Gene expression analyses in the eye and ear: recent findings and future possibilities

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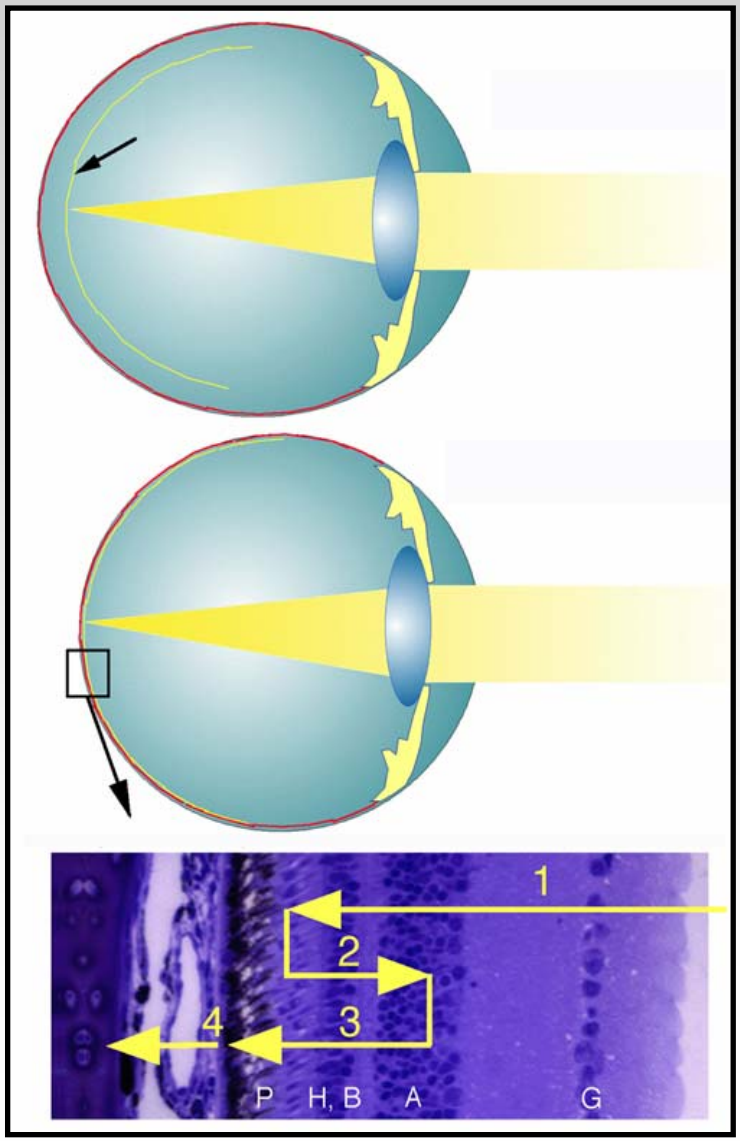
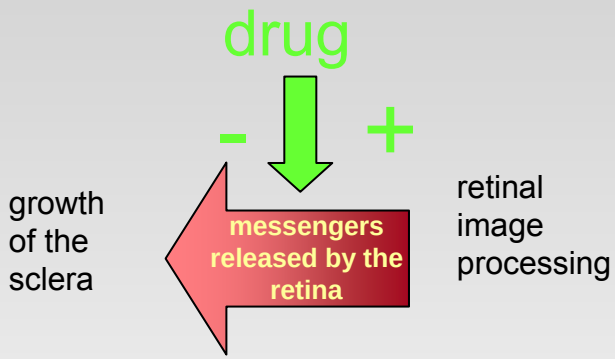
Signaling pathways during eye growth regulation



- modifying visually induced signals

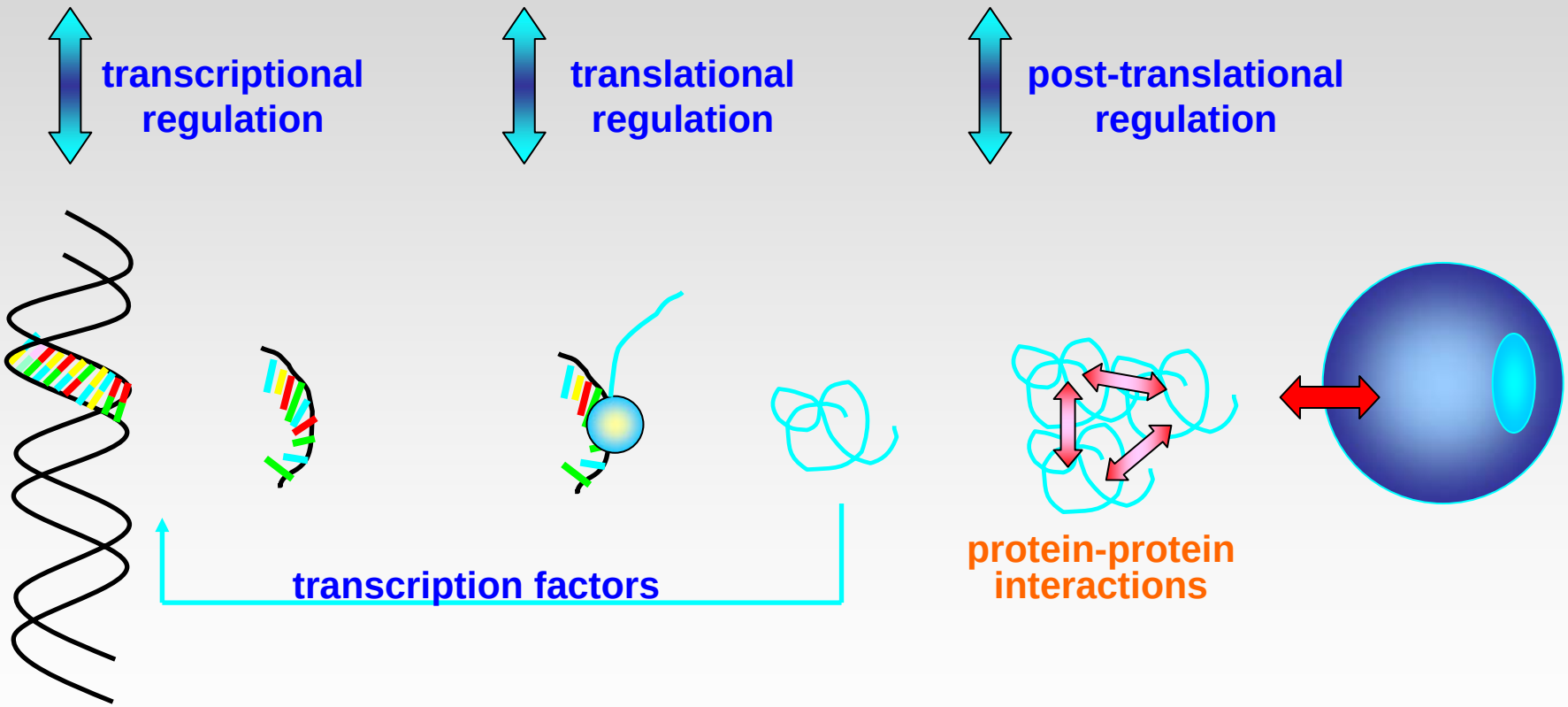


How can the best targets be identified ?

- find new targets by screening for genes that respond to experimentally induced myopia
- optimize compounds that act on already known targets

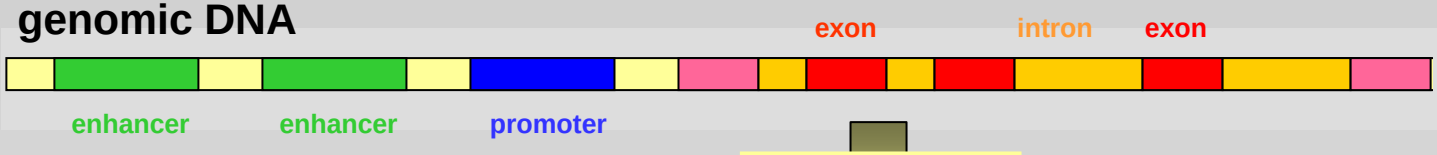
Different levels of visual and genetic control

- Eye growth is controlled at several levels



Transcription of eukaryotic genes

- **genomic DNA**



transcription

- **hnRNA**
  (hetero-nuclear, very short lived)

splicing

- **mRNA**
  (messenger)

translation

- **protein**

RNA Polymerase I transcribes ribosomal RNA (rRNA).
RNA Polymerase II transcribes messenger RNA (mRNA) and most small nuclear RNAs (snRNAs).
RNA Polymerase III transcribes transfer RNA (tRNA) and other small RNAs (including the small 5S rRNA).

Types of RNA

About 97% of the transcriptional output is non-protein-coding in eukaryotes.

Non-coding RNAs involved in translation
transfer RNA (tRNA); each kind of tRNA carries (at its 3′ end) one of the 20 **amino acids**
ribosomal RNA (rRNA):18S rRNA, 28S, 5.8S, and 5S rRNA

Non-coding RNAs involved in gene regulation
micro RNAs (miRNA, single stranded, 21-22 nucleotide): can down-regulate gene expression
small interfering RNA (siRNA, double-stranded, 20-25 nucleotides), involved in RNA interference pathway.
long noncoding RNAs that regulate genes.

Protein coding RNAs
Messenger RNA (mRNA) carries information about a protein sequence to the ribosomes. mRNA may contain regulatory elements itself, in the 5` untranslated region or 3`untranslated region. These cis-regulatory elements regulate the activity of that mRNA. The untranslated regions can also contain elements that regulate other genes.

(not complete)

Transcriptome

Transcriptome definition:

The **transcriptome** is the set of all RNA molecules, including mRNA, rRNA, tRNA and other non-coding RNA.

The term can be applied to the total set of transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type.

The transcriptome can vary with external environmental conditions.

Because it includes all *mRNA* transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time, with the exception of mRNA degradation phenomena such as transcriptional attenuation.

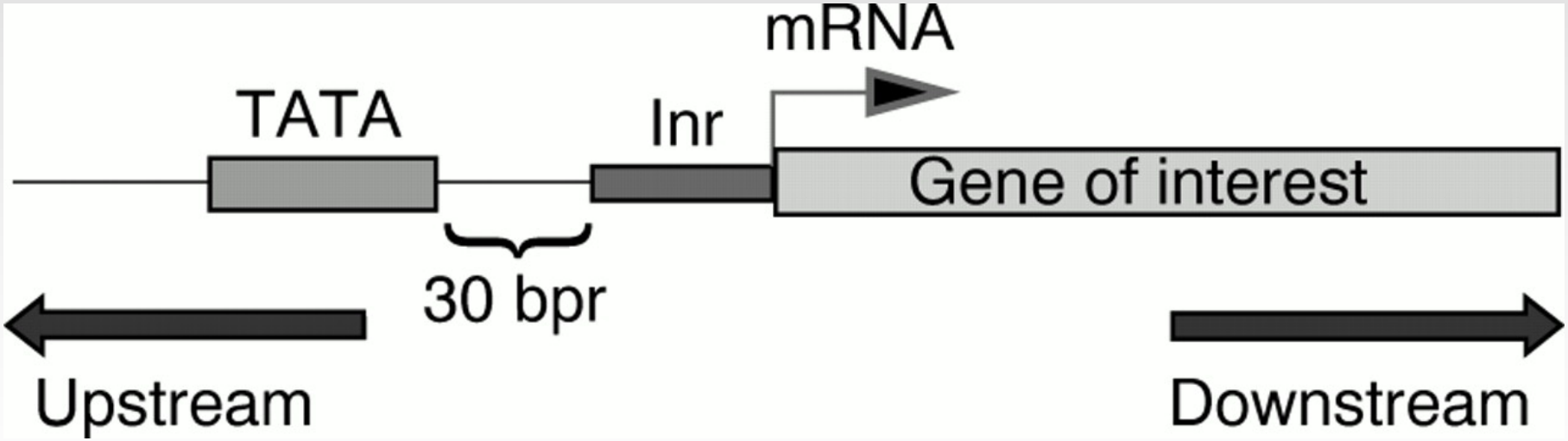
Transcriptomics is the branch of molecular biology that deals with the study of messenger RNA molecules produced in an individual or population of a particular cell type.

Control of the expression of the mRNA genes

Transcriptional initiation
This is the **most important mode for control of eukaryotic gene expression**.

Transcription start site
RNA polymerase II binds. Pol II is a complex of different proteins.

The basal promoter

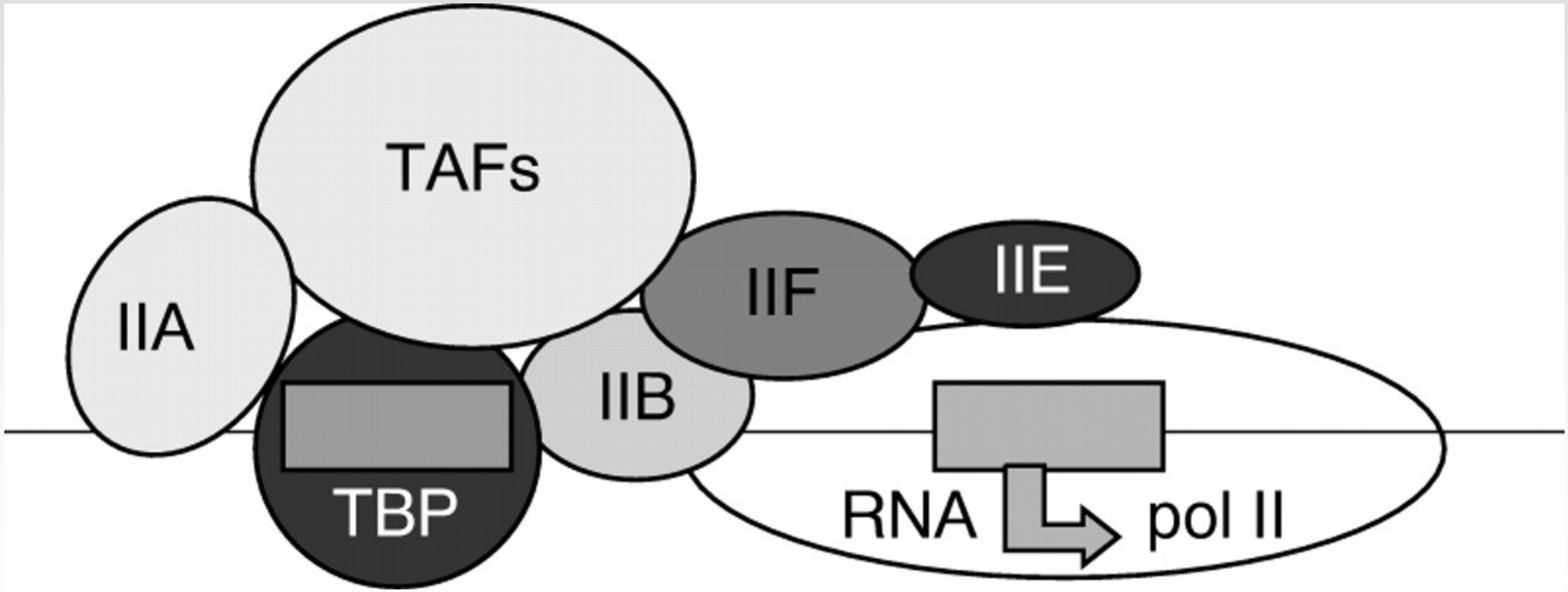


The basal promoter contains a sequence of 7 bases (TATAAAA) called the **TATA box** and an initiator element (Inr).

.

The basal promotor

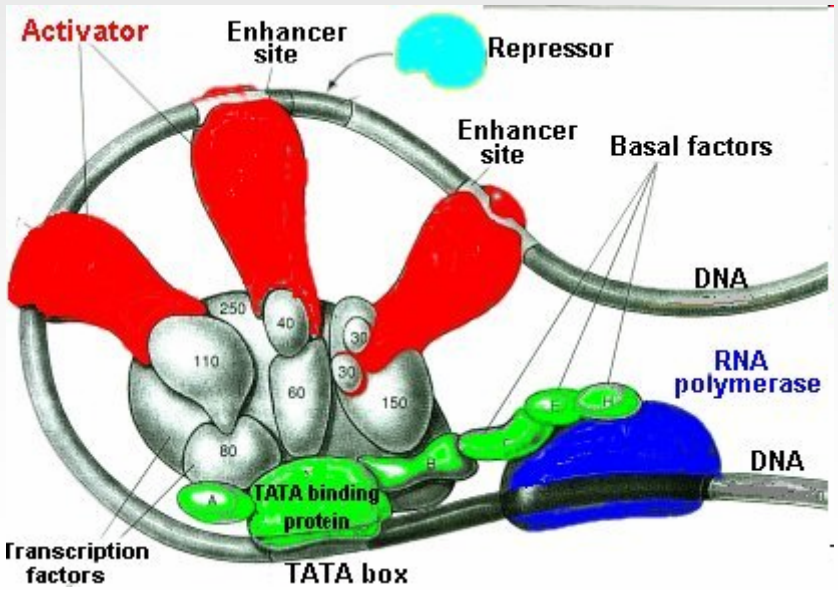
The basal promoter is bound by a large complex of some 50 different proteins, including **Transcription Factor IID** (**TFIID**), which is a complex of **TATA-binding protein** (**TBP**), which recognizes and binds to the TATA box ; 14 other protein factors which bind to TBP — and each other — but not to the DNA; **Transcription Factor IIB** (**TFIIB**) which binds both the DNA and pol II.
The basal or core promoter **is found in all protein-coding genes**.



Transcription of eukaryotic genes

The upstream enhancer/promoter

Structure and associated binding factors **differ from gene to gene**.
Regulatory sequences are predominantly located upstream (5') of the transcription initiation site, although some elements occur downstream (3') or even within the genes themselves.
The **number** and **type** of regulatory elements to be found varies with each mRNA gene.
Different combinations of transcription factors also can exert differential regulatory effects upon transcriptional initiation.
Various cell types each express **characteristic combinations** of transcription factors, this is the **major mechanism for cell-type specific regulation** of mRNA expression.



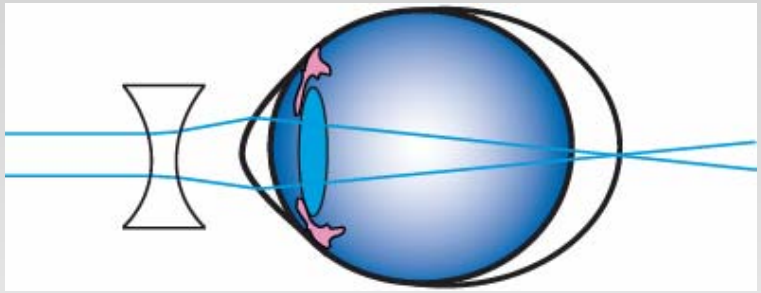
Methods used in transcriptomics studies

Analysis of induced gene expression changes

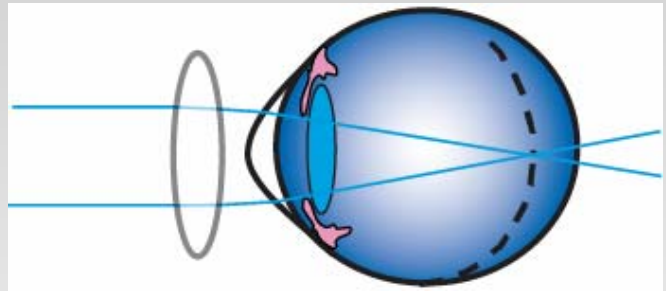
- PCR and real-time PCR
- Northern blot
- In-situ hybridisation
- Oligonucleotide microarrays
- Next generation sequencing

Measuring induced changes in transcription rate of a (known) gene in the visual system and the ear

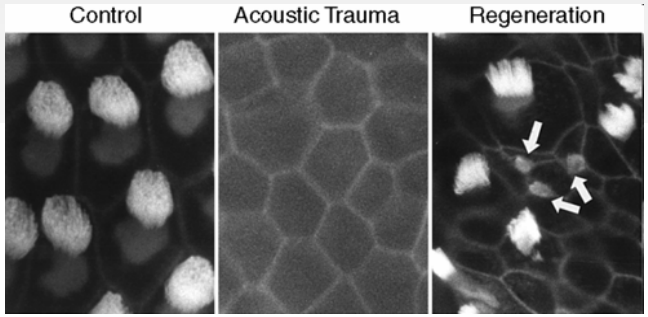
Examples for induced changes in the eye and ear:



Minus lens-wear – increased ocular growth



Plus lens-wear – decreased ocular growth



Margaret Lomax et al. (2001). Noise & Health 3 :19-35.

When noise exposure kills hair cells in birds, these cells can regenerate and hearing will recover. In mammals, however, the hair cell loss, and resulting hearing loss, is permanent.

