Basic methodologies in biotechnology

Harald H Sitte, MD

Medical University of Vienna, Austria

eurahp
European Academy of Hospital Pharmacy

European Association of Hospital Pharmacists

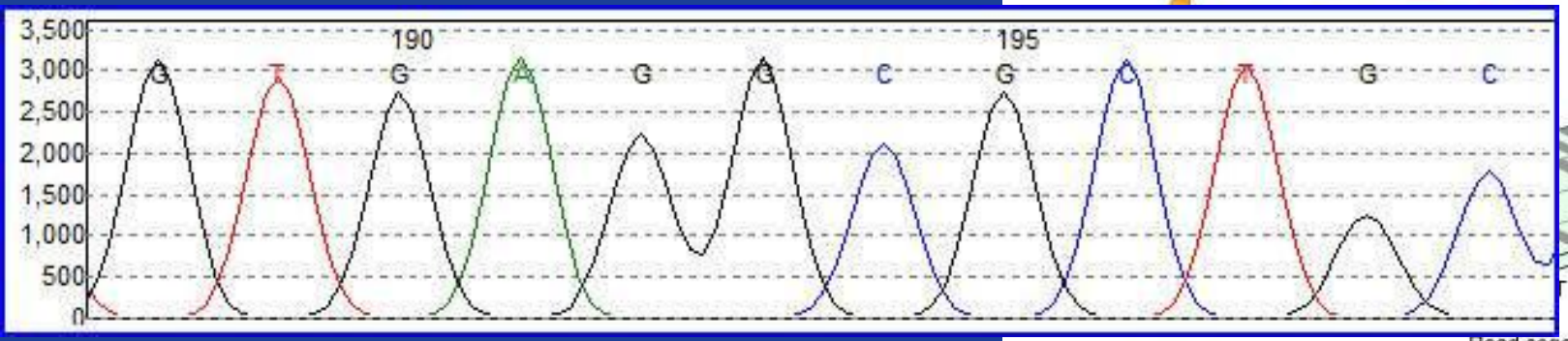
Learning outcomes

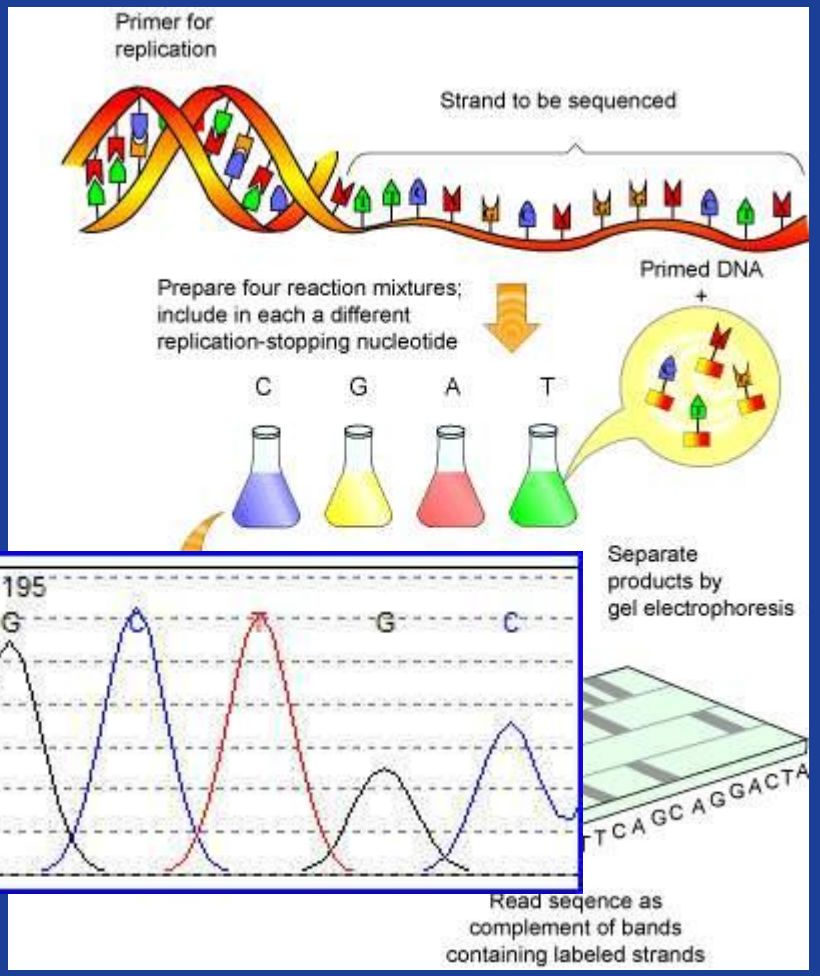
- Specific DNA techniques
- PCR technology
- Genetic engineering
- Protein engineering

