Thyroid hormone transcriptome and cerebellum development.

1) Developmental biology: General concepts

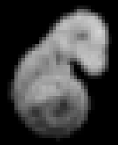
2) Genomic data: mouse and human genomes global view

3) A biological problem: mouse cerebellum and thyroid hormone
How T3 hormone acts on gene expression

4) Tools: mouse genetics + microarray technology

5) experimental design
    - data analysis
    - interpretations: direct vs indirect effects

6) future directions: data mining, online encyclopedia



Brad Smith
Elwood Linney
Center for In Vivo Microscopy
Funding: National Center for Research Resources

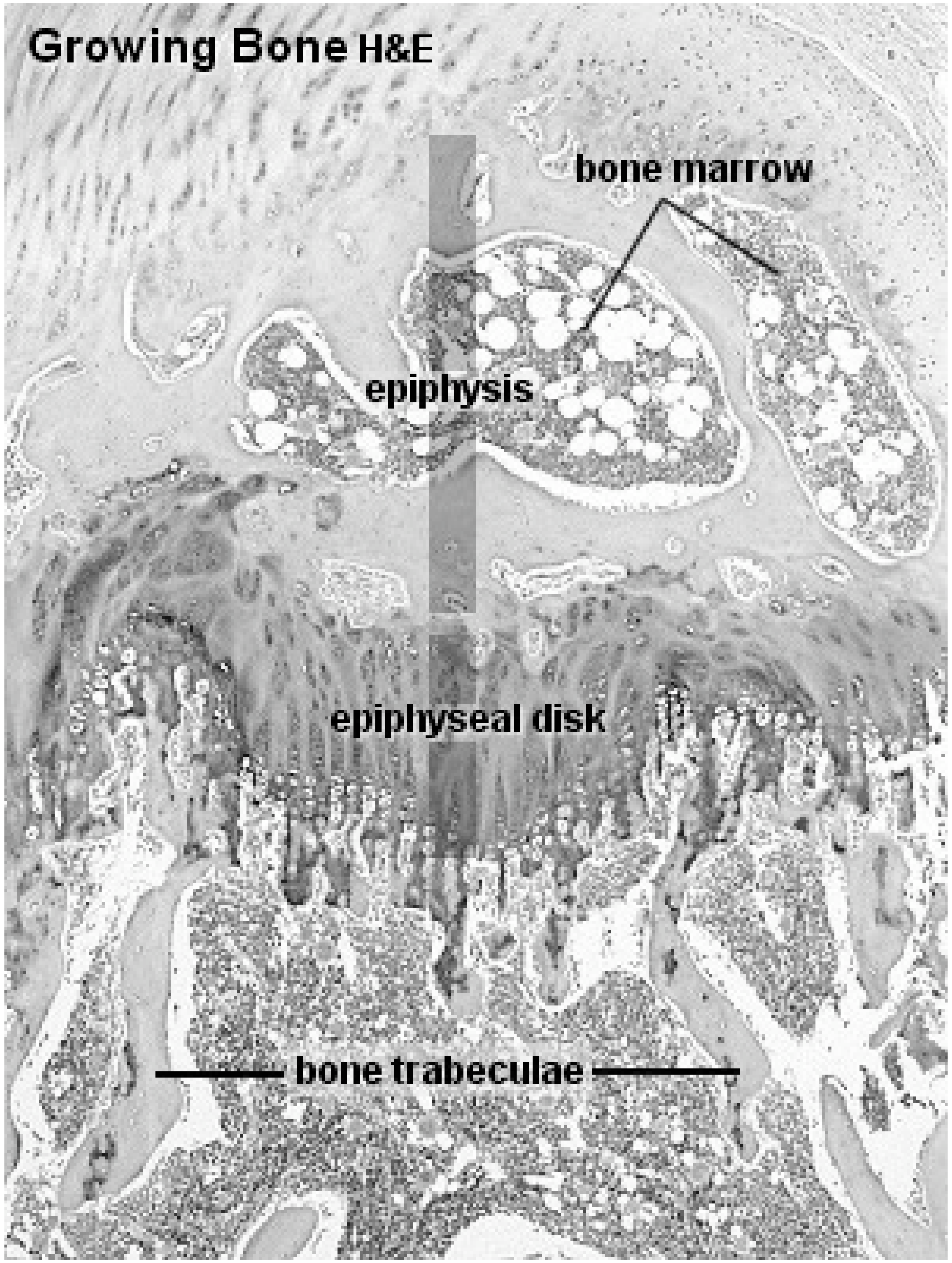
3 scales to study development:

-Histological level (>100 μm)
- Cellular level (10 μm)
- Intracellular/Molecular level (<100 nm)

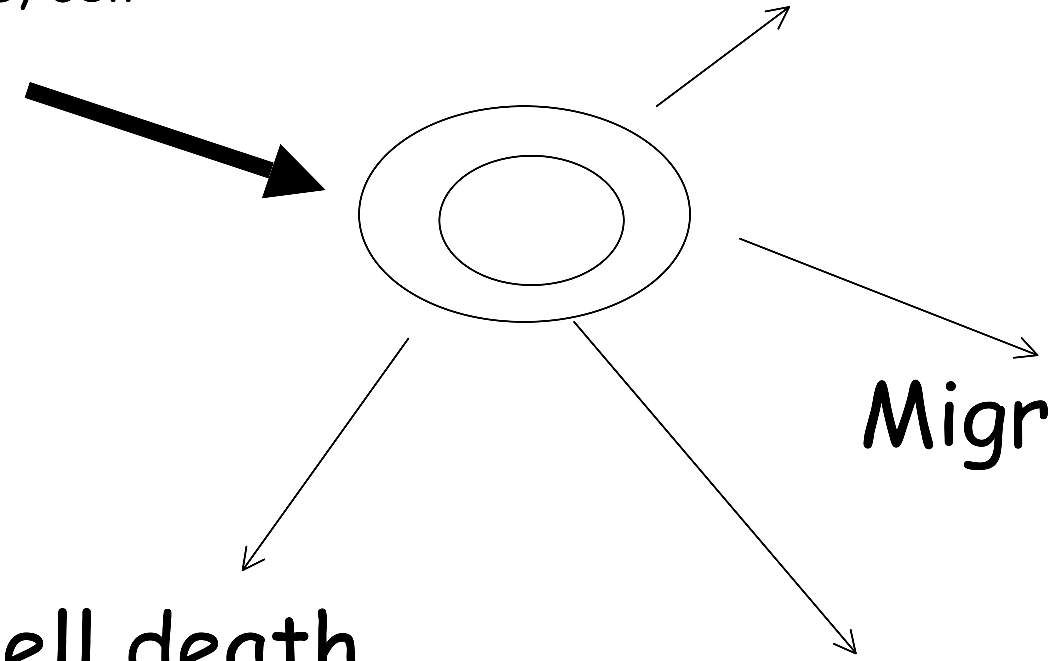
2 approaches:

-Biochemical
-Genetics (mutant studies)

Histological views: development as a self-organizing process mediated by tissues interactions



Cellular level: making decisions



External factors
(solubles factors, cell-cell contacts)

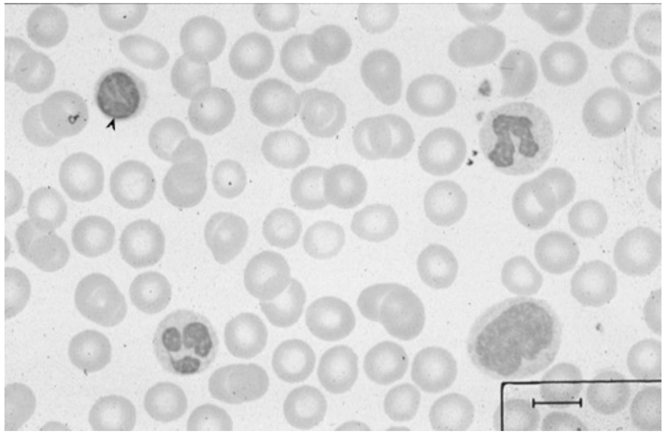
Proliferation (self-renewal)

Migration

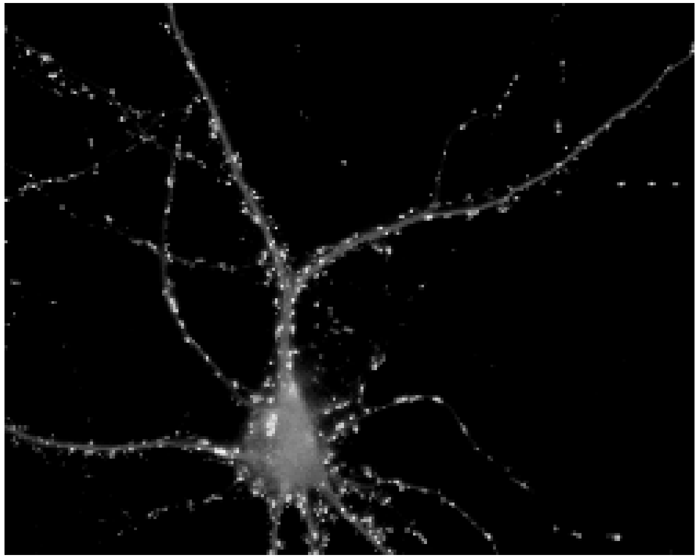
Cell death (apoptosis)

