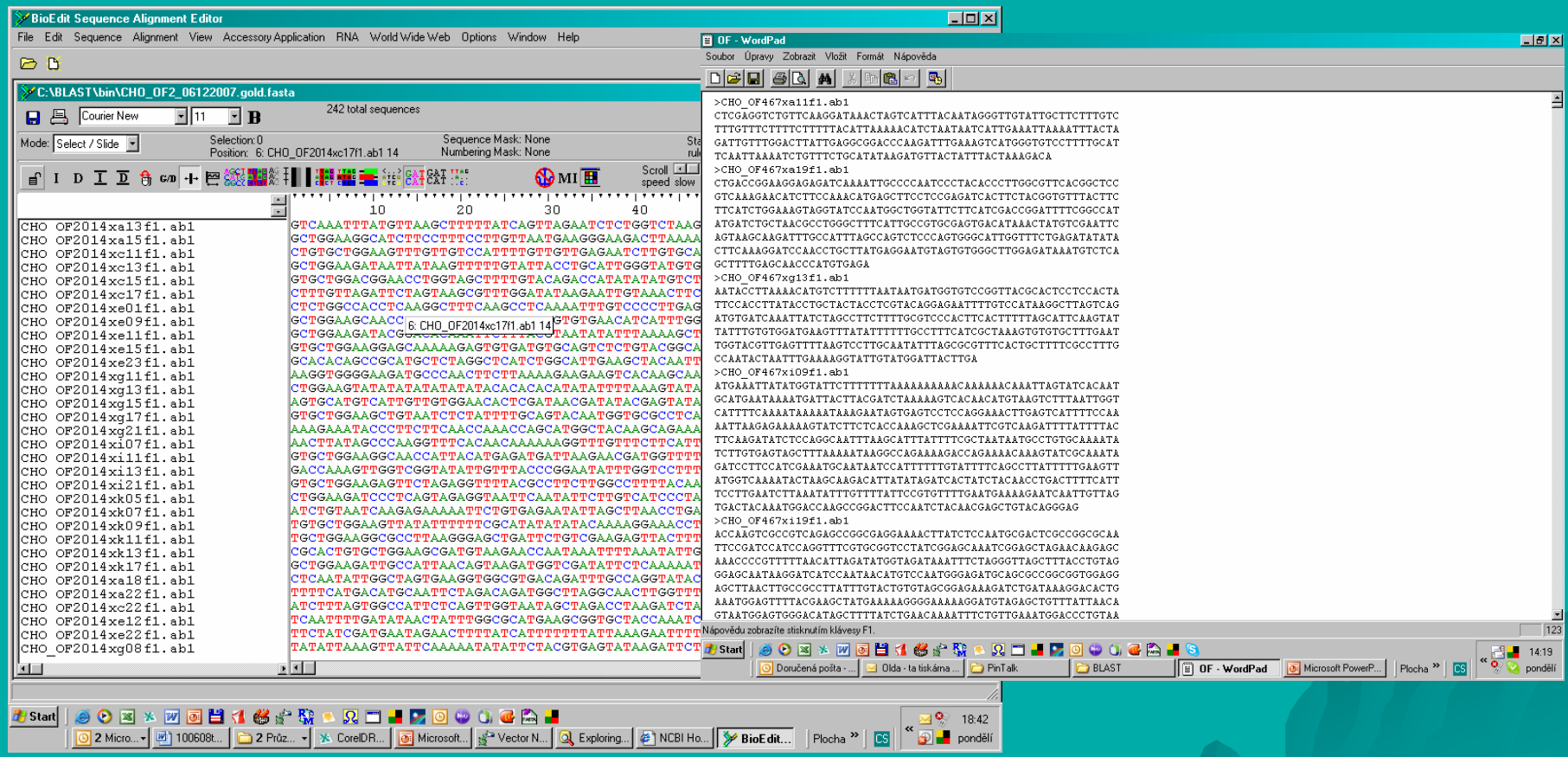
- Database: CHO_OF_2008-01-03.fasta
- 1,271,256 sequences; 706,057,077 total letters.
- Searching....10....20....30....40....50....60....70....80....90....100% done