Reverse Transcriptase Polymerase Chain Reaction (PCR)

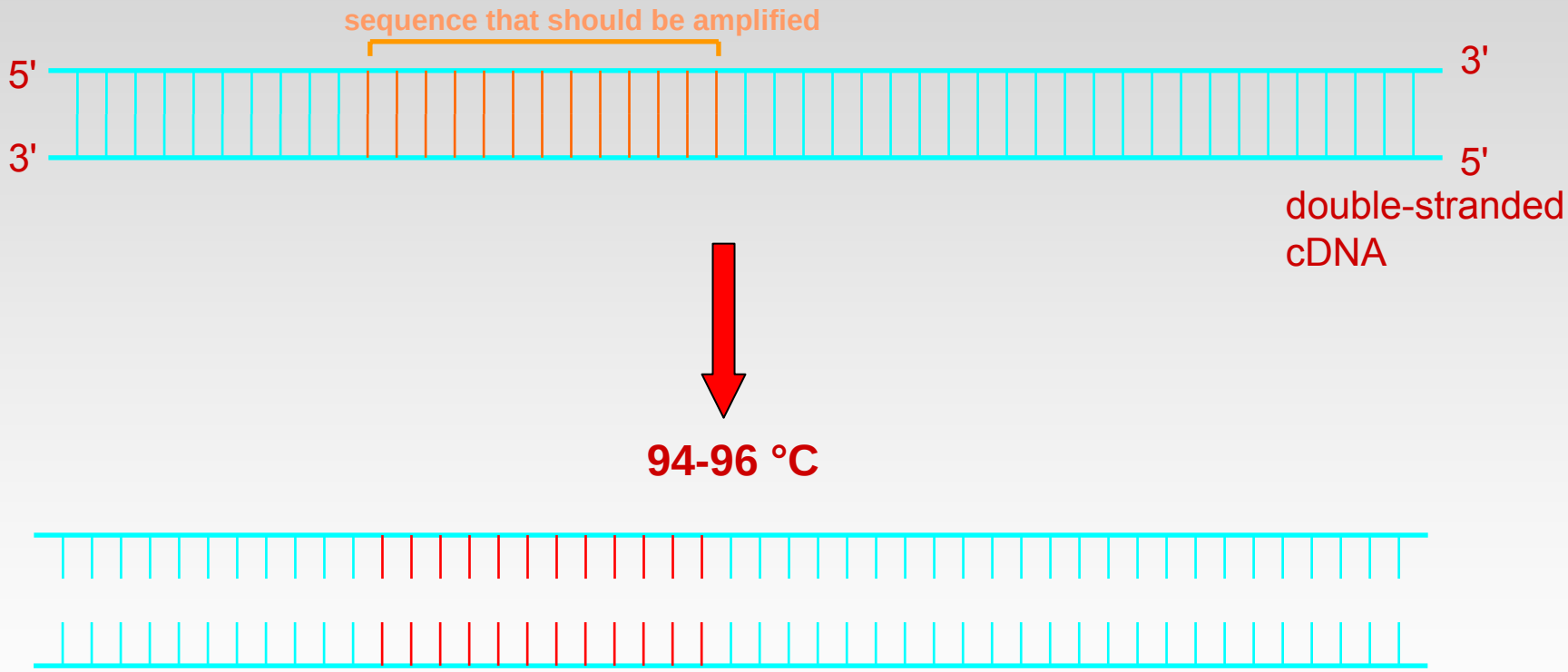
- invented by Dr. Kary Mullis 1983 (awarded with the Nobel prize 1993)
- makes it possible to produce very high numbers of copies of a sequence
- clever tricks, using repetitive "melting" of cDNA

temperature raised
to 94-96° C

Reverse Transcriptase Polymerase Chain Reaction (PCR)

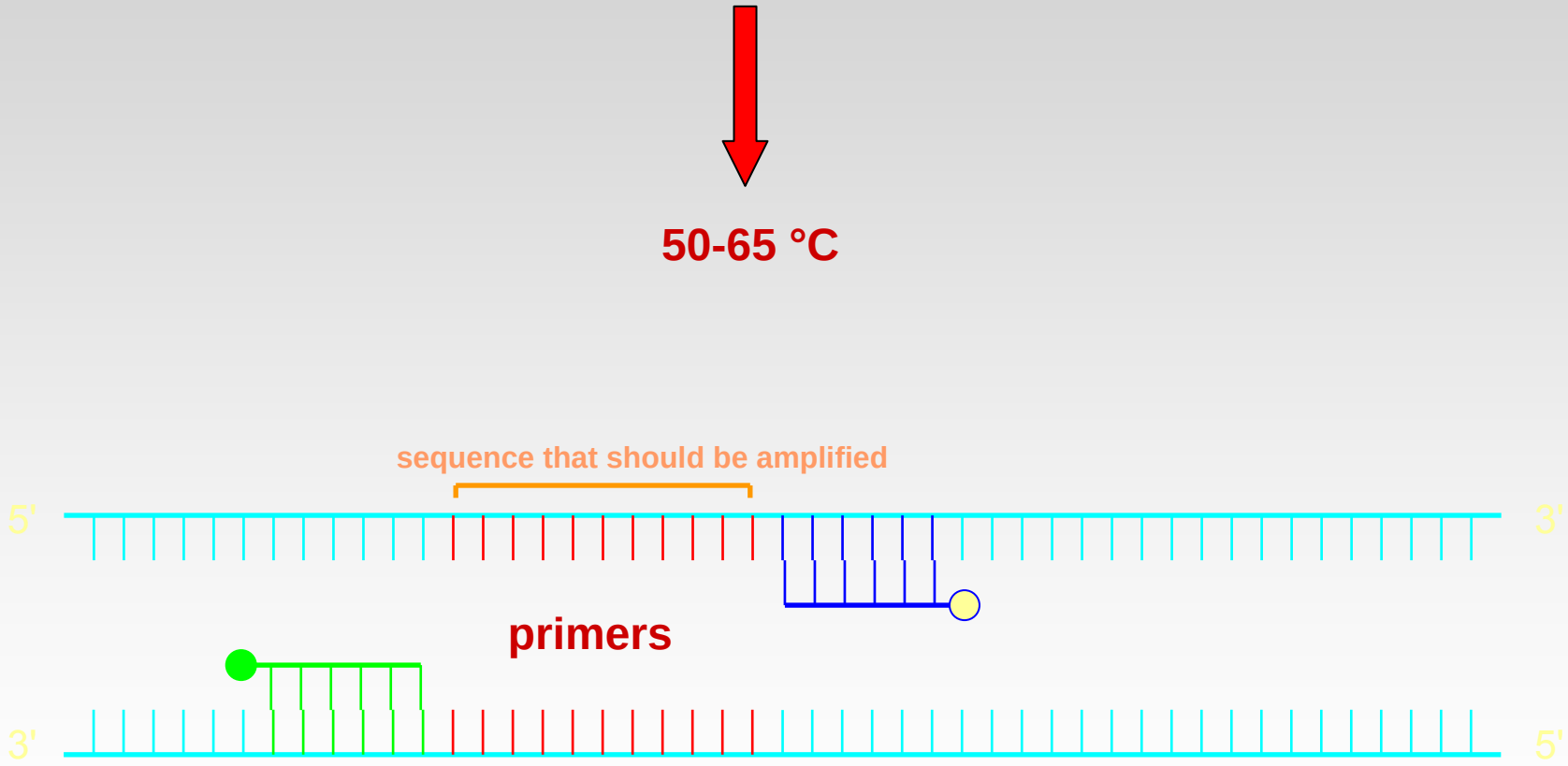
- RNA extraction, conversion into cDNA with reverse transcriptase

  STEP 1: heating and denaturation



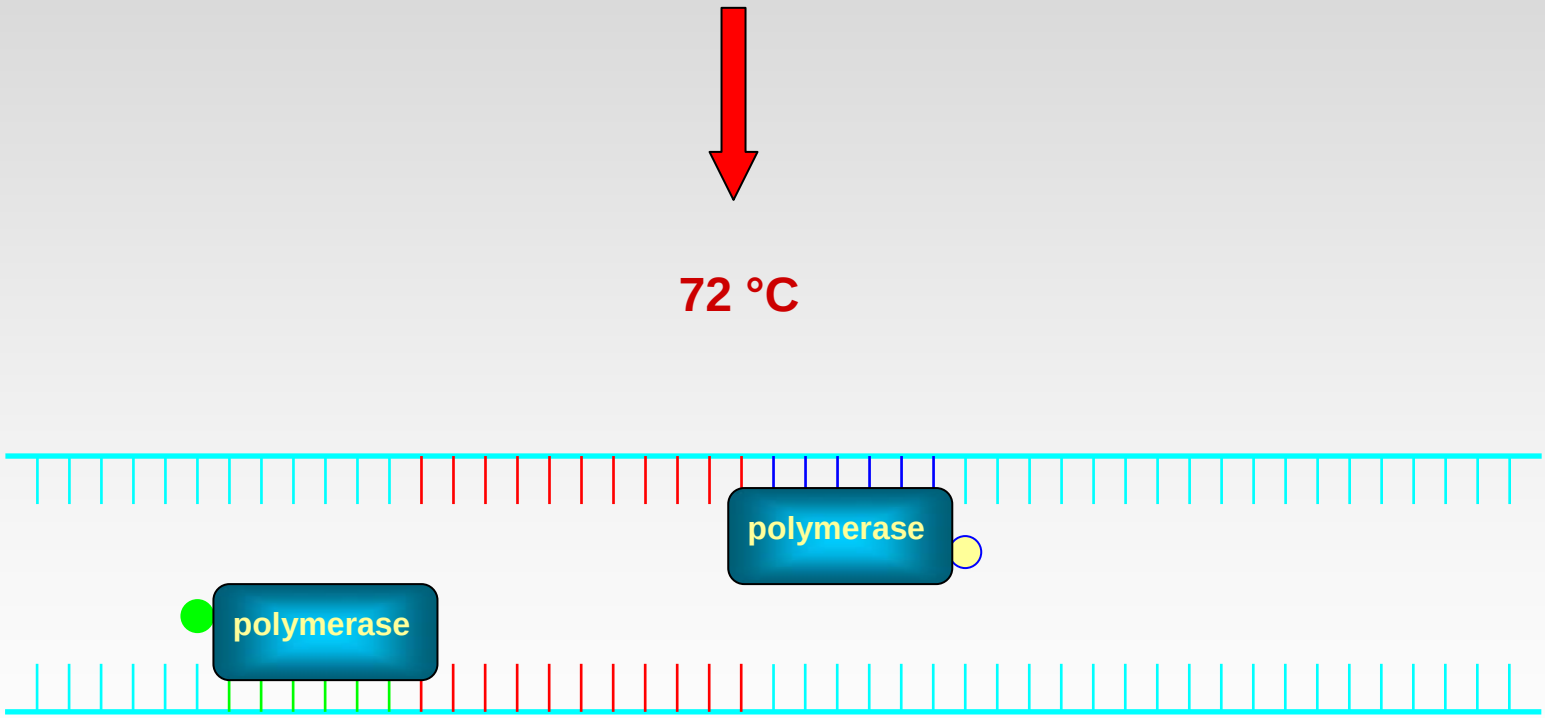
Reverse Transcriptase Polymerase Chain Reaction (PCR)

- STEP 2: annealing of primers



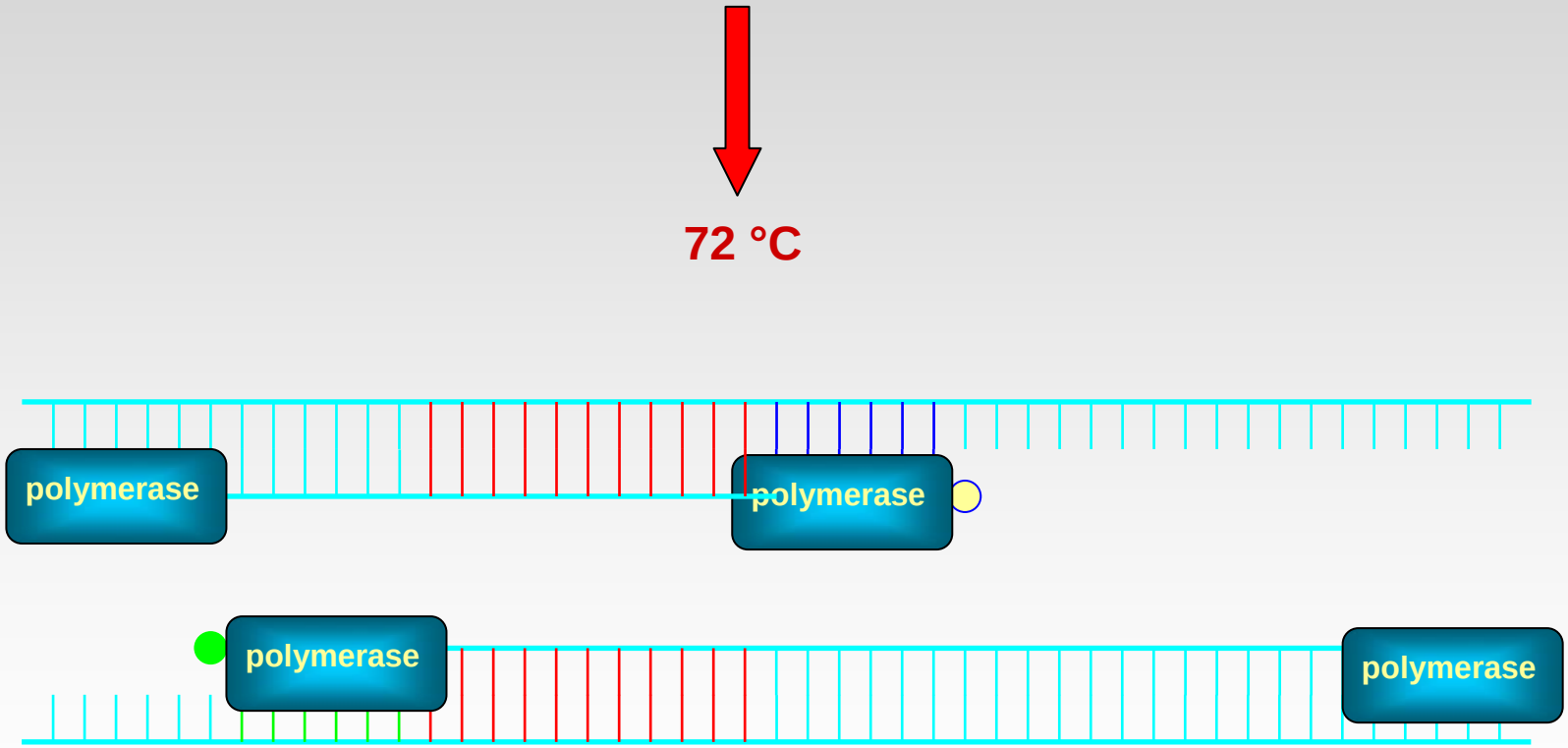
Reverse Transcriptase Polymerase Chain Reaction (PCR)

- STEP 3: action of the Taq polymerase



Reverse Transcriptase Polymerase Chain Reaction (PCR)

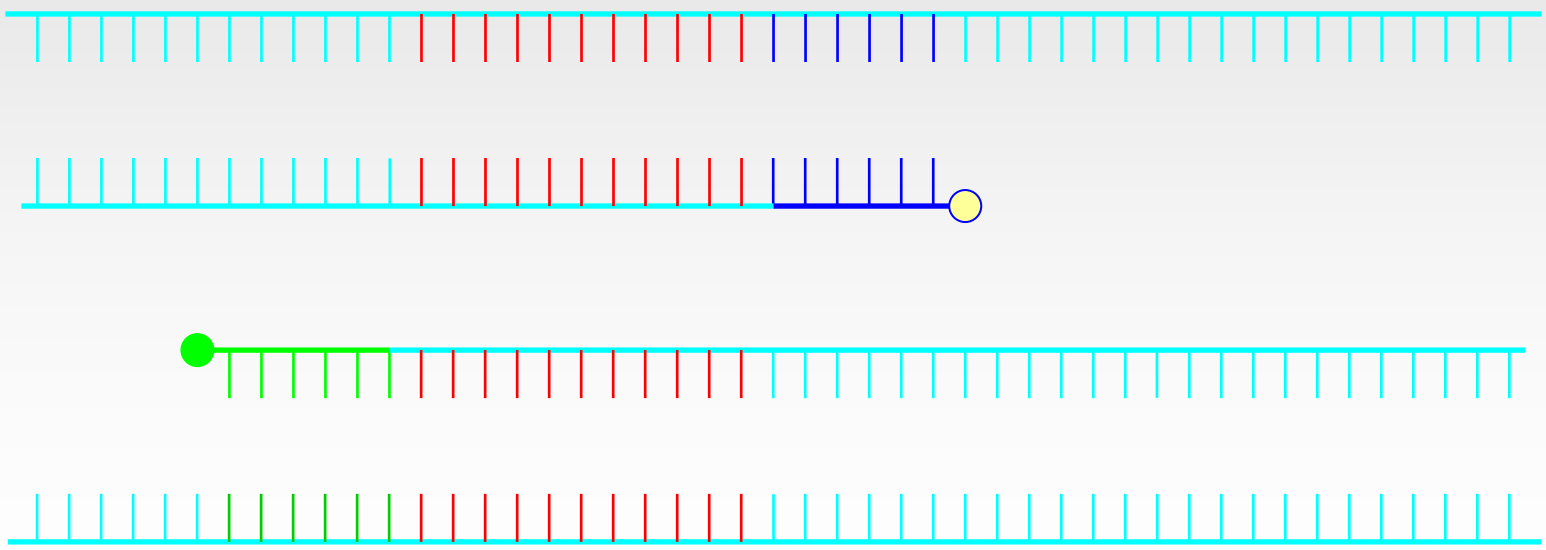
- STEP 4: synthesis (elongation)



Reverse Transcriptase Polymerase Chain Reaction (PCR)

- STEP 1
  Cycle 2: heating and denaturation

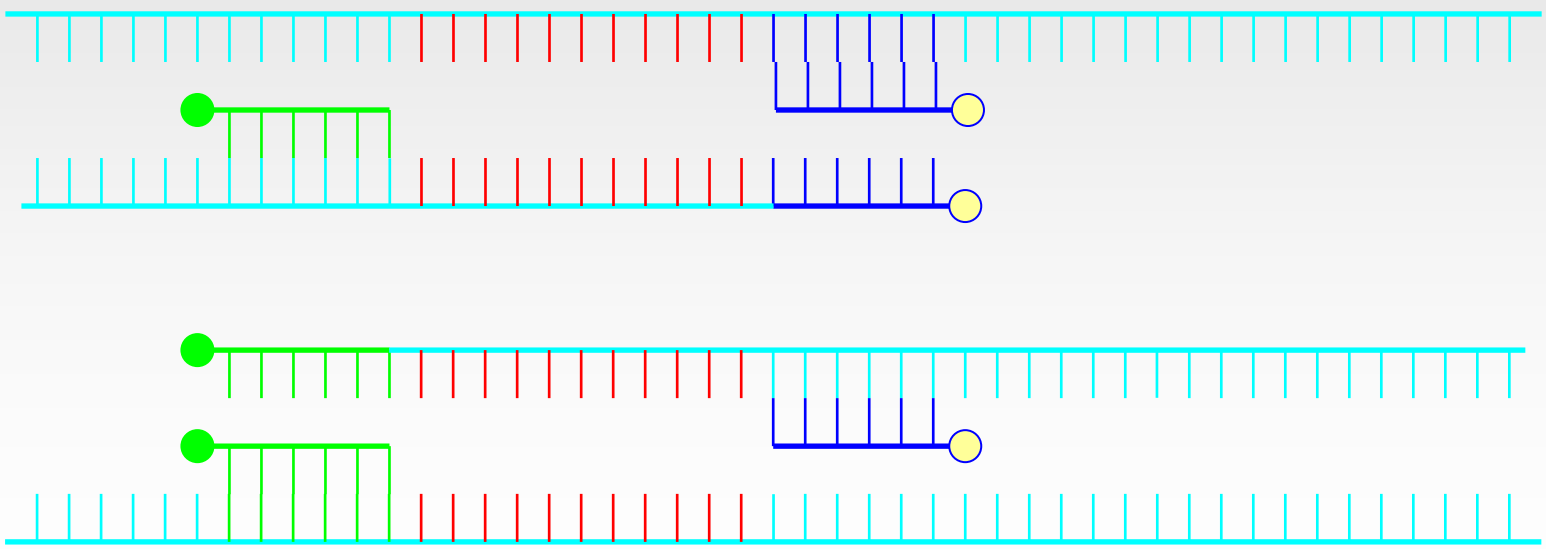
94-96 °C



Reverse Transcriptase Polymerase Chain Reaction (PCR)