Terminal differentiation

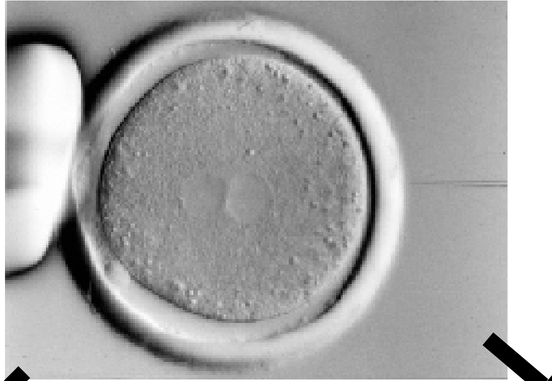
Development
Generates
>200 cell types



Lymphocytes+
erythrocytes

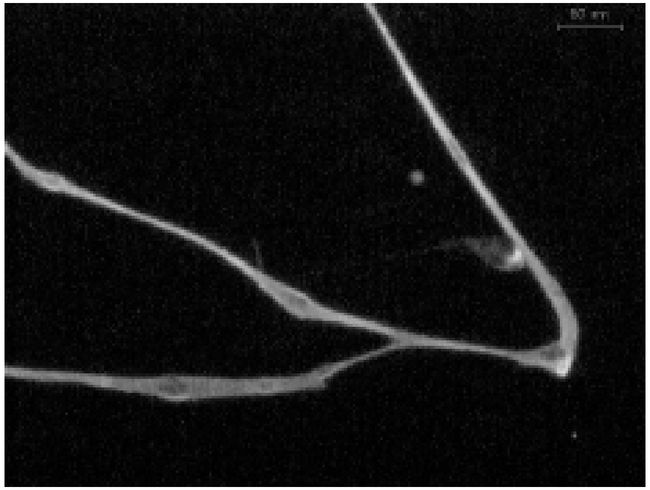


Neuron

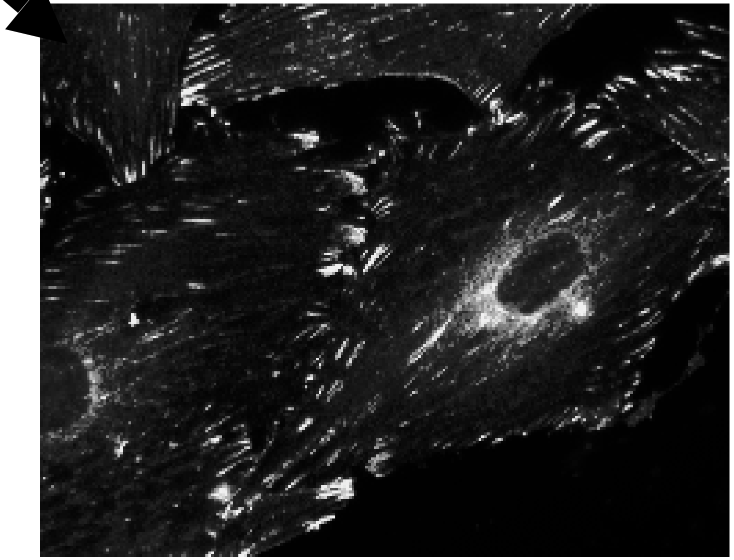


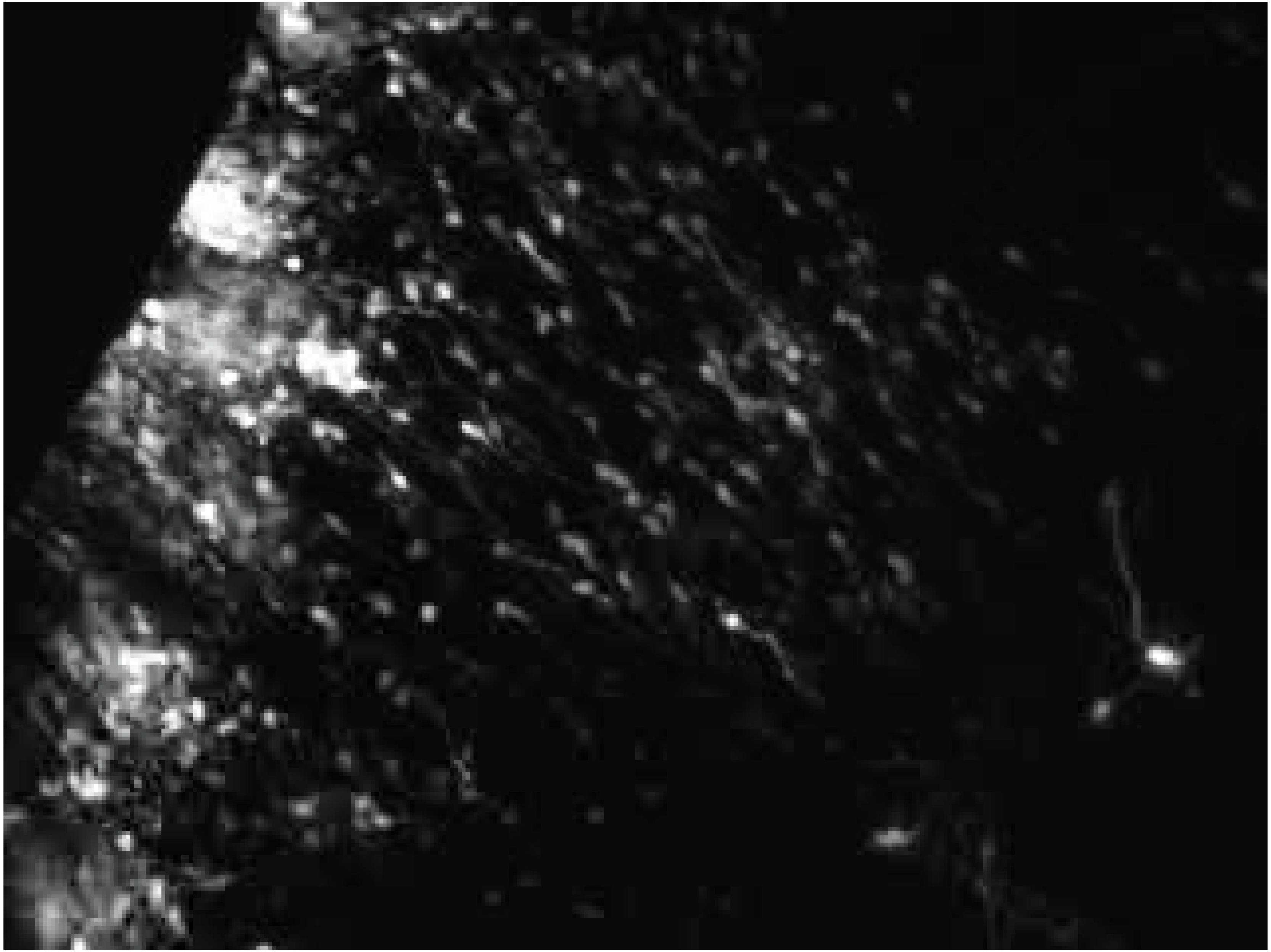
Oocyte

Muscle cells



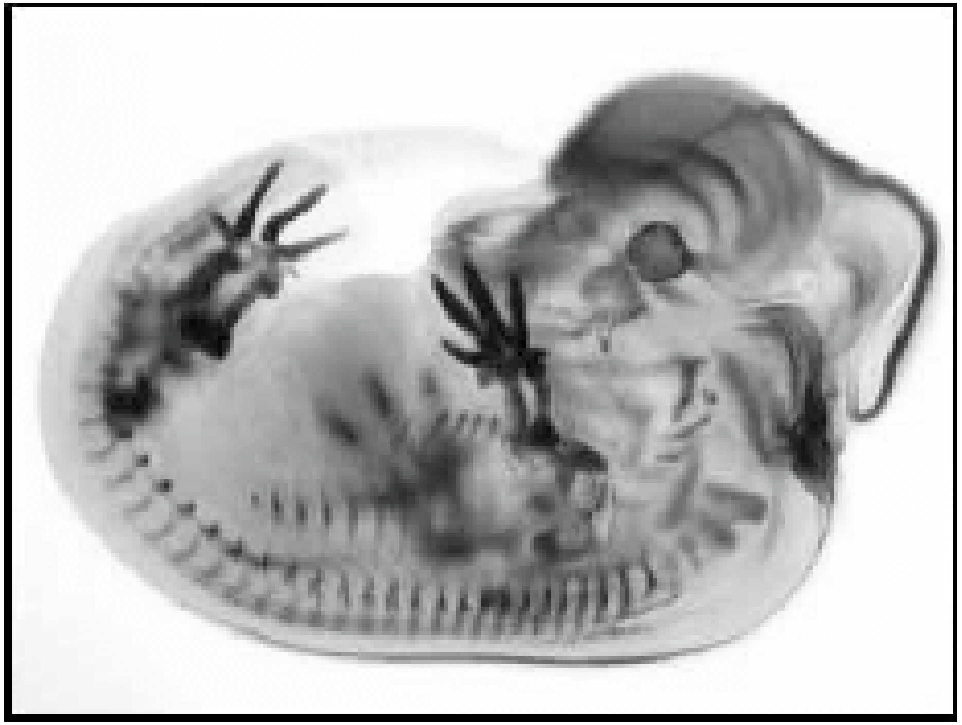
Skin epithelium





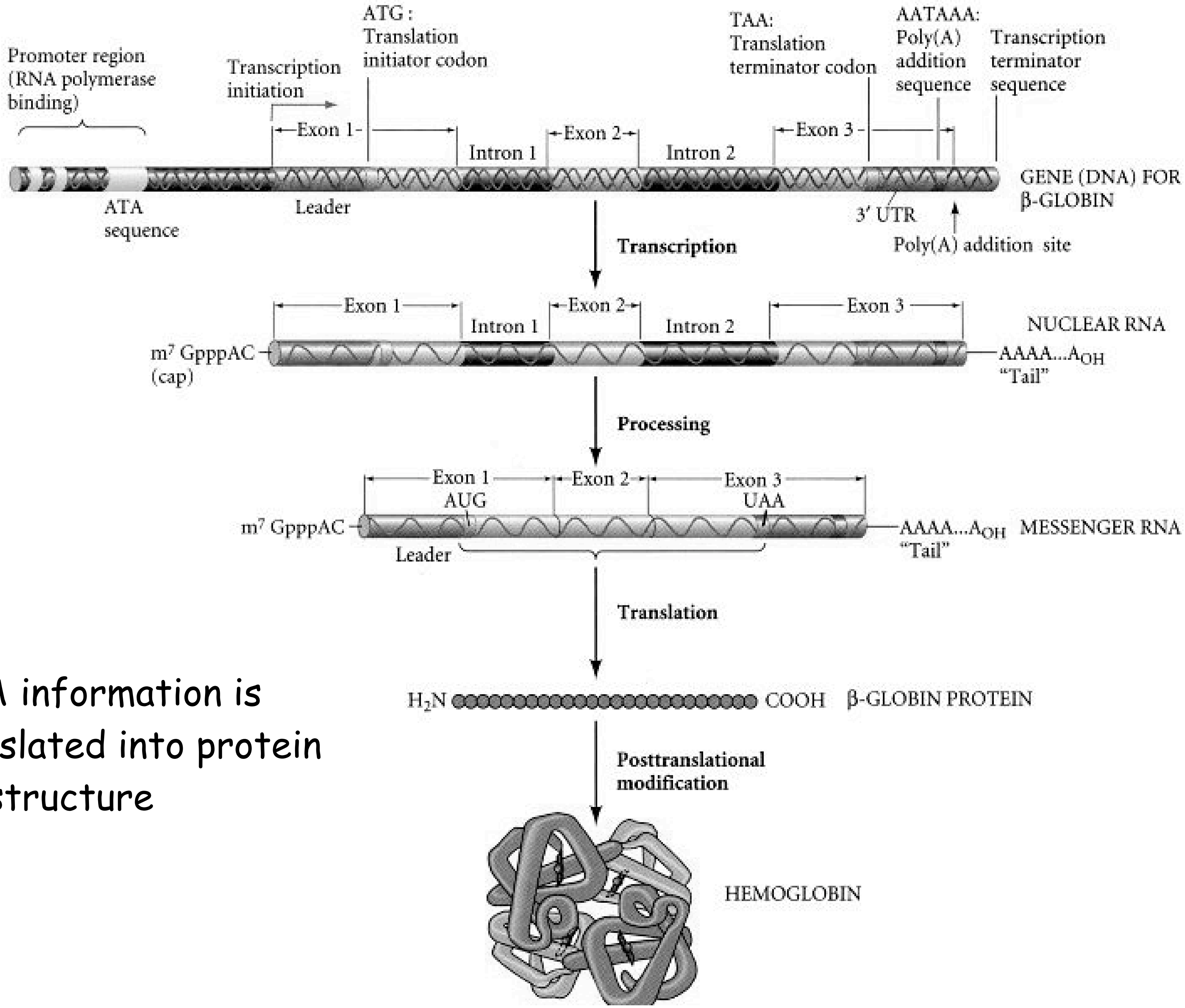
Stochastic behavior of cells

Gene expression patterns define cell types



E13.5 Noggin-lacZ transgene marks skeleton

E9.5 Fkh10 marks the otic vesicle



DNA information is translated into protein 3D structure

The developmental program is encoded by DNA

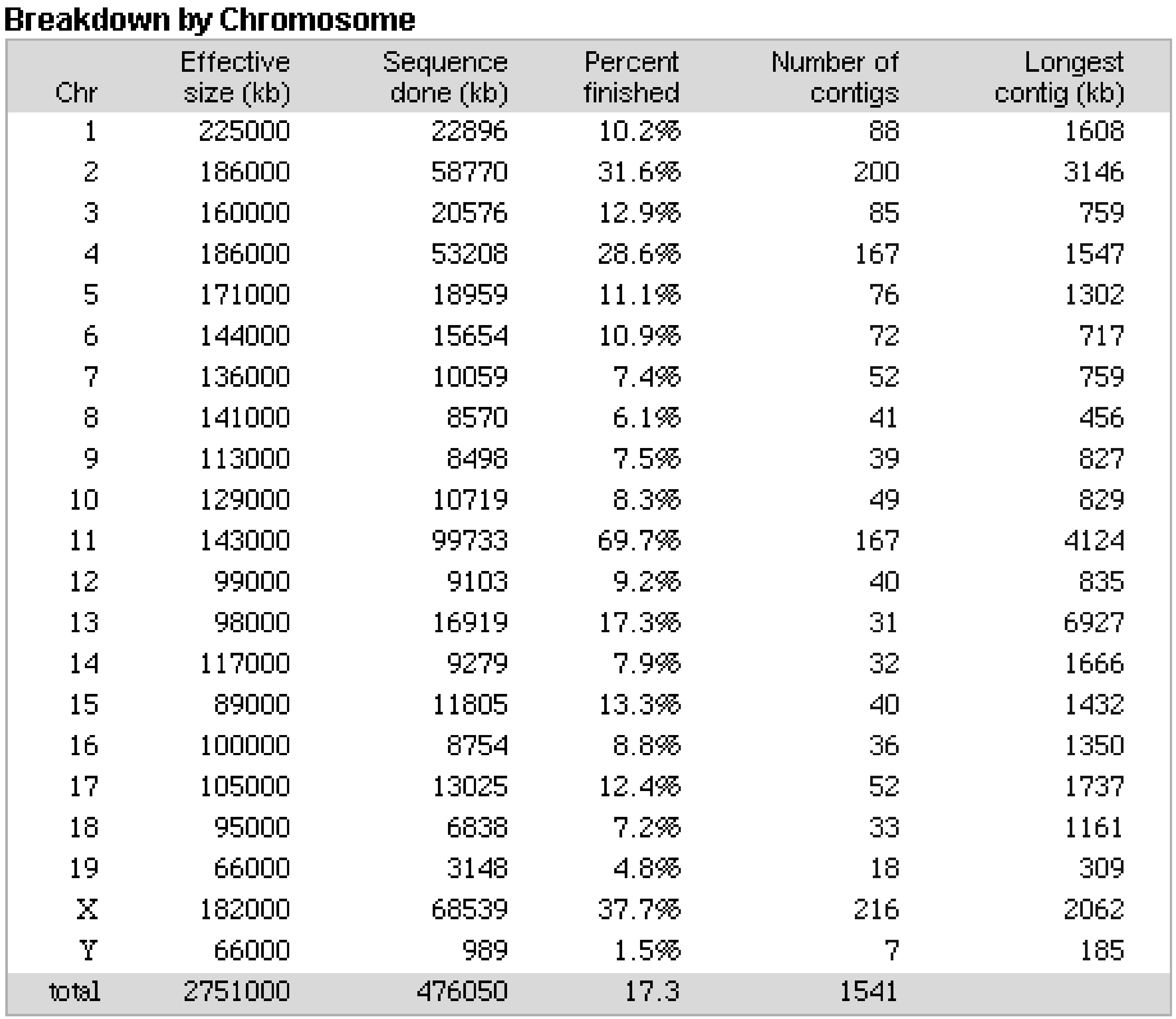
DNA
↓
mRNA
↓
Proteins

Transcription

Translation

Genome (±27 000 genes
in 3×10^9 nucleotides)
↓
Transcriptome (±50 000 mRNA
Covering ±25% of the genome)
↓
Proteome (±1 000 000 Protéines
Encoded by ±1% of the genome)

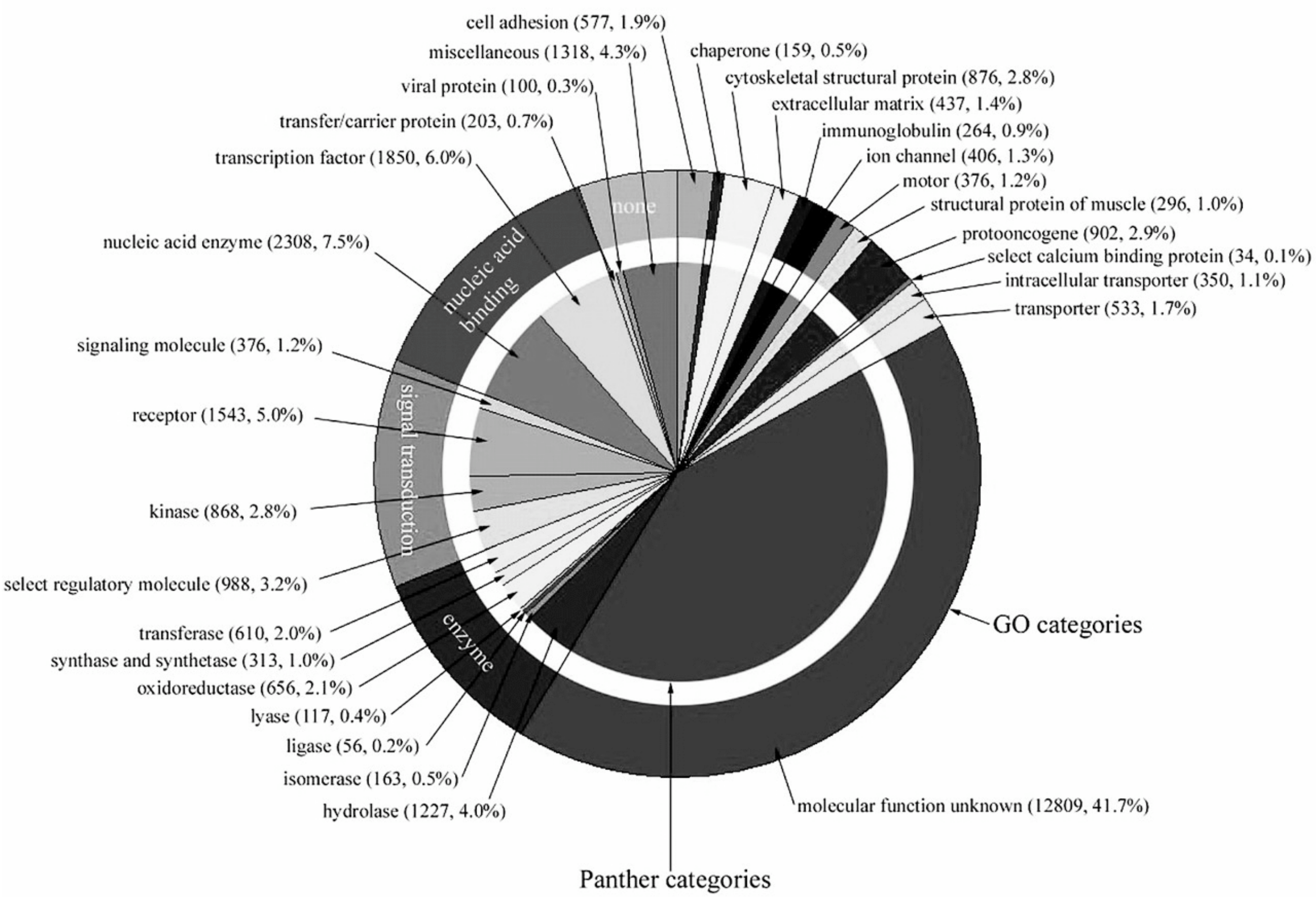
DNA sequencing center



Breakdown by Chromosome

Chr	Effective size (kb)	Sequence done (kb)	Percent finished	Number of contigs	Longest contig (kb)
1	225000	22896	10.2%	88	1608
2	186000	58770	31.6%	200	3146
3	160000	20576	12.9%	85	759
4	186000	53208	28.6%	167	1547
5	171000	18959	11.1%	76	1302
6	144000	15654	10.9%	72	717
7	136000	10059	7.4%	52	759
8	141000	8570	6.1%	41	456
9	113000	8498	7.5%	39	827
10	129000	10719	8.3%	49	829
11	143000	99733	69.7%	167	4124
12	99000	9103	9.2%	40	835
13	98000	16919	17.3%	31	6927
14	117000	9279	7.9%	32	1666
15	89000	11805	13.3%	40	1432
16	100000	8754	8.8%	36	1350
17	105000	13025	12.4%	52	1737
18	95000	6838	7.2%	33	1161
19	66000	3148	4.8%	18	309
X	182000	68539	37.7%	216	2062
Y	66000	989	1.5%	7	185
total	2751000	476050	17.3	1541	