PCR, polymerase chain reaction

DNA sequencing

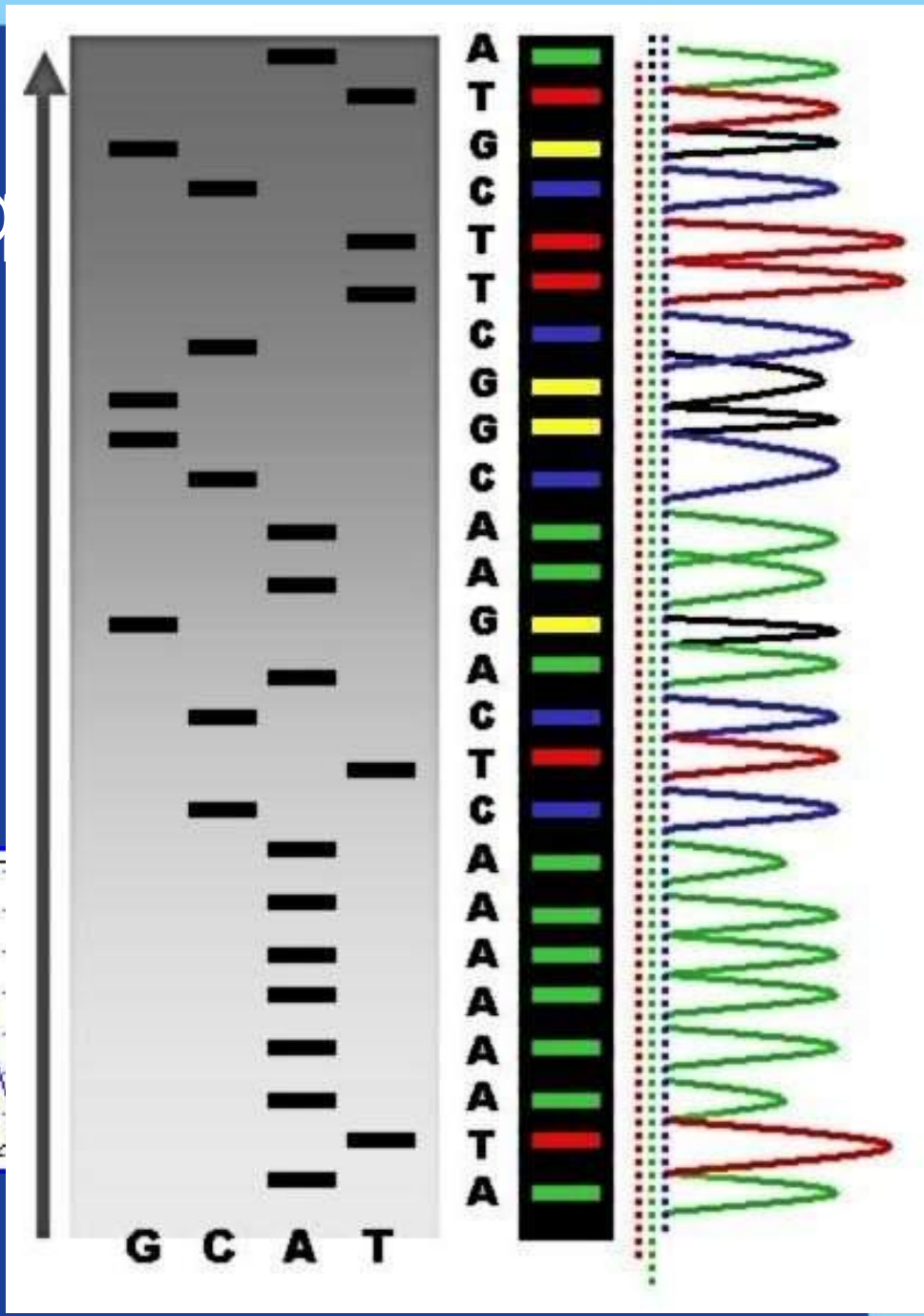
- Two methods: *after*
  - Maxam and Gilbert
  - Sanger



