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# Genetic Engineering and Cloning: Focus on Animal Biotechnology

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. What is genetic engineering?

Over the last 35 years the term genetic engineering has been commonly used not only in science but also in others parts of society. Nowadays this name is often associated by the media forensic techniques to solve crimes, paternity, medical diagnosis and, gene mapping and sequencing. The popularization of genetic engineering is consequence of its wide use in laboratories around the world and, developing of modern and efficient techniques. The genetic engineering, often used with trivia, involves sophisticated techniques of gene manipulation, cloning and modification. Many authors consider this term as synonymous as genetic modification, where a synthetic gene or foreign DNA is inserted into an organism of interest. Organism that receives this recombinant DNA is considered as genetically modified (GMO). Its production are summarized in simplified form in five steps: 1) Isolation of interested gene, 2) Construction, gene of interested is joined with promoters (location and control the level of expression), terminator (indicates end of the gene) and expression marker (identify the gene expression), 3) transformation (when the recombinant DNA is inserted into the host organism), 4) Selection (selection of those organisms that express the markers), 5) Insertion verification of recombinant DNA and its expression [1].

### 1.2. How to apply genetic engineering in our everyday

One of the main firstlings of genetic engineering is that genetic information is organized in the form of genes formed by DNA, which across some biotechnologies can be manipulated to be































































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## References

- [1] Nicholl DST. An Introduction to Genetic Engineering: Cambridge University Press; 2008.
- [2] Lewin B. Genes 8: Pearson Prentice Hall; 2004.
- [3] Satya P. Genomics and Genetic Engineering: New India Publishing Agency; 2007.
- [4] Brown TA. Genomes: Wiley-Liss; 2002.
- [5] Shapiro, J. L.; MacHattie, L. E.; Ippen, G. I. K.; Beckwith, J.; Arditti, R.; Reznikoff, W.; Mac-Gillivray, R. The isolation of pure lac operon DNA. *Nature*, 1969, 224:768-774.
- [6] Korman, A. J. Knudsen, P. J; Kaufman, J. F.; Strominger, J. L. cDNA clones for the heavy chain of HLA-DR antigens obtained after immunopurification of polysomes by monoclonal antibody. *Proceedings of National Academy of Science of United States of America*. 1982, 79:1844-1848.
- [7] Maxam, A. M.; Gilbert, W. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA*, 1977, 74 (2): 560-564.
- [8] Graham CA, Hill AJM. DNA Sequencing Protocols: Humana Press; 2001.
- [9] K GP, Gupta PPK. Biotechnology And Genomics: Rastogi Publications; 2004.
- [10] Perkin Elmer. Automated DNA Sequencing Chemistry Guide. Perkin Elmer Applied Biosystems. 1998, 3-22, 3-27
- [11] Strachan T. Human molecular genetics: Taylor & Francis; 2003.
- [12] Shinwari J. Automated DNA sequencing. *Megabace 1000*, 2005.
- [13] Kocher TD. PCR, directing sequencing and the comparative approach. *PCR methods and applications*. 1992:217.
- [14] Rao V. *Genome Res*. 1994;4:S15-S23.

- [15] Gupta PPK. *Molecular Biology and Genetic Engineering*: Rajpal And Sons Publishing; 2008.
- [16] Pease , A.C.; Solas, D.; Sullivan, E. J.; Cronin, M. T.; Holmes, C. P.; Fodor S.P. A. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. 1994; 91: 5022-5026.
- [17] Stears RL, Martinsky T, Schena M. Trends in microarray analysis. *Nat Med*. 2003 Jan; 9(1):140-5.
- [18] Chaudhuri, J.D. Genes arrayed out for you: the amazing world of microarrays. *Medical Science Monitor*, 2005; 11(2):52-62.
- [19] Rosa GJdM, da Rocha LB, Furlan LR. Estudos de expressão gênica utilizando-se microarrays: delineamento, análise, e aplicações na pesquisa zootécnica. *Revista Brasileira de Zootecnia*. 2007;36:185-209.
- [20] Jurinke C, Oeth P, van den Boom D. MALDI-TOF mass spectrometry: a versatile tool for high-performance DNA analysis. *Mol Biotechnol*. 2004 Feb;26(2):147-64.
- [21] Gut IG. DNA analysis by MALDI-TOF mass spectrometry. *Hum Mutat*. 2004 May; 23(5):437-41.
- [22] Mauger F, Bauer K, Calloway CD, Semhoun J, Nishimoto T, Myers TW, et al. DNA sequencing by MALDI-TOF MS using alkali cleavage of RNA/DNA chimeras. *Nucleic Acids Res*. 2007;35(8):e62.
- [23] Artificial gene synthesis [http://en.wikipedia.org/wiki/Artificial\\_gene\\_synthesis#cite\\_note-Edge81-2](http://en.wikipedia.org/wiki/Artificial_gene_synthesis#cite_note-Edge81-2) (accessed 1 September of 2012).
- [24] Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature*. 1970 Jun;226(5252):1211-3. PubMed PMID: 4316301. eng.
- [25] Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*: Cold Spring Harbor Laboratory Press; 2001.
- [26] Stemmer WP, Cramer A, Ha KD, Brennan TM, Heyneker HL. Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. *Gene*. 1995 Oct;164(1):49-53.
- [27] Young L, Dong Q. Two-step total gene synthesis method. *Nucleic Acids Res*. 2004;32(7):e59.
- [28] Clark DP, Pazdernik NJ. *Molecular Biology*: Academic Press; 2012.
- [29] Pratik, S. *Genomics and Genetic Engineering* New India Publishing, 2007.
- [30] Corley RB. *A Guide to Methods in the Biomedical Sciences*: Springer; 2004.