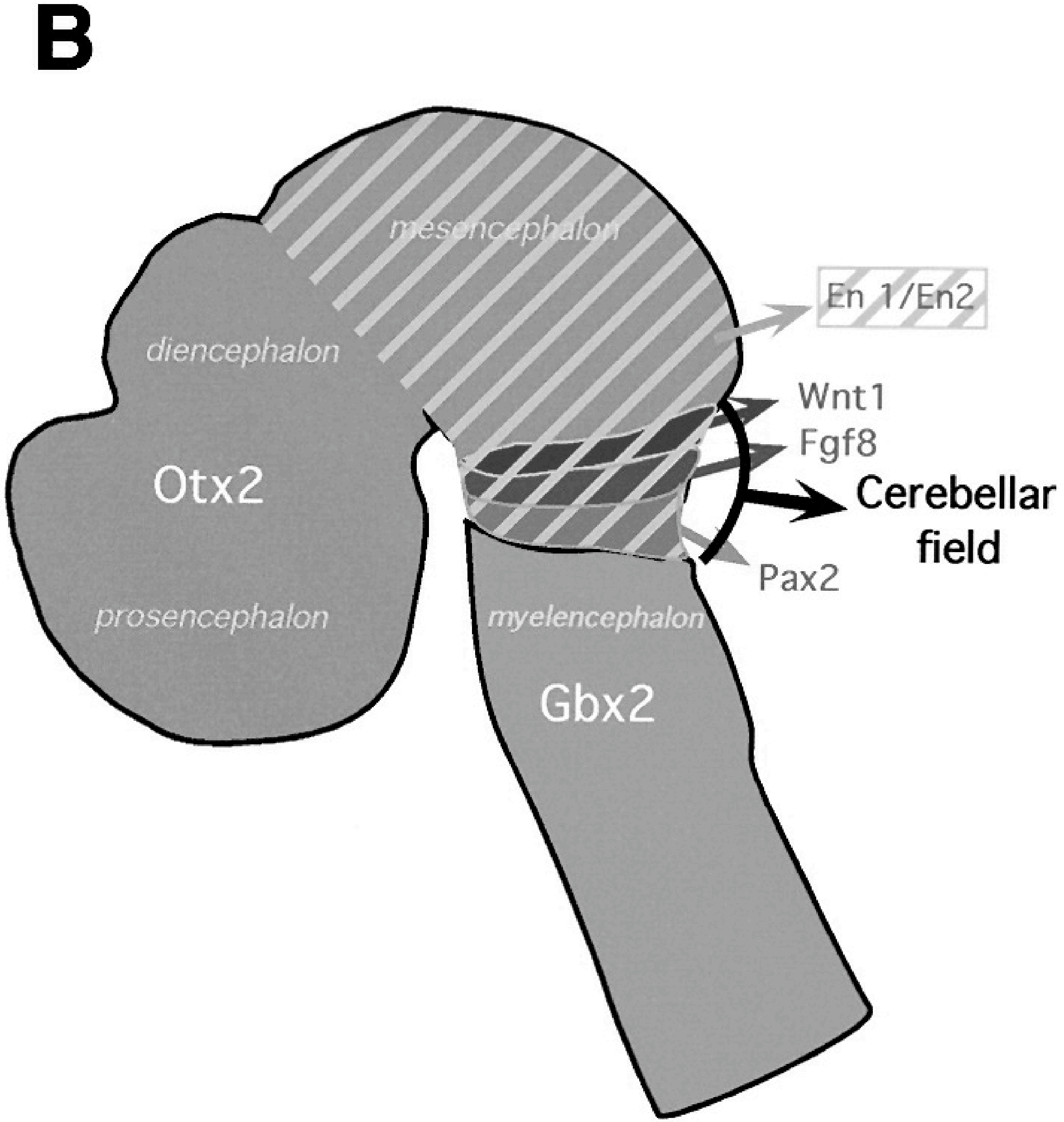
Mouse genome DNA sequencing in progress
(95% of transcribed sequences covered)



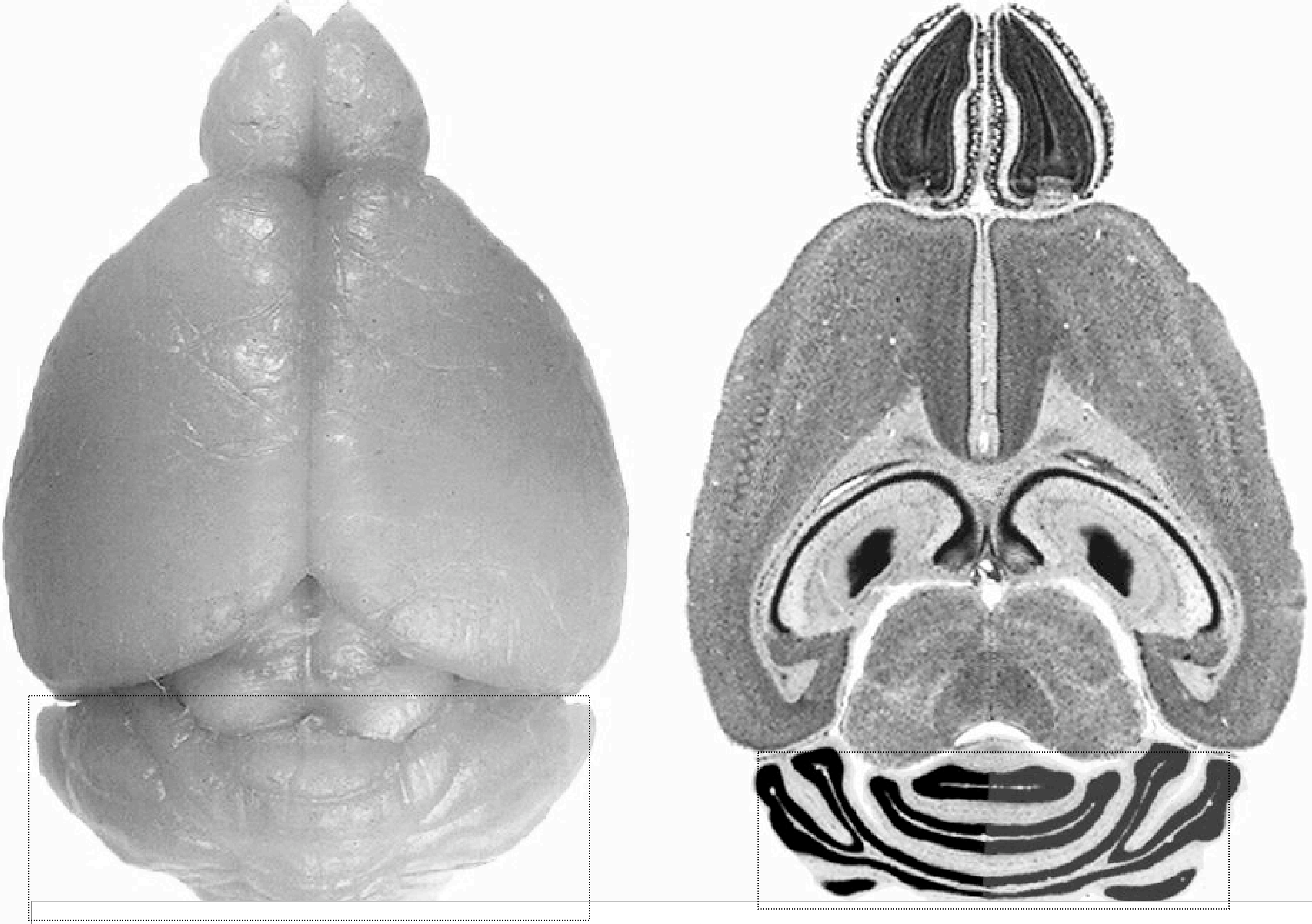
Human proteome overview

Thyroid hormone (T3) is necessary for brain development

Un crétin d'Aoste.

