THE USE OF GENE THERAPY TO TREAT CYSTIC FIBROSIS

THE PRACTICE

Cystic Fibrosis, (CF), an autosomal recessive disease, is the most common genetic disorder among Caucasians[1]. CF sufferers have two faulty *cystic fibrosis transmembrane regulator* (CFTR) genes rendering them unable to make the normal CFTR protein, consisting of 1480 amino acids[2], which functions as a channel transporting chloride ions across the cell membrane. In CF patients, cells contain high concentrations of chloride ions causing more water to transfuse into cells resulting in an accumulation of mucous. Epithelial cells lining the respiratory system are especially vulnerable to the defect in this gene[3]. CF sufferers' malfunctioning exocrine glands assist in causing problems such as the production of mucus in their lungs, resulting in predisposition to respiratory and lung infection.

One approach to compensate for the defective CFTR genes, is gene therapy, which endeavours to introduce normal CFTR genes into CF patients lung epithelial cells[4], using non-replicating adenovirus (cold virus). The adenovirus infects human epithelial cells with the CFTR gene, allowing them to produce the chloride-regulating protein restoring chloride balance[5]. The procedure in humans is continually questioned as the adenovirus could cause serious infection in the gene therapy recipient; rendering the treatment futile. Moreover, introduced cells are gradually lost so the effect diminishes and repeat treatments are necessary[6]. Gene therapy is still developing and its success rate remains unproven[7].

---

[1] Beardsley, T. "Clearing the airways- Cystic Fibrosis may be treated with gene therapy", *Scientific American*, volume 263 (6), 1990, p 14

[2] Bennington, T. & Propert, D, et al. "Cystic fibrosis handbook (1996 edition)", *Cystic Fibrosis Association of Victoria*, p 12.

[3] Glausiusz, J. "Hunting down genes", *Sunday Herald-Sun*, 18 Feb 1996, p 80.

[4] Welsh, M. & Smith, A. "Cystic Fibrosis", *Scientific American*, December 1995, p42.

[5] Lewin, R. "Gene therapy promises cure for cystic fibrosis", *New Scientist*, 18 Jan 1992, p 5.

[6] Ibid.

[7] Coghlan, A. "Gene dream fades away", *New Scientist*, 25 Nov 1995, p 15.

RELEVANT GENETICS

CF is an autosomal disorder, caused by a defective CFTR gene on the non-sex-determining chromosome 7 at locus 7q3 (top half) **(Fig. 1)**. A chromosome (thread-like structure found in nuclei) consists of deoxyribonucleic acid (DNA) and protein. Genes are messages encoded on DNA strands and are composed of deoxyribose sugar, phosphate and the bases adenine(A), thymine(T), guanine(G) and cytosine(C) which pair as A-T and G-C to form a double helix[8] **(Fig. 1)**. The arrangement of these bases encodes the information for the synthesis of proteins[9].

[8] Evans, B. et al. "Biology Two (2nd edition)", *Heinemann Educational Australia*, Australia, 1995, p 230.
[9] "Genetic engineering and protein synthesis", *CSIRO Australia*, 1992.