Sequences producing High-scoring Segment Pairs:	Smallest Sum High Probability Score	P(N)	N
CHO_OF3427xa15f1.ab1 CHROMAT_FILE: CHO_OF3427xa15f1.ab1 P...	277	1.7e-06	1
CHO_OF429xl03f1.ab1 CHROMAT_FILE: CHO_OF429xl03f1.ab1 PHD...	277	1.9e-06	1
CHO_OF4990xj04f1.ab1 CHROMAT_FILE: CHO_OF4990xj04f1.ab1 P...	277	2.2e-06	1
CHO_OF772xl10r1.ab1 CHROMAT_FILE: CHO_OF772xl10r1.ab1 PHD...	277	3.2e-06	1
CHO_OF445xj13f2.ab1 CHROMAT_FILE: CHO_OF445xj13f2.ab1 PHD...	277	4.1e-06	1
CHO_OF4953xl12r1.ab1 CHROMAT_FILE: CHO_OF4953xl12r1.ab1 P...	259	1.1e-05	1
CHO_OF4893xe08r1.ab1 CHROMAT_FILE: CHO_OF4893xe08r1.ab1 P...	259	2.2e-05	1
CHO_OF5079xf09f1.ab1 CHROMAT_FILE: CHO_OF5079xf09f1.ab1 P...	254	2.2e-05	1
CHO_OF4414xm03r1.ab1 CHROMAT_FILE: CHO_OF4414xm03r1.ab1 P...	259	2.9e-05	1
CHO_OF5125xb09r1.ab1 CHROMAT_FILE: CHO_OF5125xb09r1.ab1 P...	254	3.3e-05	1
CHO_OF3034xl08f1.ab1 CHROMAT_FILE: CHO_OF3034xl08f1.ab1 P...	254	3.4e-05	1
CHO_OF4710xf02r1.ab1 CHROMAT_FILE: CHO_OF4710xf02r1.ab1 P...	249	7.8e-05	1
CHO_OF3417xl07f1.ab1 CHROMAT_FILE: CHO_OF3417xl07f1.ab1 P...	245	8.9e-05	1
CHO_OF181xh09r1.ab1 CHROMAT_FILE: CHO_OF181xh09r1.ab1 PHD...	211	0.0045	1
CHO_OF686xf17f1.ab1 CHROMAT_FILE: CHO_OF686xf17f1.ab1 PHD...	211	0.0058	1