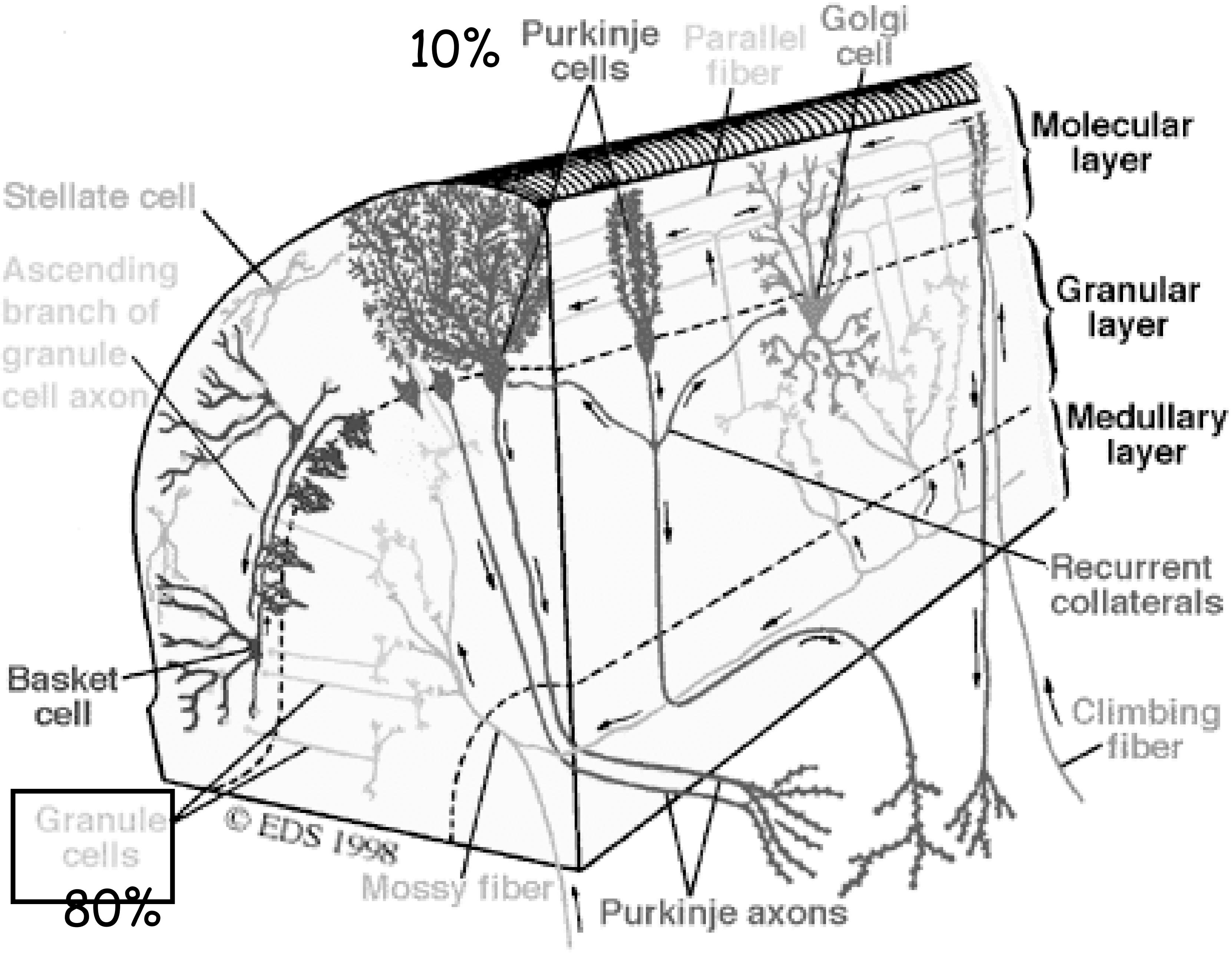


Cerebellum as a model for brain development



Mouse cerebellum as model of human cerebellum

Histological organisation of the adult cerebellum

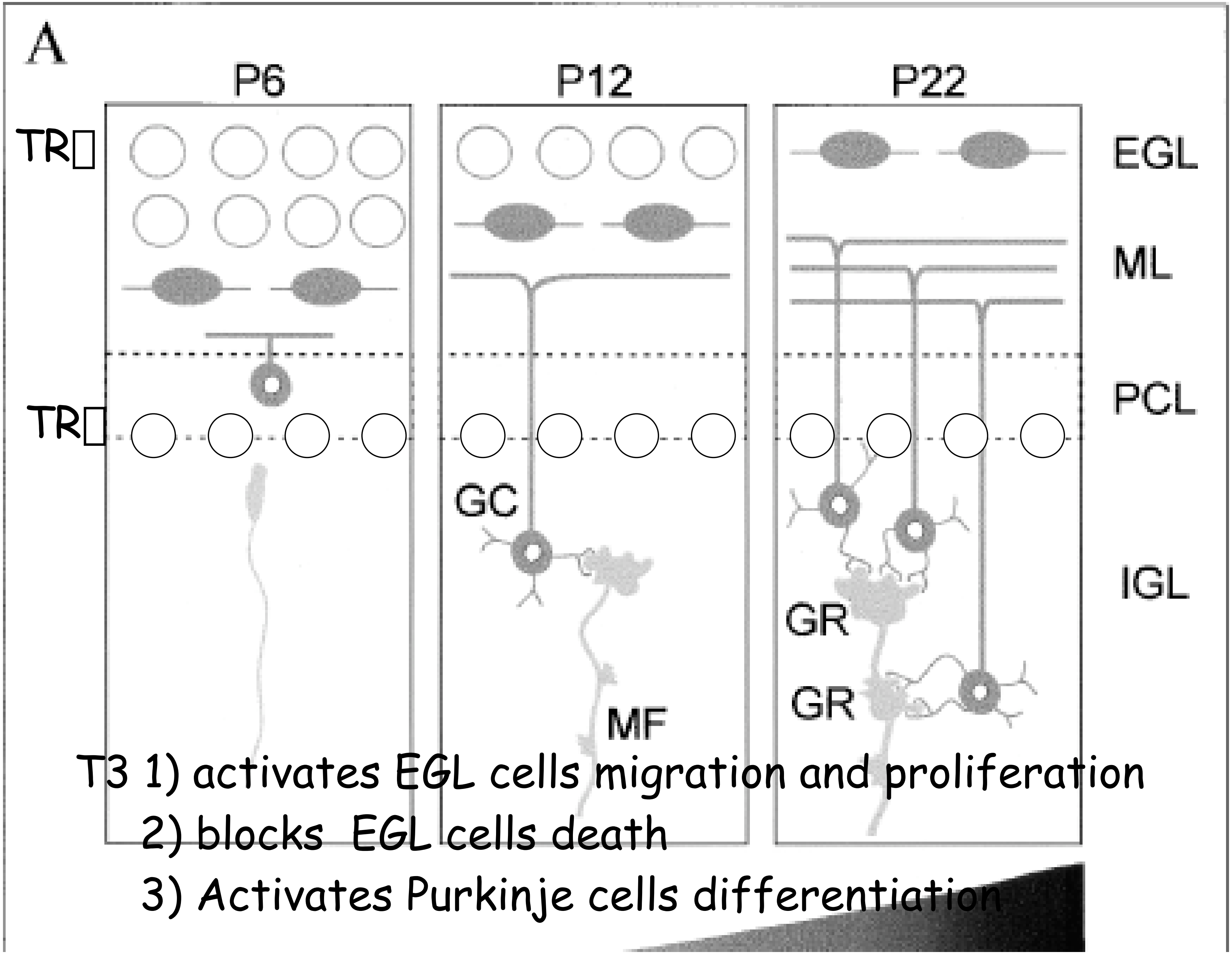


Euthyroid mice

(P15)

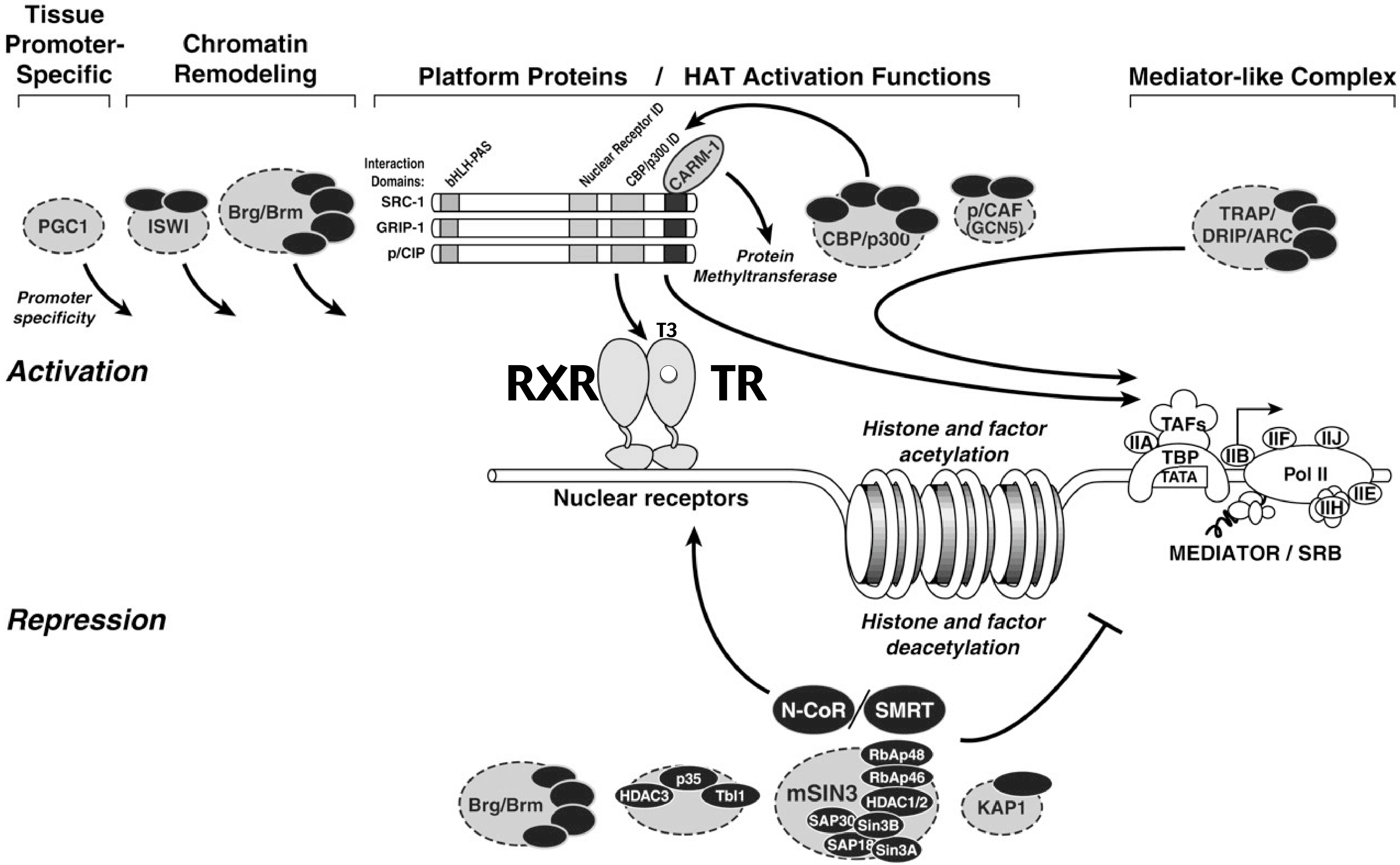
Hypothyroïd mice

(low T3 level = thicker granular cells, +disorganized Purkinje cell layer)

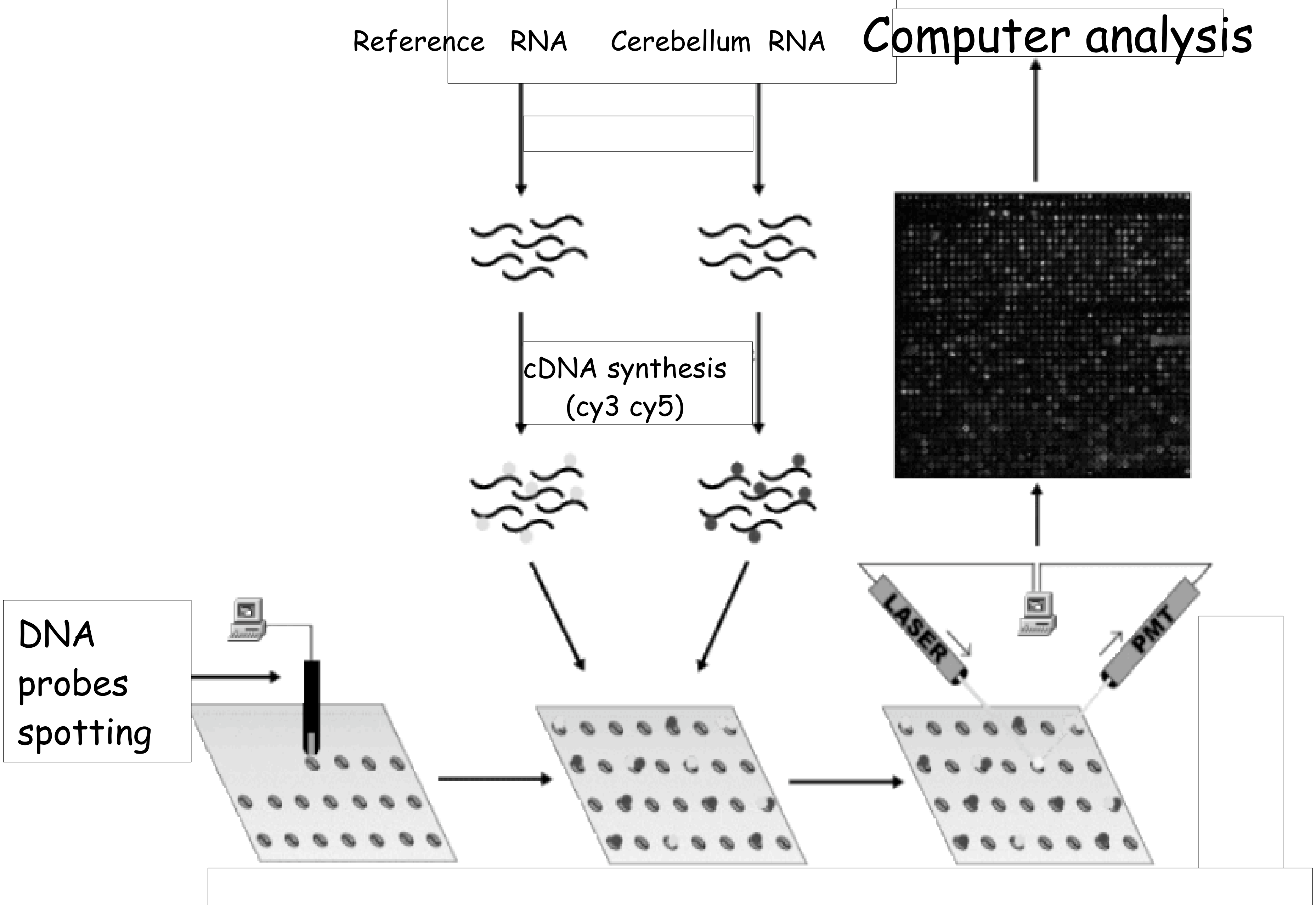


T3 1) activates EGL cells migration and proliferation
2) blocks EGL cells death
3) Activates Purkinje cells differentiation

Molecular level: T3 acts directly on gene transcription



Tool#1: Microarrays



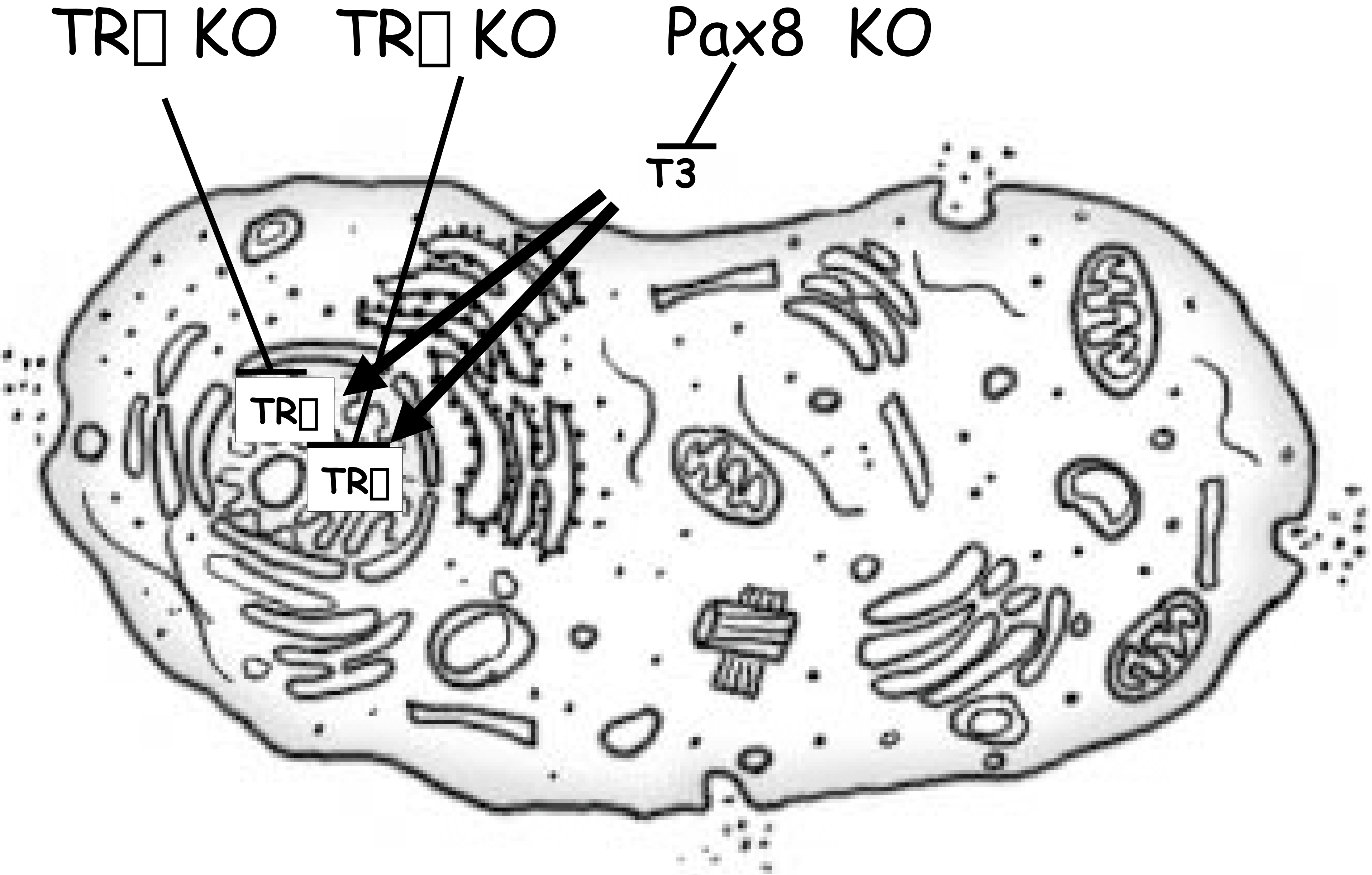
Reference RNA

Cerebellum RNA

Computer analysis

cDNA synthesis
(cy3 cy5)