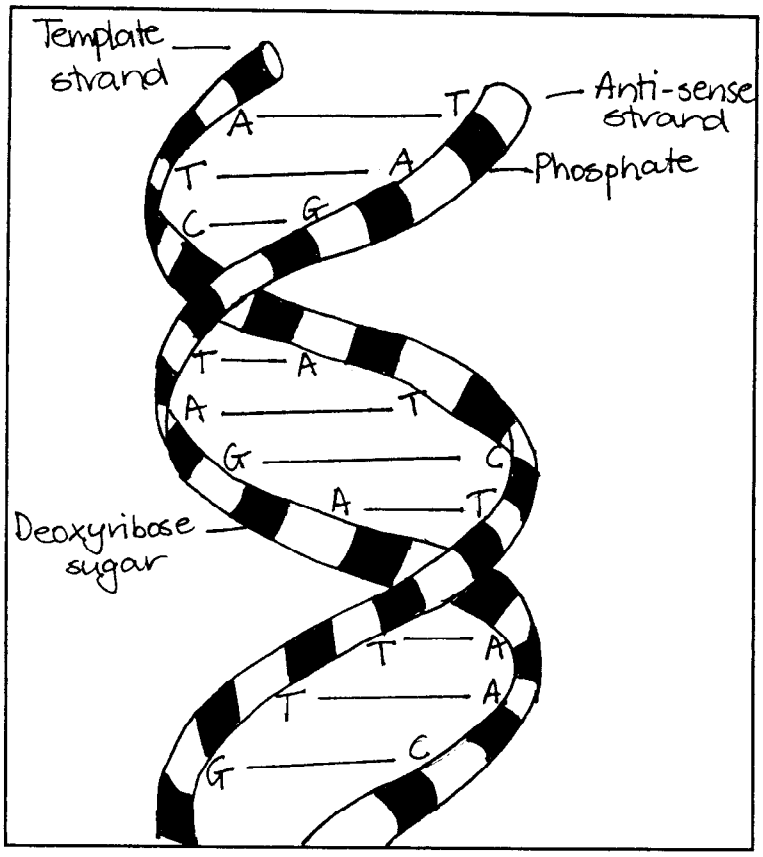

Figure 1[10]: DNA molecule including part of the base sequence of the template strand of the CFTR gene.

The three types of mutation of genes are: base substitution, addition and deletion.

Over 500 mutations of the CFTR gene causing CF have been identified[11]. The most common mutation, the deletion of the amino acid phenylalanine ΔF508 **(Fig. 2 & 3),** results from deletion of the nucleotide C from codon 507(isoleucine) and two T nucleotides from codon 508(phenylalanine) [12]. Therefore, the isoleucine 507 remains encoded for by the mutated sequence ATT, but phenylalanine is omitted from the CFTR protein, resulting in the loss of its function and subsequently CF.

[10] Fung, S. "Biology Study Guide- Units 3 and 4", *Longman Cheshire*, Australia, 1993, p 108.
[11] Bennington, T. & Propert, D. et al. "Cystic fibrosis handbook (1996 edition)", p 1, Op. Cit.
[12] Welsh, M. & Smith, A. "Cystic fibrosis", p 39, Op. Cit.

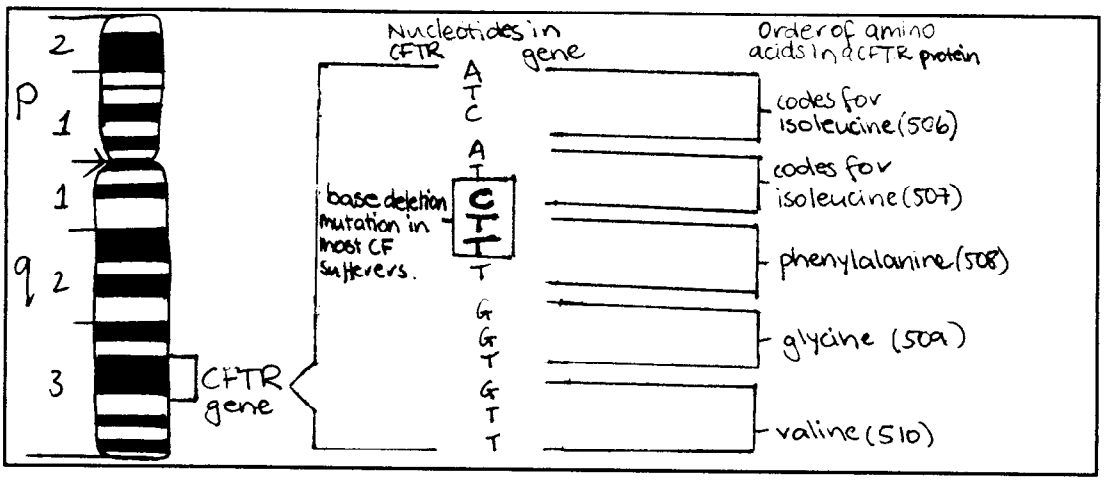

Figure 2[13]: Chromosome 7.
Figure 3[14]: The mutation which causes CF.

The aim of gene therapy, for CF sufferers, is introducing a functioning CFTR gene into lung epithelial cells, resulting in the production of CFTR, to compensate for the defective protein produced by CF sufferers. The CFTR protein is produced during transcription and translation in protein synthesis.

