

NTC NEWSLETTER

SPRING/SUMMER 2011

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HYPERGRO™ PROCESS PATENTED

A US Patent issues May 17 for NTC's industry leading HyperGRO™ fermentation process, which is used to make plasmid DNA, the active ingredient in genetic medicines and vaccines. The HyperGRO™ process produces up to 2.6 grams per liter of highly purified DNA.

Previously, the industry leading methods reported only 50-200mg/L, less than 1/10th the yields attainable with HyperGRO™, according to, (cont. next page)

NTC/JHU BAG \$920K PHASE II SBIR GRANT

NTC has been granted a major research award, aimed at overcoming transgene silencing, a phenomenon that has dogged gene therapists and DNA vaccinologists for many years. The award, from the National Institutes of Health, came as a result of a successful Phase I study that resulted in improvements to non-viral gene therapy, including: anti-silencing elements (ASEs); gene translation enhancers; antibiotic-free vectors; a novel vaccine adjuvant; and DNA production enhancers, according to Clague Hodgson, NTC's President. Cont, pg. 3

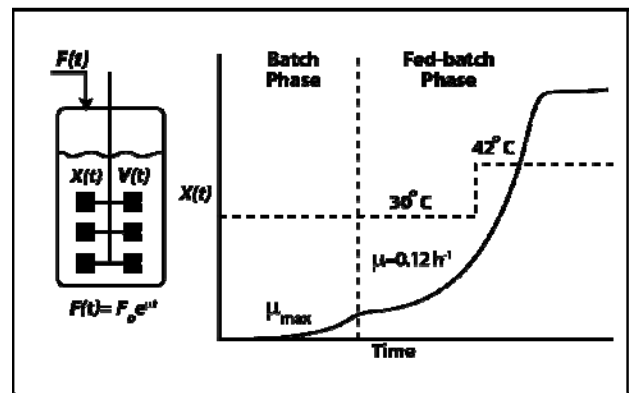
HYPERGRO™, CONT.

Aaron Carnes, who, along with NTC's Chief Scientific Officer, Dr. Jim Williams, are the inventors of the HyperGRO™ technology. "Already, government and contract manufacturing facilities have licensed the HyperGRO™ process to make GMP-grade plasmid DNA," said Carnes.

As of April, 2011, at least five of NTC's partner organizations are planning to make GMP-grade DNA for clinical trials with NTC's vectors. HyperGRO™ provides them with the purest DNA at the lowest possible price.

Plasmids are made in living bacterial cell factories by the gut bacterium *E. coli*, which produces up to a thousand copies of the small, circular DNA molecules per cell (see sidebar illustration). The key to the HyperGRO™ process is restricted exponential growth in optimized medium, followed by temperature shift, according to NTC's President, Dr. Clague Hodgson.

"A big advantage of HyperGRO™ is cost saving," said Williams, "However, the most important thing is improved product purity, which is the natural result of processing less biomass with HyperGRO™ than is required with other methods." Another advantage is reduced use of chemicals for processing the biomass, which makes HyperGRO™ the most environmentally sensitive plasmid process available, Williams said.



More about HyperGRO:

<http://natx.com/HyperGRO.html>

NTC IN PRESS (2011)

Luke, J., Vincent, J.M., Du, S.X., Whalen, B., Leen, A., Hodgson, C.P., and Williams, J.A. (2011). Improved Antibiotic-free plasmid vector design by incorporation of transient expression enhancers. *Gene Ther.* 18: 334-343

Luke, J., Simon, G.G., Söderholm, J., Errett, J.S., August, J.T., Gale, M. Jr., Hodgson, C.P., and Williams, J.A. (2011) Coexpressed RIG-I Agonist Enhances Humoral Immune Response to Influenza DNA Vaccine. *J Virol.* 85: 1370-1383

Carnes, A.E., Luke, J., Vincent, J.M., Schukar, A., Anderson, S., Hodgson, C.P., and Williams, J.A. (2011). Plasmid DNA Fermentation Strain and Process-Specific Effects on Vector Yield, Quality and Transgene Expression. *Biotechnol. Bioeng.* 108: 354-363

Luke, J., Carnes, A.E., Sun, P., Hodgson, C.P., Waugh, D.S., and Williams, J.A. (2011) Thermostable Tag (TST) protein expression system: engineering thermotolerant recombinant proteins and vaccines. *J. Biotechnol.* 151:242-50

Luke, J.M., Carnes, A.E., Hodgson, C.P., Williams, J.A. (2011) Vector Insert-Targeted Integrative Antisense Expression System for Plasmid Stabilization. *Mol. Biotechnol.* 47:43-49

NTC/JHU Bag \$920K Phase II SBIR Grant

"The work is reported in several publications, currently in press, from the R&D group led by Dr. Jim Williams, NTC's Vice President, Chief Scientific Officer and the Principal Investigator on the project, who also wrote the grant," Hodgson said.