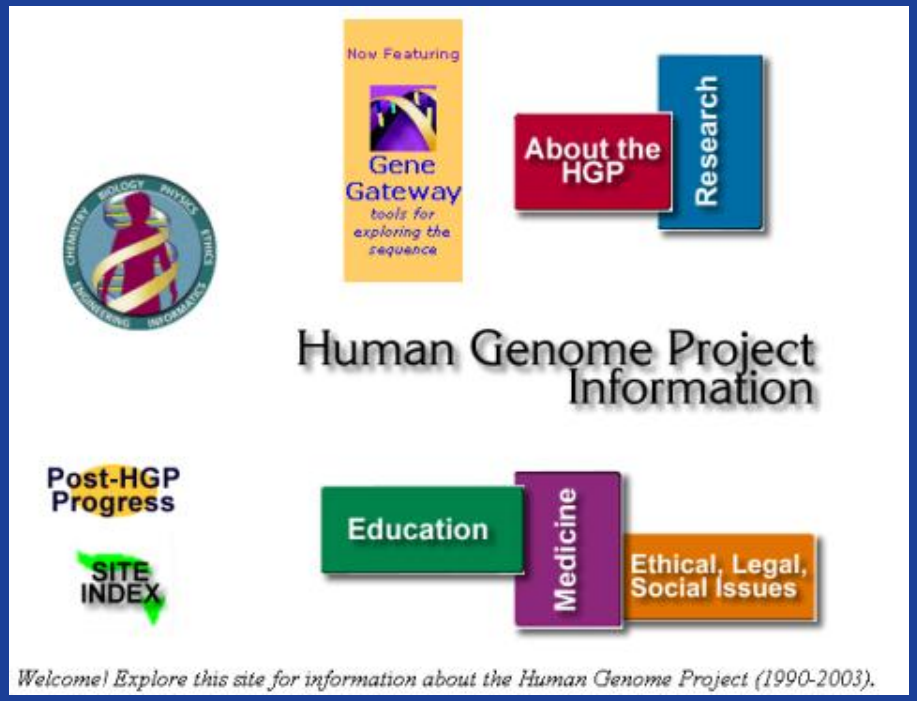


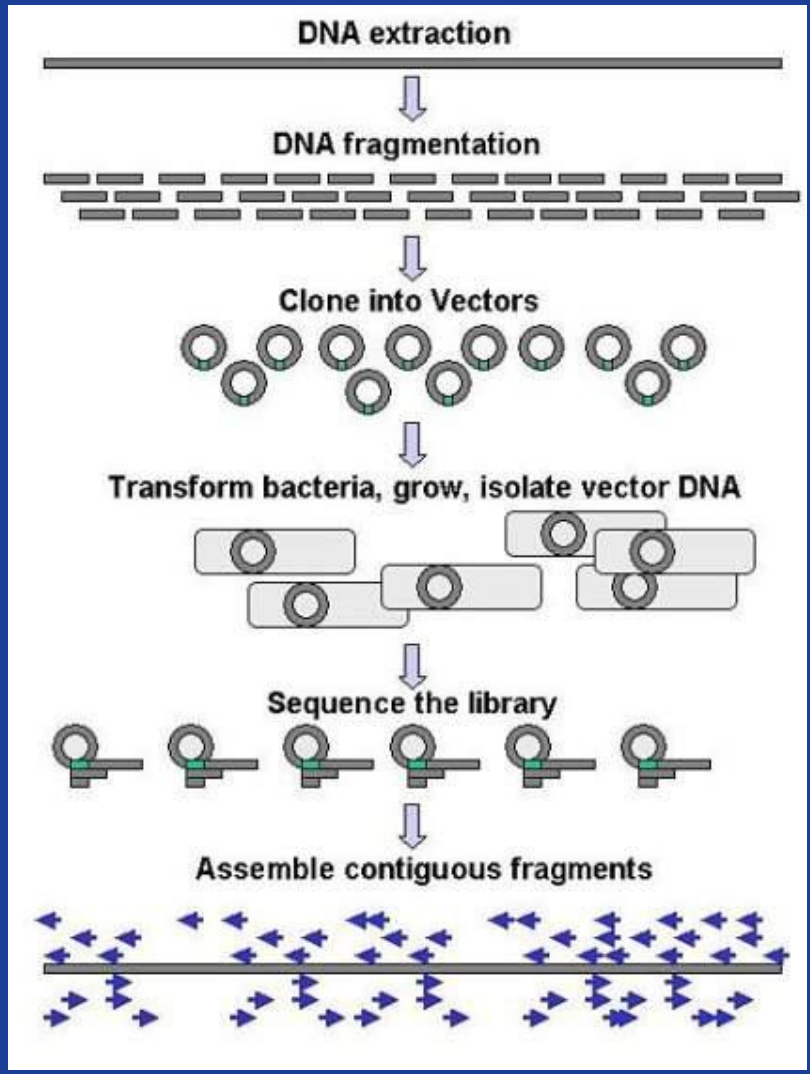
DNA seq

- Two methods: *after*
  - Maxam and Gilbert
  - Sanger

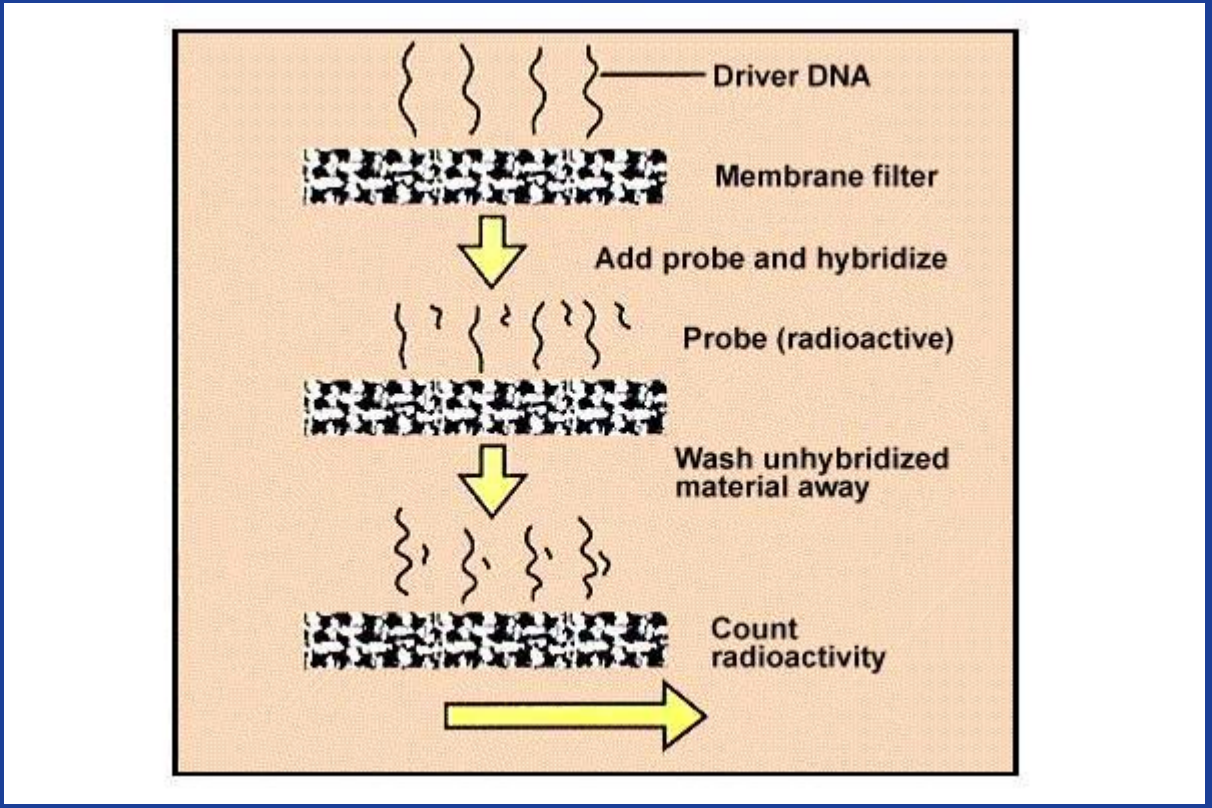


Large-scale sequencing

