

seconds or microseconds) to a media containing the cells. The electrical pulses typically facilitate the non-lethal transport of extracellular nucleic acids into the cells. The exact electroporation protocols (such as the number of pulses, duration of pulses, pulse waveforms, etc.), will depend on factors such as the cell type, the cell media, the number of cells, the substance (s) to be delivered, etc., and can be determined by one of ordinary skill in the art.

[0159] In yet another set of embodiments, the nucleic acid may be delivered to the cell in a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the nucleic acid to the cell such that the nucleic acid can be processed and/or expressed in the cell. Preferably, the vector transports the nucleic acid to the cells with reduced degradation, relative to the extent of degradation that would result in the absence of the vector. The vector optionally includes gene expression sequences or other components able to enhance expression of the nucleic acid within the cell. The invention also encompasses the cells transfected with these vectors. Host cells include, for instance, cells and cell lines, e.g. prokaryotic cells (e.g., *E. coli*) and eukaryotic cells (e.g., dendritic cells, CHO cells, COS cells, yeast expression systems, and recombinant baculovirus expression in insect cells). Other cells have been previously described.

[0160] In general, vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the nucleotide sequence (or precursor nucleic acid) of the invention. Viral vectors useful in certain embodiments include, but are not limited to, nucleic acid sequences from the following viruses: retroviruses such as Moloney murine leukemia viruses, Harvey murine sarcoma viruses, murine mammary tumor viruses, and Rouse sarcoma viruses; adenovirus, or other adeno-associated viruses; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio viruses; and RNA viruses such as retroviruses. One can readily employ other vectors not named but known to the art.

[0161] Some viral vectors can be based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the nucleotide sequence of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA.

[0162] Genetically altered retroviral expression vectors may have general utility for the high-efficiency transduction of nucleic acids. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the cells with viral particles) can be found in Kriegler, M., *Gene Transfer and Expression, A Laboratory Manual*, W.H. Freeman Co., New York (1990) and Murry, E. J. Ed., *Methods in Molecular Biology*, Vol. 7, Humana Press, Inc., Clifton, N.J. (1991), both hereby incorporated by reference.

[0163] Another example of a virus for certain applications is the adeno-associated virus, which is a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. The adeno-associated virus

further has advantages, such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and/or lack of superinfection inhibition, which may allow multiple series of transductions.

[0164] Another vector suitable for use with the invention is a plasmid vector. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See e.g., Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. These plasmids may have a promoter compatible with the host cell, and the plasmids can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRC/CMV, SV40, and pBlueScript. Other plasmids are well-known to those of ordinary skill in the art. Additionally, plasmids may be custom-designed, for example, using restriction enzymes and ligation reactions, to remove and add specific fragments of DNA or other nucleic acids, as necessary. The present invention also includes vectors for producing nucleic acids or precursor nucleic acids containing a desired nucleotide sequence (which can, for instance, then be expressed or otherwise processed within the cell to produce antibodies). These vectors may include a sequence encoding a nucleic acid and an in vivo expression element, as further described below. In some cases, the in vivo expression element includes at least one promoter.

[0165] The nucleic acid, in one embodiment, may be operably linked to a gene expression sequence which directs the expression of the nucleic acid within the cell (e.g., to produce antibodies). The nucleic acid sequence and the gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription of the nucleic acid sequence under the influence or control of the gene expression sequence. A "gene expression sequence," as used herein, is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the nucleotide sequence to which it is operably linked. The gene expression sequence may, for example, be a eukaryotic promoter or a viral promoter, such as a constitutive or inducible promoter. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription, for instance, as discussed in Maniatis, T. et al., *Science* 236:1237 (1987), incorporated herein by reference. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in plant, yeast, insect and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes).

[0166] The selection of a particular promoter and enhancer depends on what cell type is to be used and the mode of delivery. For example, a wide variety of promoters have been isolated from plants and animals, which are functional not only in the cellular source of the promoter, but also in numerous other plant and/or animal species. There are also other promoters (e.g., viral and Ti-plasmid) which can be used. For example, these promoters include promoters from the Ti-plasmid, such as the octopine synthase promoter, the nopaline synthase promoter, the mannopine synthase promoter, and promoters from other open reading frames in the T-DNA, such as ORF7, etc. Promoters isolated from plant viruses include the 35S promoter from cauliflower mosaic virus (CaMV). Promoters that have been isolated and reported for