Through collaboration with investigators at Johns Hopkins University (JHU) and other institutions, the effectiveness of non-viral gene therapy and DNA vaccination using NTC's vectors was demonstrated in mouse, rabbit, and pig models, as well as in the human *ex vivo* (cell therapy) mode.

NTC's collaborators on the Phase II work include two JHU spinoff companies: Gene Facelift, LLC; and Canton Biotechnologies, Inc.

Gene Facelift (led by JHU graduate and entrepreneur, Dr. Aaron Tabor) is a cosmetic treatment for ageing skin, aimed at stimulating skin cell growth and restoration. Dr. Tabor is also the inventor of a cutaneous delivery method and formulations, which are used

in combination with NTC's vectors to deliver skin growth factor genes.

"The genetic cosmetic for anti-aging is just our first step," said Dr. Tabor, "Our goal is to use the same gene transfer technology to help children battling inherited blistering skin disorders; paralyzed patients fighting pressure ulcers; and, burn victims struggling to control infection and grow new skin."

Canton, under the leadership of JHU investigator and surgeon, Dr. John Harmon, is developing treatments for wound healing and diabetic ulcers, combining proprietary JHU gene discoveries with electroporation delivery of the vectors. Dr. Harmon's laboratory has been working with DNA expression vectors to enhance wound healing for a decade.

"The NTC vectors are the best we have ever seen," said Dr. Harmon, "They have high transfection efficiency and extraordinary safety features. We are excited to be working with NTC to bring these vectors to the clinic."

Dr. John Harmon

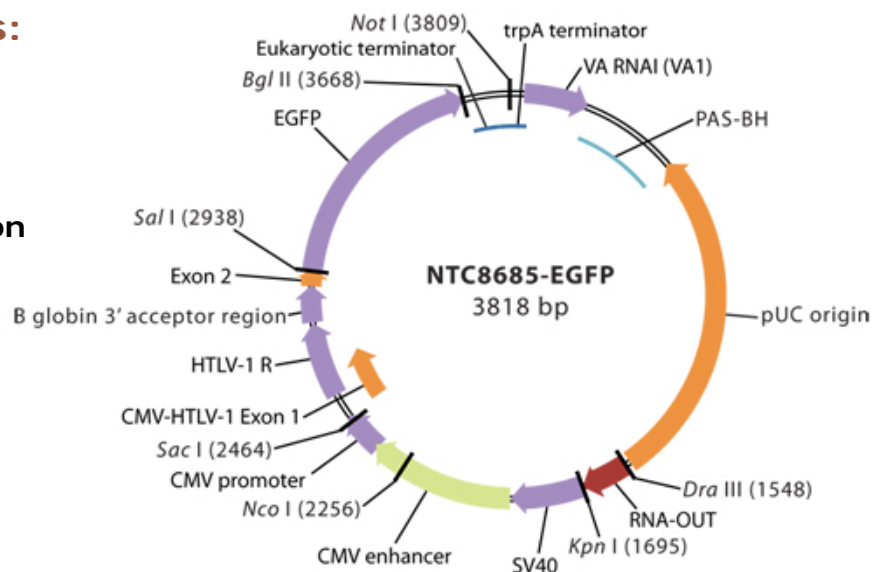
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NTC Vector Advantages:

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THERMOSTABLE PROTEIN EXPRESSION SYSTEM 'CHILLS-OUT' COLD CHAIN PROBLEMS FOR VACCINE MAKERS

Cold storage is a problem that nags the vaccine industry, especially in the third world, where vaccines are most badly needed. Ideally, it would be desirable to make protein 'subunit' vaccines that are thermo-tolerant, especially if it also makes the proteins more soluble, easier to purify, and more economical - all without negative effects on immunogenicity.

Researchers at Nature Technology Corp. (NTC) report (Luke *et al.*, *J. Biotechnol.*, 151:242-50, 2011) two discoveries to facilitate the above. First, a thermostable fusion partner (tag) has been created, based on Malto-dextrin Binding Protein (MBP), from the extremophile bacteria, *Pyrococcus furiosus*.