DNA
probes
spotting



Tool#2: Knockout mice

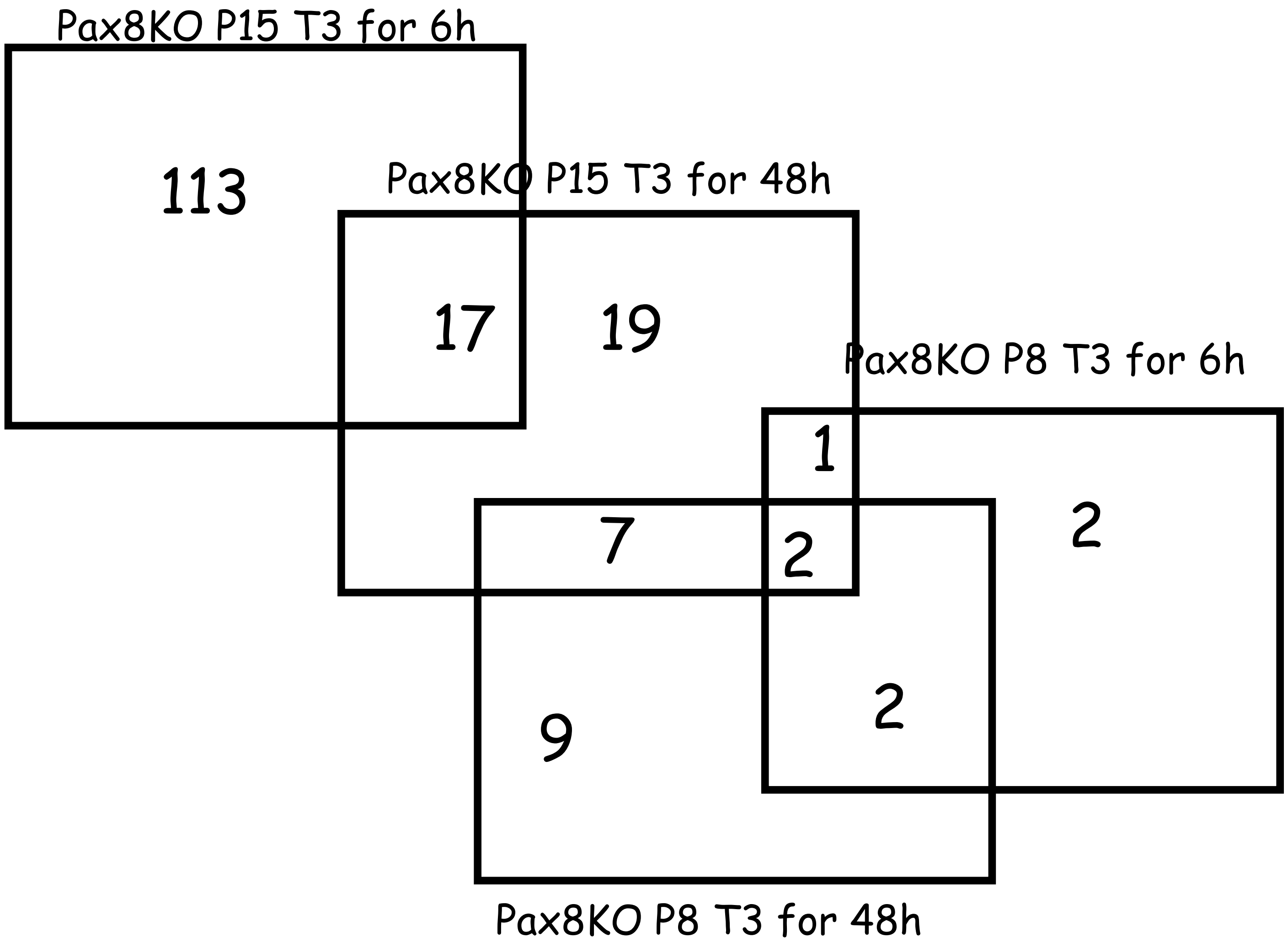
A database project:

1) 20 000 spotted DNA probes
2) Triplicate experiments =10^6 data points

Genotype	Age	Treatment
Wild type	P8	
	P15	
	P15	15 days PTU
	P15	15 days PTU+ 2days T3
TR□ KO	P15	15 days PTU
	P15	15 days PTU+ 2days T3
TR□ KO	P15	15 days PTU
	P15	15 days PTU+ 2days T3
Pax8 KO	P8	
	P8	6 hours T3
	P8	1 day T3
	P8	2 days T3
	P15	
	P15	6 hours T3
	P15	1 day T3
	P15	2 days T3

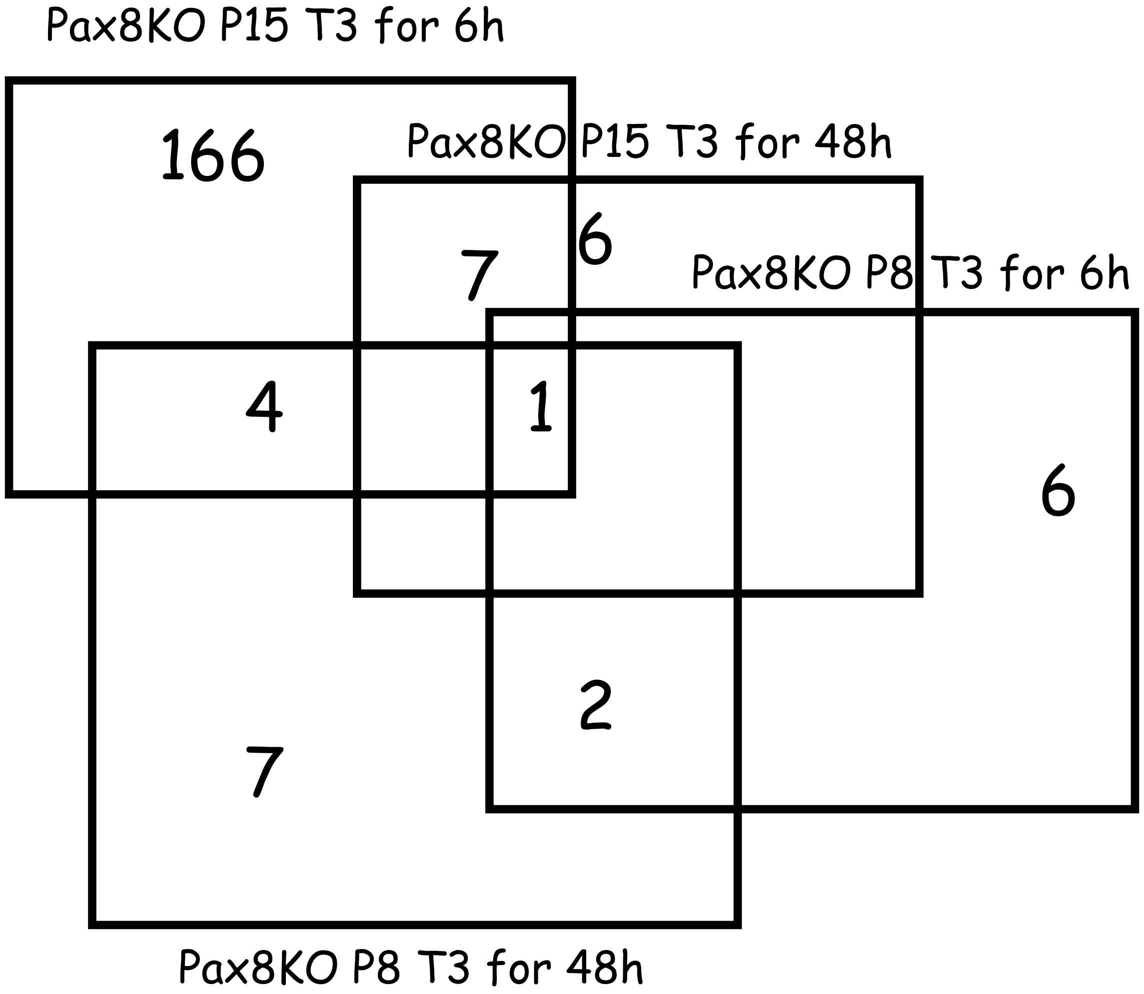
(days of PTU treatment remove most endogenous T3)

UG		**P8**	**P8.6h**	**P8.48h**	**P15.WT**	**P15**	**P15.6H**	**P15.48H**
	45054	1,03	0,07	1,60	0,93	1,71	3,26	0,05
	14012	4,31	3,41	6,12	5,83	5,92	0,72	1,59
	23178	1,88	2,20	2,46	6,62	3,73	0,62	1,03
	18502	1,84	1,40	3,17	1,86	2,62	0,35	0,73
	28479	2,49	2,18	3,80	4,16	4,41	0,45	1,25
data		3,20	2,99	3,80	3,85	3,65	0,57	1,20
	196110	2,35	2,27	3,14	3,41	3,53	0,69	1,16
	40802	2,11	1,67	2,58	3,74	2,50	0,33	0,89
	21500	1,94	1,63	2,70	1,90	2,36	0,39	0,87
	103185	3,49	2,52	3,61	3,87	3,51	0,63	1,31
	154915	1,71	1,64	2,16	2,56	2,37	0,52	0,99
	197479	0,43	0,33	0,38	0,60	0,68	0,28	0,29
	46019	1,52	1,40	2,02	3,02	2,29	0,32	1,04
data		1,26	1,62	1,68	1,41	1,48	0,43	0,70
	29717	2,57	1,97	2,70	3,31	2,43	0,49	1,20
	70573	0,85	0,51	0,51	0,36	0,76	0,64	0,39
	11964	0,38	0,27	0,18	0,15	0,27	0,24	0,14
	2952	0,67	0,55	0,36	0,37	0,54	0,46	0,29
data		0,96	0,83	0,64	0,93	1,10	1,41	0,60
	17917	1,48	1,28	0,78	1,05	1,02	1,44	0,56
	20922	0,61	0,46	0,54	1,18	1,00	0,78	0,56
data		0,54	0,44	0,59	0,88	0,97	0,88	0,55
	1396	0,38	0,31	0,36	0,60	0,65	0,47	0,37
data		1,14	1,21	1,04	1,04	1,23	0,99	0,71
	10727	0,91	0,78	0,58	0,96	0,90	1,26	0,52
	29586	1,02	0,67	0,56	0,48	0,74	0,66	0,45
	14555	0,07	0,08	0,10	0,10	0,15	0,12	0,09
	29855	0,30	0,27	0,31	0,81	0,69	0,61	0,42
	1059	0,28	0,22	0,26	0,49	0,52	0,53	0,32
	198802	0,16	0,14	0,17	0,20	0,24	0,13	0,15
data		0,39	0,30	0,31	0,36	0,44	0,35	0,28
	15571	0,40	0,33	0,37	0,40	0,57	0,40	0,36
	30412	1,48	1,05	1,68	1,45	1,62	0,37	1,01
	17610	0,68	0,57	0,70	0,82	0,83	0,69	0,52
	199817	1,17	1,41	1,31	1,09	1,12	0,40	0,71
	157648	0,44	0,41	0,30	0,33	0,48	0,45	0,31
	30244	0,38	0,34	0,41	0,60	0,73	0,50	0,46
	26091	1,29	1,31	0,74	0,99	1,04	1,19	0,67
data		0,45	0,36	0,44	0,50	0,57	0,40	0,36
	35854	0,28	0,27	0,33	0,97	0,97	1,10	0,63
	22242	1,13	0,96	1,21	1,77	1,49	0,83	0,97
	22086	0,52	0,42	0,58	0,63	0,61	0,42	0,40
	27302	0,29	0,30	0,30	0,66	0,52	0,45	0,34
	4263	0,58	0,42	0,49	0,56	0,70	0,76	0,46



Down regulated genes (Pax8 KO+T3/Pax8 KO)

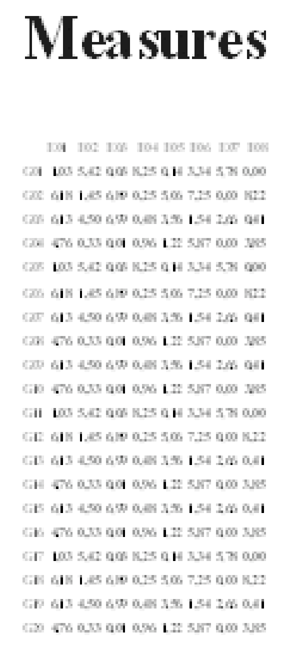
Threshold: 1.5



Up regulated genes (Pax8KO+T3/Pax8KO)

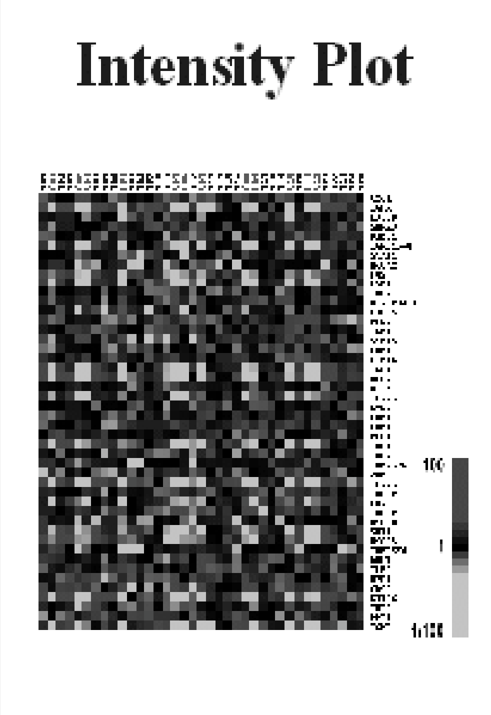
Threshold: 1.5

Eisen Clustering

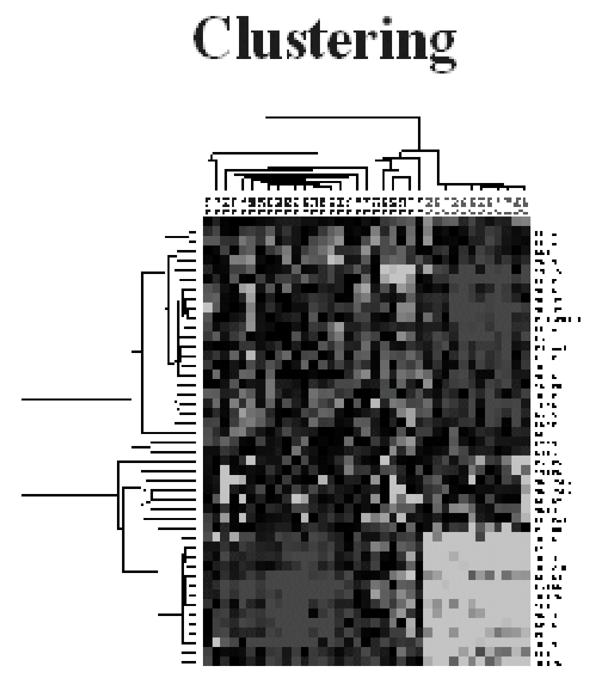


Measures

	E01	E02	E03	E04	E05	E06	E07	E08
G01	1,03	5,42	0,08	8,25	0,14	3,34	5,78	0,00
G02	6,18	1,65	6,89	0,25	5,06	7,25	0,00	8,22
G03	6,13	4,50	6,59	0,48	3,56	1,54	2,46	0,41
G04	4,76	0,33	0,01	0,96	1,22	5,87	0,00	3,85
G05	1,03	5,42	0,08	8,25	0,14	3,34	5,78	0,00
G06	6,18	1,65	6,89	0,25	5,06	7,25	0,00	8,22
G07	6,13	4,50	6,59	0,48	3,56	1,54	2,46	0,41
G08	4,76	0,33	0,01	0,96	1,22	5,87	0,00	3,85
G09	6,13	4,50	6,59	0,48	3,56	1,54	2,46	0,41
G10	4,76	0,33	0,01	0,96	1,22	5,87	0,00	3,85
G11	1,03	5,42	0,08	8,25	0,14	3,34	5,78	0,00
G12	6,18	1,65	6,89	0,25	5,06	7,25	0,00	8,22
G13	6,13	4,50	6,59	0,48	3,56	1,54	2,46	0,41
G14	4,76	0,33	0,01	0,96	1,22	5,87	0,00	3,85
G15	6,13	4,50	6,59	0,48	3,56	1,54	2,46	0,41
G16	4,76	0,33	0,01	0,96	1,22	5,87	0,00	3,85
G17	1,03	5,42	0,08	8,25	0,14	3,34	5,78	0,00
G18	6,18	1,65	6,89	0,25	5,06	7,25	0,00	8,22
G19	6,13	4,50	6,59	0,48	3,56	1,54	2,46	0,41
G20	4,76	0,33	0,01	0,96	1,22	5,87	0,00	3,85