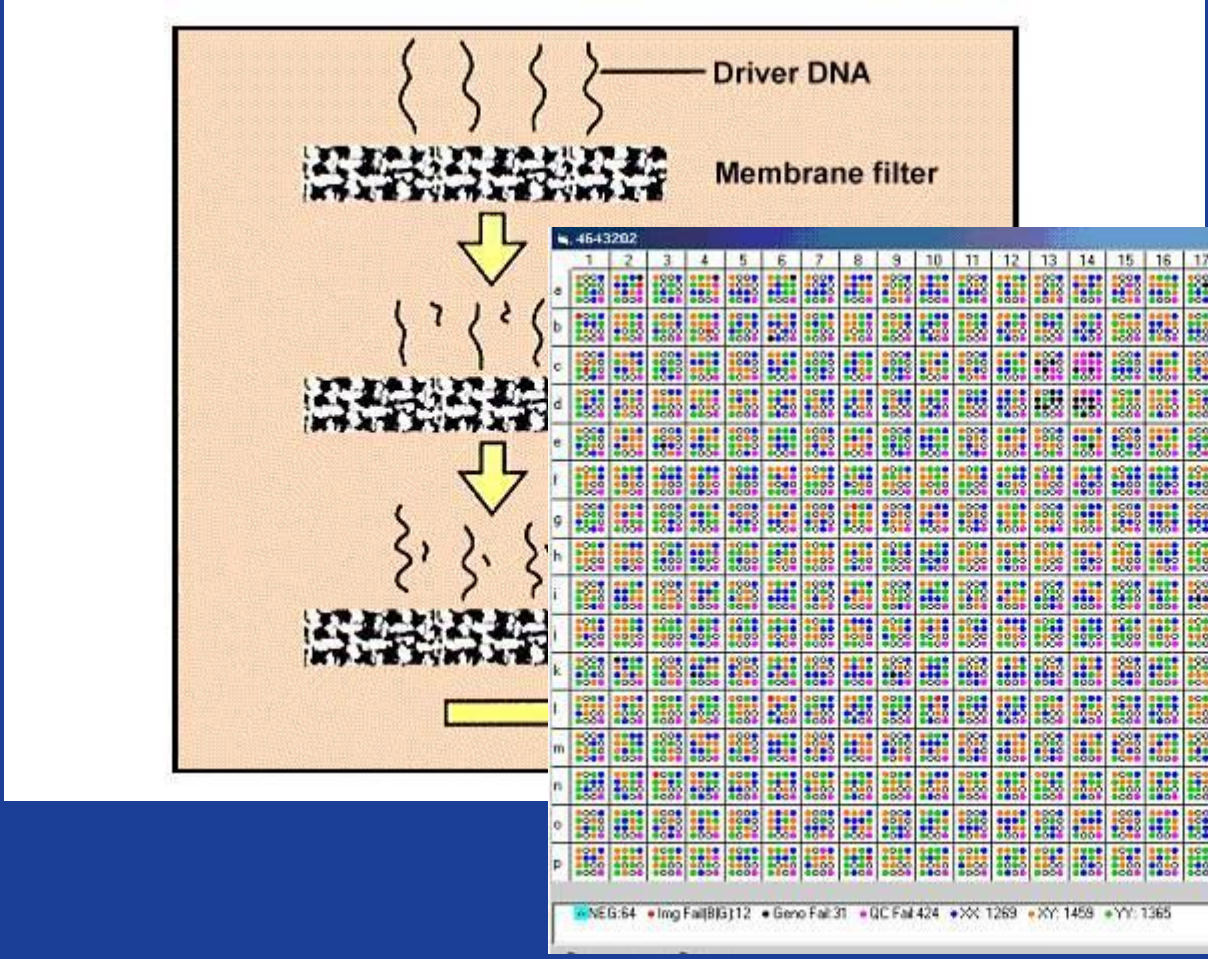




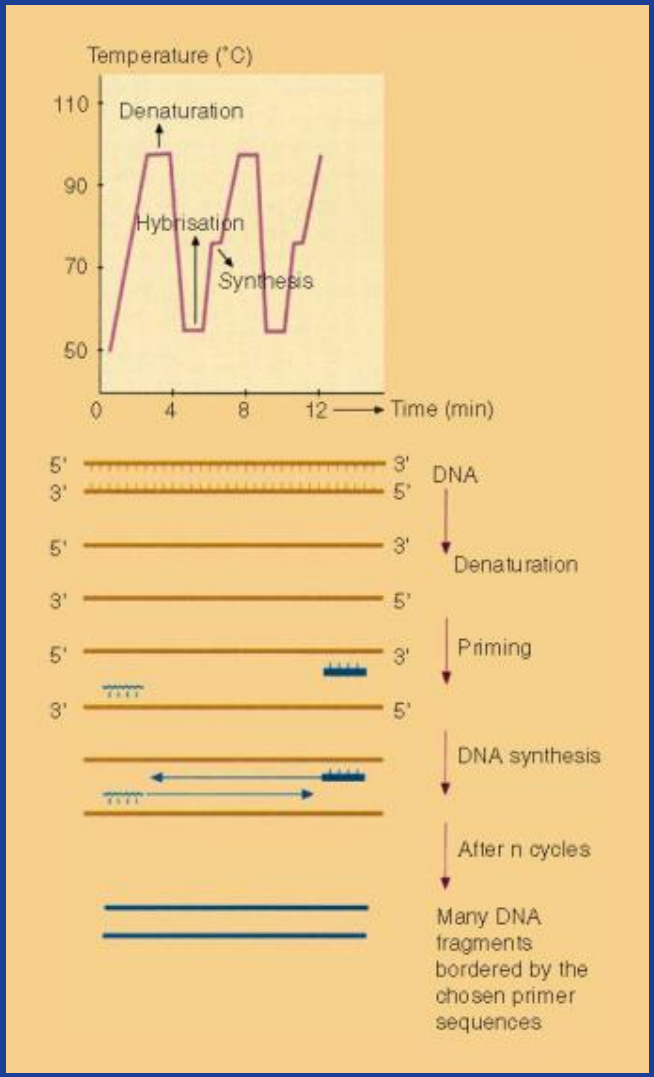
DNA hybridization



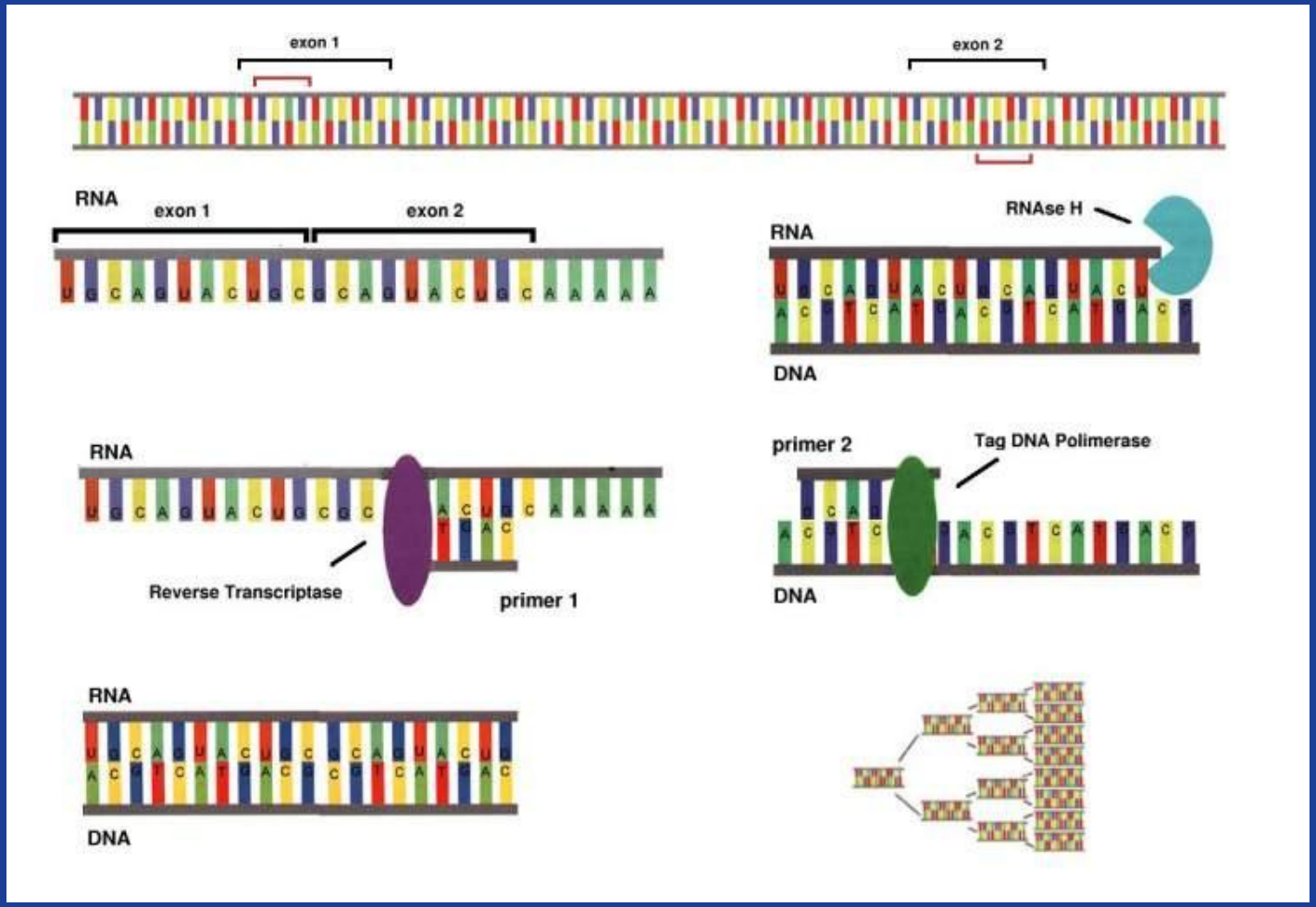
DNA hybridization



Methodology: principles