"To be effective, vaccines should activate both the innate and the adaptive arms of the immune system," said NTC president, Clague Hodgson. "Ideally, both the antigen and the adjuvant should be thermostable," he added.

The N-terminal tagged *Pfu*-MBP-antigens have increased solubility, impart thermostability on the conjugate antigens, can be easily purified by heat flocculation from *E. coli* production host cells, and are highly immunogenic. HA flu antigen made this way was superior in antibody production, compared to a DNA immunogen.

"To be effective, vaccines should activate both the innate and the adaptive arms of the immune system," said Dr. Clague Hodgson. "Ideally, both the antigen and the adjuvant should be thermostable," he added.

The second part of the invention is thermostable flagellin (adjuvant), the bacterial protein responsible for motility, which activates innate immunity through toll like receptor 5 (TLR5). The above cited article reports both thermostable *S. typhimurium* flagellin (native, no tag) as well as *Pfu*-MBP

-flagellin, both of which strongly activated TLR5-mediated innate immunity.

The antigen/adjuvant proteins were made using NTC's flagship protein expression system, pVEX. Surprisingly good product yields of 6g/L and 5g/L were obtained from fermentation cultures of the MBP-conjugate and flagellin proteins, respectively, according to Dr. Jim Williams, inventor of the technology.

NTC's thermostable flagellin products are available for immediate delivery.

Call 1 888 WIZ-BANG for more details.

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Thermostable Vaccine Components (larger quantities available by quotation):

HN-flagellin	1mg protein	\$335
HN-TST-Flagellin	1mg protein	\$335
HN-TST-HA2	1mg protein	\$335

'Impaired' Gene Constructs go to 'Plasmid Detox'

One of the most common and vexing problems in DNA manufacturing is the so-called 'toxic plasmid'. This is a plasmid that either contains genes that are expressed as toxic products in *E. coli*; or else they may contain cryptic promoters that express unintended toxic polypeptides from the DNA insert. In either case, they kill host cells, restrict growth, and are unstable.

The best solution, advocated by NTC's Chief Scientist and inventor, Jim Williams, is to make plasmids that are nontoxic. However, one of the most frequent mistakes in genetic engineering is failure to consider vector development at the earliest stages, according to Williams.

For those who plan ahead, NTC offers a selection of vectors and rapid cloning services. The vectors are optimized for regulatory compliance and gene expression. However, if the vector is now 'locked', the only option may be to use 'Plasmid-Detox'.

Plasmid Detox involves creating a new host strain expressing antisense RNA directed against the toxic product. The antisense construct is inserted into the host chromosome (NTC service using host chromosome engineering). Expression of the antisense is intended to block inappropriate expression of the toxic gene in the host cell during plasmid growth.

"In many cases, the toxic sequences can be determined empirically by looking at the construct design and considering the genes therein" said Williams, "However, if nothing 'jumps out' of the design as a candidate toxic gene, it is possible that it is a cryptic, promoter-driven toxic polypeptide." You may not know what parts are toxic, therefore, NTC will make detox construct covering the eukaryotic portion of the plasmid, and possibly two constructs will be made, in order to determine which strand of DNA is responsible.

Usually, therefore, NTC makes a preliminary assessment and screens for improved expres-

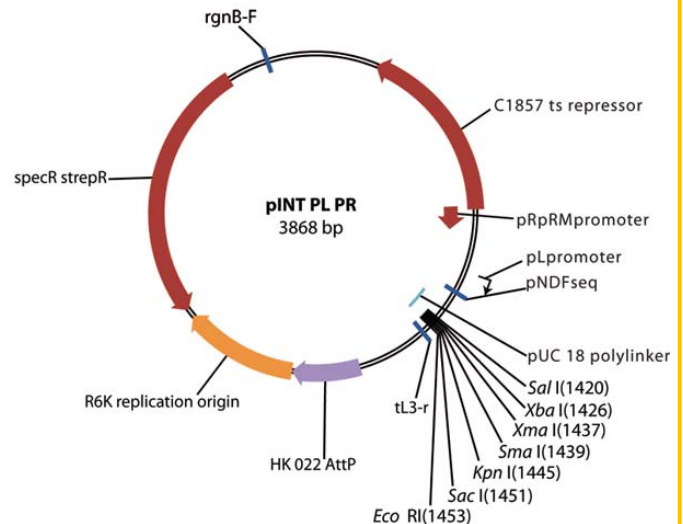
"NTC has an economical service for creating host strains designed to overcome plasmid-specific toxicity and instability."

sion. Often, approximately a four fold improved yield is possible, as for example, with a retroviral *gag-pol* plasmid or an influenza H1 antigen plasmid (both are known to be toxic when expressed in *E. coli*; Luke *et al.*, *Mol. Biotechnol.* 47:43-49, 2011).