[13] Kinnear, J. & Martin, M. "Nature of Biology Book Two", p 219, Op. Cit.
[14] Welsh, M. & Smith, A. "Cystic Fibrosis", p 39, Op. Cit.

The DNA double helix separates into two strands by the enzyme RNA polymerase, a temporary copy of the template strand (contains encoded information) replaces it in transcription**(Fig. 4)**. The temporary copy, pre-messenger RNA (pre-mRNA) is single-stranded and replaces the base thymine on the DNA strand for uracil, which bonds to adenine[15]. As the pre-mRNA is released, the two DNA strands recoil. The introns (non-coding regions) are discarded leaving a shorter mRNA molecule which is capped and tailed so it can carry the information from the DNA to the ribosomes[16].

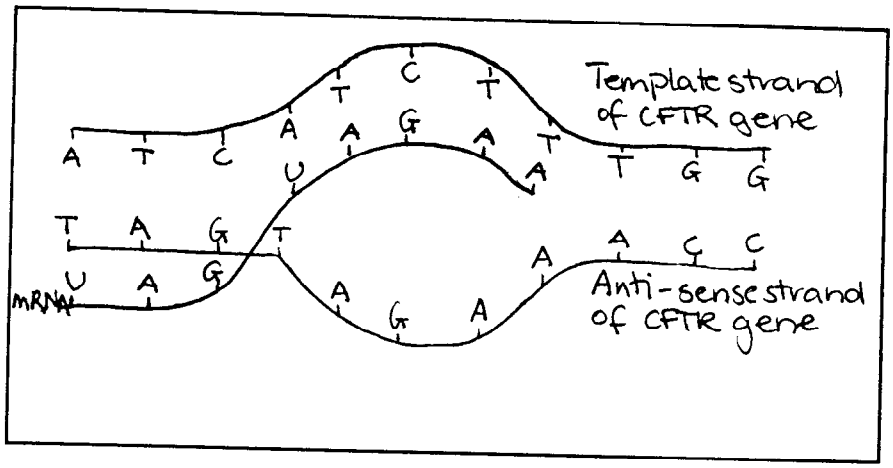

Figure 4[17]: The bases of the CFTR gene are being transcribed into mRNA.

Translation occurs at the ribosomes, the location of protein manufacture, where the mRNA is assembled and the codes of the bases are read. The information in the mRNA is translated by transfer RNA (tRNA) into a sequence of linked amino acids. Each codon (three bases) translates for a particular amino acid to be made. The deletion of the amino acid, phenylalanine at codon 508 in the original DNA means the mutation is transcribed into mRNA, translated into tRNA resulting in it being absent from the amino acid chain. This causes the CFTR protein to be defective and the symptoms of CF.

[15] "Genetic Engineering and Protein Synthesis", Op. Cit.
[16] Kinnear, J. & Martin, M. "Nature of Biology Book Two", p 270, Op. Cit.
[17] "Genetic Engineering and Protein Synthesis", Op. Cit.

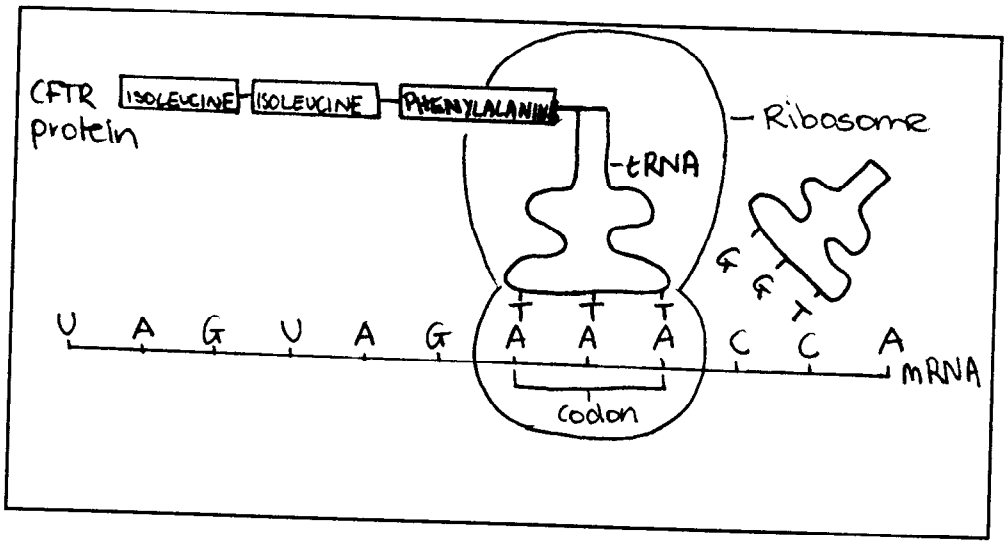

Figure 5[18]: The CFTR protein is assembled as translation occurs.