- [31] Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, et al. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proc Natl Acad Sci U S A*. 1992 Sep;89(18):8794-7.
- [32] BUY hpucbth. BUY, <http://people.ucalgary.ca/~browder/transgenic.html>. (accessed 3 September of 2012).
- [33] Working with viral vector [http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working\\_with\\_Viral\\_Vectors.pdf](http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf) (accessed 3 September of 2012).
- [34] Gene Cloning, chapter 7. <http://www.blackwellpublishing.com/genecloning/pdfs/chapter7.pdf>. (accessed 3 September of 2012).
- [35] Grabundzija I, Irgang M, Mátés L, Belay E, Matrai J, Gogol-Döring A, et al. Comparative analysis of transposable element vector systems in human cells. *Mol Ther*. 2010 Jun;18(6):1200-9.
- [36] Meir YJ, Wu SC. Transposon-based vector systems for gene therapy clinical trials: challenges and considerations. *Chang Gung Med J*. 2011 Nov-Dec;34(6):565-79. 37.
- [37] Giaca, M. *Gene Therapy*, Springer, 2010.
- [38] Pinkert, C.A., *Transgenic Animal Technology. A laboratory handbook*. 2<sup>nd</sup> Ed. Academic press, 2002.
- [39] Mello, M.R.B. ; Caetano, H. V. A.; Marques, M. G.; Padilha, M. S.; Garcia, J. F.; Assumpção, M.E.O.A.; Lima, A. S.; Nicácio, A. C.; Mendes, C. M.; Oliveira, V. P.; Visintin, J. A. Production of cloned calf from fetal fibroblast cell line. *Brazilian Journal of Medical and Biological Research*, 2003, 36: 1485-1489.
- [40] Kato, Y.; Tani, Y.; Sotomaru, Y.; Kurokawa, K.; Kato, J.; Doguchi, H.; Yasue, H.; Tsunoda, Y. Eight calves cloned from somatic cells of a single adult. *Science*, 1998, 282: 2095-2098.
- [41] Onishi, A.; Iwamoto, M.; Akita, T.; Mikawa, S.; Takeda, K.; Awata, T.; Hanada, H.; Perry, A.C. Pig cloning by microinjection of fetal fibroblast nuclei. *Science*, 2000, 289: 1188-1190.
- [42] Shin, T.; Kraemer, D.; Pryor, J.; Rugila, J.; Howe, L.; Buck, S.; Muphy, K.; Lyons, L.; Westhusin, M. A cat cloned by nuclear transplantation. *Nature*, 2002, 415: 6874-6859
- [43] Wakayama, T.; Perry, A.C.; Zuccotti, M.; Johnson, K.R.; Yanagimachi, R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*, 1998, 23:369-74.
- [44] Woods, G. L.; White, K. L.; Vanderwall, D. K.; Li, G.; Aston, K. I.; Bunch, T. D.; Meerdo, L. N.; Pate, B. J. A Mule Cloned from Fetal Cells by Nuclear Transfer. *Science*, 2003, 22: 1063.