NTC, has an economical service for creating host strains designed to overcome plasmid-specific toxicity and instability.

http://www.natx.com/pINT_PL_PRVectors.html

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Easy ~ NTC's pINT PL PR shuttle makes it fast and simple to knock down expression of toxic genes in *E. coli* during plasmid DNA production

Economical ~ \$1,249 includes: vector; cloning; strain engineering; and testing, Vector, \$299

Effective ~ Typical four-fold improvement in plasmid yield

Personalized DNA THERAPEUTICS FROM *E. COLI*

In addition to vectors for gene therapy and DNA vaccination, NTC offers complete vector development services, including insert design and optimization, vector construction using NTC's vectors, and production of DNA (or protein) products for preclinical testing. According to business development specialist and scientist, Justin Vincent, "NTC has a process for design, construction and production of custom gene biologics in the timeframe of a month."

Recently, the 'big pharma' model of drug discovery has failed to provide a viable pipeline of blockbuster drugs. "We are seeing increasing numbers of investigators from small biotech companies, and from academia, who are approaching NTC with exciting plans for treating infectious and metabolic diseases," said Vincent. "For those who are creating or developing plasmid-based DNA vaccines, gene therapeutics, or expressed recombinant proteins, NTC is an excellent consultant and partner," he said. "NTC's unique combination of technology and experience can be a valuable resource, from concept to pre-clinicals."

Once the test objects are in your hands, NTC will assist you in locating a GMP manufacturing facility that is best suited to your product needs, and will transfer the technologies (such as pVEX [protein expression] or HyperGRO [plasmid fermentation] to the facility, if necessary. "There are numerous monogenic diseases that are potentially amenable to gene therapy," said Vincent, "and NTC is prepared to assist those developing these therapies, both in design and manufacture."

"For those who are creating or developing plasmid-based DNA vaccines, gene therapeutics, or expressed recombinant proteins, NTC is an excellent consultant and partner. NTC's unique combination of technology and experience can be a valuable resource, from concept to pre-clinicals."

Justin Vincent
NTC Business Development Specialist
402.472.6530

Meetings

NTC is scheduled to participate at various scientific and industry meetings during 2011:

Vaccines and Adjuvants for Emerging and Infectious Diseases, Montego Bay, Jamaica
May 11-13, (Booth, Sponsor, Oral presentation by Jim Williams)

American Society for Gene and Cell Therapy (ASGCT), Seattle, WA
May 18-21, (Booth, Poster presentation)

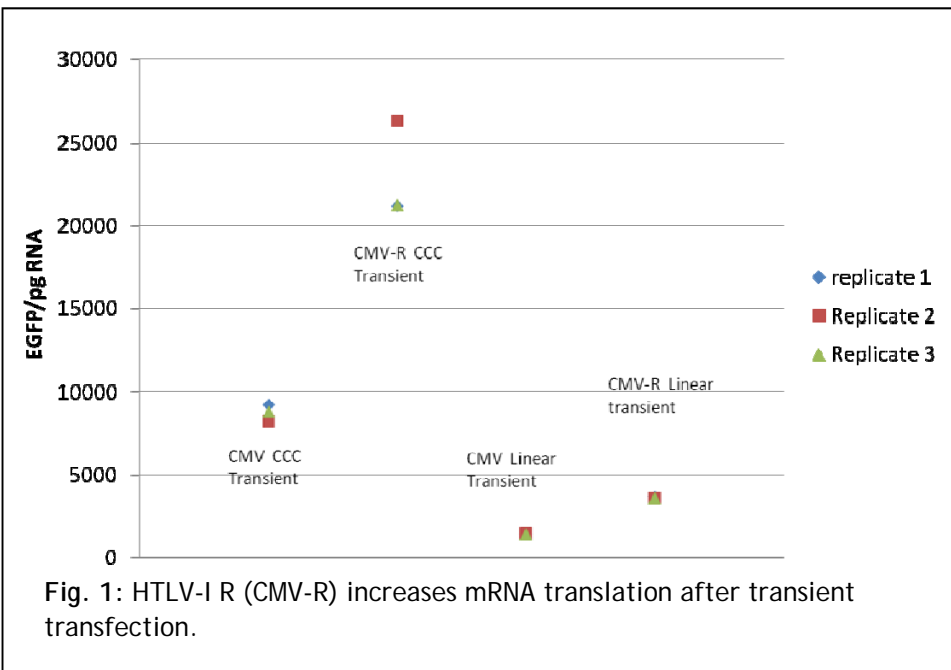
BIO 2011, Washington, DC
June 27-30, (NTC will be in the Nebraska Pavillion)