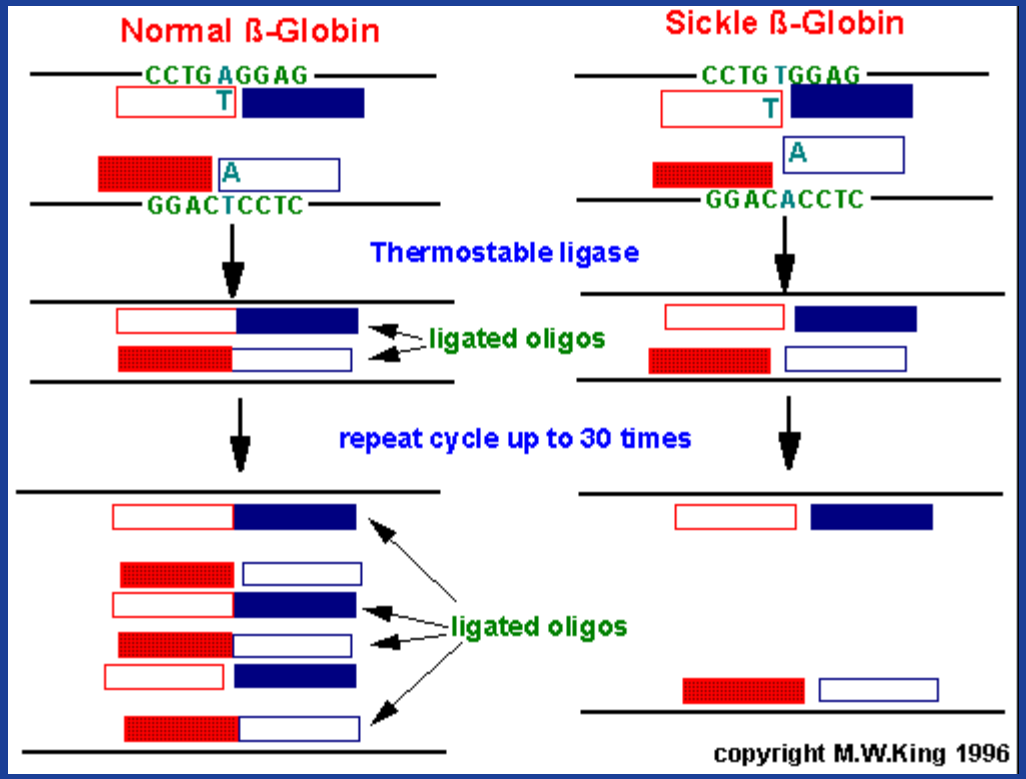


Modified PCR methodologies: RT-PCR

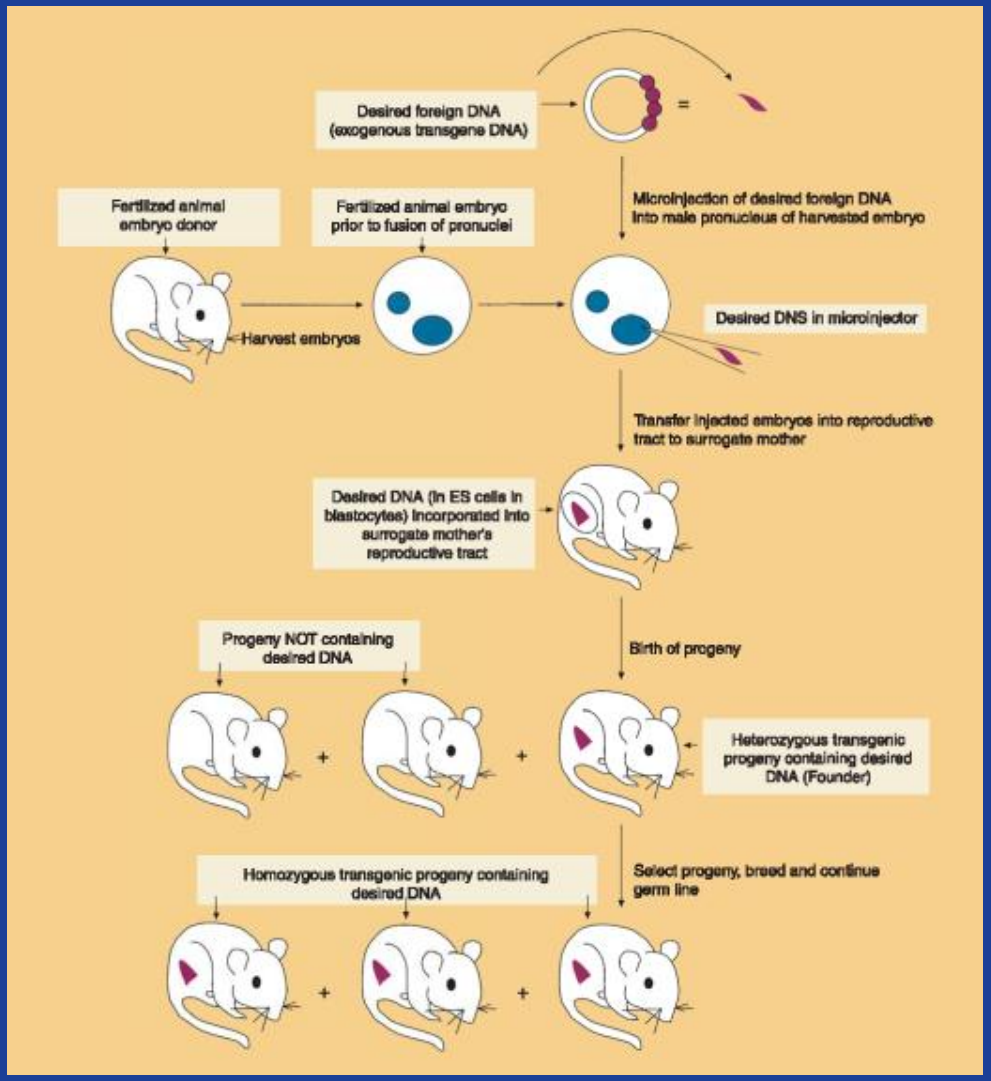


RT-PCR, reverse transcriptase PCR

Ligase chain reaction



What are transgenic mice?