[18] Kinnear, J & Martin, M. "Nature of Biology Book Two",p 273, Op. Cit.

THE APPLICATION

Gene therapy involves the insertion of the normal CFTR gene into the defective epithelial cells of the CF patient's bronchial tubes[19] so the functioning CFTR protein can be made. Only 5-10% of the epithelial cells need express the normal CFTR gene for the procedure to be successful and results in reverting the symptoms seen[20]. Repeated treatments are necessary for continued results.

The CFTR gene was isolated from a human genomic library in 1989[21] employing molecular biology techniques such as gel electrophoresis (separates DNA fragments by size[22]) and a specific probe(single stranded segment of DNA) with a base sequence complementary to the CFTR gene[23]. The probe is labelled with a radioactive marker so the CFTR gene can be located easily from other fragments.

Once the normal CFTR gene was isolated, it could be inserted into a plasmid (small DNA rings found in bacteria[24]) which had been split using the same restriction enzyme (cuts DNA molecules only at a specific base sequence[25]) so that the sticky ends of the plasmid and the normal CFTR gene will correspond and are able to join together **(Fig 6)**. They are joined together using DNA ligase(joins DNA fragments together).

---

[19] Bennington, T. & Propert, D. "Cystic Fibrosis handbook (1996 edition)", p30, Op. Cit.
[20] Ibid.
[21] Welsh, M.J. & Smith, A.E. "Cystic Fibrosis", p 38, Op. Cit.
[22] "Gel Electrophoresis- student worksheet", *CSIRO Australia*, 1996.
[23] Kinnear, J. & Martin, M. "Nature of Biology Book Two", p 295, Op. Cit.
[24] "Genetic Engineering and Protein Synthesis", Op. Cit.
[25] Heffernan, D.A. "The Australian Biology Dictionary", *Longman Australia Pty Ltd*, 1995, p 248.

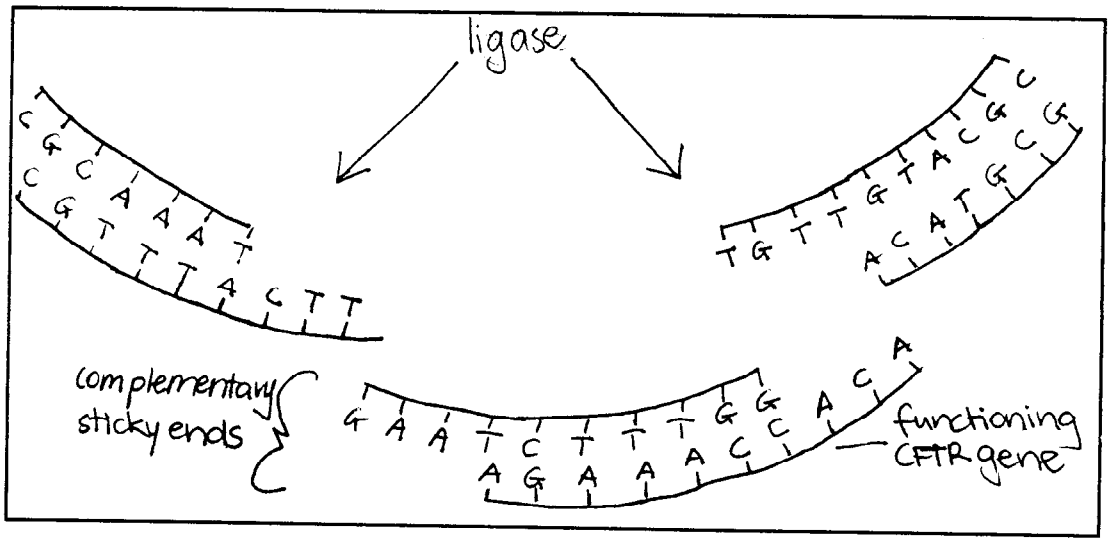

Figure 6[26]: The normal CFTR gene is being inserted into the plasmid.