DNA Vaccines, San Diego, CA
July 12-14, (Booth, Sponsor)

Vaccine and ISV Annual Global Congress, Seattle, WA
October 2-4 (Booth, Sponsor)

TRANSIENT EXPRESSION ENHANCERS (TEEs): GETTING GENES TEE'D OFF

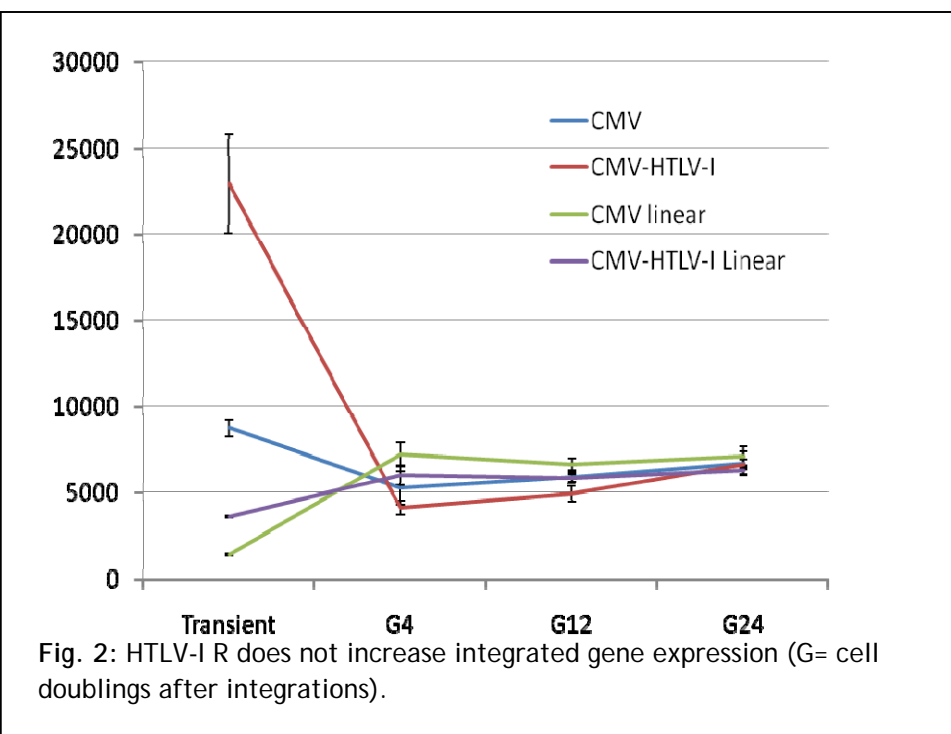
Improved, non-viral, plasmid DNA vectors are needed for safe and effective gene therapy and DNA vaccination. Now, NTC introduces highly effective vectors with TEEs, to further boost the magnitude and duration of transient expression in mammalian cells, using potent, minimal, antibiotic-free expression vectors with rationally designed, additive combinations of expression enhancers (Luke *et al.*, *Gene Ther.*, 18:334-343, 2011).



Combining an SV40-boosted CMV enhancer with HTLV-1 R region (downstream from the CMV enhancer) and Viral Associated 1 (VA1) RNA boosted the levels of transient expression (Fig. 1). However, HTLV-1 R and VA1 sequences did not boost expression after forced integration into genomic DNA (Fig. 2).

When combined with electroporation delivery, the vector platform further increased transient expression and improved HIV-1 gp120 vaccine-induced neutralizing antibody titers in rabbits.

“The antibiotic-free vectors incorporating TEEs are safer, more potent alternatives to improve transgene expression for DNA therapy or vaccination,” said Dr. Jim Williams, NTC’s CSO.





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NTC VECTORS: PRECISION CLONING

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Choice of NTC Vector	N/A	\$420
1g research grade DNA Prep	\$12,500*	\$9,995

TOTAL COST:	\$12,000*	\$10,835
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