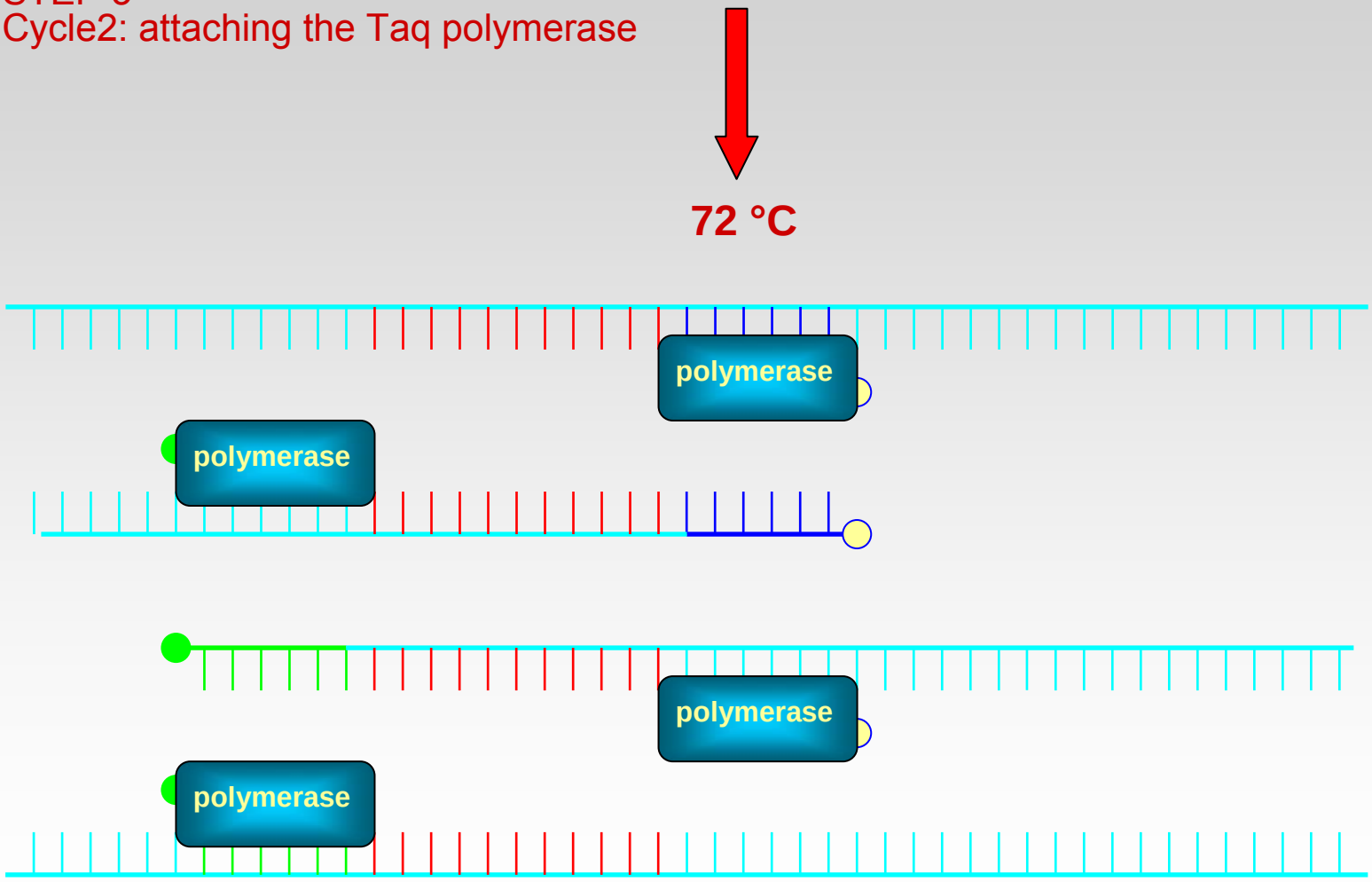
- STEP 2:
  Cylcle 2: annealing of primers

50-65 °C



Reverse Transcriptase Polymerase Chain Reaction (PCR)

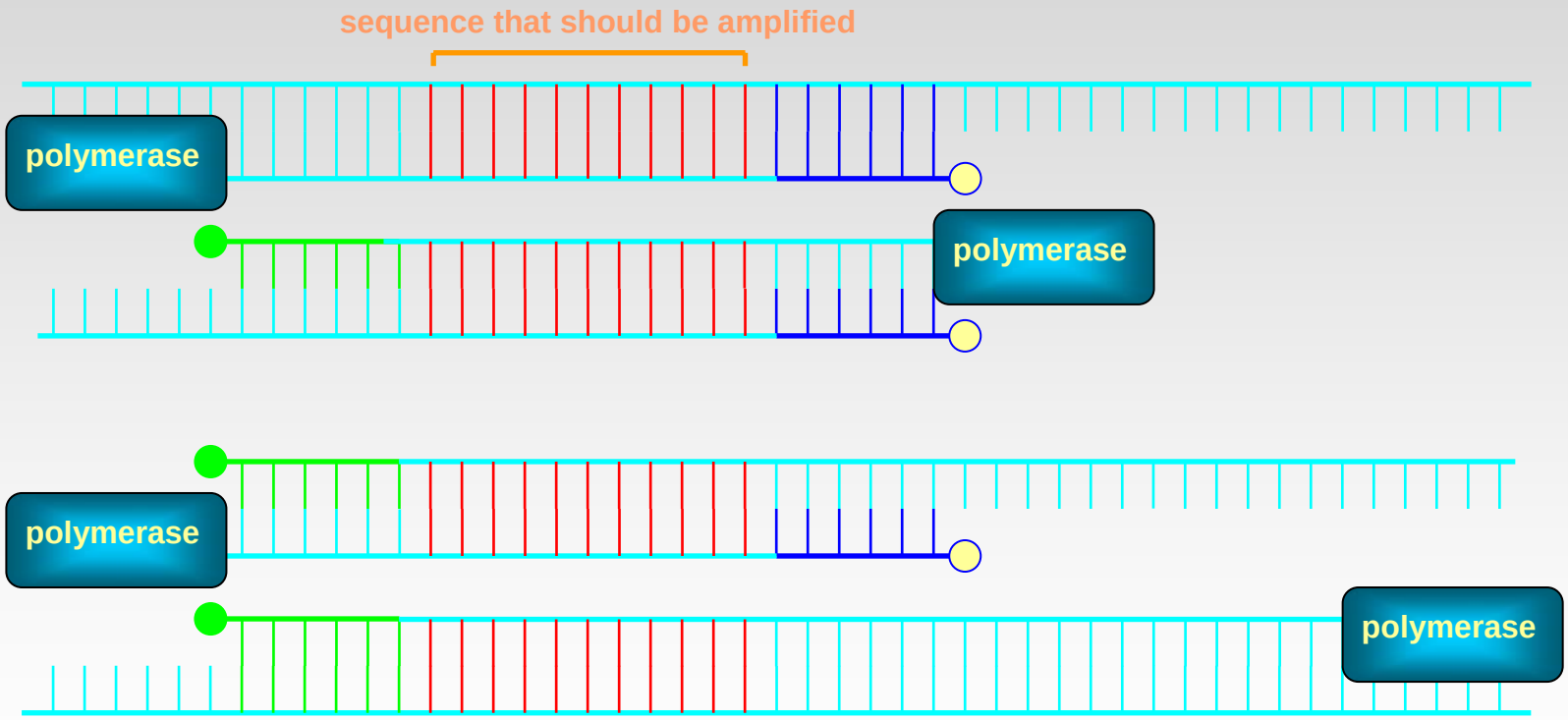
- STEP 3
  Cycle2: attaching the Taq polymerase



Reverse Transcriptase Polymerase Chain Reaction (PCR)

- STEP 4
  Cycle 2: synthesis

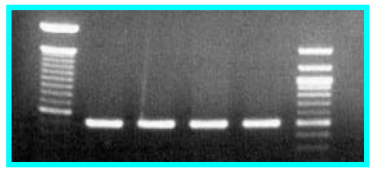
72 °C



after 35-45 cycles:

Ethidiumbromide-stained agarose gel

$2^{40} = 10^{12}$ amplification factor



Reverse Transcriptase Polymerase Chain Reaction (PCR)

- Problems of standard PCR: not exact quantification possible
- Amplification functions may have different shapes and they saturate

Quantitative methods:

a) Northern blot

b) Real-time PCR

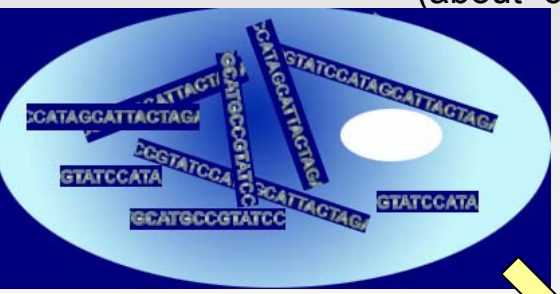
Quantitative measurement of RNA: Northern Blot

area ~ amount RNA

tissue

total RNA

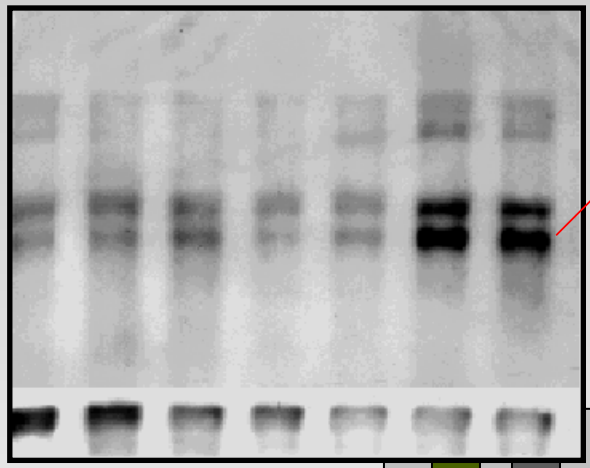
1-10 µg !
(about 6 pg/cell)



Large fragments

Small fragments

+



18S-rRNA

enzyme-linked
antibody against DIG:
amplification !

Digoxigenin

probe

different
treatments

blotting paper

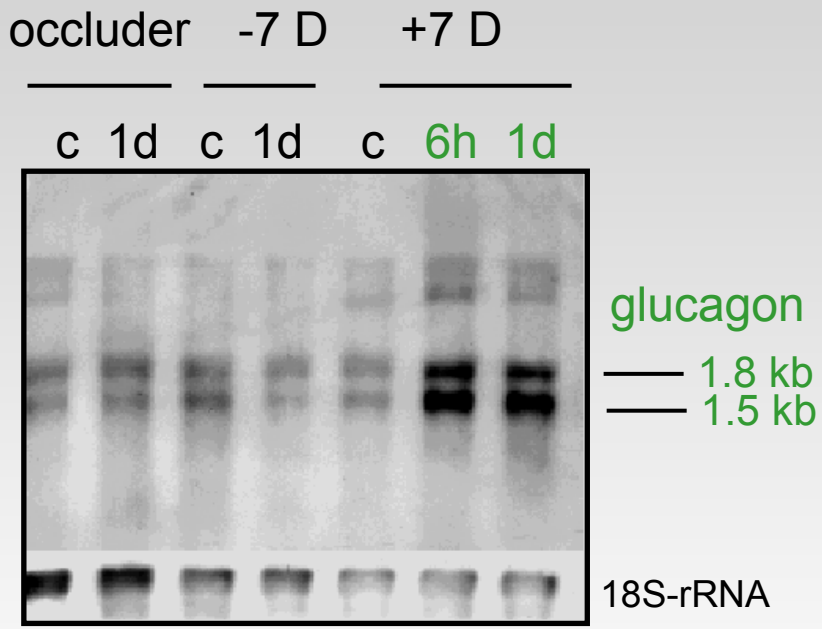
blotting membrane

gel

transfer solution

Northern Blot: examples of results

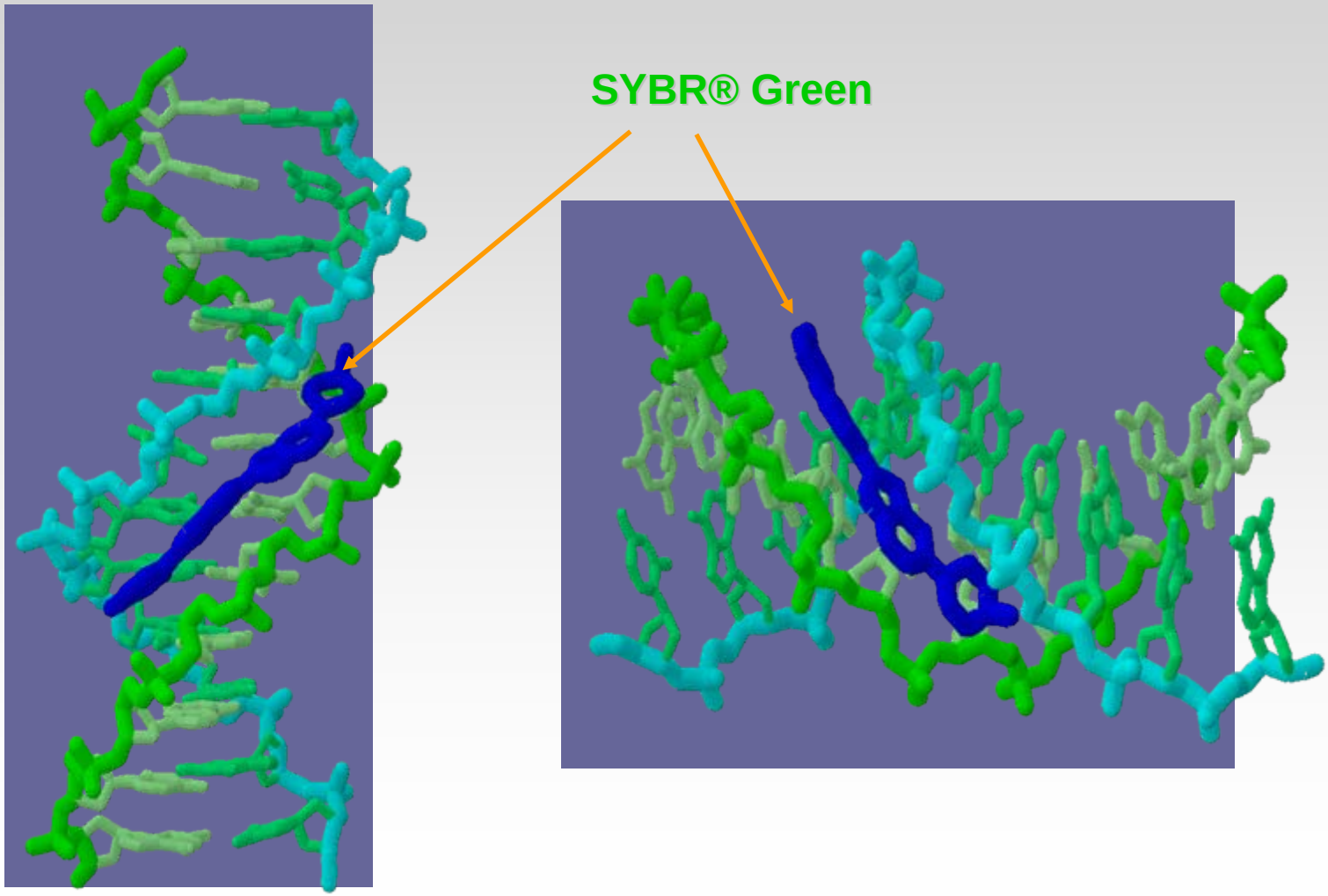
After 6 hours with positive lenses,
glucagon mRNA is increased
(Feldkaemper et al, IOVS 41, 1623-1628, 2002).



- Advantages:
  additional information about the size of the mRNA transcripts
- Disadvantages:
  need of high amount of RNA

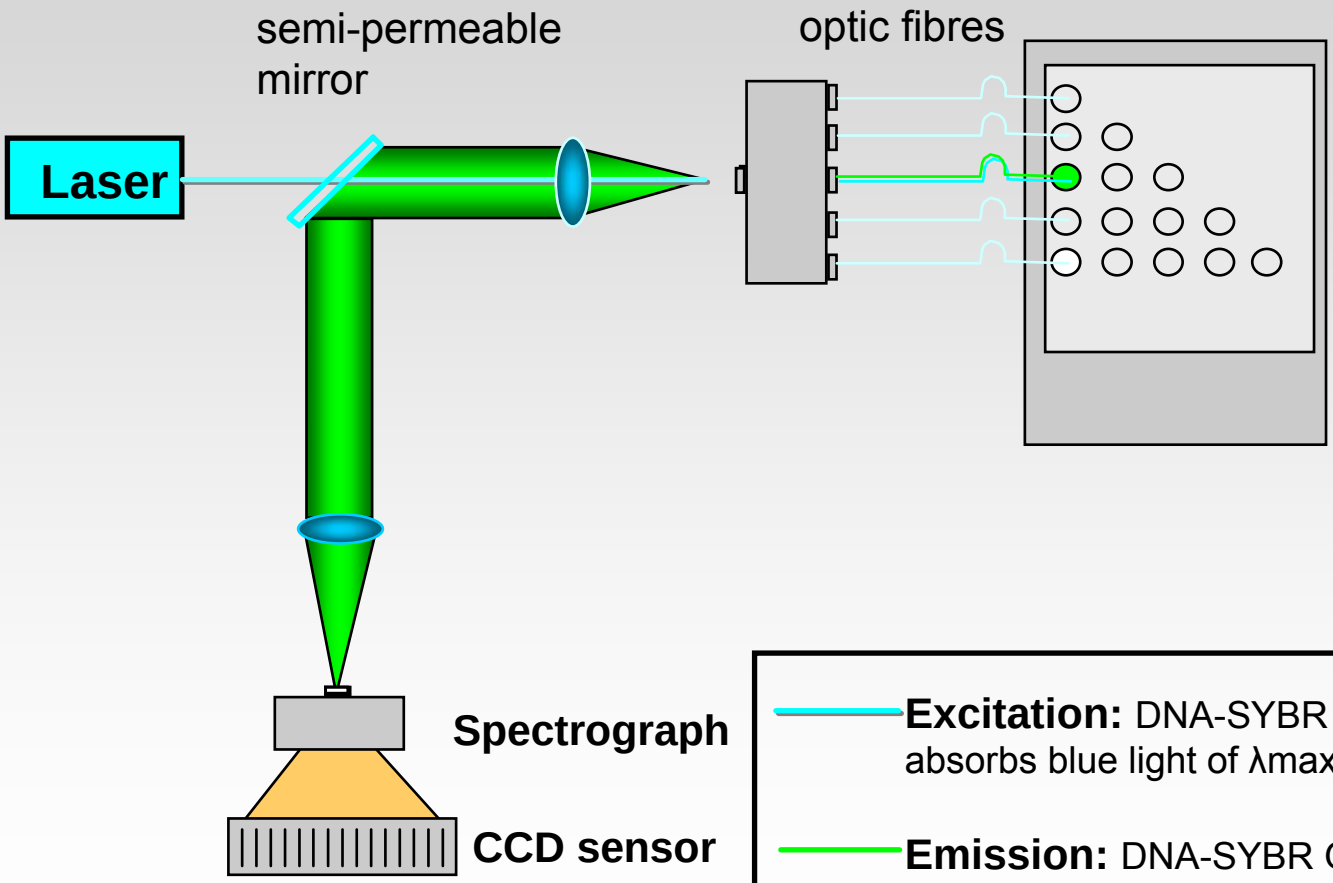
Real Time Polymerase Chain Reaction (real-time PCR)

- simplest method: SYBR® Green fluoresence ~ **double-stranded** DNA



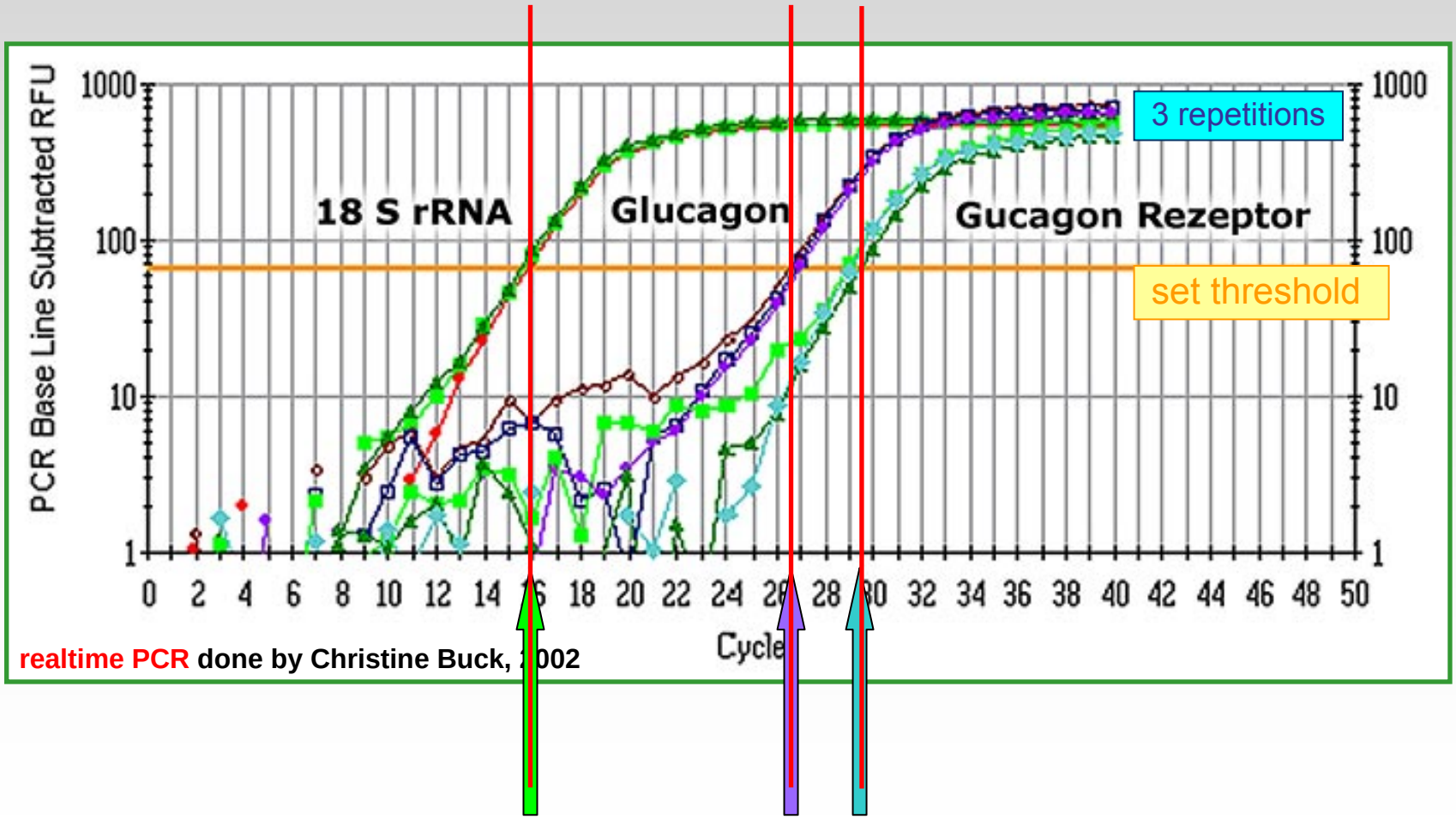
Real Time Polymerase Chain Reaction (real-time PCR)