- [45] Lee, B.C.; Kim, M.K.; Jang, G. O. H. J.; Yuda, F.; Kim, H.J.; Shamim, M.H.; Kim, J.J.; Kang, S.K.; Schatten, G.; Hwang, W.S. Dogs cloned from adult somatic cells. *Nature*, 2005, 436: 641-646.
- [46] Mello, M. R. B. Clonagem em Bovinos. In: Palhan, H.B. (ed.) *Reprodução em Bovinos. Fisiopatologia, terapêutica e Biotecnologia*. 2nd Ed. LF livros, 2008. p.225-233
- [47] Foote, H.R. Historical Perspective. In: Cibelli, J.; Lanza, P.R.; Campbell, K.H.S.; West, M.D. (ed.) *Principles of Cloning*. Academic Press, 2002. p. 3-14
- [48] Bordignon, V.; Henkes, L.E. Clonagem Animal: a busca da amplificação de cópias geneticamente modificadas. In: Collares, T. (ed.) *Animais Transgênicos. Princípios e métodos*. São Carlos, Suprema, 2005. p137-166.
- [49] Vajta, G.; Lewis, I.M.; Trounson, A.O.; Purup, S.; Maddox-Hyttel, P.; Schmidt, M.; Pedersen, H.G.; Greve, T.; Callesen, H. Handmade somatic cell cloning in cattle: analysis of factors contributing to high efficiency in vitro. *Biology of Reproduction*, 2003, 68: 571-578.
- [50] Transgenic Animals [http://en.wikipedia.org/wiki/Transgenic\\_animal#Transgenic\\_animals](http://en.wikipedia.org/wiki/Transgenic_animal#Transgenic_animals) (accessed 3 September of 2012).
- [51] Genetically modified mouse [http://en.wikipedia.org/wiki/Genetically\\_modified\\_mouse](http://en.wikipedia.org/wiki/Genetically_modified_mouse). (accessed 1 September of 2012).
- [52] Transgenesis history <http://www.transgenicmouse.com/transgenesis-history.php>. (Accessed 1 September of 2012).
- [53] Nagy, A.; Gertsenstein, M.; Vintersten, K.; Behringer, R. *Manipulating the mouse embryo. A laboratory manual*, 3<sup>rd</sup> ed. Cold Spring harbor Laboratory Press, 2003.
- [54] Houdebine, L.M. Métodos de gerar animais transgênicos e controle da expressão gênica In: Collares, T. (ed.) *Animais Transgênicos. Princípios e métodos*. São Carlos, Suprema, 2005. p 81-113.
- [55] Montoliu, L.; Lavado, A. Animais transgênicos na biologia, na biomedicine e na biotecnologia. In: Collares, T. (ed.) *Animais Transgênicos. Princípios e métodos*. São Carlos, Suprema, 2005. p 114-136.
- [56] Williams RS, Wagner PD. Transgenic animals in integrative biology: approaches and interpretations of outcome. *J Appl Physiol*. 2000 Mar;88(3):1119-26.
- [57] Sutovsky, P. *Somatic Cell Nuclear Transfer*, Springer, 2007, 591.
- [58] Williams, R. S.; Wagner, P. D. Transgenic animals in integrative biology: approaches and interpretations of outcome *J. Appl. Physiol*, 2000, 88: 1119–1126.
- [59] Gordon I. *Reproductive Technologies in Farm Animals*: CABI; 2005.
- [60] Houdebine LM. Cloning by numbers. *Nat Biotechnol*. 2003;21(12):1451-2.

- [61] Yanagimachi R. Cloning: experience from the mouse and other animals. *Mol Cell Endocrinol.* 2002;187(1-2):241-8. 62.
- [62] Miyashita N, Shiga K, Fujita T, Umeki H, Sato W, Suzuki T, et al. Normal telomere lengths of spermatozoa in somatic cell-cloned bulls. *Theriogenology.* 2003;59(7):1557-65.
- [63] FOX MW. Genetic Engineering Biotechnology 1: Animal Welfare and Environmental Concerns. *Applied Animal Behaviour Science.* 1988;20:83-94. Epub 94.
- [64] Frewer LJ, Howard C, Shepherd R. Public concerns in the United Kingdom about general and specific applications of genetic engineering: risk, benefit, and ethics. *Sci Technol Human Values.* 1997;22(1):98-124. PubMed PMID: 11654686. eng.
- [65] Hilgartner S. The Dominant View of Popularization: Conceptual Problems, Political Uses. *Social Studies of Science.* 1990;20(3):519-39. Epub 539.
- [66] Barth WL. Engenharia Genética e bioética .*Rev. Trim.,* 2005; v.35(149): 361-391.