[26] "Genetic Engineering and Protein Synthesis", Op. Cit.

The plasmids are inserted into bacteria which is plated out onto the antibiotics tetracycline and ampicillin agar plates respectively[27]. The non-recombinant plasmids are resistant to ampicillin and tetracycline. Bacteria with plasmids containing the CFTR gene, contain a gene resistant to ampicillin, but not tetracycline. When the plates are compared, successful recombinant plasmids (survived on ampicillin and not tetracycline) can be identified[28]. The bacterial colony resistant to ampicillin is selected and grown so replicas of the CFTR gene are obtained in plasmids.

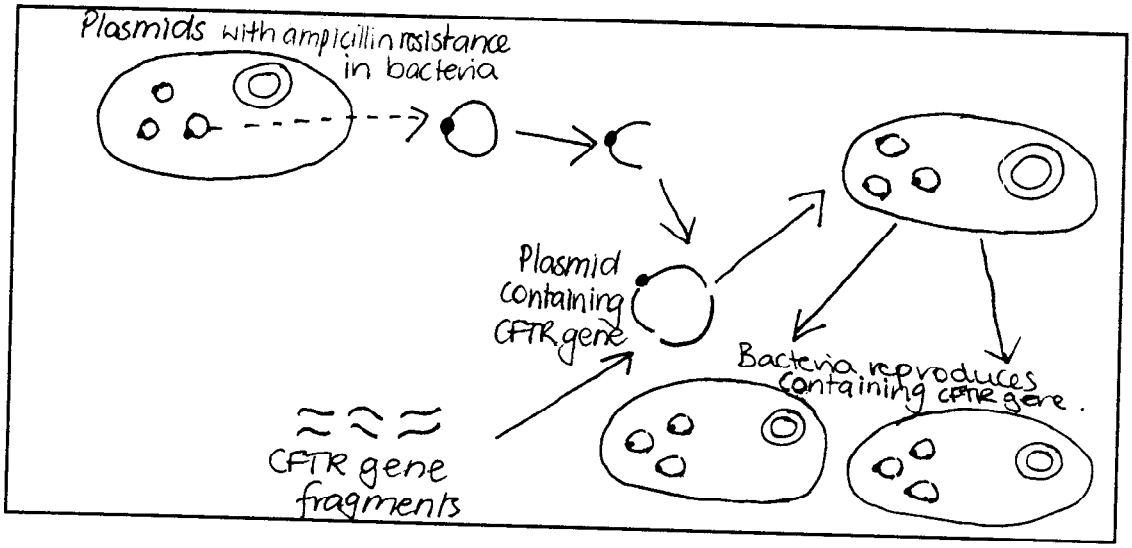

Figure 7[29]: The CFTR gene in the plasmid is multiplied as the bacteria undergoes binary fission.

[27] "Genetic engineering and protein synthesis", Op. Cit.
[28] Ibid.
[29] Verma, S. "Weird science looms", *The Education Age*, 30/7/96, p 10.

The CFTR gene is spliced into the adenovirus, after being debilitated by removing its genes controlling replication[30], using ligase and is introduced into the CF patient via a nasal spray[31]. The adenovirus, attracted to the epithelial cells of the lungs naturally[32], breaks down and releases the CFTR gene. The DNA is incorporated into the epithelial cells' genome where it transcribed and translated. The cells are now able to produce the normal CFTR protein and the amount of mucous is controlled[33].