Impressive example: GFP/nude mouse

GFP, green fluorescent protein

Transgenic animal models of human disease

Molecular Psychiatry (2004) 9, 664–683

www.nature.com/mp

FEATURE REVIEW

Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy

J Götz, JR Streffer, D David, A Schild, F Hoerndli, L Pennanen, P Kurosinski and F Chen

Division of Psychiatry Research, University of Zürich, Zürich, Switzerland

Alzheimer's disease (AD) is a devastating neurodegenerative disease that affects more than 15 million people worldwide. Within the next generation, these numbers will more than double. To assist in the elucidation of pathogenic mechanisms of AD and related disorders, such as frontotemporal dementia (FTDP-17), genetically modified mice, flies, fish and worms were

Transgenic animals and plants as ‘bioreactors’

nature biotechnology

Human antibodies from transgenic animals

Nils Lonberg

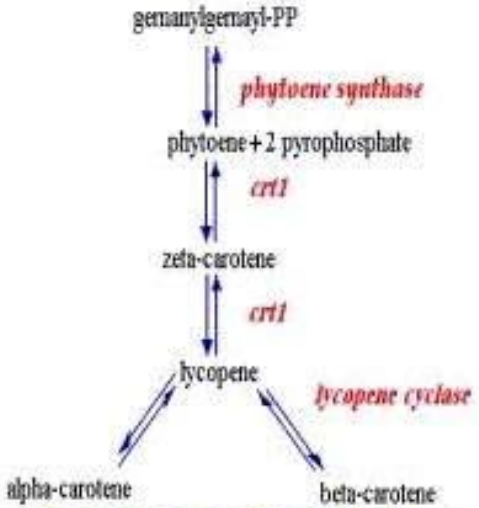
Laboratory mice provide a ready source of diverse, high-affinity and high-specificity monoclonal antibodies (mAbs). However, development of rodent antibodies as therapeutic agents has been impaired by the inherent immunogenicity of these molecules. One technology that has been explored to generate low immunogenicity mAbs for *in vivo* therapy involves the use of transgenic mice expressing repertoires of human antibody gene sequences. This technology has now been exploited by over a dozen different pharmaceutical and biotechnology companies toward developing new therapeutic mAbs, and currently at least 33 different drugs in clinical testing—including several in pivotal trials—contain variable regions encoded by human sequences from transgenic mice. The emerging data from these trials provide an early glimpse of the safety and efficacy issues for these molecules. Nevertheless, actual product approval, the biggest challenge so far, is required to fully validate this technology as a drug discovery tool. In the future, it may be possible to extend this technology beyond rodents and use transgenic farm animals to directly generate and produce human sequence polyclonal sera.

Title: **Transgenic animals as urinary bioreactors for the production of polypeptide in the urine, recombinant DNA construct for kidney-specific expression, and method of using same**

Document Type and Number: United States Patent 6888047 **Link to this page:** http://www.freepatentsonline.com/6888047.html

Abstract: The invention relates to recombinant DNA constructs, a method for producing a recombinant biologically active protein in vivo in the urine of a non-human mammal using a kidney-specific promoter, such as the uromodulin promoter, and the transgenic non-human mammals that serve as urine-based bioreactors for protein production.

Transgenic animals and plants as ‘bioreactors’



Boosting heterologous protein production in transgenic dicotyledonous seeds using *Phaseolus vulgaris* regulatory sequences

Geert De Jaeger, Stanley Scheffer, Anni Jacobs, Mukund Zambre, Oliver Zobell, Alain Goossens, Ann Depicker*, and Geert Angenon[1]