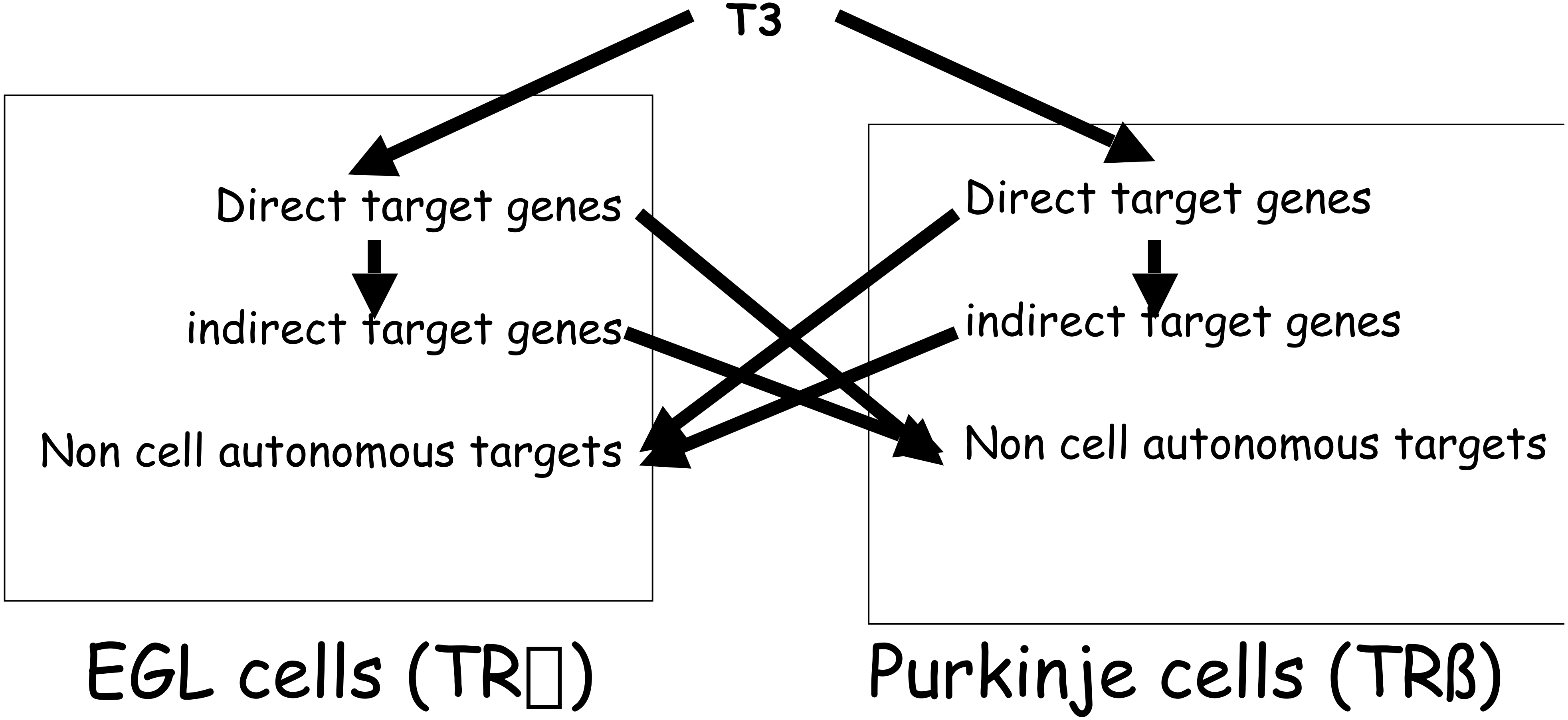


Intensity Plot



Clustering

Data interpretation considering only two cell types (requires knowledge of expression pattern)



T3

Direct target genes

indirect target genes

Non cell autonomous targets

Direct target genes

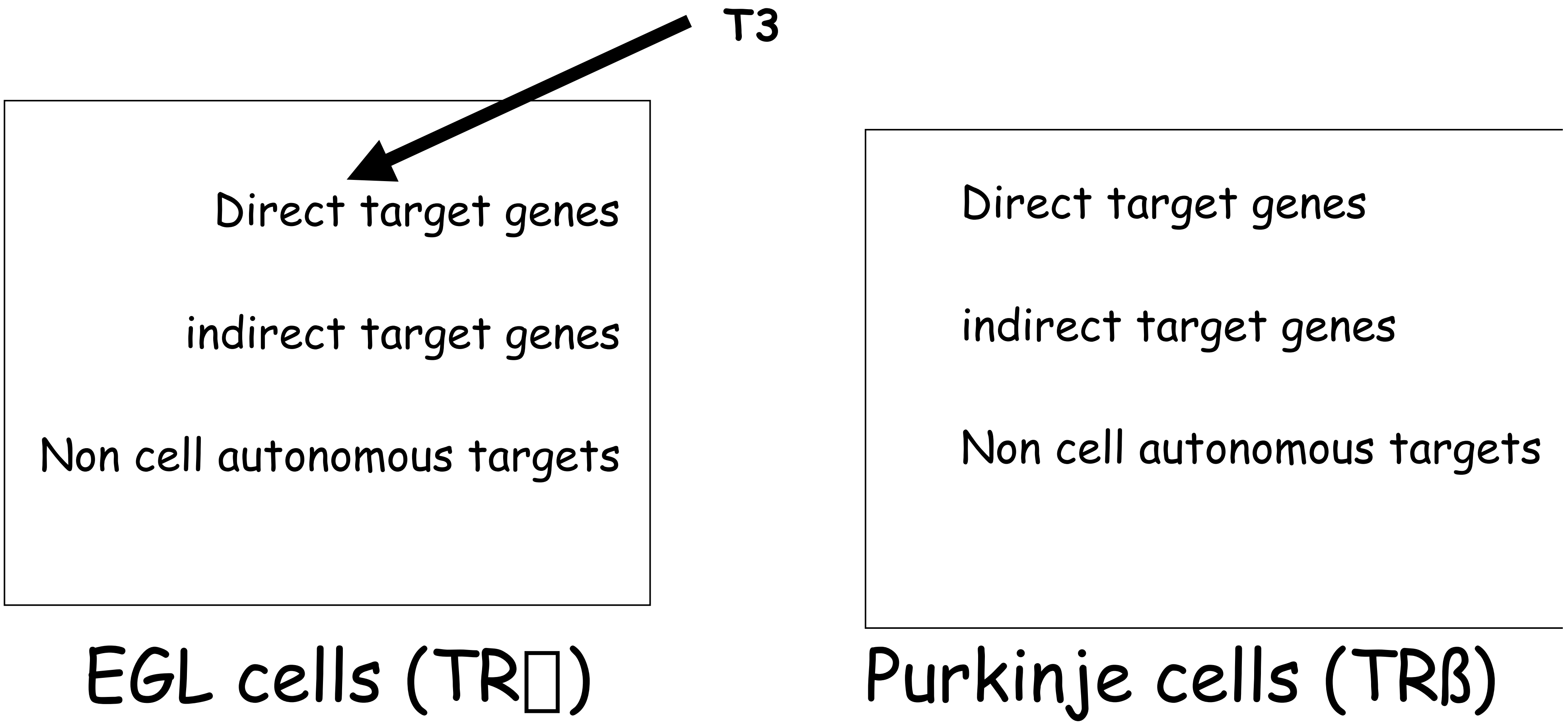
indirect target genes

Non cell autonomous targets

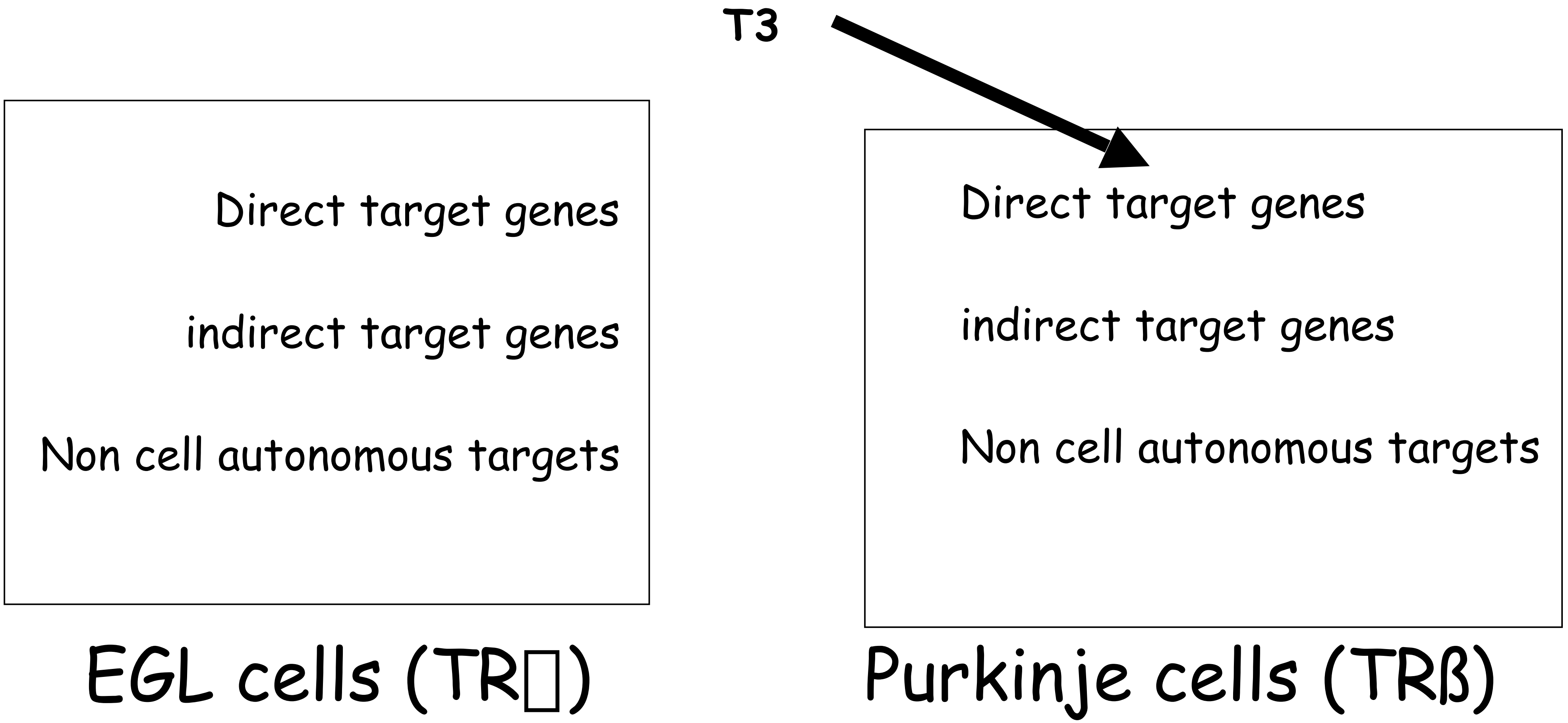
EGL cells (TR□)

Purkinje cells (TRß)

Data interpretation 1) Identify direct target genes in EGL cells =
Expressed in EGL+immediate response+blunted in TR□KO



Data interpretation 2) Identify direct target genes in Purkinje cells = Expressed in Purkinje cells+immediate response+blunted in TR□KO



T3

Direct target genes

indirect target genes

Non cell autonomous targets

EGL cells (TR□)

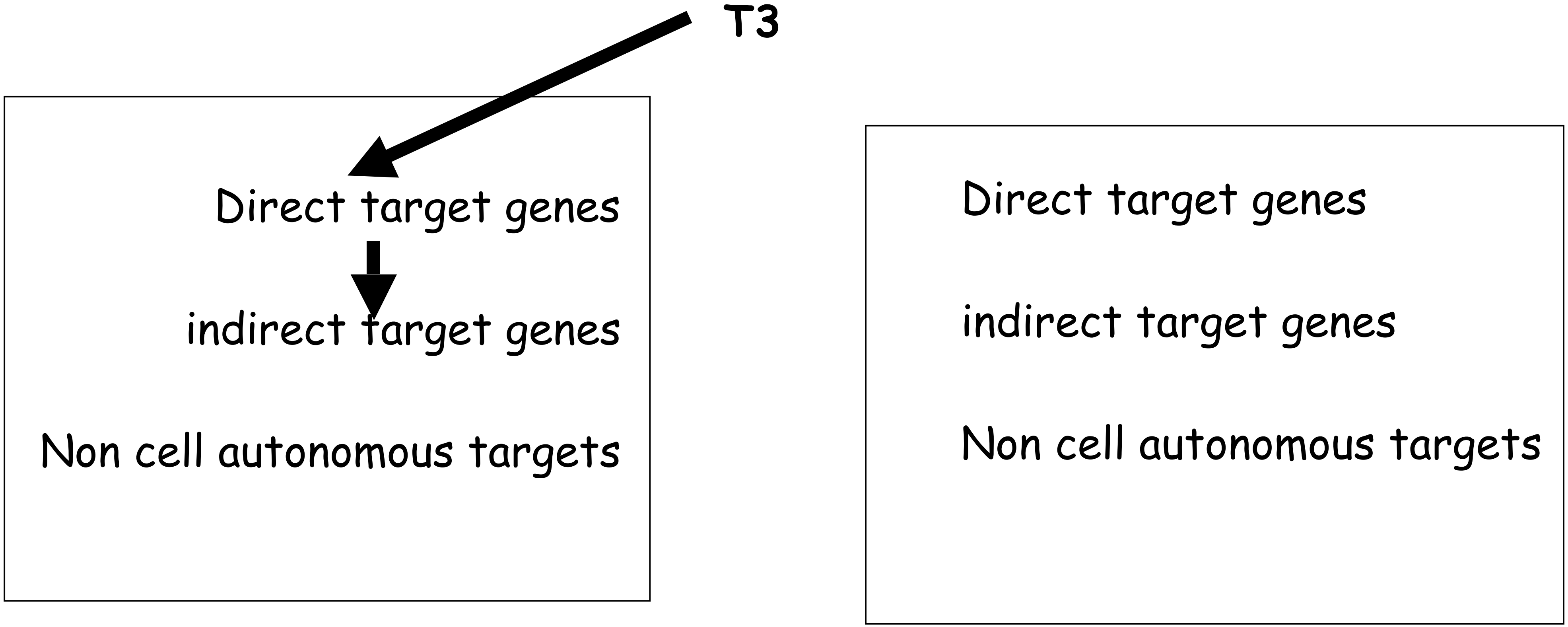
Direct target genes

indirect target genes

Non cell autonomous targets

Purkinje cells (TRß)