- quantitative Real Time PCR: thermocycler with integrated photometer



Real time Polymerase Chain Reaction (real-time PCR)

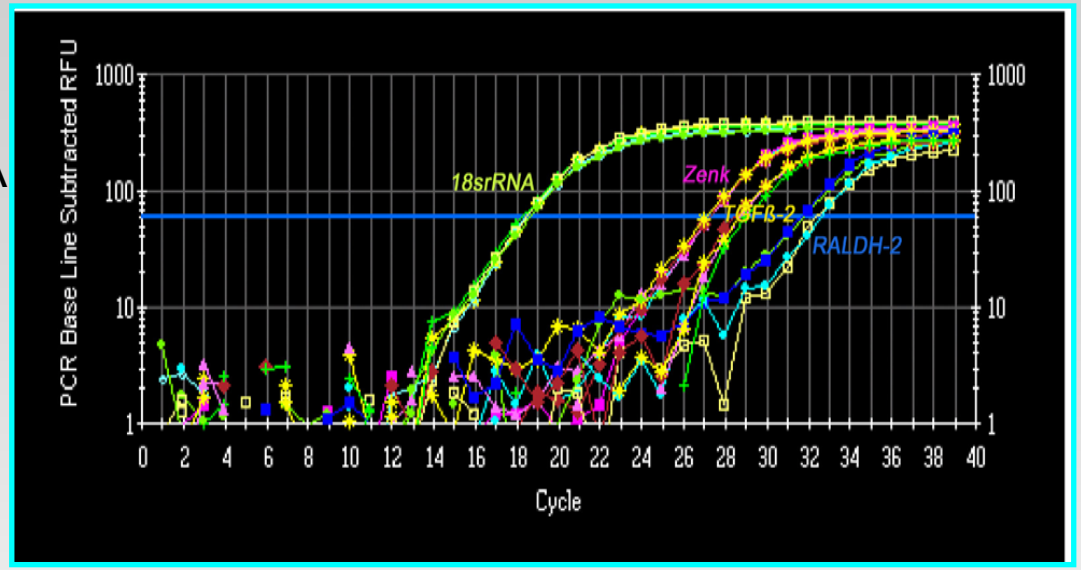
- "on-line" measurement of the increase in copies of the target sequence

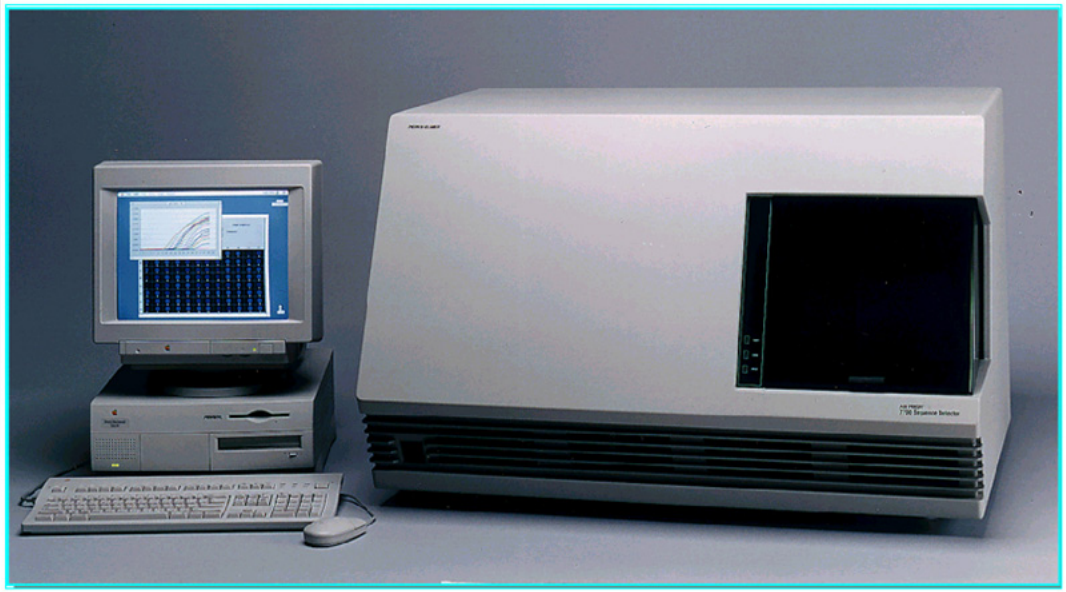


Real Time Polymerase Chain Reaction (real-time PCR)

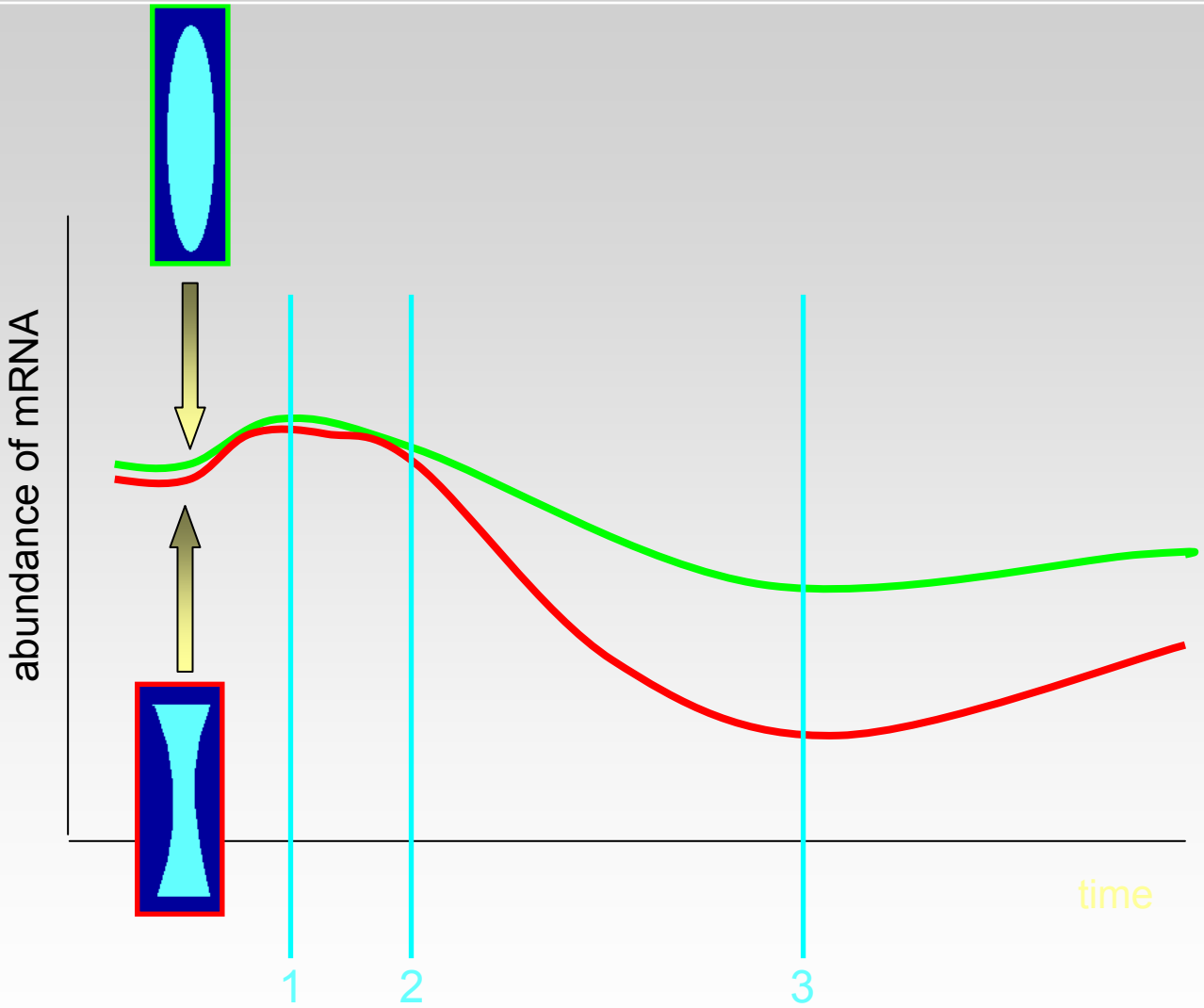
- Advantages
  only very low amount of RNA/cDNA is needed (1 ng)
  quick

- Disadvantages:
  relatively expensive





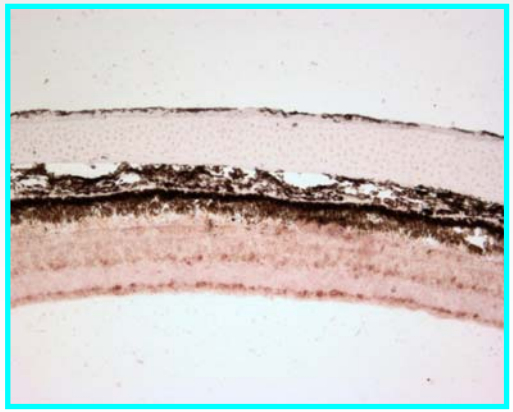
Time course problem



- compare effects of lenses at 1,2, 3

Localizing the transcript
in the tissue

Localisation of mRNA transcripts: In-situ hybridisation



Digoxigenin

RNA Probe

in vitro transcription

with Digoxigenin incorporation depending on primer for transcription

anti-sense sense

(2 primers and sequencing)

linearization with restriction endonucleases

cDNA

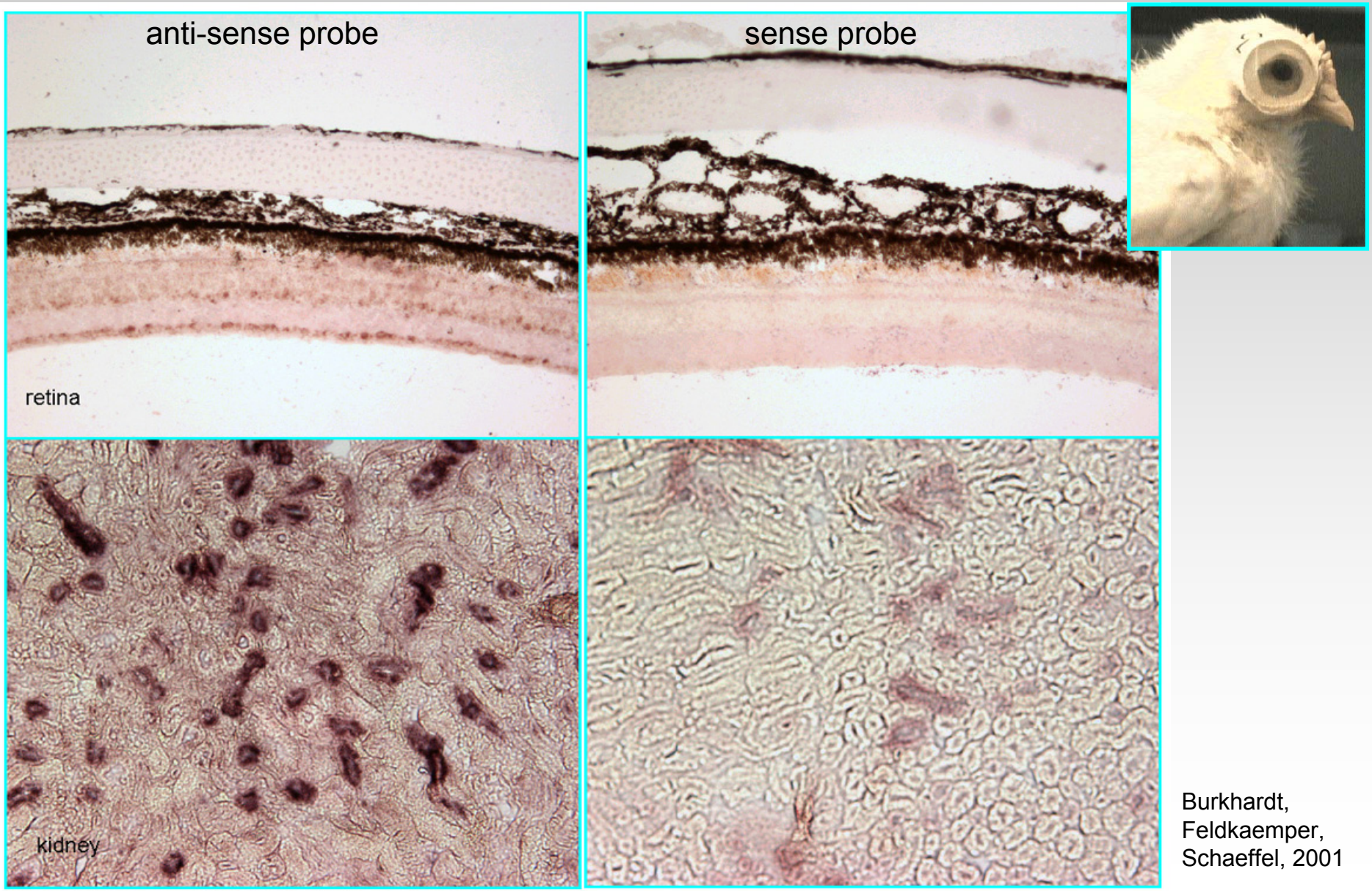
Plasmid

PCR with primers to amplify the target sequence (i.e. glucagon receptor)

enzyme-linked antibody against DIG: amplification !

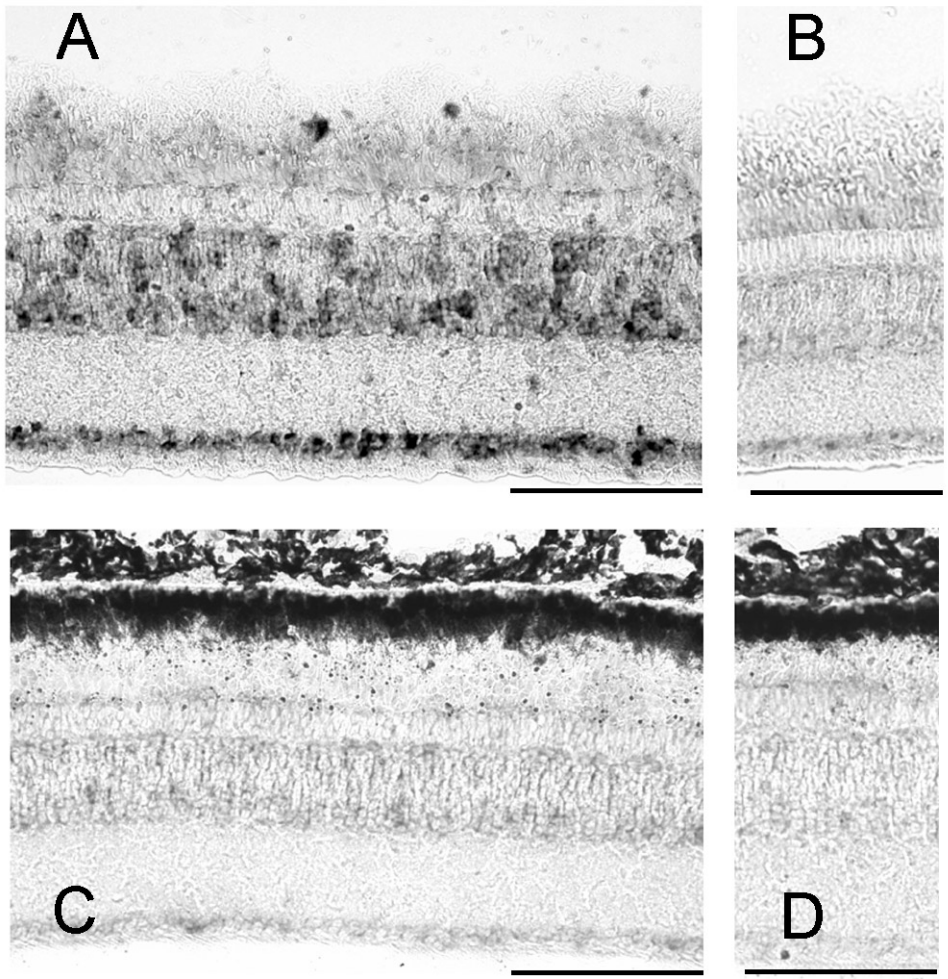
localization of target mRNA

In-situ hybridisation: the chicken glucagon receptor



Burkhardt,
Feldkaemper,
Schaeffel, 2001

In-situ hybridisation: the chicken glucagon receptor



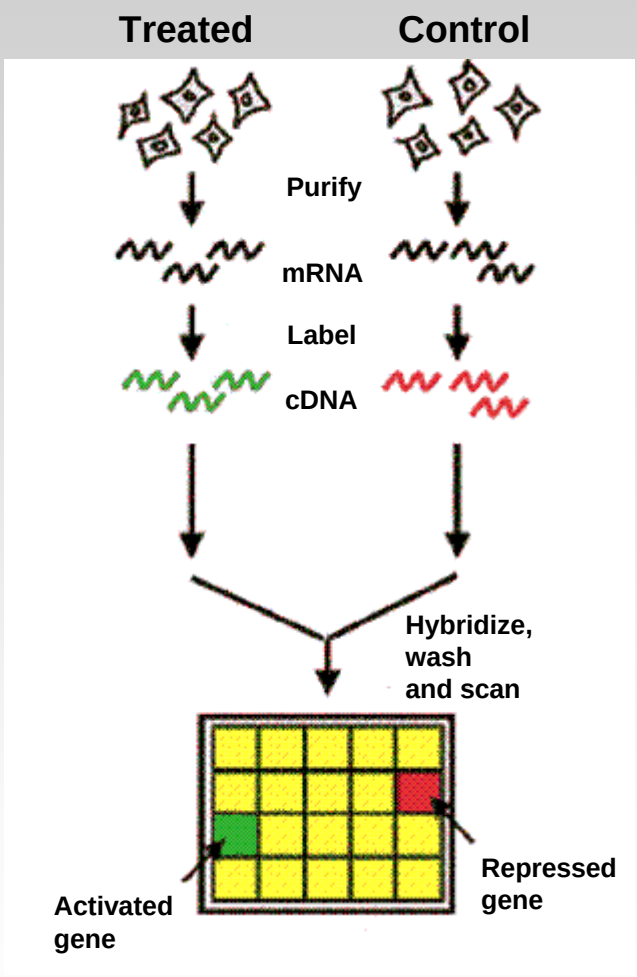
The scale bar represents 100 µm.

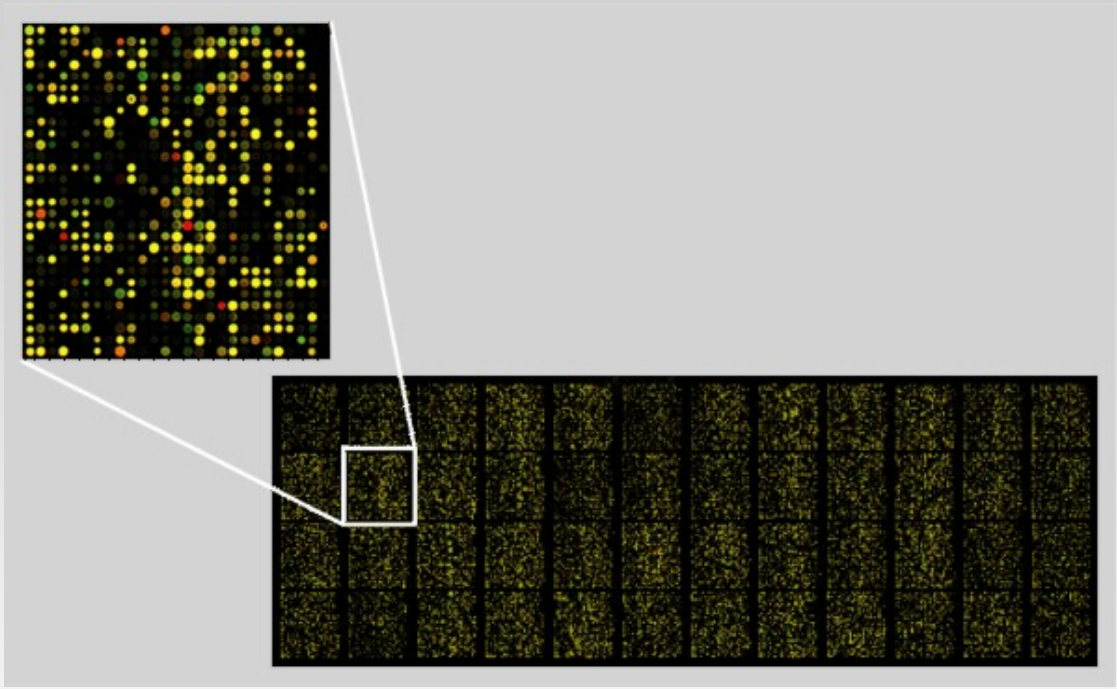
(A) Glucagon receptor expression after injection of water, antisense probe. (B) Same section as in (A) labelled with the sense probe.

(B) (C) Glucagon receptor expression after injection of a glucagon antagonist.
(D) Same section as in (C) labelled with the sense probe.