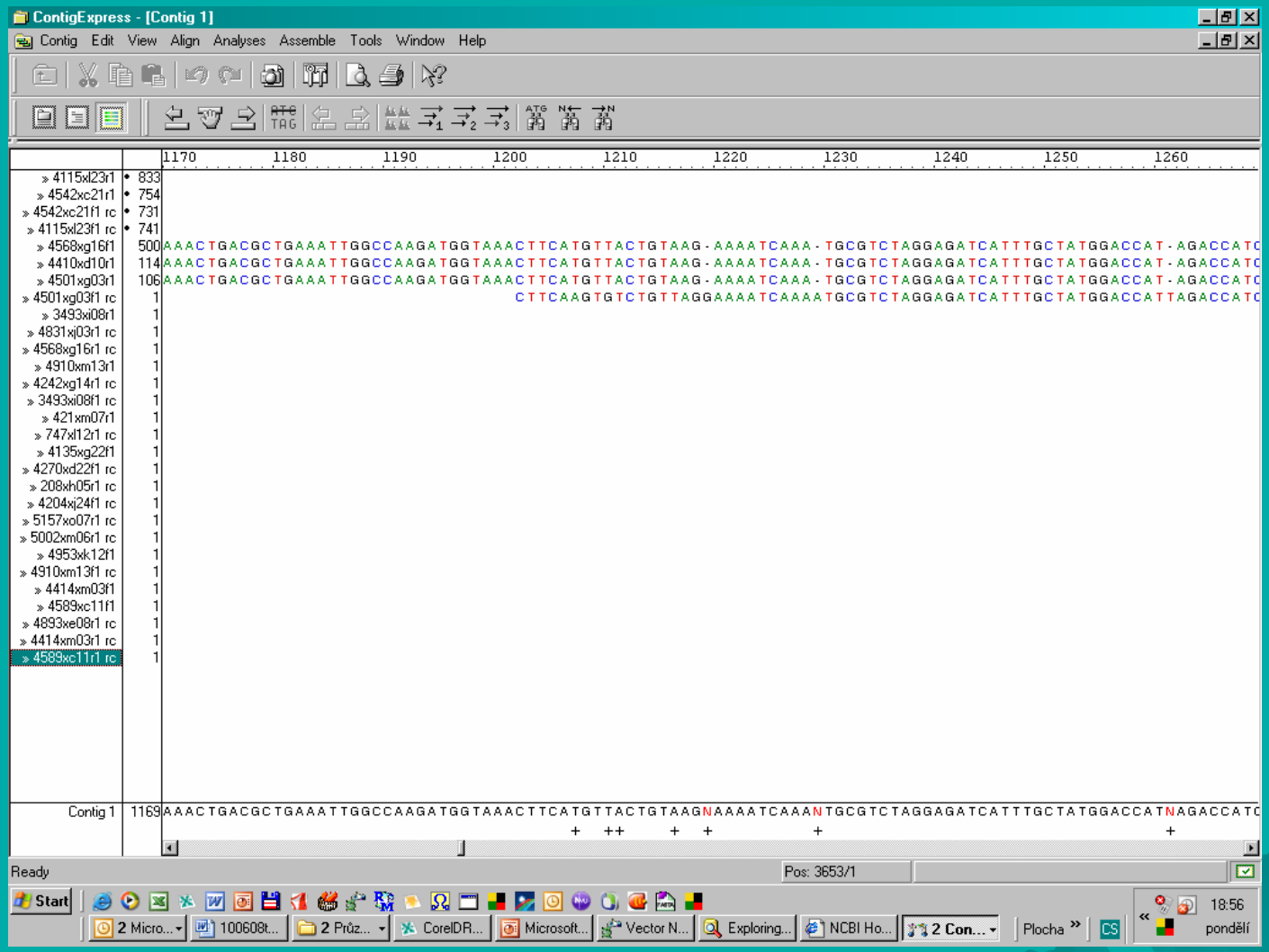
TGI blast does not seem to work properly anymore 🙀

⇒ ncbi blast or DOS blasting through stored TGI data

retrieved data in Bioedit – transfer into simple .txt file – easier search by „find“