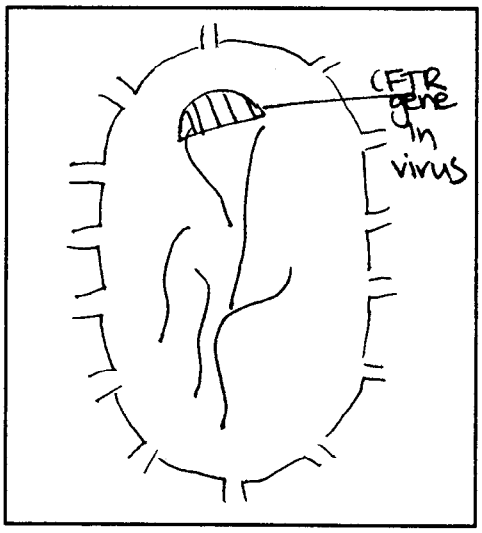

Figure 8[34]: Virus **containing the CFTR gene.**

[30] Lewin, R. "Gene therapy promises cure for cystic fibrosis", p 5, Op. Cit.
[31] Rosenfeld, M. "Gene therapy for cystic fibrosis", *CHEST*, Jan 1996, p249.
[32] Beardsley, T. "Clearing the airways- cystic fibrosis may be treated with gene therapy", p16, Op. Cit.
[33] Lewin, R. "Gene therapy promises cure for cystic fibrosis", p 5, Op. Cit.
[34] Bennington, T. & Propert, D. "Cystic Fibrosis Handbook (1996 edition)", p 31, Op. Cit.

BIOLOGICAL IMPLICATION

CF, a life threatening and crippling disorder involves intense pain and suffering inhibiting patients from participating in many of life's experiences such as sport and education[35]. With advancements in the development of gene therapy for humans, CF patients may be offered longer and more fulfilling lives. The quality of life for CF sufferers would be vastly improved by the introduction of a healthy gene into their cells, freeing them of the deleterious effects of the mucous build up in their lungs. Patients may even survive to adulthood if gene therapy is successful[36] encouraging them to lead active and positive lifestyles with plans for the future[37].

Although gene therapy sounds like the solution to CF, there are safety risks.The adenovirus(cold virus) used to introduce the CFTR gene into the patient may be harmful for although although the virus has been treated so it cannot replicate to cause infection, it is possible it will revert back to its disease-causing state[38] and cause inflammation in the lungs which is disastrous for a CF patient[39]. Also, part of the virus's protein is required to administer the CFTR gene, our immune system may have antibodies which would respond to the adenovirus and each treatment may result in a more severe immune response[40]. This may be more damaging than the CF.

---

[35] Bennington, T. & Propert, D. "Cystic Fibrosis Handbook (1996 edition)", p39, Op. Cit.
[36] Beardsley, T. "Clearing the airways- cystic fibrosis may be treated with gene therapy", p 16, Op. Cit.
[37] Bennington, T. & Propert, D. "Cystic Fibrosis Handbook (1996 edition)", p 39, Op. Cit.
[38] Brown, P. "Britain blazes an alternative trail for gene therapy", *New Scientist*, 15 Feb 1996, p 5.
[39] Aldhous, P. "Safer gene therapy in sight for cystic fibrosis", *New Scientist*, 7 Jan 1995, p 6.
[40] Crisp, J. "CYSTIC-L archive: re- gene therapy", [... it.edu/people/mernst/cf/cystic-l/950506/0161.html]

THE ISSUE

Should gene therapy trials involving the adenovirus as a treatment for CF be conducted on humans?

As CF is only found in humans, trials are necessary to develop and refine gene therapy for effective use in humans. However, the Cystic Fibrosis Association of Victoria, harbours concerns believing that such trials exploit the already vulnerable position that CF patients must live[41] and as implementation of gene therapy is at least 5 years away, money could be better spent improving the lives of current sufferers[42]. France's National Institute for Health and Medical Research believes more research needed to justify the risks of gene therapy trialed on humans especially the involvement of the adenovirus[43] and that rapid development of medical techniques like gene therapy means assessment of risks is inadequate.

Scientist, Ronald Crystal, leading trials at the National Heart, Lung and Blood Institute of the US, believes problems are inevitable as CF is a 'difficult disorder' and they are doing things 'nobody has tried to do before'[44]. Successful tests have involved mice, cotton rats, rhesus monkeys and baboons[45] and have all been deemed successful. Currently the patients trialing gene therapy at the University of Pennsylvania, have no side effects[46]. The information and insight obtained from the trials on humans is fundamental to curing CF which will be of immeasurable benefit to future generation sufferers.