Data interpretation 3) Identify indirect target genes in EGL cells = Expressed in EGL+late response+blunted in TR□KO



T3

Direct target genes

indirect target genes

Non cell autonomous targets

Direct target genes

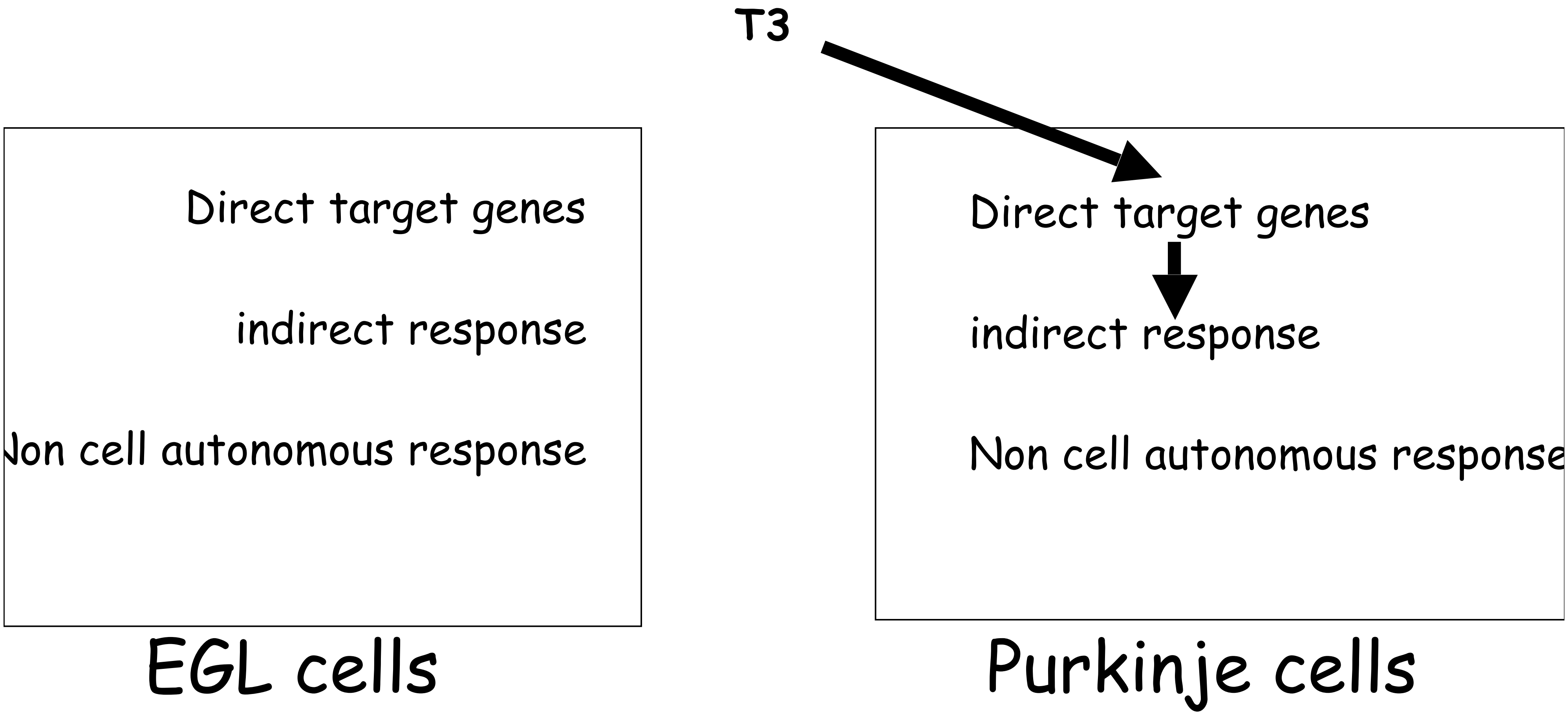
indirect target genes

Non cell autonomous targets

EGL cells (TR□)

Purkinje cells (TRß)

Data interpretation 3) Identify indirect target genes in Purkinje cells = Expressed in Purkinje cells+late response+blunted in TR□KO



T3

Direct target genes

indirect response

Non cell autonomous response

EGL cells

Direct target genes

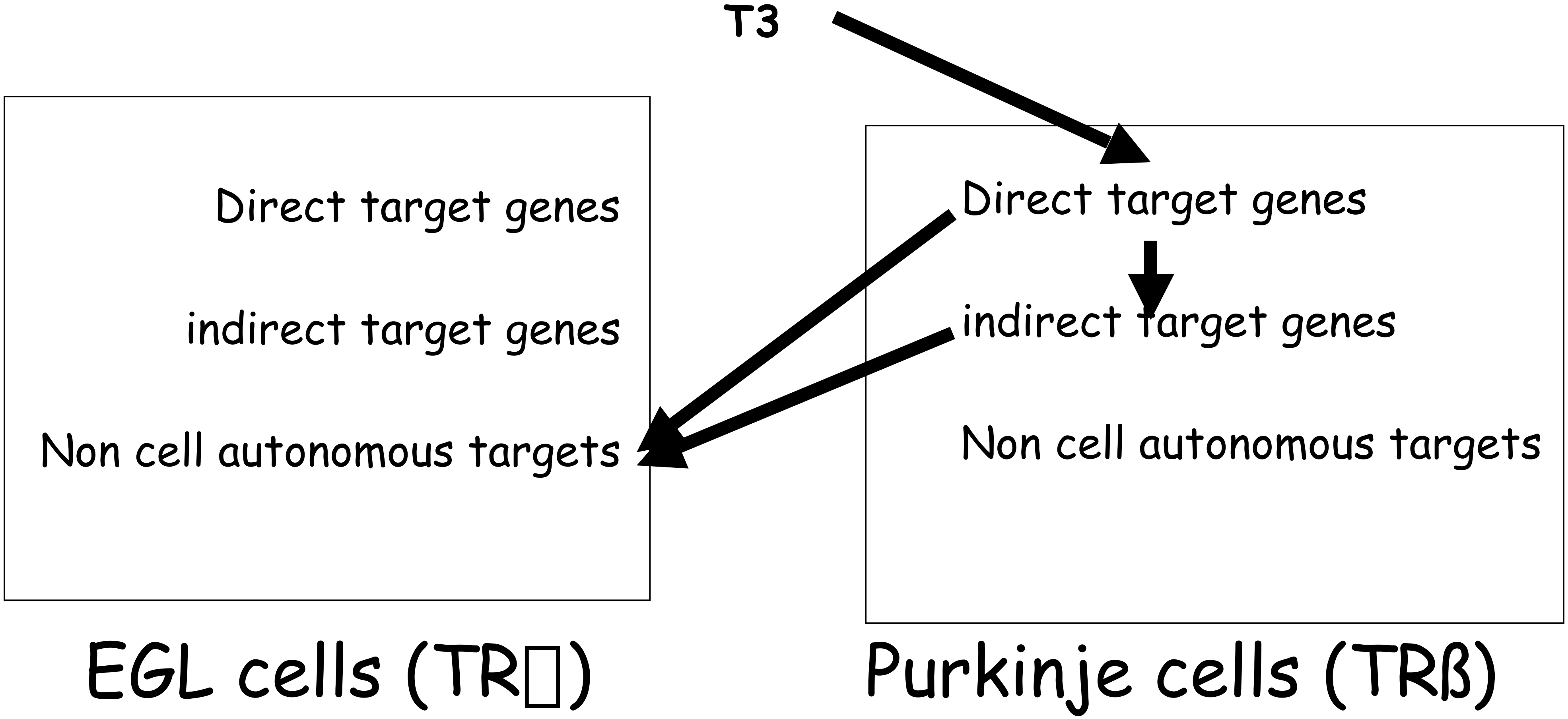
indirect response

Non cell autonomous response

Purkinje cells

Data interpretation 4) Identify non cell autonomous effects in EGL

Expressed in EGL cells+ response blunted in TRßKO



T3

Direct target genes

indirect target genes

Non cell autonomous targets

EGL cells (TR□)

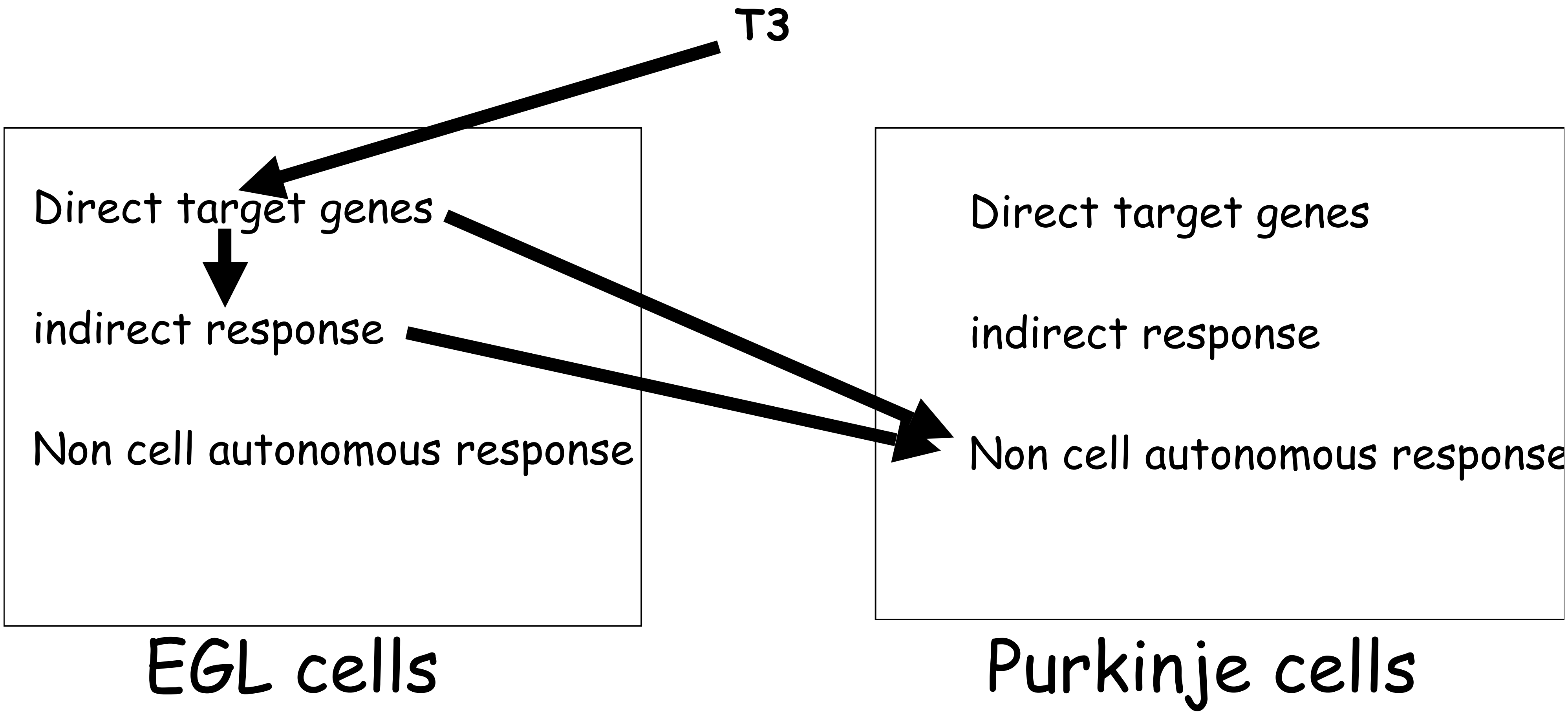
Direct target genes

indirect target genes

Non cell autonomous targets

Purkinje cells (TRß)

Data interpretation 5) Non cell autonomous effects in Purkinje cells
Expressed in Purkinje cells+ response blunted in TR□KO



T3

Direct target genes

indirect response

Non cell autonomous response

EGL cells

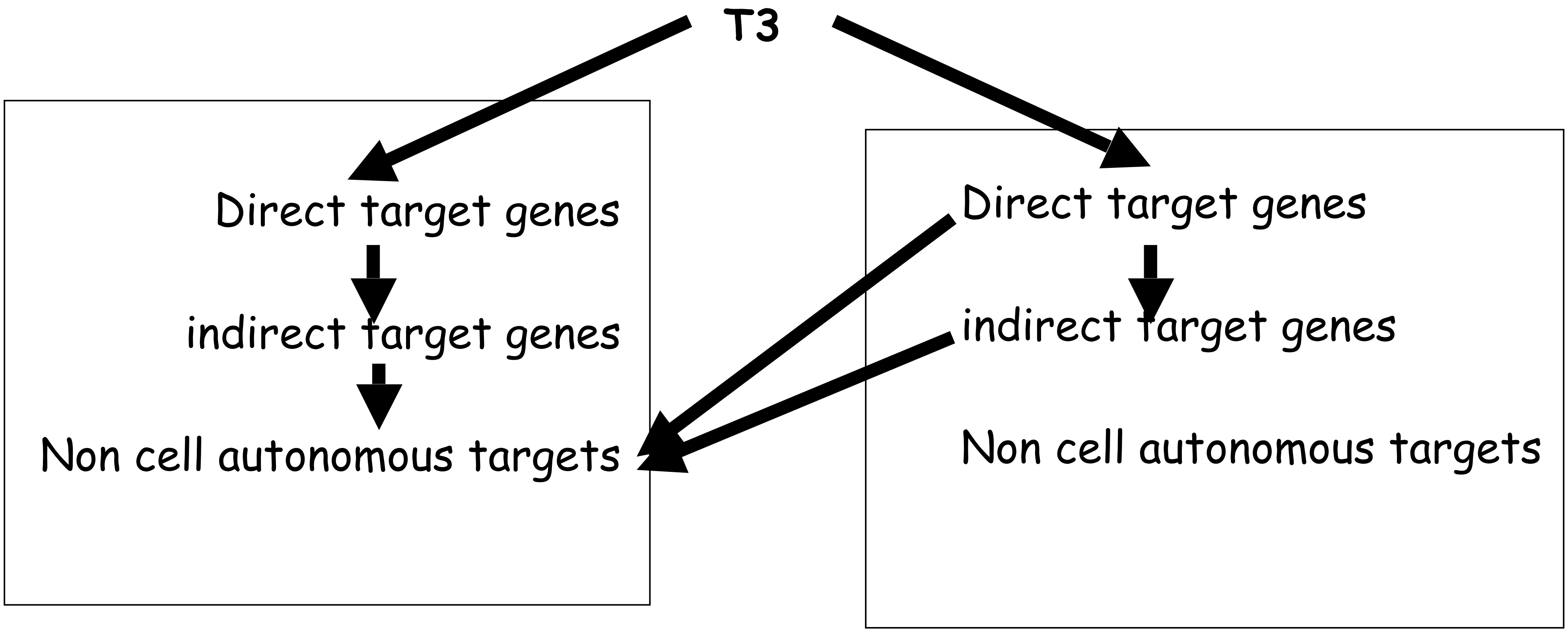
Direct target genes

indirect response

Non cell autonomous response

Purkinje cells

Data interpretation 6) requires cell-cell interaction: for example expressed in EGL cells+ blunted in both TR□KO and TRßKO