Vector NTI Assembly 5

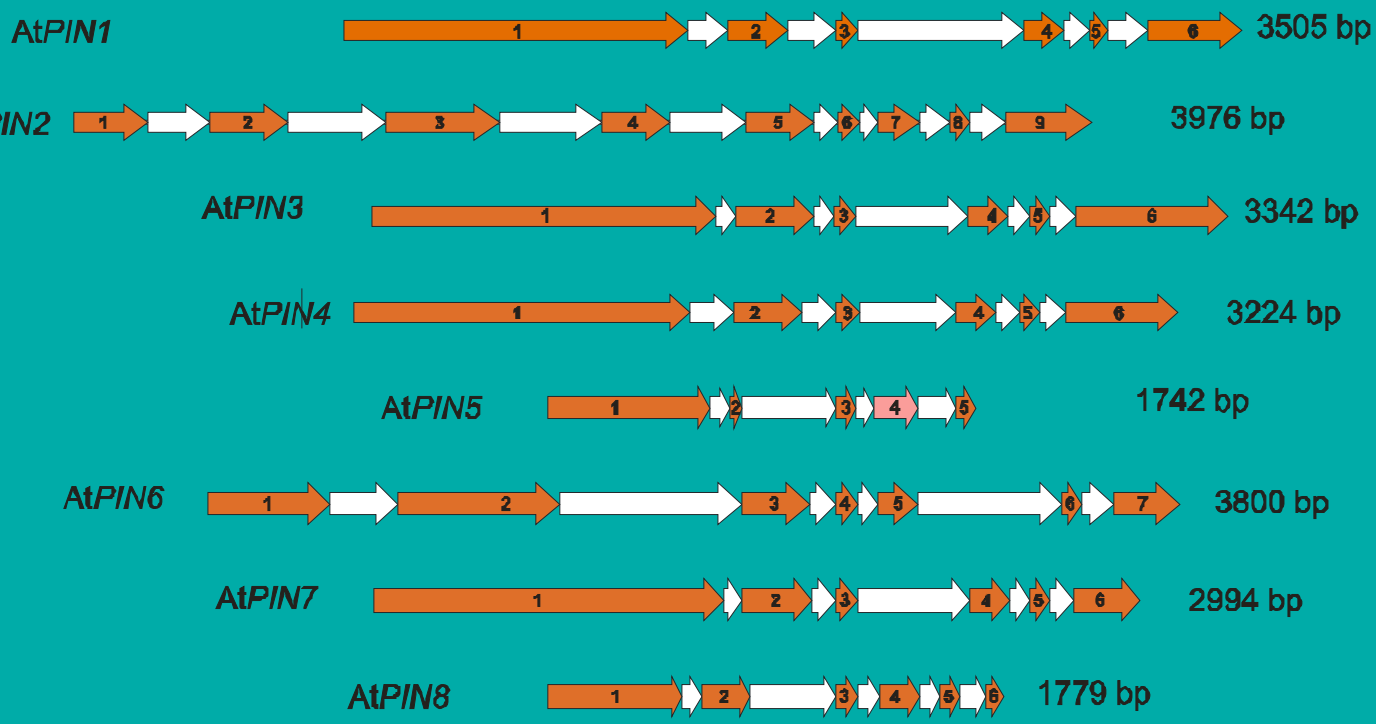


Exons „3, 4 and 5" in the *PINs* (and not just *At*)

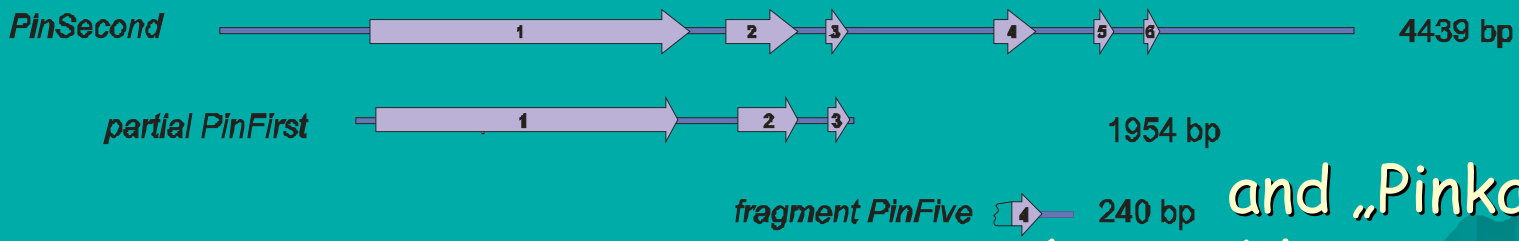
- conserved sizes (86 bp, 158 bp, 77 bp)...unlike introns
- this feature applies to at least other 3 putative tobacco *PINs*