Published online 4 November 2002; doi:10.1038/nbt755

Over the past decade, several high-value proteins have been pro-

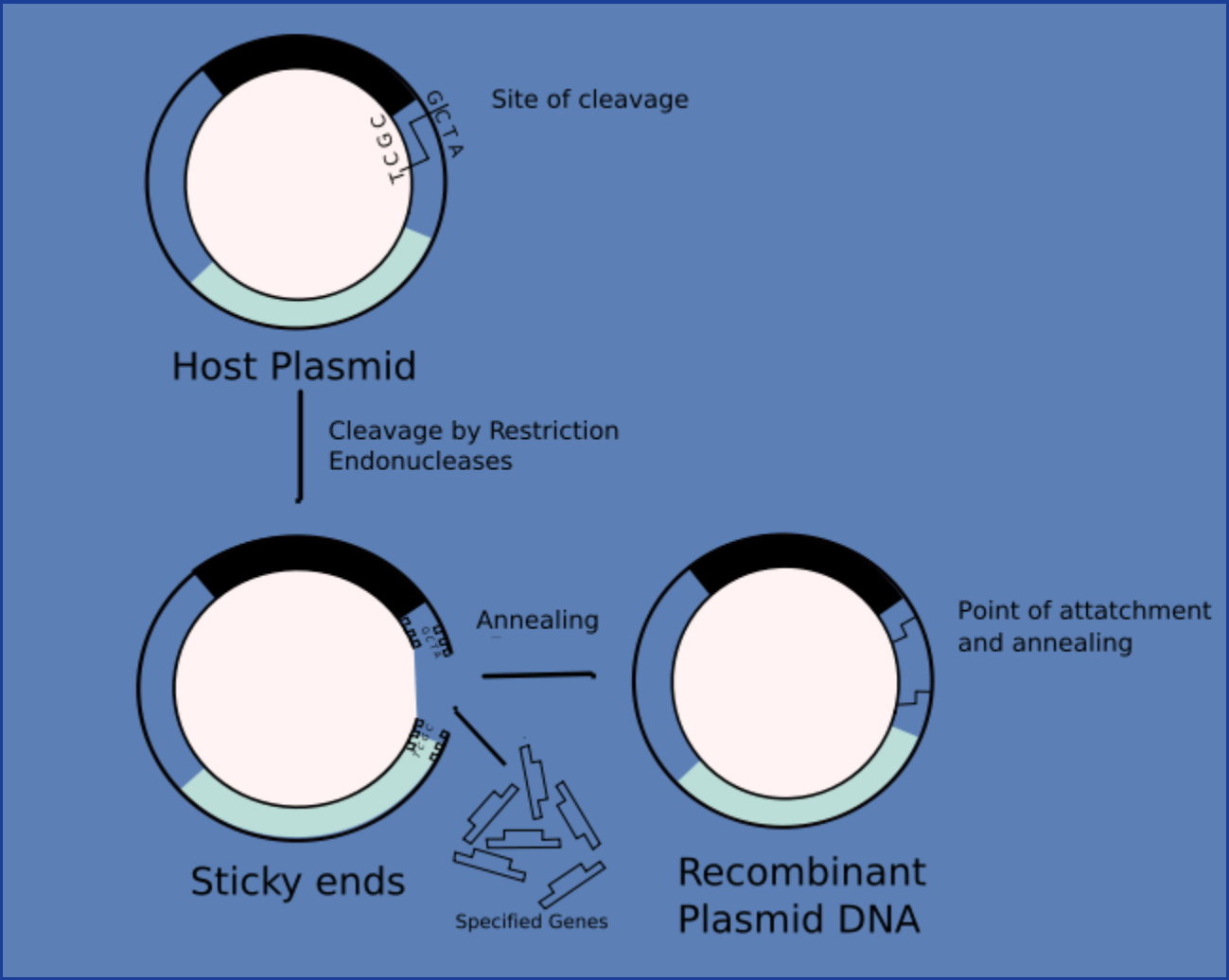
http://www.nature.com/naturebiotechnology

Title: **Transgenic animals as urinary bioreactors for the production of polypeptide in the urine, recombinant DNA construct for kidney-specific expression, and method of using same**

Document Type and Number: United States Patent 6888047 **Link to this page:** http://www.freepatentsonline.com/6888047.html

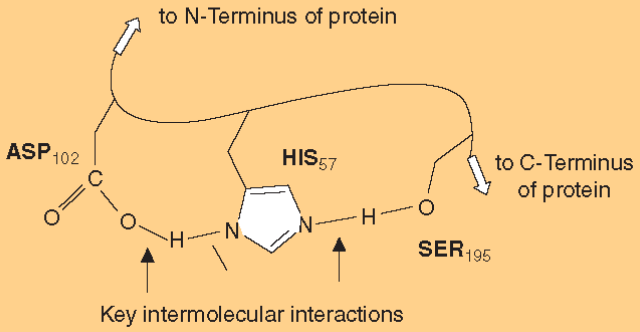
Abstract: The invention relates to recombinant DNA constructs, a method for producing a recombinant biologically active protein in vivo in the urine of a non-human mammal using a kidney-specific promoter, such as the uromodulin promoter, and the transgenic non-human mammals that serve as urine-based bioreactors for protein production.

Production of engineered proteins

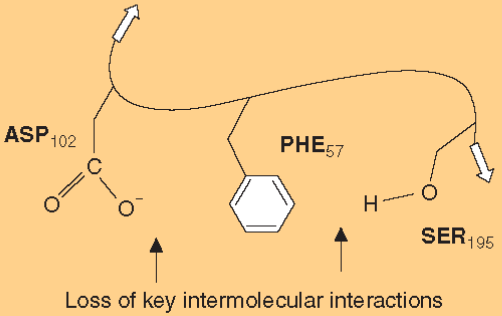


Mutagenesis

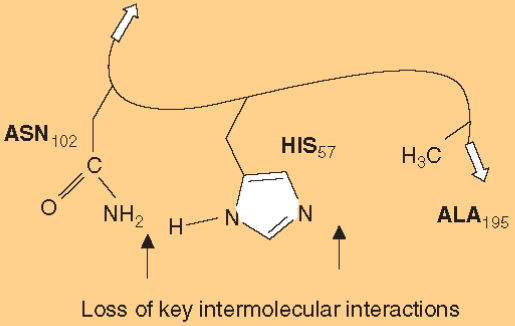
A. Catalytic triad (the catalytic machinery) at active site of a wild type, parent serine protease



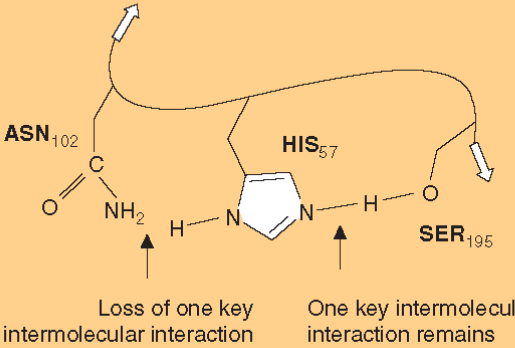
B. Theoretical site-directed mutagensis studies HIS_{57} to PHE_{57} mutant