T3

Direct target genes

indirect target genes

Non cell autonomous targets

Direct target genes

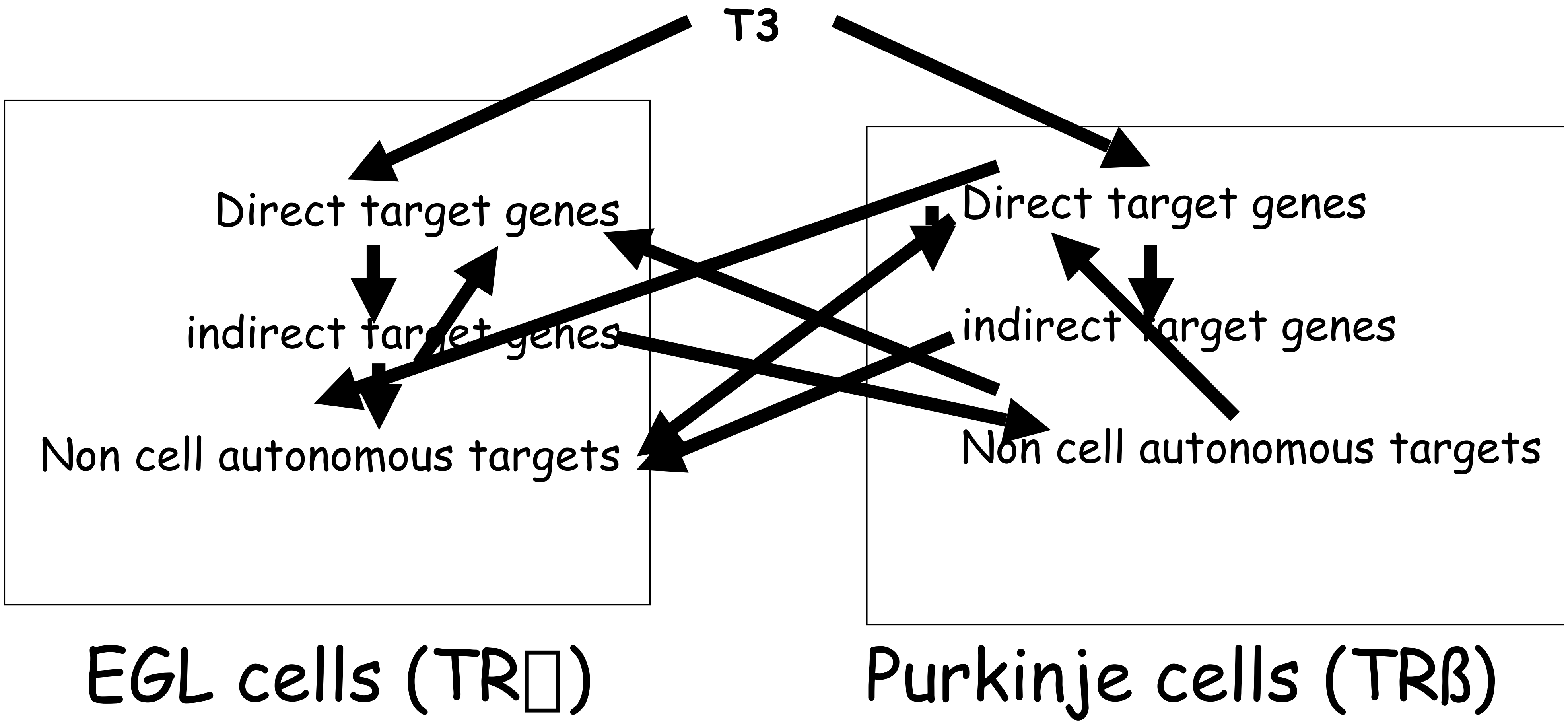
indirect target genes

Non cell autonomous targets

EGL cells (TR□)

Purkinje cells (TRß)

No possible interpretation



T3

Direct target genes

indirect target genes

Non cell autonomous targets

EGL cells (TR□)

Direct target genes

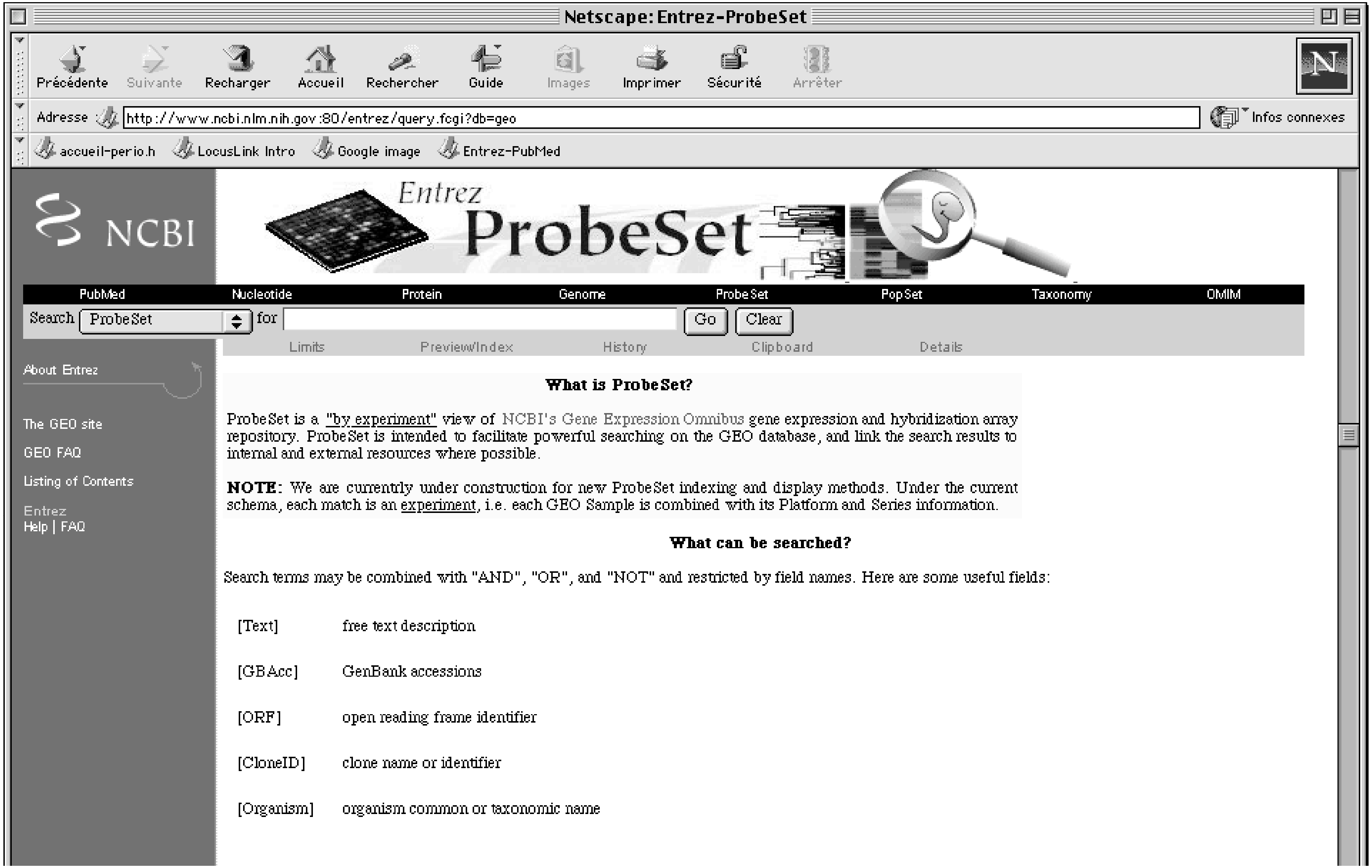
indirect target genes

Non cell autonomous targets

Purkinje cells (TRß)

Future directions

1) Microarray/SAGE public databases



Adresse: http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=geo

Search: ProbeSet for

What is ProbeSet?

ProbeSet is a "by experiment" view of NCBI's Gene Expression Omnibus gene expression and hybridization array repository. ProbeSet is intended to facilitate powerful searching on the GEO database, and link the search results to internal and external resources where possible.

NOTE: We are currently under construction for new ProbeSet indexing and display methods. Under the current schema, each match is an experiment, i.e. each GEO Sample is combined with its Platform and Series information.

What can be searched?

Search terms may be combined with "AND", "OR", and "NOT" and restricted by field names. Here are some useful fields:

[Text]	free text description
[GBAcc]	GenBank accessions
[ORF]	open reading frame identifier
[CloneID]	clone name or identifier
[Organism]	organism common or taxonomic name

Future directions

2) Encyclopaedia of gene expression

What Genes are expressed in selected Components in stage TS15 ?

Genes found in EMAGE

Gene Symbol	Display related sub...
En1	☑
En2	☑
Fgf8	☑
Gbx2	☑
Hoxb1	☑
Hoxb6	☑
Hoxd4	☑
Otx2	☑
Pax1	☑
Pax2	☑
Pax5	☑
Pax6	☑
Shh	☑

Assays with positive results for each gene

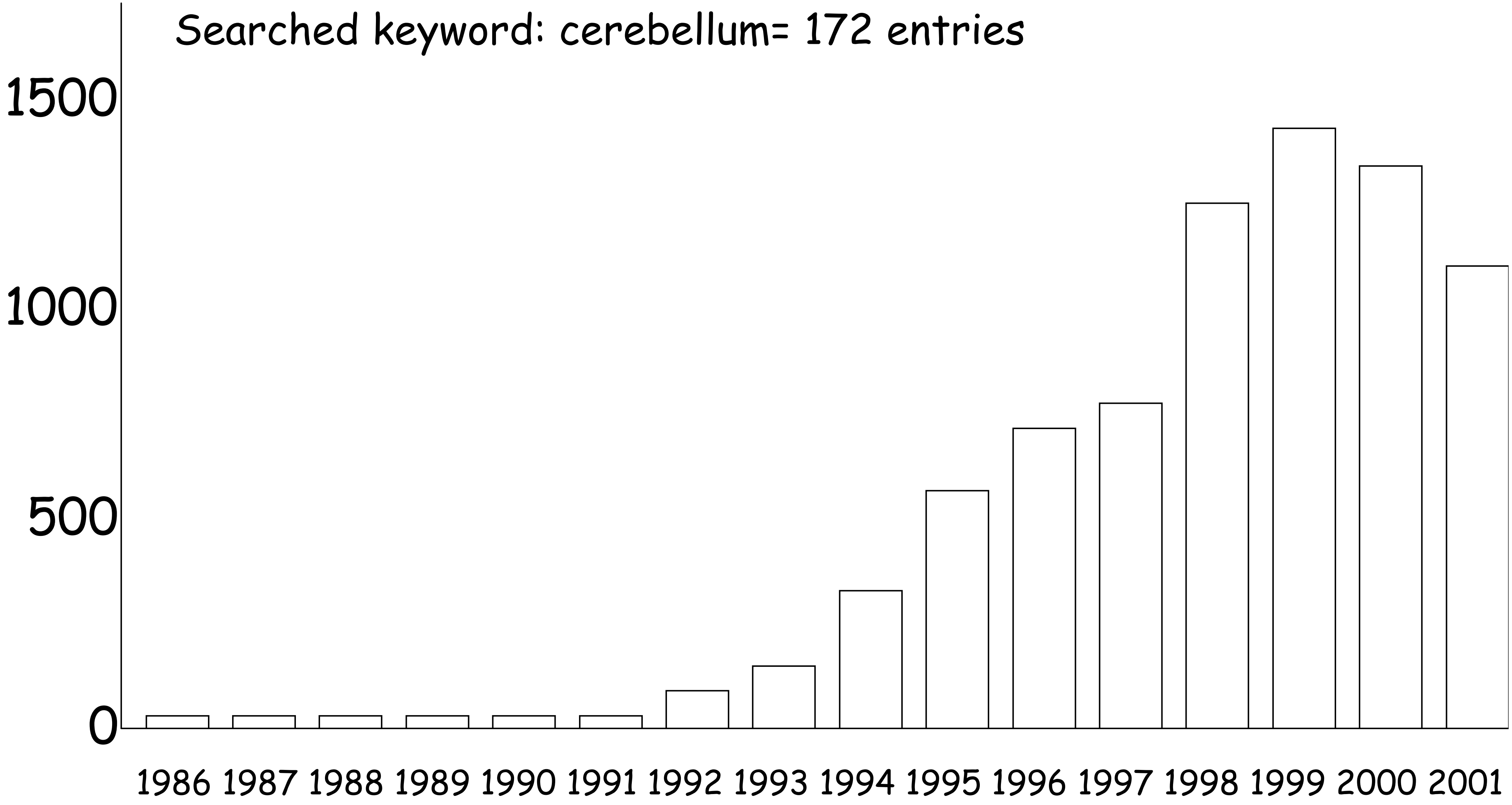
Gene	Submitte...	Display image	ID
En1	EMAGE ED...	☑	EMAGE:73
En2	EMAGE ED...	☑	EMAGE:74
Fgf8	EMAGE ED...	☑	EMAGE:71
Fgf8	EMAGE ED...	☑	EMAGE:70
Gbx2	EMAGE ED...	☑	EMAGE:77
Gbx2	EMAGE ED...	☑	EMAGE:75
Hoxb1	EMAGE ED...	☑	EMAGE:79
Hoxb6	EMAGE ED...	☐	EMAGE:8
Hoxd4	EMAGE ED...	☐	EMAGE:48
Otx2	EMAGE ED...	☐	EMAGE:59
Pax1	EMAGE ED...	☐	EMAGE:61
Pax2	EMAGE ED...	☐	EMAGE:62
Pax5	EMAGE ED...	☐	EMAGE:64

En1 En2 Fgf8 Fgf8 Gbx2 Gbx2 Hoxb1

Status : no database open.

Future directions

3) More mouse mutations.



Searched keyword: cerebellum= 172 entries

Entries in the mouse knockout and mutations database