PIN genes ex-intr

in Arabidopsis



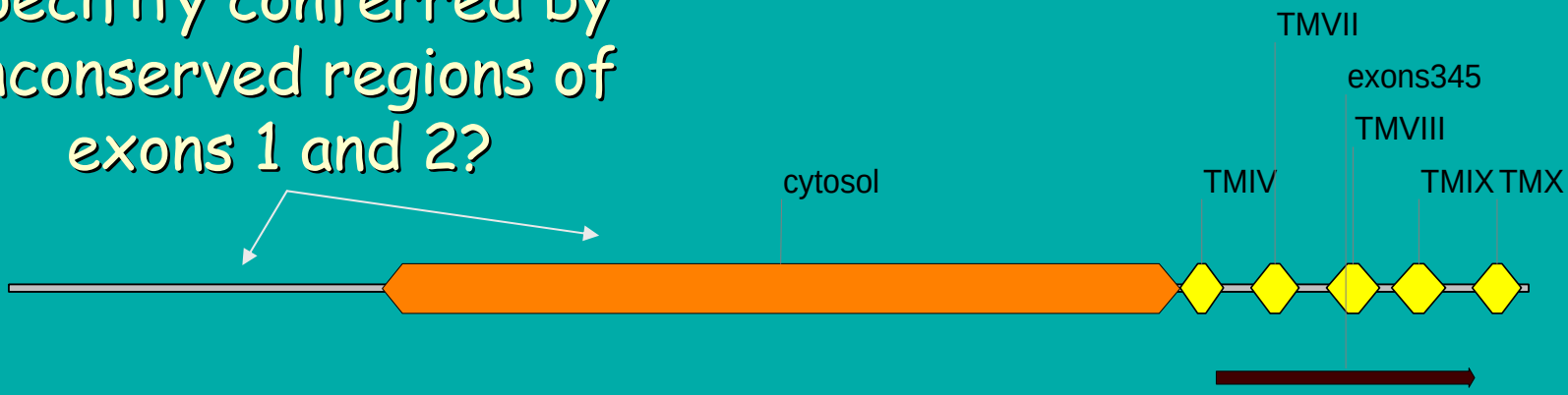
in tobacco BY-2



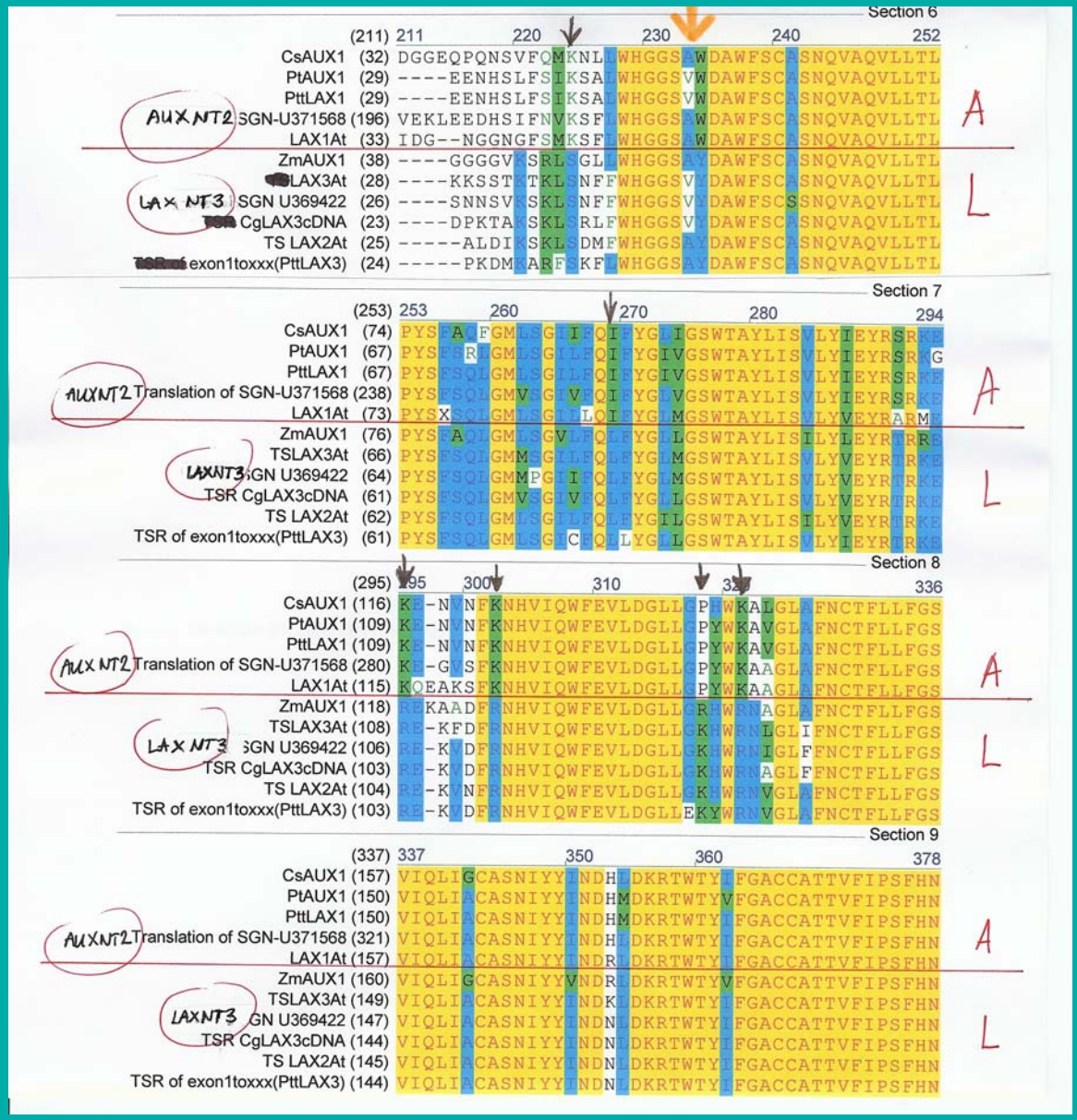
and „Pinka2"
(resemblance to AtPIN2)