C. Theoretical site-directed mutagenesis studies ASP_{102} to ASN_{102} and SER_{195} to ALA_{195} mutant

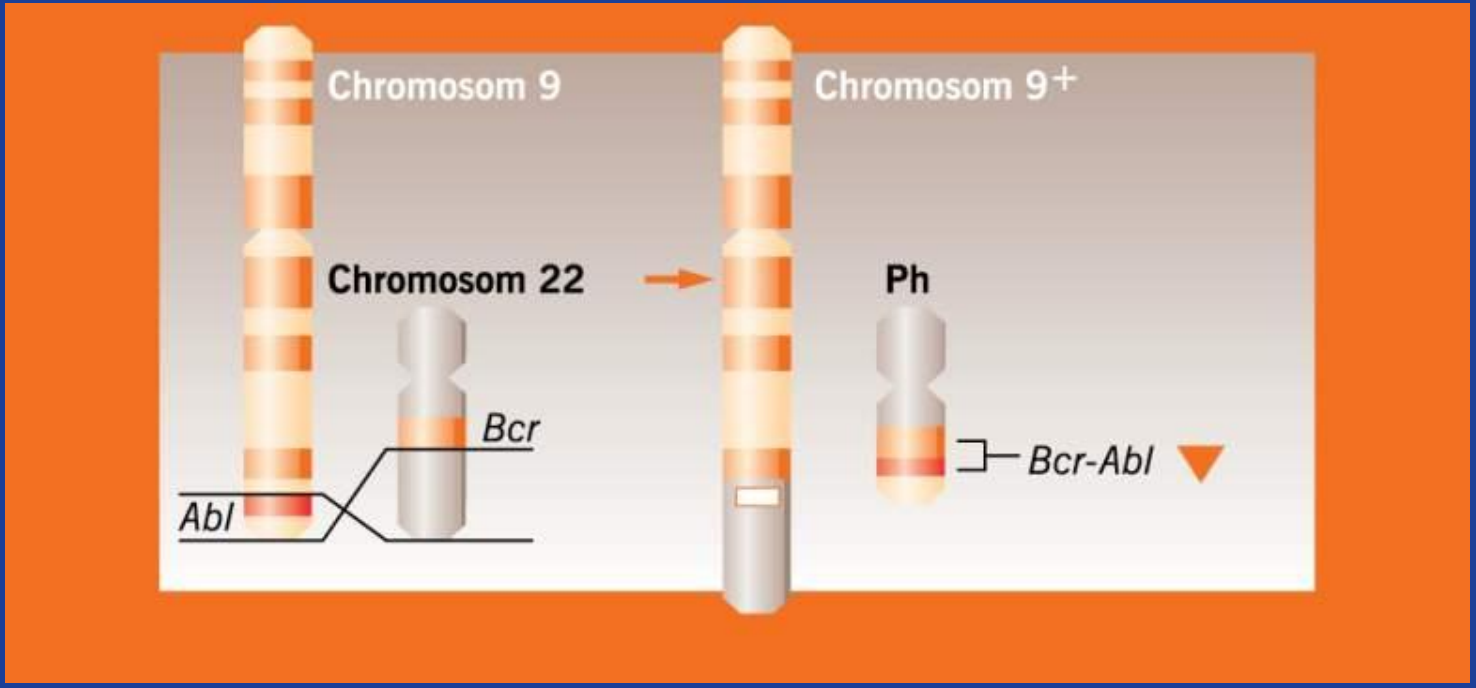


D. ASP_{102} to ASN_{102} Mutant from site-directed mutagenesis (from Craik et al. 1987)



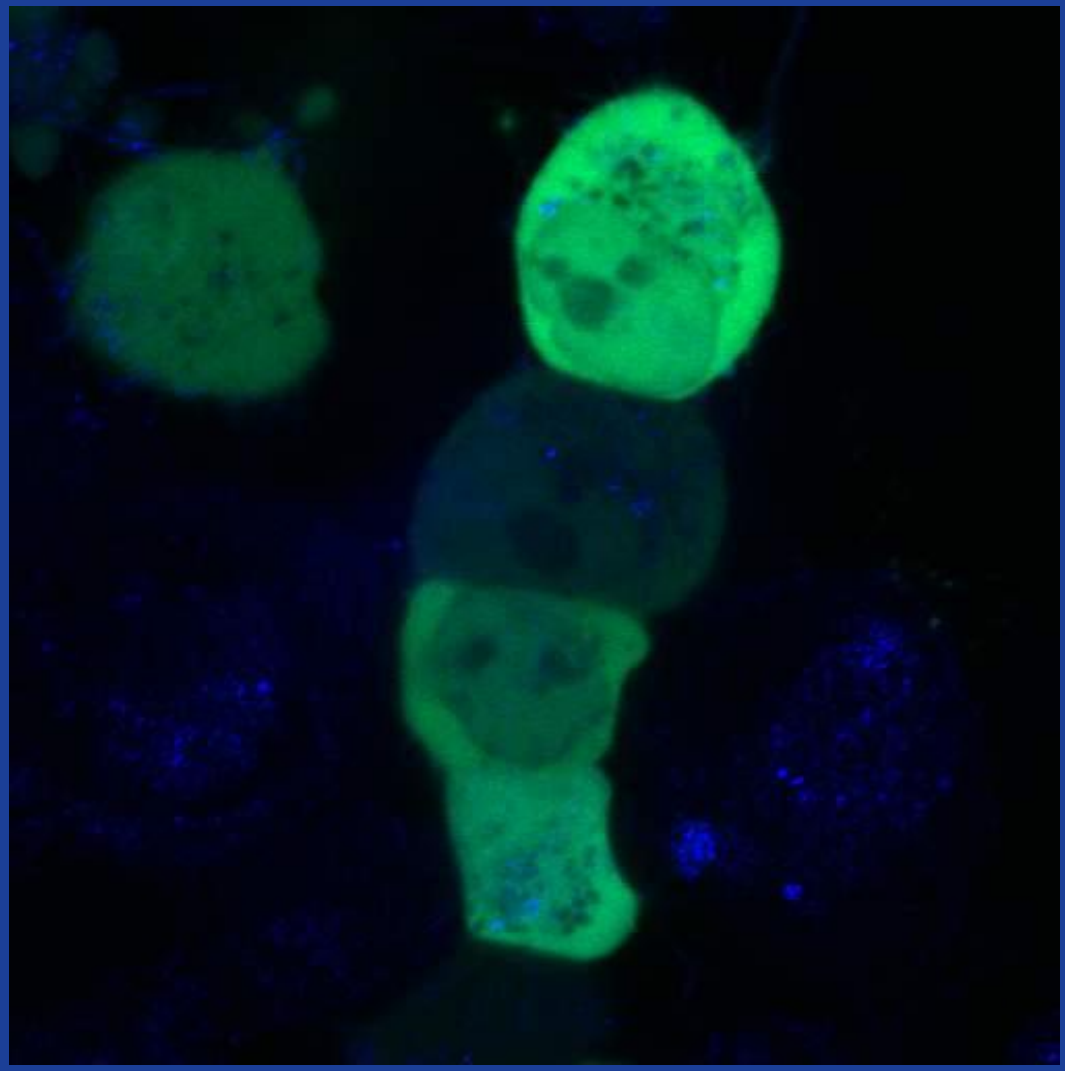
Fusion proteins

- A naturally occuring example:
  the Philadelphia chromosome



Fusion proteins

- A genetically engineered fusion protein: a GFP-tagged human transporter protein



Conclusion slide

- Specific DNA techniques
  - *Sequencing, hybridization etc*
- PCR technology
  - *Polymerase chain reaction and advanced…*
- Genetic engineering
  - *Transgenic animals*
- Protein engineering
  - *How can we modify proteins*