Searching for new genes
that are controlled by visual experience
(e.g. defocus) or hearing loss

Microarray Workflow

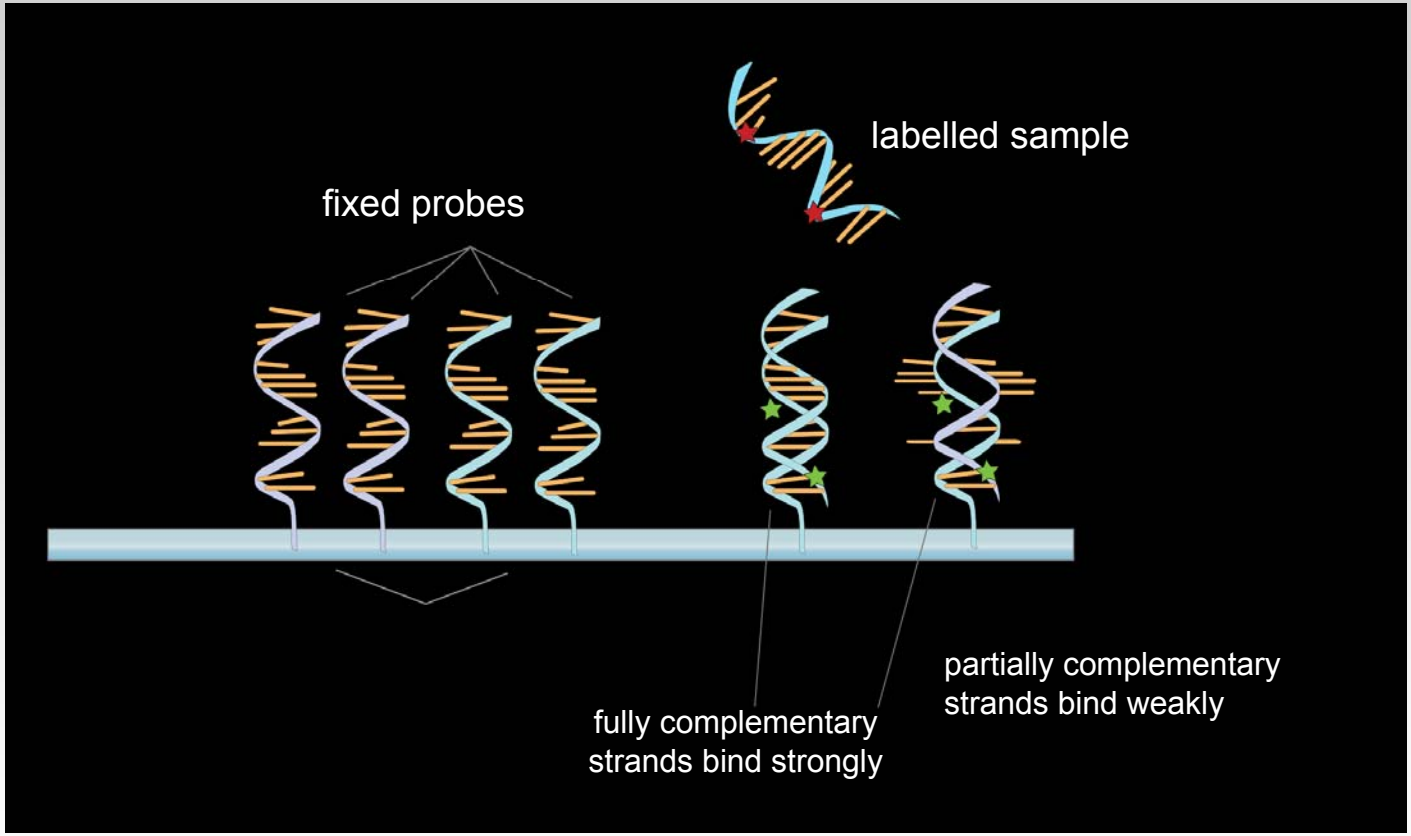




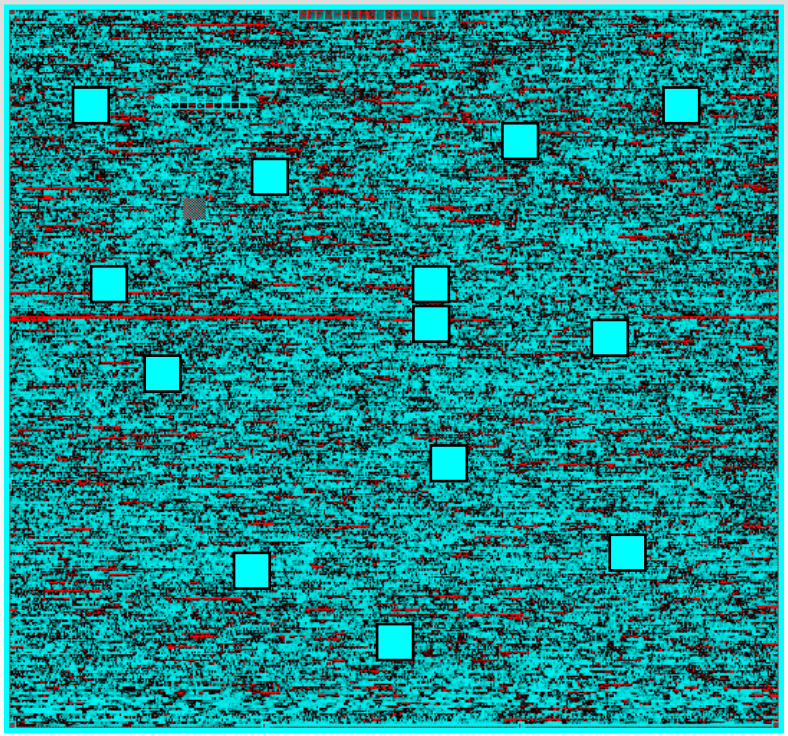
Core principle behind microarrays is hybridization between two DNA strands

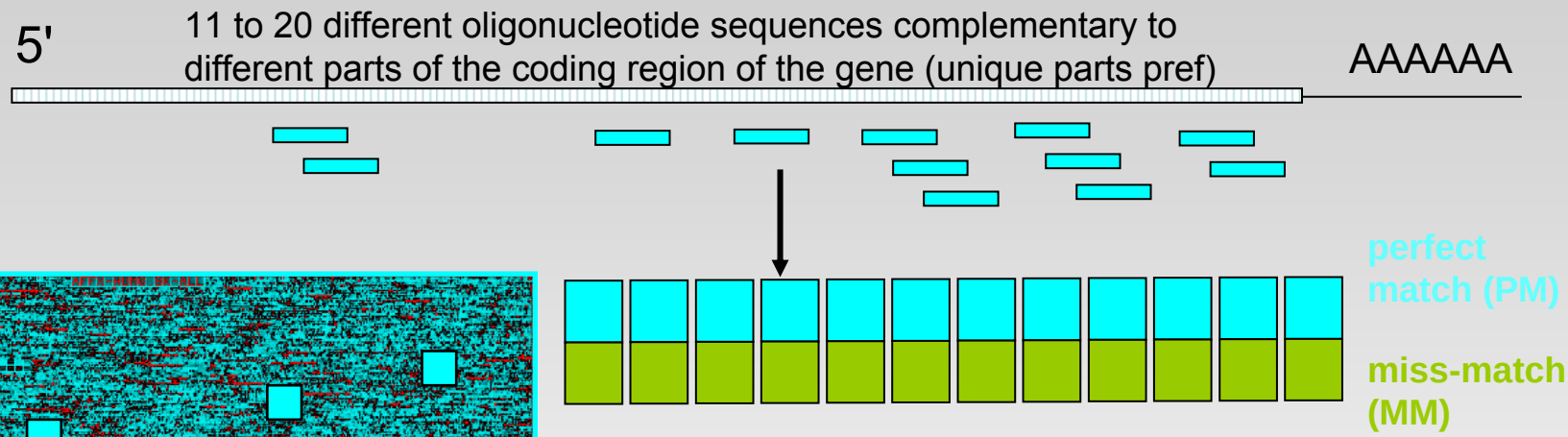
Outcome: Relative signal intensities for each transcript → Correlated to mRNA levels in the samples

Hybridization of the target to the probe



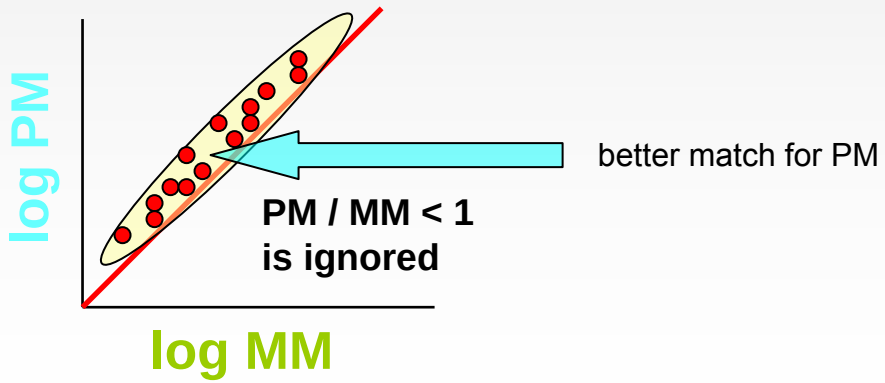
Microarray Technique (Affymetrix chips)



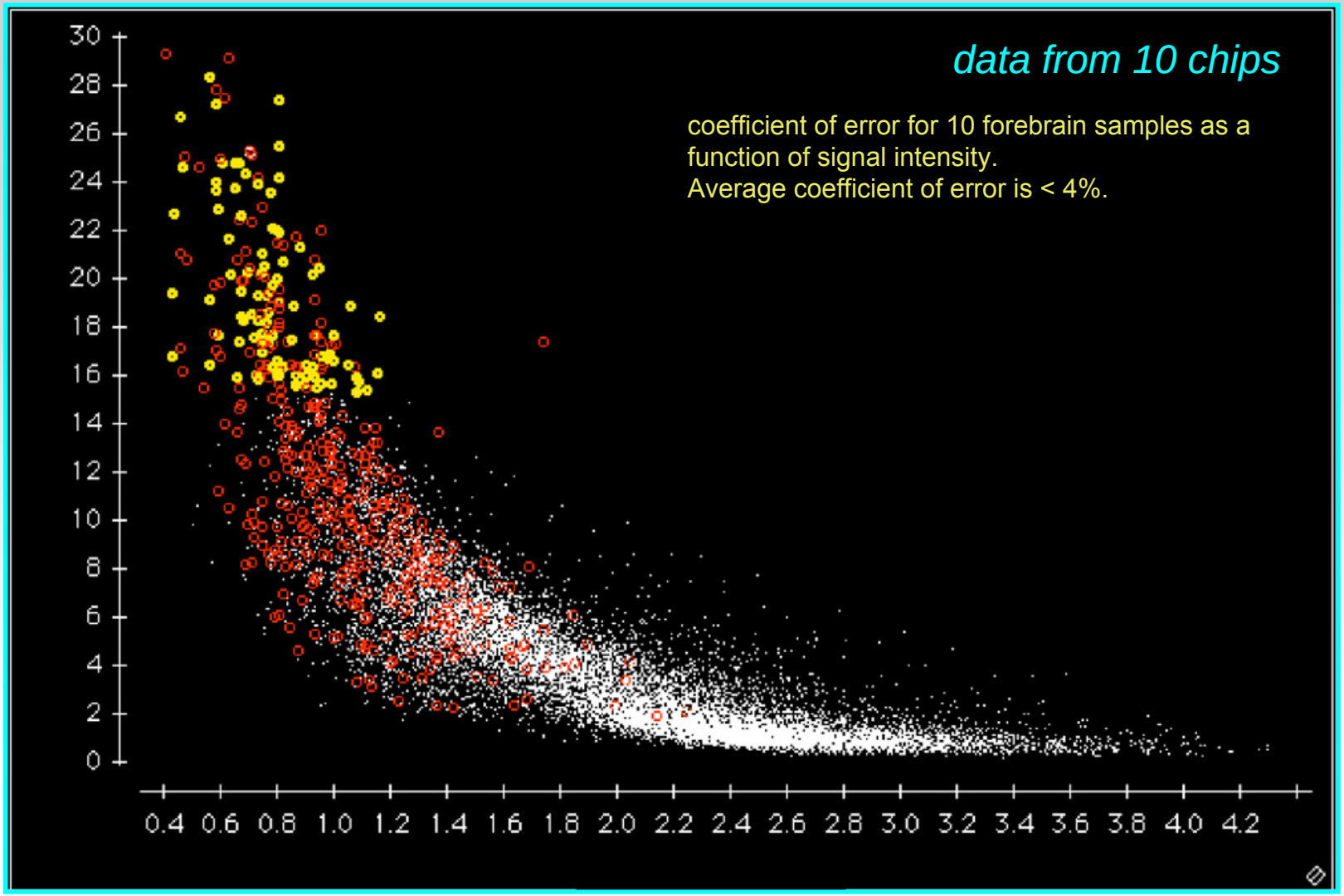


PM - 25 bases complementary to mRNA

MM - 13th base is AT or GC swap



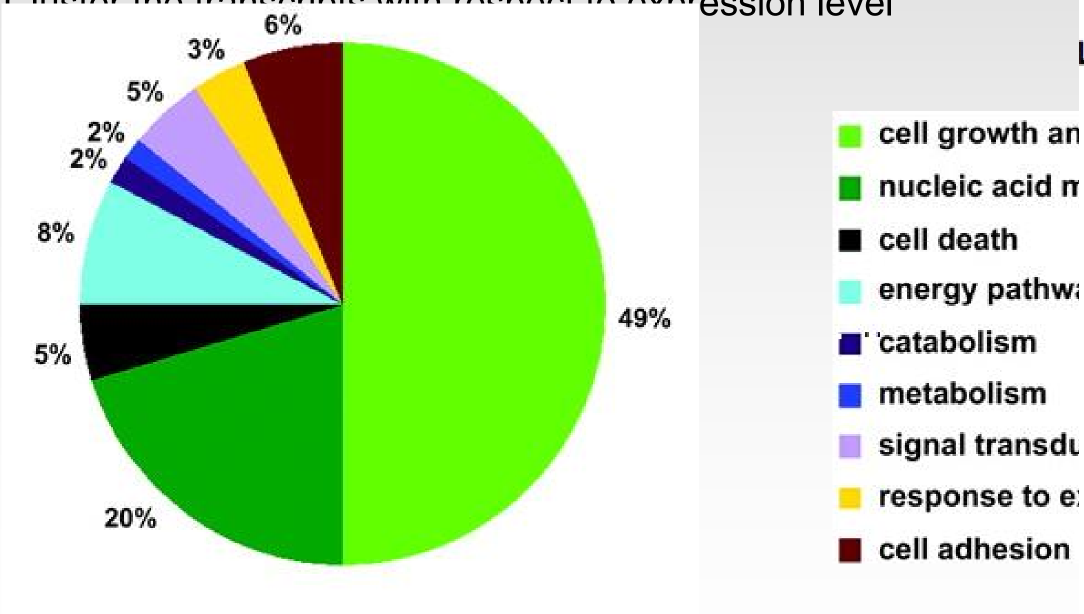
Microarray technique - increase in detection reliability with expression rate

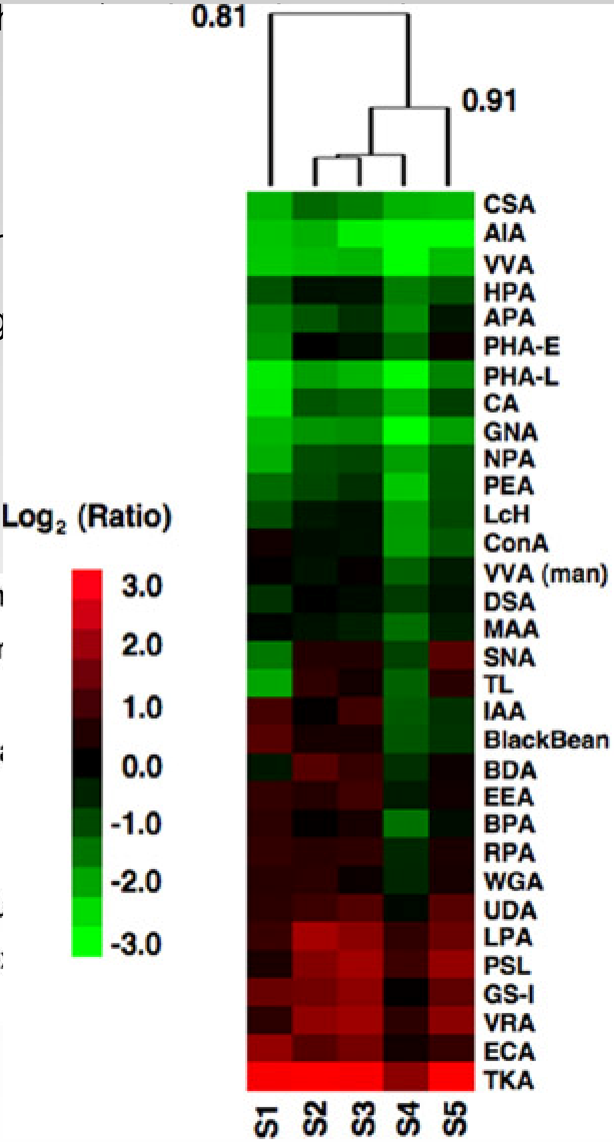


after Robert Williams, Memphis

Microarray Workflow

- Statistics: correction for multiple testing. Threshold-setting (fold-ch
  of transcripts
- Scaling/normalisation of the intensity
- Validation of selected transcripts (usually real-time PCR, Northern
- Cluster the transcripts with respect to function (e.g. Gene Ontolog
  function, cellular component)
- Cluster the transcripts with respect to expression level



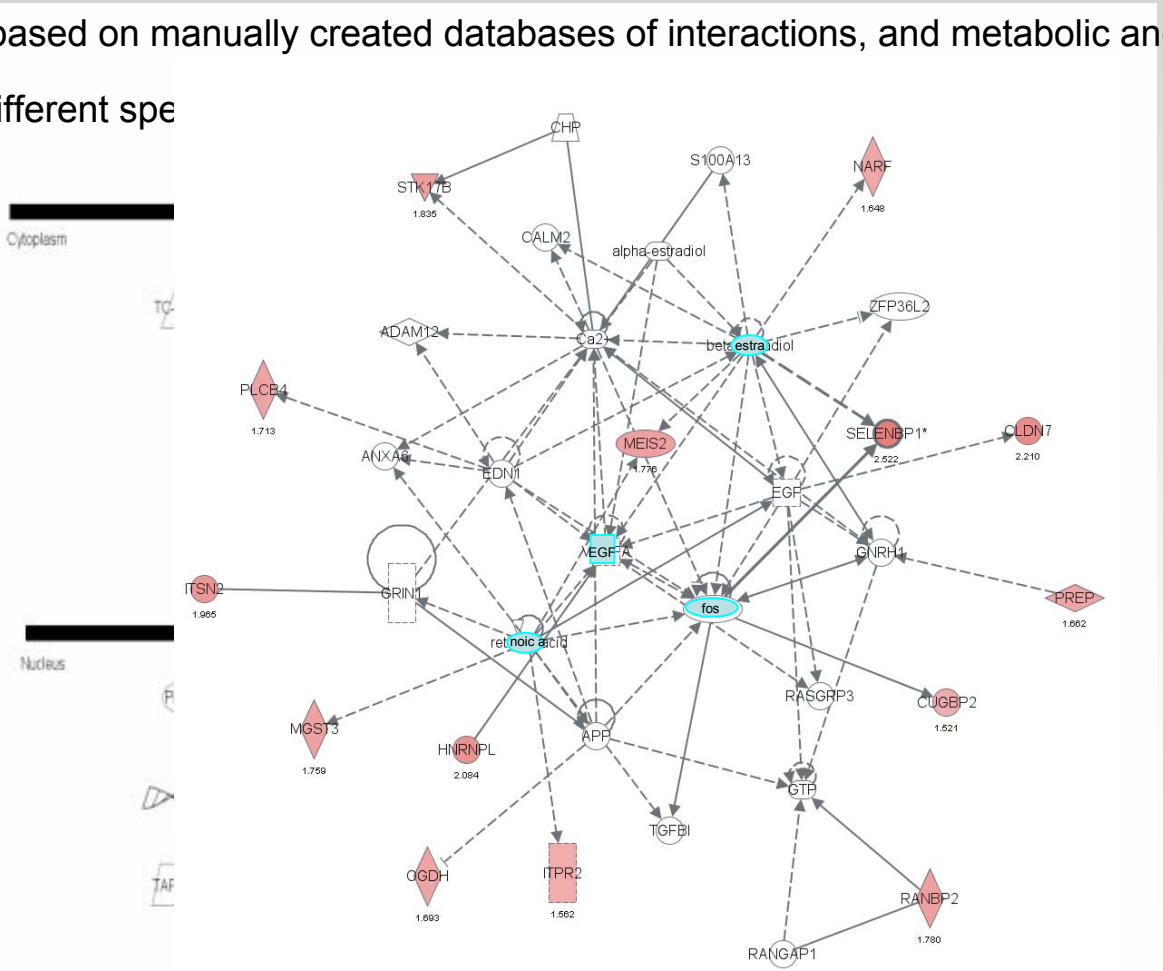


Figures from: Tkatchenko et al. PNAS 2006; 103: 12 / Schippel et al. *Mol.Vis.* 2009; 15