---

[41] Bennington, T. & Propert, D. "Cystic Fibrosis Handbook (1996 edition)", p 44, Op. Cit.
[42] Ibid.
[43] Patel, T. "'Risks ignored' as medicine rushes ahead", *New Scientist*, 18 March 1995 p10.
[44] Brown, P. "Surprise illness halts gene therapy trial", *New Scientist*, 28 Aug 1996, p 5
[45] Wilson, J. "Gene therapy for cystic fibrosis: challenges and future directions", The American Society for Clinical Investigation, December 1995, p 2552.
[46] "Gene therapy update", [http://www.med.upenn.edu/~mednews/may94/7203-4.html]

BIBLIOGRAPHY

STANDARD BIOLOGY TEXTBOOKS

- Evans, B. et al, "Biology Two-survival mechanisms - continuity and change", *Heinemann Educational Australia*, Australia, 1995.
- Fung, S. "Biology study guide-units 3 and 4", *Longman* Cheshire, Australia, 1993.
- Heffernan, D.A. "The Australian biology dictionary", *Longman*, Australia, 1995.
- Kinnear, J. & Martin, M. "Nature of biology - book two", *The Jacaranda Press*, Queensland, Australia, 1993.
- Suzuki, D. & Knudtson, P. "Genetics - the clash between the new genetics and human values", *New Delta Enterprises*, USA, 1989.

ELECTRONIC MEDIA

- "Genetic engineering and protein synthesis video", CSIRO, 1992.

INTERNET ADDRESSES

- Crisp, J. "re-gene therapy", [...it.edu/people/mernst/cf/cystic-l/950506/0161.html]
- "Gene therapy update", [http://www.med.upenn.edu/~mednews/may94/7203_4.html]

HANDBOOK

- Bennington, T. and Propert, D., et al, "Cystic fibrosis handbook 1996 edition", *Cystic Fibrosis Association of Victoria*, The Jacaranda Press, 1995.

ARTICLES FROM SCIENTIFIC PUBLICATIONS

- Aldhous, P. "Safer gene therapy in sight for cystic fibrosis", *New Scientist*, 7 Jan 1995.
- Beardsley, T. "Clearing the airways-cystic fibrosis may be treated with gene therapy", *Scientific American*, volume 263(6), 1990, pp 14-16.
- Brown, P. "Surprise illness halts gene therapy trial", *New Scientist*, 28 Aug 1993, p 5.
- Brown, P. "Britain blazes at alternative trial for gene therapy", *New Scientist*, 15 Feb 1992, p5.
- Coghlan, A. "Gene dream fades away", *New Scientist*, 25 Nov 1995, pp 14-15.
- Coghlan, A. "Hidden costs of a clean inheritance", *New Scientist*, 14 May 1994, pp 14-15.
- Davies, K. "Cystic fibrosis: a quest for a cure", *New Scientist*, 7 Dec 1991, pp 22-26.
- Larrick, J.W. and Burck, K.L. "Gene therapy-Application of molecular biology", *Elsevier Science Publishing*, New York, 1991.
- Lewin, R. "Gene therapy promises cure for cystic fibrosis", *New Scientist*, 18 Jan 1992.
- Patel, T. "Risks ignored' as medicine rushes ahead", *New Scientist*, 18 Mar 1995, p 10.
- Rosenfeld, M.A. & Collins, F.S. "Gene therapy for cystic fibrosis", *CHEST*, January 1996, pp 241-252.
- Theodore, F. "Progress towards human gene therapy", *Science*, June 1989, pp1275-1280.

- Verma, I.M. "Gene therapy", *Scientific American*, November 1990, pp34-41.
- Webb, J. "Optimism over CF gene therapy", *New Scientist*, volume 137, 20 Mar 1993, p 7.
- Welsh, M.J. & Smith, A.E. "Cystic Fibrosis", *Scientific American*, December 1995, pp 36- 43.
- Wilson, J.M. "Gene therapy for cystic fibrosis: Challenges and future directions", *The American Society for Clinical Investigation*, Inc., volume 96, December 1995, pp2547-2554.

HANDOUTS

- CSIRO student worksheets - Gel electrophoresis and Transforming bacteria, 1996.

NEWSPAPER ARTICLES

- Chen, E. "Panel approves experimental gene therapy for cystic fibrosis", *Los Angeles Times*, 4 Dec 1992, p 3.
- Glausiusz, J. "Hunting down genes", *Sunday Herald Sun*, 18 Feb 1996, p 80.
- Verma, S. "Weird science looms", *The Education Age*, 30 July 1996, p 10.