PIN 3,4,5 exons and TM regions

specificity conferred by unconserved regions of exons 1 and 2?



AtPIN1
622 aa



AUX/LAX 9

- conserved sizes and positions of exons 2, 3, 4 and 5 in AUX/LAX (not shown)
- exons 6 to 8 can be linked or can be separated by introns - !!! not relevant to AUXxLAX
- consistent differences in some aminoacids AUXxLAX (arrows)

Table of so far found genes and gene fragments

	pin					aux/lax			
	PinFirst	PinSecond	PinThird	PinFive	Pinka2	auxNT1	auxNT2 A2/AS2	auxNT2-like AS1	LAXNT3 L3/LS3
complete contig	n	y	n	(Petr)	y	n	y	n	y
short oligos: band genomic/cDNA; Ta							357bp/257bp; 55,4°C-62,8°C	269bp/???; 67°C-69,6°C	736bp/736bp; 63,7°C-64,6°C
cloning oligos: band genomic/cDNA; Ta		3200bp/xxx; 65°C					4296bp/1500bp; 65°C/63,7°C-65,4°C	*	???/1496bp
EST confirmation	y	n			y (NCBI)		? (NT2-like)?	y	y
parts	ex1to 3	ex 1 to 6			ex 1 to 6	ex 1 and 2	ex 1 to 8	ex 6 to 8	ex 1 to 8
PCR whole genomic cell							y	*	???(11.8.08)
PCR short genomic		y (10.-11.7.08)					y	*	y (6.8.08)
PCR plant cDNA whole							y (13.8.08)	*	*
PCR plant cDNA short		y (6.8.08)					n (6.8.08)	y (6.8.08)	*
PCR cell cDNA whole							y (13.8.08)	*	*
PCR cell cDNA short		n (11.8.08) ???					n (11.8.08)	y (11.8.08)	n (11.8.08)
promoter contig		y							

Conclusions

- The „auxin transport" genes were identified in silico and their presence confirmed in BY-2 genome. Their expression has been detected in BY-2 plants and cell culture

- There seem to be many members of both families (PIN and AUX/LAX) in tobacco. It is necessary to clone and sequence whole genes (including introns) to
  - **eliminate differences caused by:**
    - possible gene duplications or rearrangements
    - wrongly spliced mRNAs
    - differentially spliced mRNAs
  - **and identify true members of each family with different genomic sequence as well as functions**