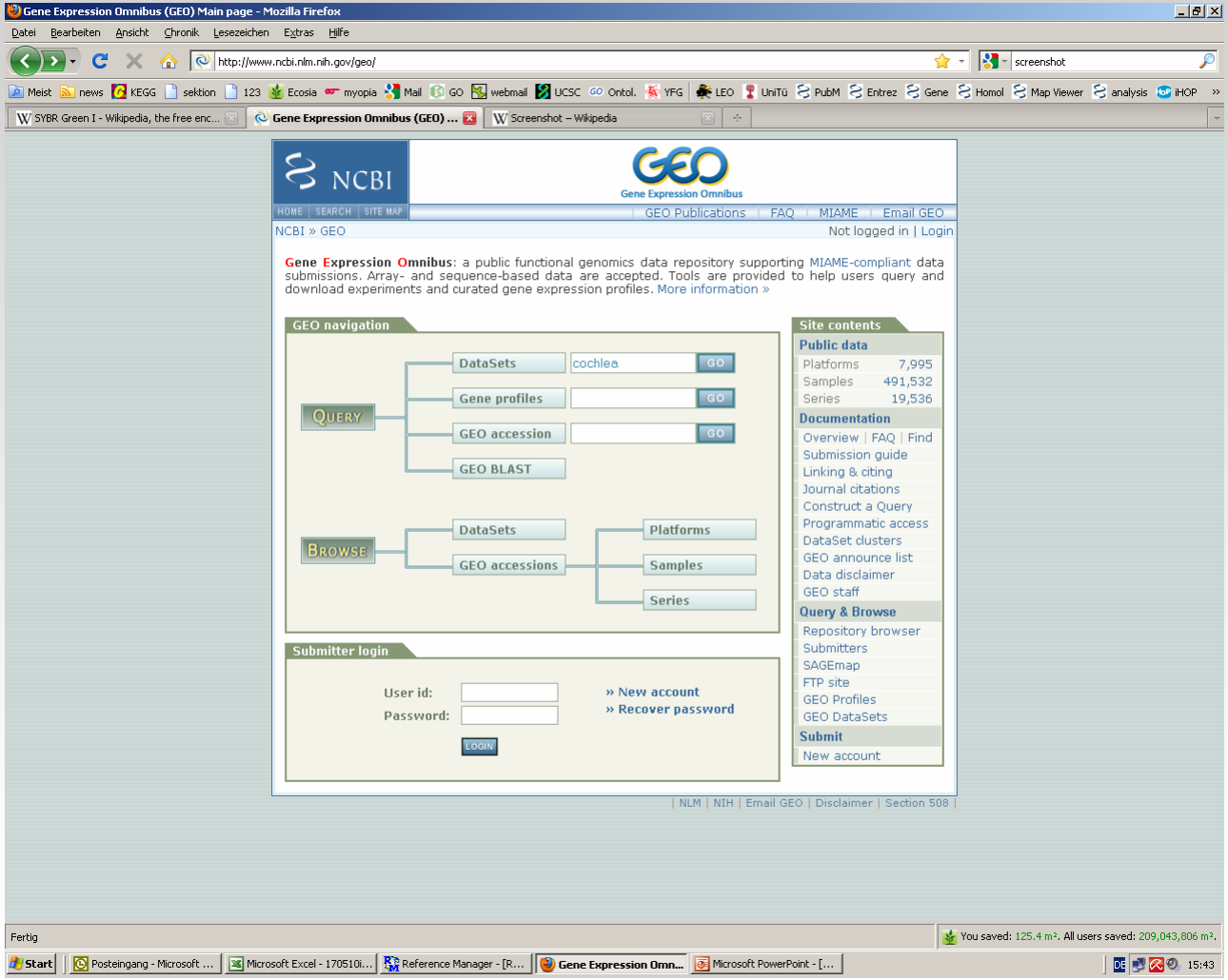
Microarray Workflow

• **Canonical pathway** analysis: e.g. interferon signalling, glutamate receptor signalling

• **Network analysis**: creating large and highly complex graphic representations of cellular and molecular processes, and the integration of a variety of biological data, including RNA expression. Information is based on manually created databases of interactions, and metabolic and signalling pathways for different spe



Databases



http://www.ncbi.nlm.nih.gov/geo/

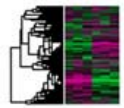
Gene expression omnibus

GSM109465: Cochlea_WT_9mo_5
GSM109466: Cochlea_WT_9mo_2
GSM109467: Cochlea_D257A_9mo_1

☐ **2:** GDS3028 record: **Age-related hearing loss model: cochlea** [*Mus musculus*] GEO Profiles, Links

Summary:	Analysis of cochleas of 7 and 36 week old DBA/2J animals. The DBA/2J animal suffers from age-related hearing loss (AHL), a progressive disease characterized by an age-associated loss of hair cells and spiral ganglion cells in the cochlea. Results provide insight into the molecular basis of AHL. Parent Platform: GPL339 Reference Series: GSE6045
Type:	Expression profiling by array, count
Subsets:	2 age, 2 disease state sets.
Supplementary Files:	CEL download...
Samples:	6

GSM140222: Cochlea DBA2J 7 weeks 1
GSM140223: Cochlea DBA2J 7 weeks 2
GSM140224: Cochlea DBA2J 7 weeks 3
GSM140225: Cochlea DBA2J 36 weeks 1
GSM140226: Cochlea DBA2J 36 weeks 2
GSM140227: Cochlea DBA2J 36 weeks 3

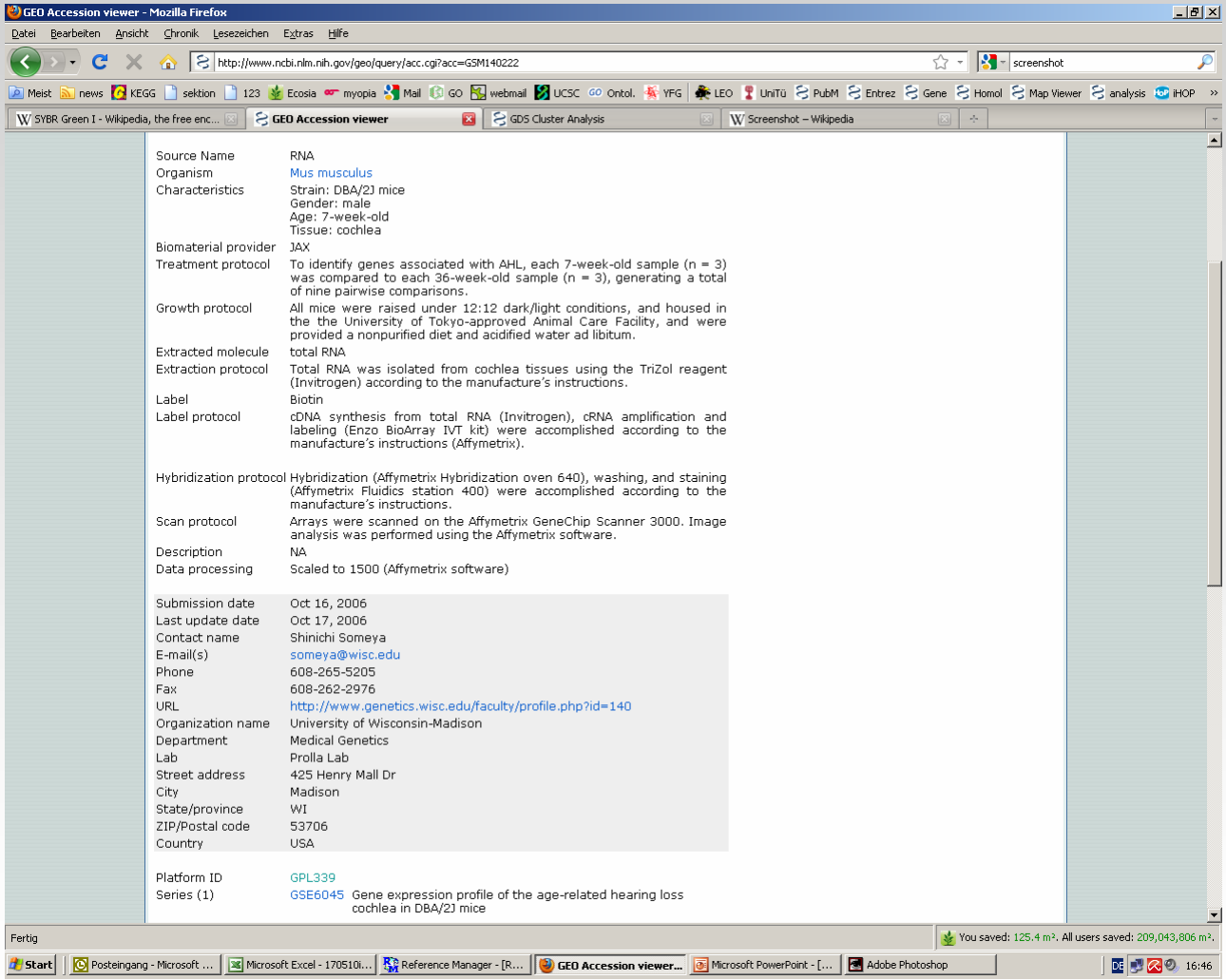


☐ **3:** GDS2681 record: **Caloric restriction effect on aged cochlea** [*Mus musculus*] GEO Profiles, Links

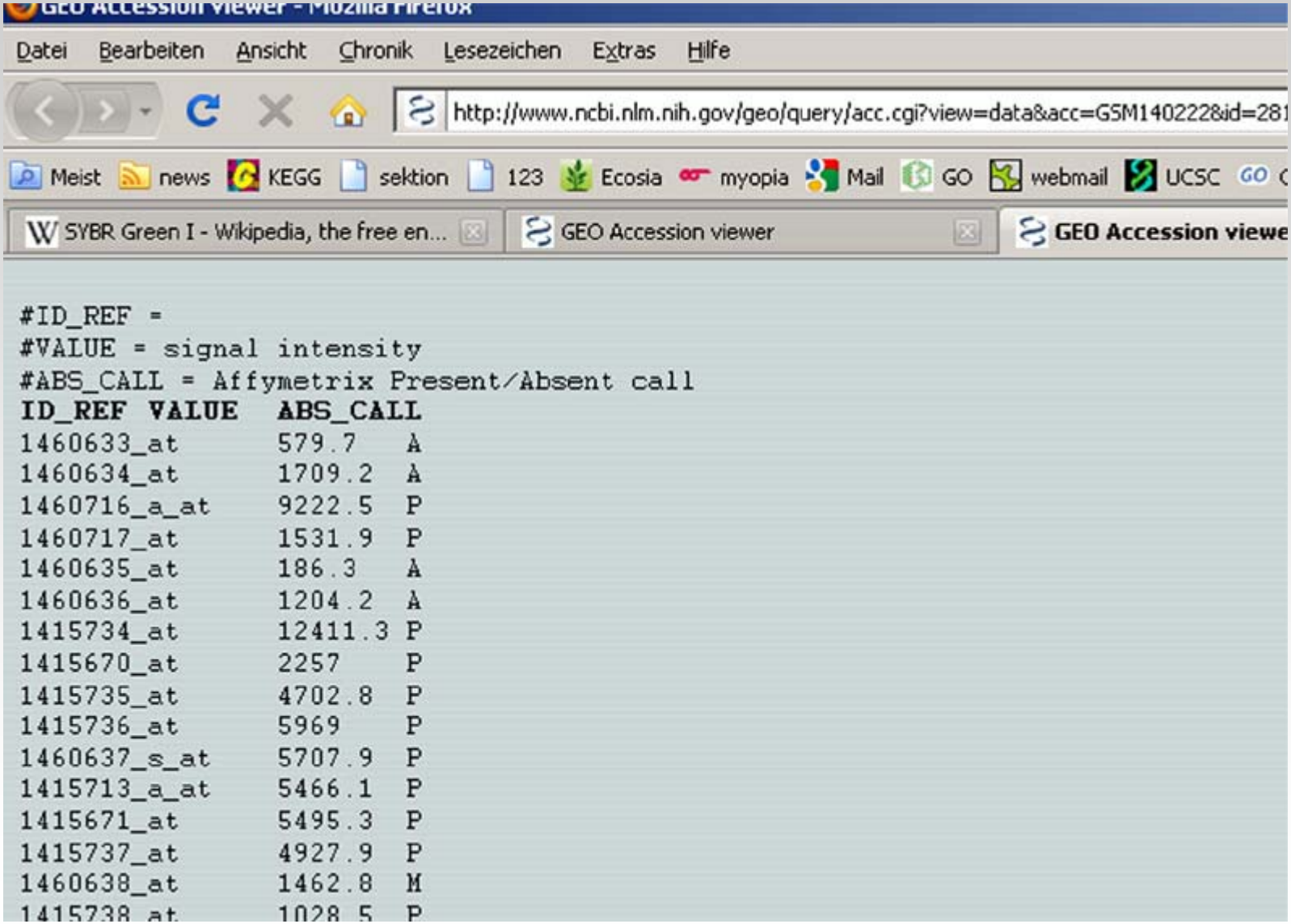
Summary:	Analysis of cochleas of 15 month old C57BL/6 (B6) animals on a calorie restricted diet. The B6 strain is a model of age-related hearing loss or presbycusis. Caloric restriction (CR) prevents late-onset presbycusis in B6. Results provide insight into the molecular basis of this effect of CR. Parent Platform: GPL1261 Reference Series: GSE4786
Type:	Expression profiling by array, count
Subsets:	2 age, 2 protocol sets.
Supplementary Files:	CEL download...
Samples:	9

GSM108106: Cochlea_YC_4mo_1

Gene expression omnibus



Gene expression omnibus



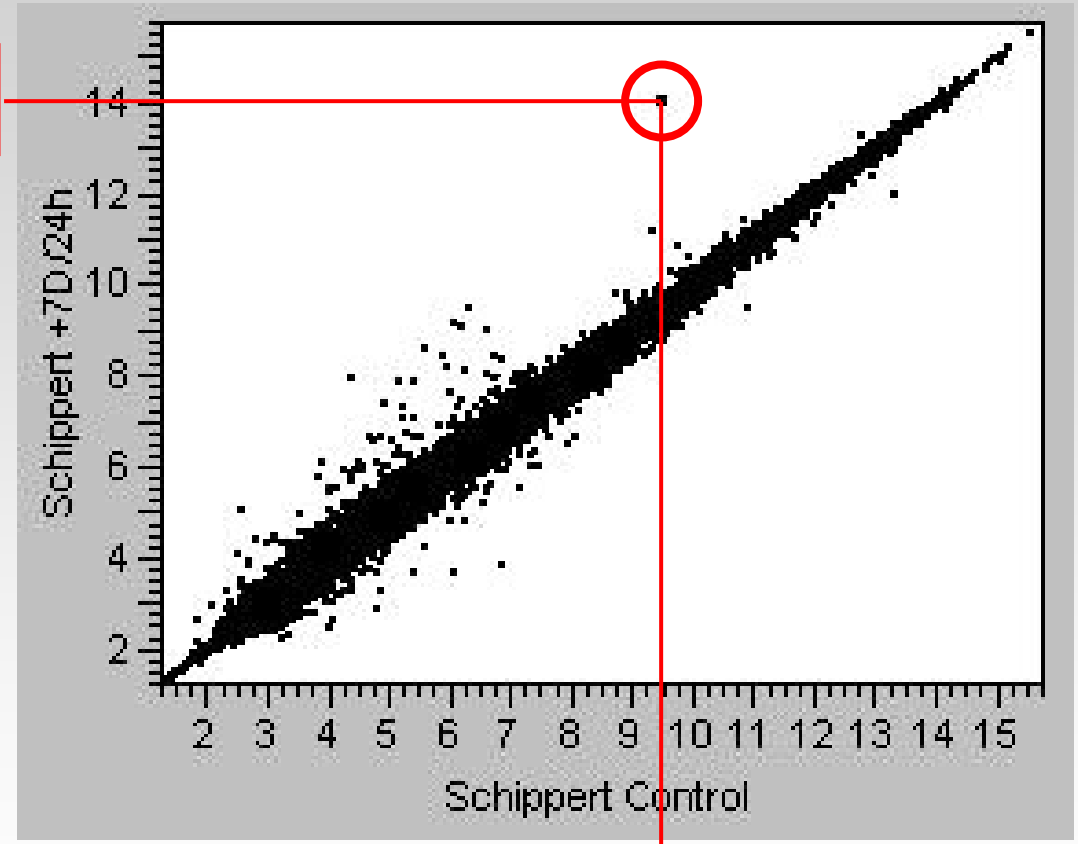
Recent Experiments: Myopia Research

Tissue	Animal	Treatment	Duration	Author
* Retina	Monkey	Lid-fusion	2-4 months	Tkatchenko et al. *PNAS* 2006; 103:12
* Retina	Mouse	FDM	30min / 4h / 1d	Brand et al. *Mol.Vis.* 2007; 13
* Retina	KO-mouse	Age-difference WT vs. HM	30d / 42d -	Schippert et al. *Mol.Vis.* 2009; 15
* Retina	Chick	LIH	1d	Schippert et al. *Mol.Vis.* 2008; 14
Retinal AC	Chick	LIM and LIH	1d	Ashby et al. *IOVS* 2010; 51:7
* R/RPE	Chick	FDM	6h / 3d	McGlinn et al. *IOVS* 2007; 48:8
R/RPE	Chick	LIM and LIH	6h / 3d	R. Stone yesterday
* R/RPE/Ch	Chick	Recovery	1d / 4d	Rada et al. *Mol.Vis.* 2009; 15
R/RPE/Ch	Chick	Recovery	0h / 6h / 24h	Giummarra et al. ARVO 2010
* RPE/Ch	Marmoset	LIM vs. LIH	92d	Shelton et al. *Mol.Vis.* 2008; 14
RPE	Albino Chick	WT vs. HM	-	Rymer et al. *Exp.Eye Res.* 2007; 85
RPE	Chick	LIM	38d	Zhang et al. ARVO 2010
Sclera	Mouse	Age-difference	21d / 56d	Zhou et al. *IOVS* 2006; 47:5
Sclera	Human	no Treatment	-	Young et al. *Mol.Vis.* 2004; 10
Scleral FB	Human	Mechanical Stretching	30min / 24h	Cui et al. *Exp.Eye Res.* 2004; 78

* Reviewed in “Gene profiling in experimental models of eye growth: Clues to myopia pathogenesis”. Stone et al., *Vis.Res.* 2010

Expression pattern comparison

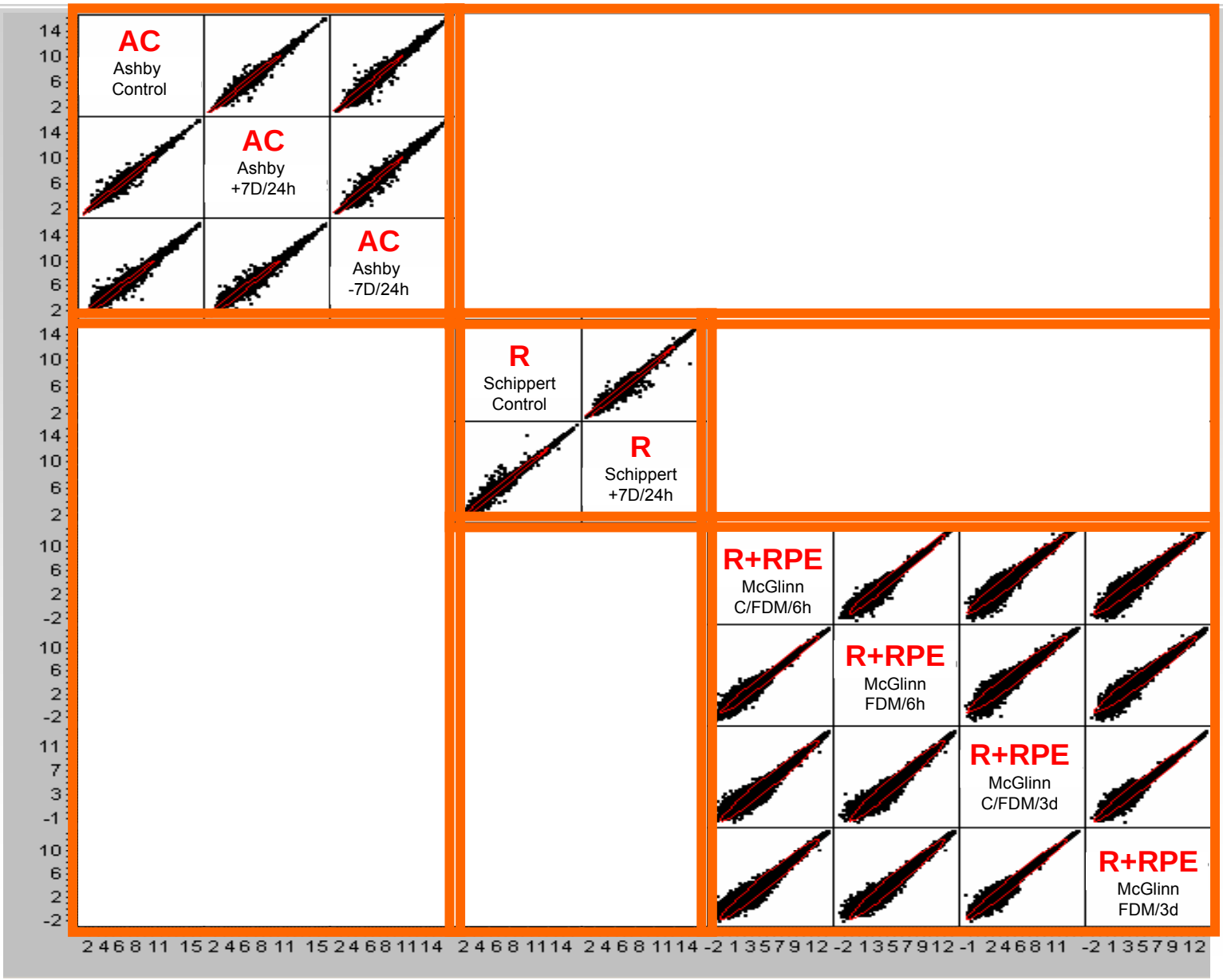
LOG_2 of the mean relative signal intensities



Would mean that the signal intensity of this mRNA D-dopachrome tautomerase (enzyme involved in melanin synthesis) is 23-times higher in the retina of treated animals compared to control animals.

Expression pattern comparison

LOG of the mean relative signal intensities



Data replotted from Ashby et al. *IOVS* 2010, Schippert et al. *Mol.Vis.* 2008 and McGlinn et al. *IOVS* 2007

Overlap between studies

Little overlap between studies so far can be explained by:

- Tissue type

  Amacrine cells / Retina / Retina+RPE / Retina+RPE+Choroid / RPE / RPE+Choroid / Sclera

- Methods to induce eye growth / arrest eye growth

  Lid suture / Deprivation myopia / Lens induced myopia / Lens induced hyperopia / Recovery / genetically modified animals

- Species

- Data analysis (normalisation, threshold-settings…)

Recent Experiments, Outcomes

Genes identified by lens or deprivation experiments:

"Usual suspects"

- VIP (Tkatchenko et al. *PNAS* 2006, McGlinn et al. *IOVS* 2007, Ashby et al. *IOVS* 2010)
- ZENK or Egr-1 (Brand et al. *Mol.Vis.* 2007, Schippert et al. *Mol.Vis.* 2008, Ashby et al. *IOVS* 2010)
- Ovotransferrin (Rada et al. *Mol.Vis.* 2009)
- Pre-pro glucagon (Schippert et al. *Mol.Vis.* 2008, Ashby et al. *IOVS* 2010)
- FGF2 (Shelton et al. *Mol.Vis.* 2008)

New candidate genes

- Protein kinase Akt-2 (Brand et al. *Mol.Vis.* 2007)
- Oxoglutarate dehydrogenase (Schippert et al. *Mol.Vis.* 2009)
- Angiopoietin 2 (Ashby et al. *IOVS* 2010)
- Avian thymic hormone (Rada et al. *Mol.Vis.* 2009)
- Noggin (Zhang et al. ARVO 2010)
- Bone morphogenetic protein-2 and -7 (Cui et al. *Exp.Eye Res.* 2004, McGlinn et al. *IOVS* 2007, Zhang et al. ARVO 2010)
- Prepro-urotensin II-related peptide (McGlinn et al. *IOVS* 2007, Schippert et al. *Mol.Vis.* 2008)

Recent Experiments, outcomes

Unexpected relationships:

- Hint that RPE/choroid in LIH shares similarities to hypoxia-induced pathways
  (Shelton et al. *Mol.Vis.* 2008)
- Different pathways involved in LIM/LIH and in initiation / sustained eye growth in R/RPE
  (R.Stone, talk IMC 2010)

Recent Experiments: cochlea after noise trauma

Control versus noise-damaged cochleae: (Margaret Lomax et al. (2001). Noise & Health 3 :19-35.)

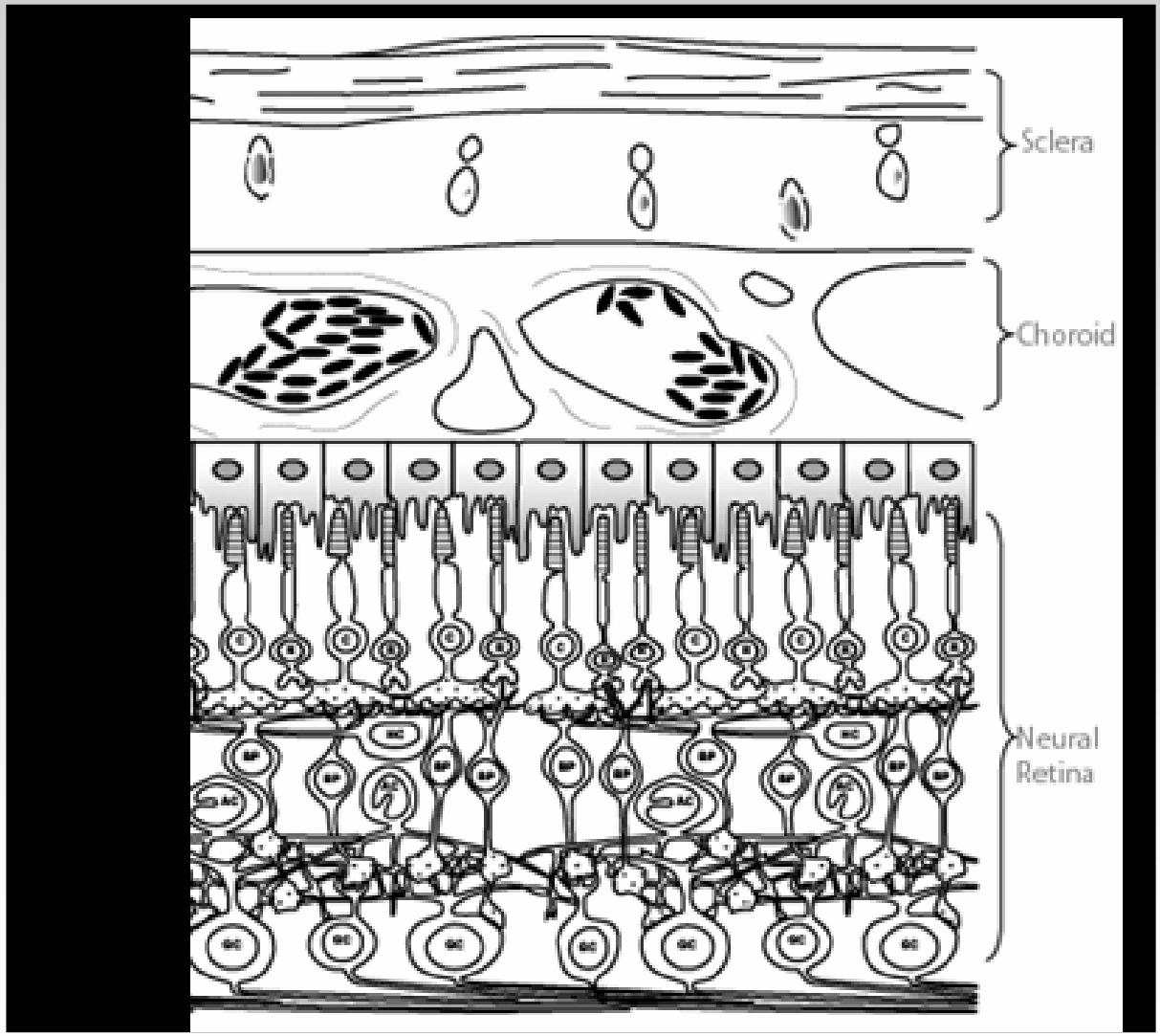
Early growth response genes:

egr-1

NGF-inducible anti-proliferative protein

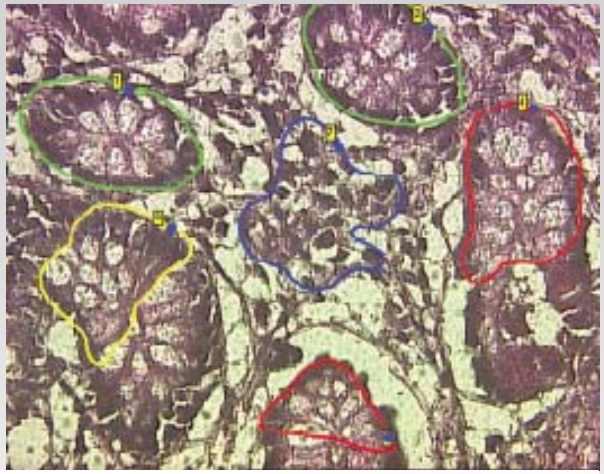
c-fos

Problem: Retinal Cell Population is Heterogeneous



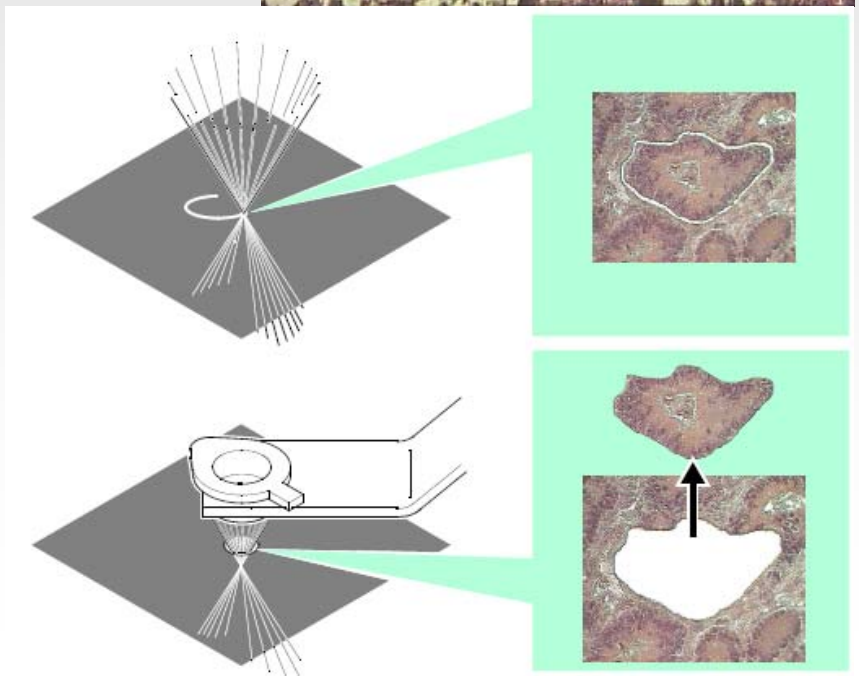
Laser Capture Microdissection

1. Draw new element that is to be extracted

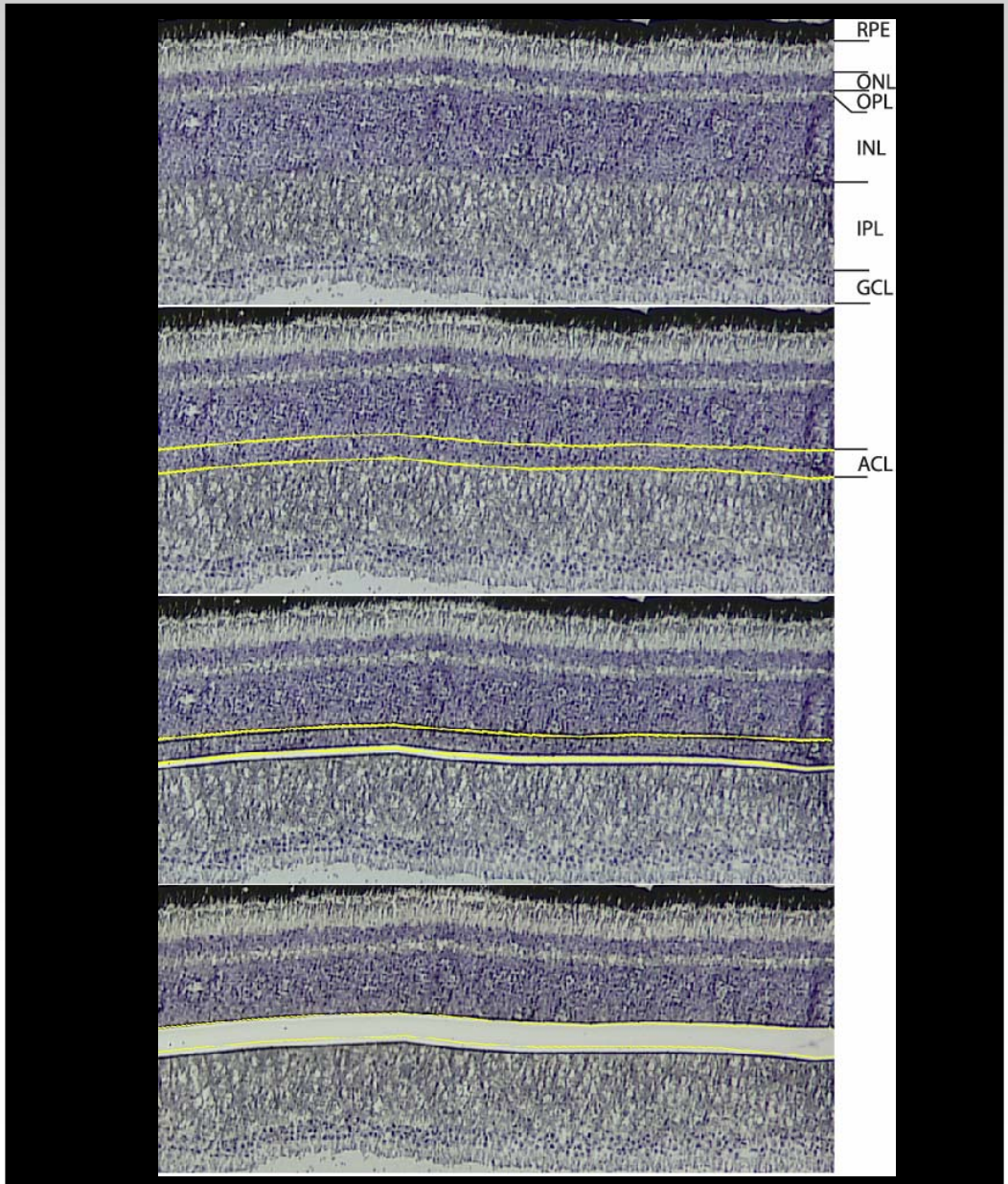


2. Initiate laser cutting

3. Catapult cut section into collection tube



Laser Capture Microdissection



Microarray and possibilities beyond Microarray

- Hundreds of potential signals have been identified with Microarrays, we “just” need to make sense of them
- Couple of potential signalling pathways have been proposed
- Concordance between studies is still poor, repetition using the same treatment paradigms is necessary

Next generation sequencing (NGS)

Determines the sequence of thousands of DNA sequences in a single run (High throughput sequencing (35-400bp/transcript based on platform)

PRO:	CON:
- Very sensitive	- Expensive
- Quantitative	- Complex sample preparation
- No knowledge of genome required	- Data analysis very demanding

Next generation sequencing

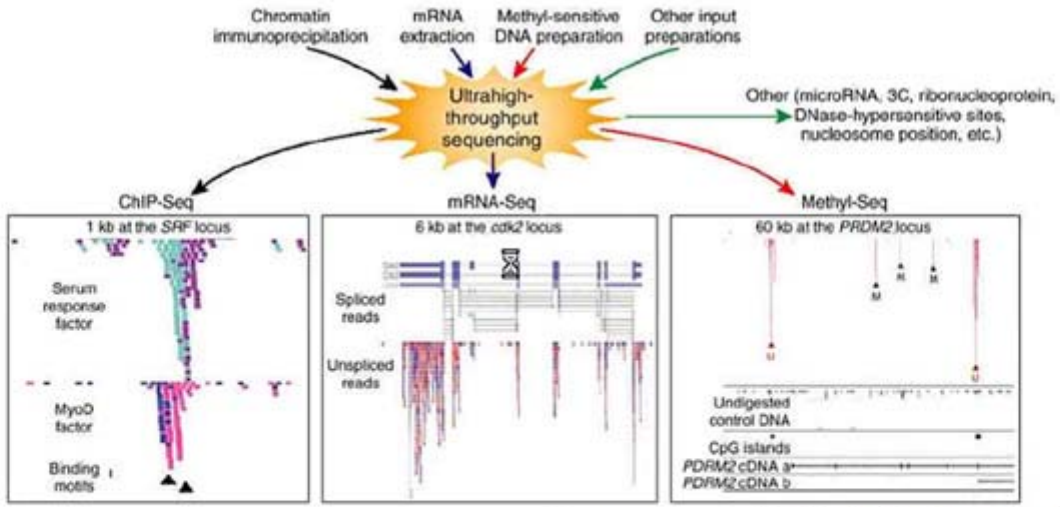
"Seq-based" Functional Genomics Applications

ABI SOLiD

>200M 35-50bp reads/run, 1-256 samples/run

Illumina Genome Analyzer

>80M 35-70bp reads/run, 1-8 samples/run



Sequence census methods for functional genomics. Wold B, Myers RM. Nat Methods. 2008 Jan;5(1):19-21.

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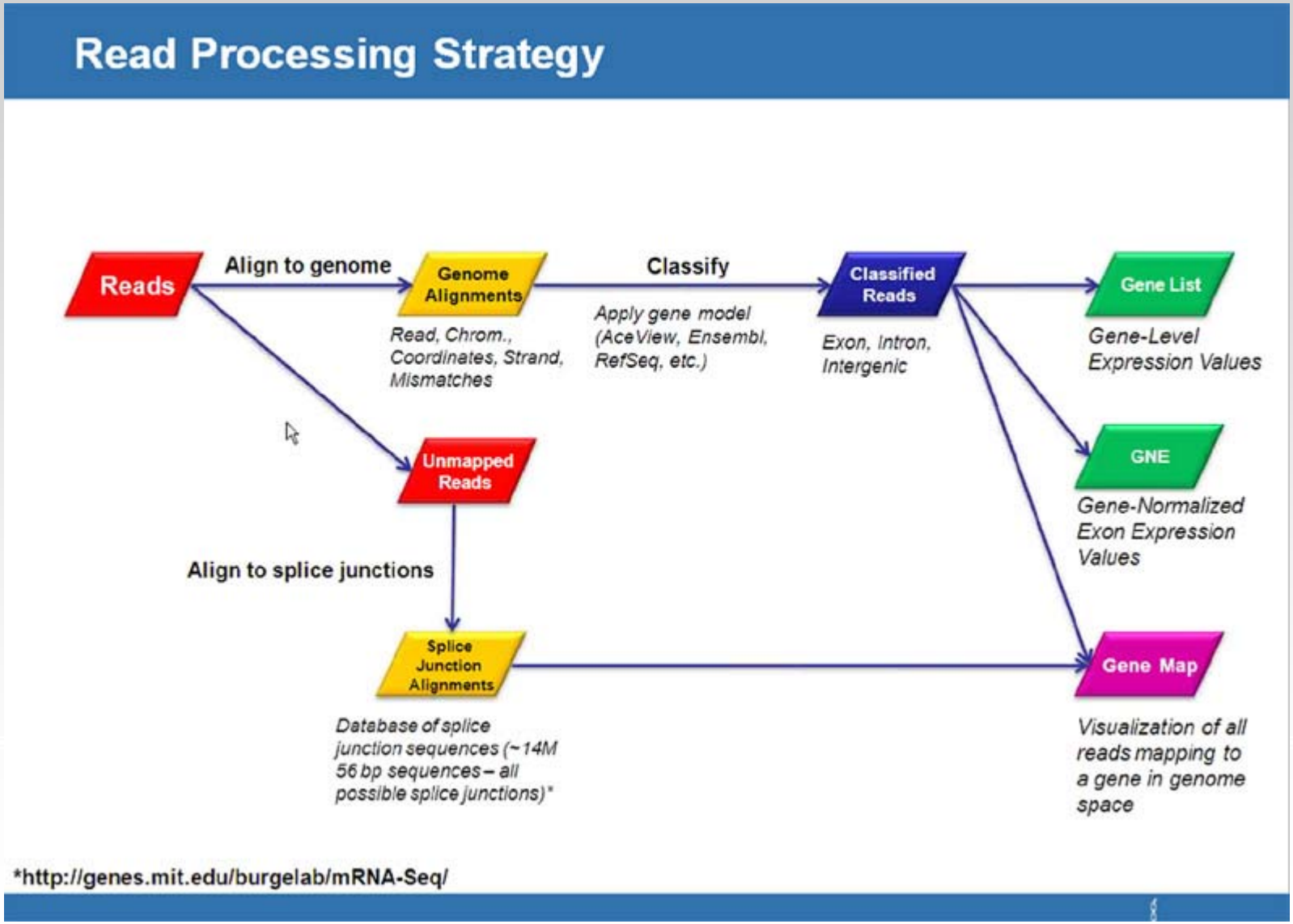
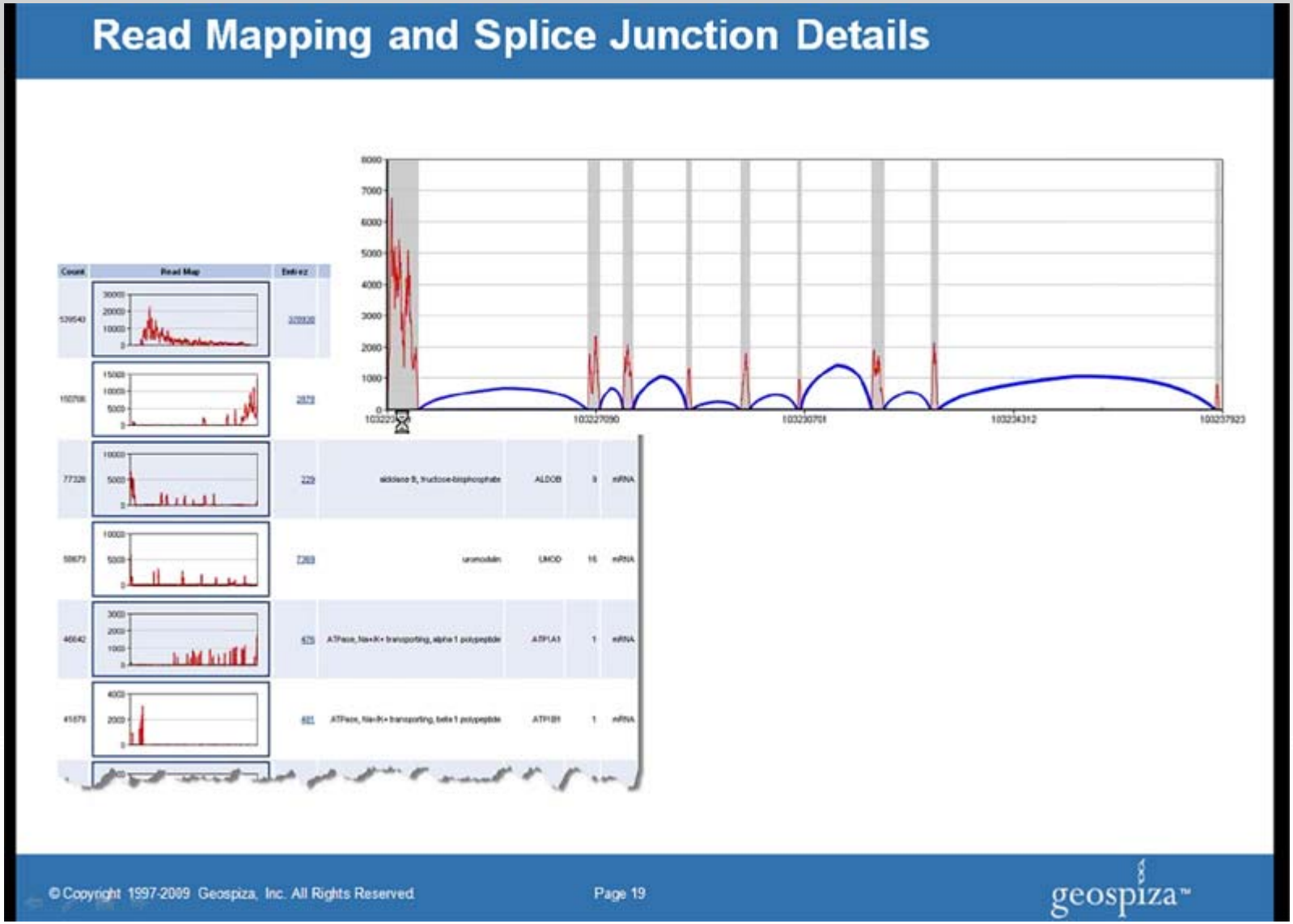
Next generation sequencing

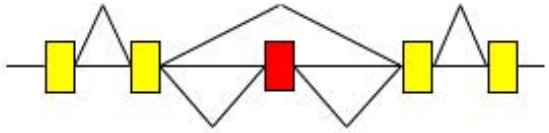


Next generation sequencing

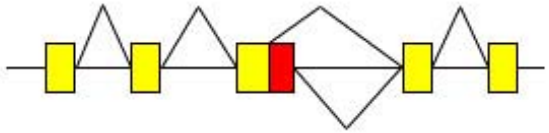


Alternative splicing

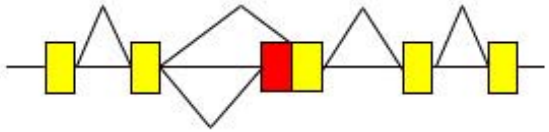
Exon skipping



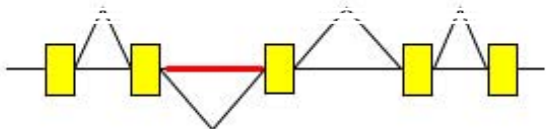
Alternative 5`donor sites



Alternative 3`donor sites



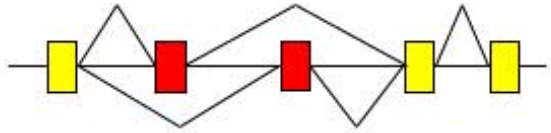
Intron retention



Constitutive exon

Alternative exon

Mutually exclusive exons



Exon skipping: an exon may be spliced out of the primary transcript or retained. This is the most common mode in mammalian pre-mRNAs.

Mutually exclusive exons: One of two exons is retained in mRNAs after splicing, but not both.

Alternative donor site: An alternative 5' splice junction (donor site) is used, changing the 3' boundary of the upstream exon.

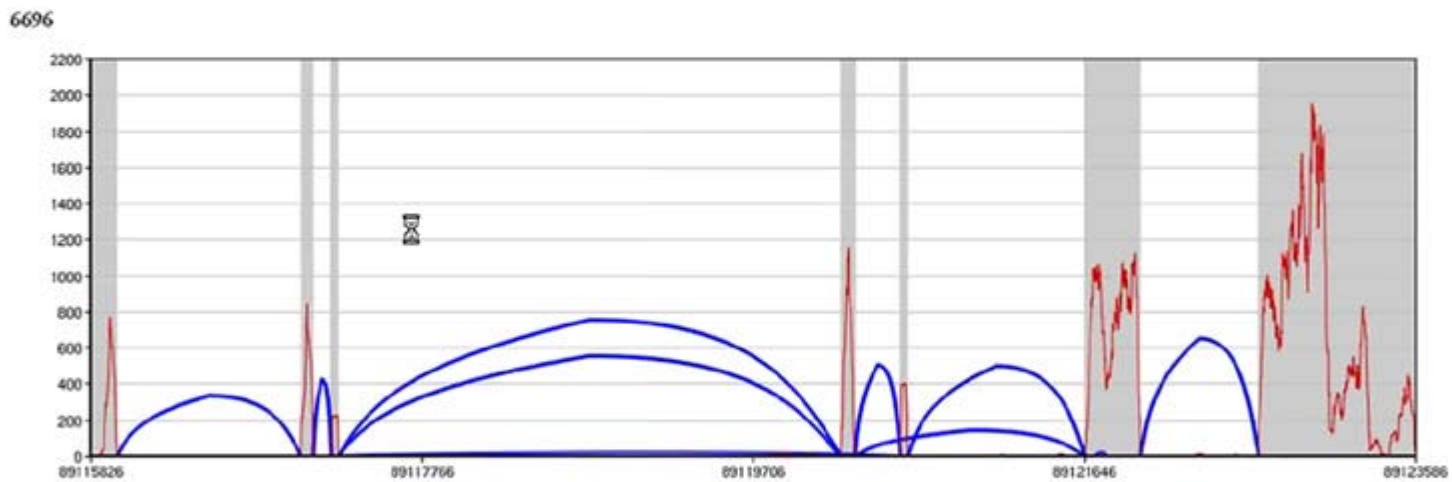
Alternative acceptor site: An alternative 3' splice junction (acceptor site) is used, changing the 5' boundary of the downstream exon.

Intron retention: A sequence may be spliced out as an intron or simply retained. This is distinguished from exon skipping because the retained sequence is not flanked by introns. If the retained intron is in the coding region, the intron must encode amino acids in frame with the neighboring exons, or a stop codon or a shift in the reading frame will cause the protein to be non-functional. This is the rarest mode in mammals.

Next generation sequencing

Read Mapping with Splice Junctions

Secreted phosphoprotein 1 (SPP1)



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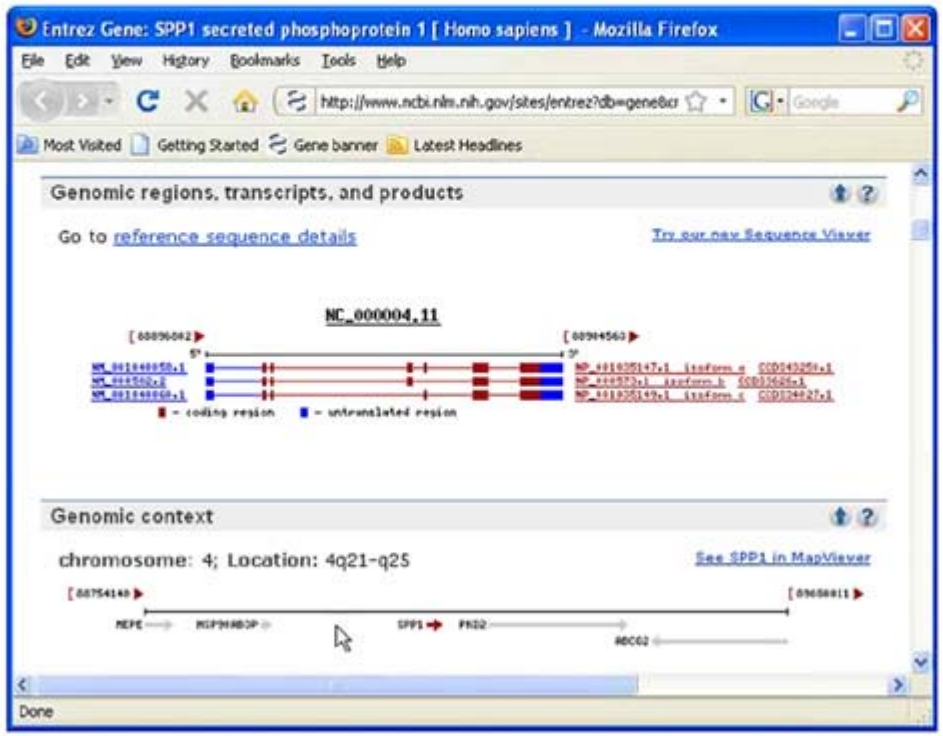
Next generation sequencing

Gene Mapping with Splice Junctions

Secreted phosphoprotein 1 (SPP1)



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Next generation sequencing

Sequences to Biological Interpretation – Gene Level Expression Values


```
>2357_2012_1445_F3
T211020211010001222202201011000 2001
>2357_2012_1665_F3
T211110330302121200012022122321 22111
>2357_2012_1761_F3
T213102112012103021110121220220 23222
>2357_2012_1816_F3
T100220012103002222202210001112 2201
>2357_2012_1835_F3
T101123112100212222022012201013 21322
...
```

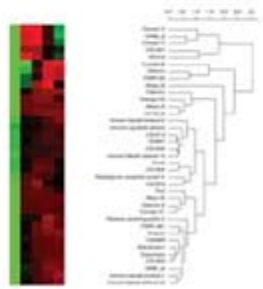
Secondary Analysis

Alignments
Annotation
Summaries

# Reads	Reference Definition
626672	hsa-miR-517a MIMAT0002852 Homo sapiens miR-517a pir:miR-517a
599613	gga-miR-451 MIMAT0003771 Gallus gallus miR-451, oan-miR-451 MIMAT0006001 Ornithorhynchus anatinus miR-451
337507	hsa-miR-515-3p MIMAT0002827 Homo sapiens miR-515-3p, pir:miR-515
209025	hsa-miR-521 MIMAT0002854 Homo sapiens miR-521, pir:miR-521
146329	hsa-miR-29c MIMAT0000681 Homo sapiens miR-29c, mmu-miR-29c, rno-miR-29c, dre-miR-29a, aac-miR-29c, fru-miR-29a, bta-miR-29a, bta-miR-29c, mmi-miR-29c, cfa-miR-29c, ptr-miR-29c
131695	hsa-miR-517c MIMAT0002868 Homo sapiens miR-517c, rno-miR-517a

Tertiary Analysis

Comparison Statistics
Clustering
Pathway Analysis



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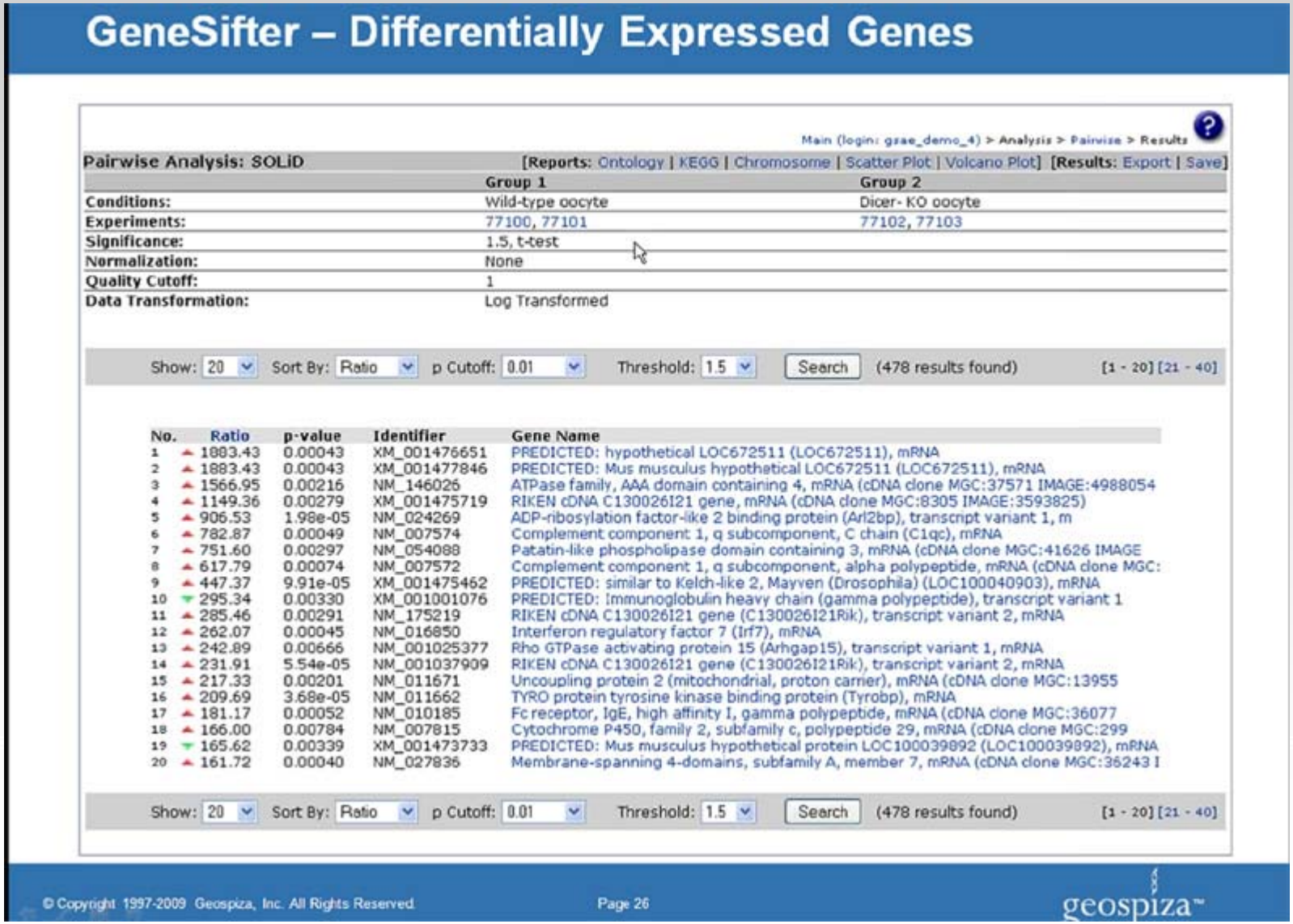
Next generation sequencing



Next generation sequencing

Sequences to Biological Interpretation


```
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T211020211101000122220220101100020001
>2357_2012_1665_F3
T211110330302121200012022122322122111
>2357_2012_1761_F3
T213102112012103021110121220220232222
>2357_2012_1816_F3
T100220012103002222202221000111222201
>2357_2012_1835_F3
T101123112100212222022012201013213222
...
```

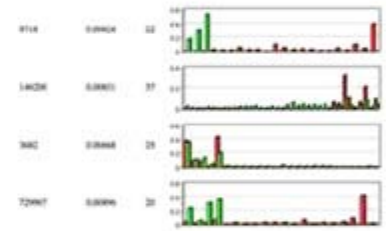
Secondary Analysis

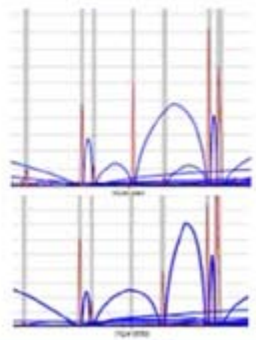
Alignments
Annotation (Gene Model)
Summaries

# Reads	Reference Definition
626672	hsa-miR-517a MIMAT0002852 Homo sapiens miR-517a, ptr-miR-517a
589613	gga-miR-451 MIMAT0003775 Gallus gallus miR-451, oan-miR-451 MIMAT0006001 Ornithorhynchus anatinus miR-451
227937	hsa-miR-519-3p MIMAT0002827 Homo sapiens miR-519-3p, ptr-miR-519
209625	hsa-miR-521 MIMAT0002834 Homo sapiens miR-521, ptr-miR-521
146525	hsa-miR-29c MIMAT0000681 Homo sapiens miR-29c, mmu-miR-29c, rno-miR-29c, dre-miR-29a, xtr-miR-29c, fru-miR-29a, tni-miR-29a, bta-miR-29c, mdo-miR-29c, cfa-miR-29c, ptr-miR-29c
131196	hsa-miR-517c MIMAT0002856 Homo sapiens miR-517c, ssc-miR-517a

Tertiary Analysis

Splicing Indicators
Gene Viewer





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Next generation sequencing

Differential Splicing

Main (login: eric_test) > Analysis > Pairwise > Results > Gene Summary

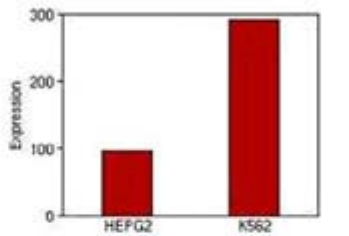
» Gene Summary: Cathepsin C

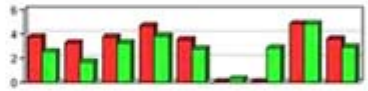
• By Group

Group	Condition	N	Mean	SEM	SEM/Mean	Quality Mean
1	HEPG2	1	95.7900	-	-	95.7900
2	K562	1	291.460	-	-	291.460

• By Target

Group	Sample	Expression	Quality
1	s_002067_sequence	95.7900	95.79
2	s_007332_sequence	291.460	291.46





» One-Click Gene Summary™

Accession No.: NM_148170
Cluster ID: Hs.128065
UG Title: Cathepsin C
Gene ID: CTSC
Homologene: Mm.322945, Rn.122504
Chromosome: 11
Cytoband: 11q14.1-q14.3
Seq Count: 948
Entrez Gene: 1075
Gene Name: cathepsin C
Synonyms: CPPI | DPP1 | DPPI | HMS | JP | JPD | PALS | PLS
OMIM: 170650
RefSeq mRNA: NM_148170 (FASTA)
RefSeq Prot: NP_680475 (FASTA)
Summary: The protein encoded by this gene, a member of the peptidase C1 family, is a lysosomal cysteine proteinase that appears to be a central coordinator for

Gene Ontologies:

Biological Process
- immune response
- proteolysis

Molecular Function
- chloride ion binding
- cysteine-type endopeptidase activity
- cysteine-type peptidase activity
- peptidase activity

Cellular Component
- lysosome

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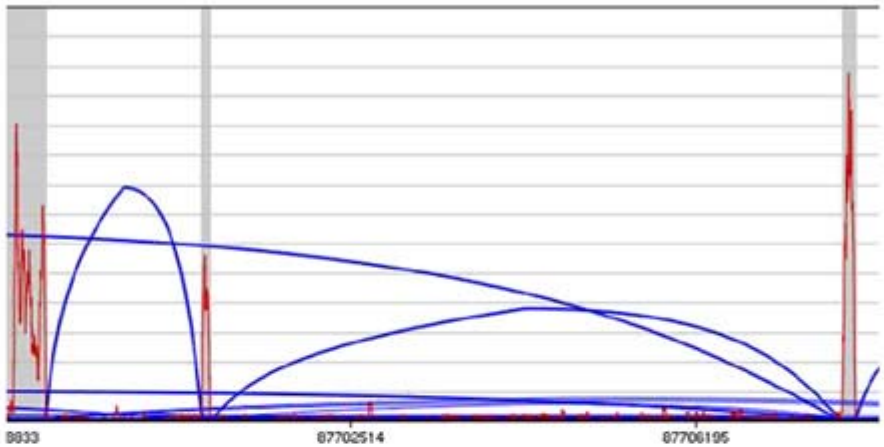
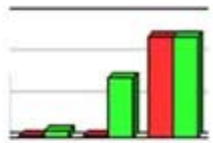
Next generation sequencing

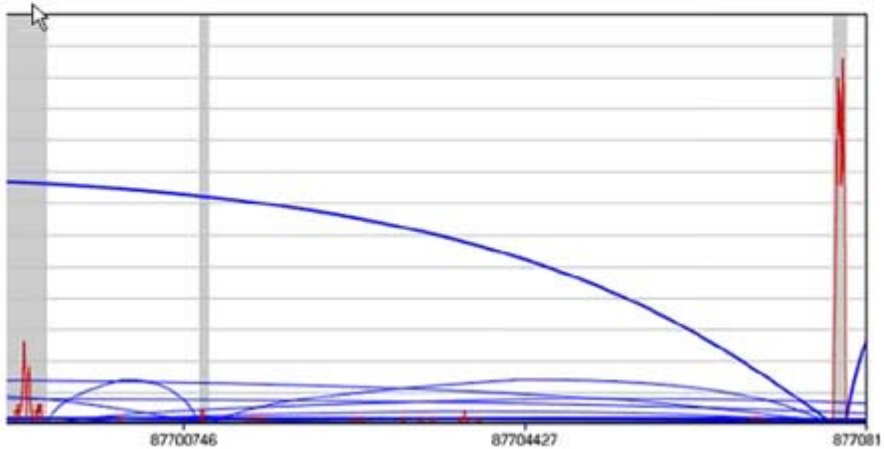
Differential Splicing – Cathepsin C

HEPG2





K562



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Next generation sequencing

- Alternative to microarrays
- Can be used to profile mRNA as well as non-protein coding RNAs without prior information
- Can survey the entire transcriptome, including novel, un-annotated regions
- Can determine gene structure and isoform levels by mapping read density across transcript or gene and can use reads to map splice junctions
- Can identify and quantify both rare and common transcripts, with over six orders of magnitude of dynamic range.

Conclusions

techniques of molecular biology can be used to:

- identify (PCR) and characterize genes whose transcription is controlled by specific visual experience (defocus) (northern blot, quantitative PCR)
- identify gene products that could provide potential targets for pharmacological intervention of myopia (microarray)
- survey the entire transcriptome, including novel, un-annotated regions (next generation sequencing)

(not complete)