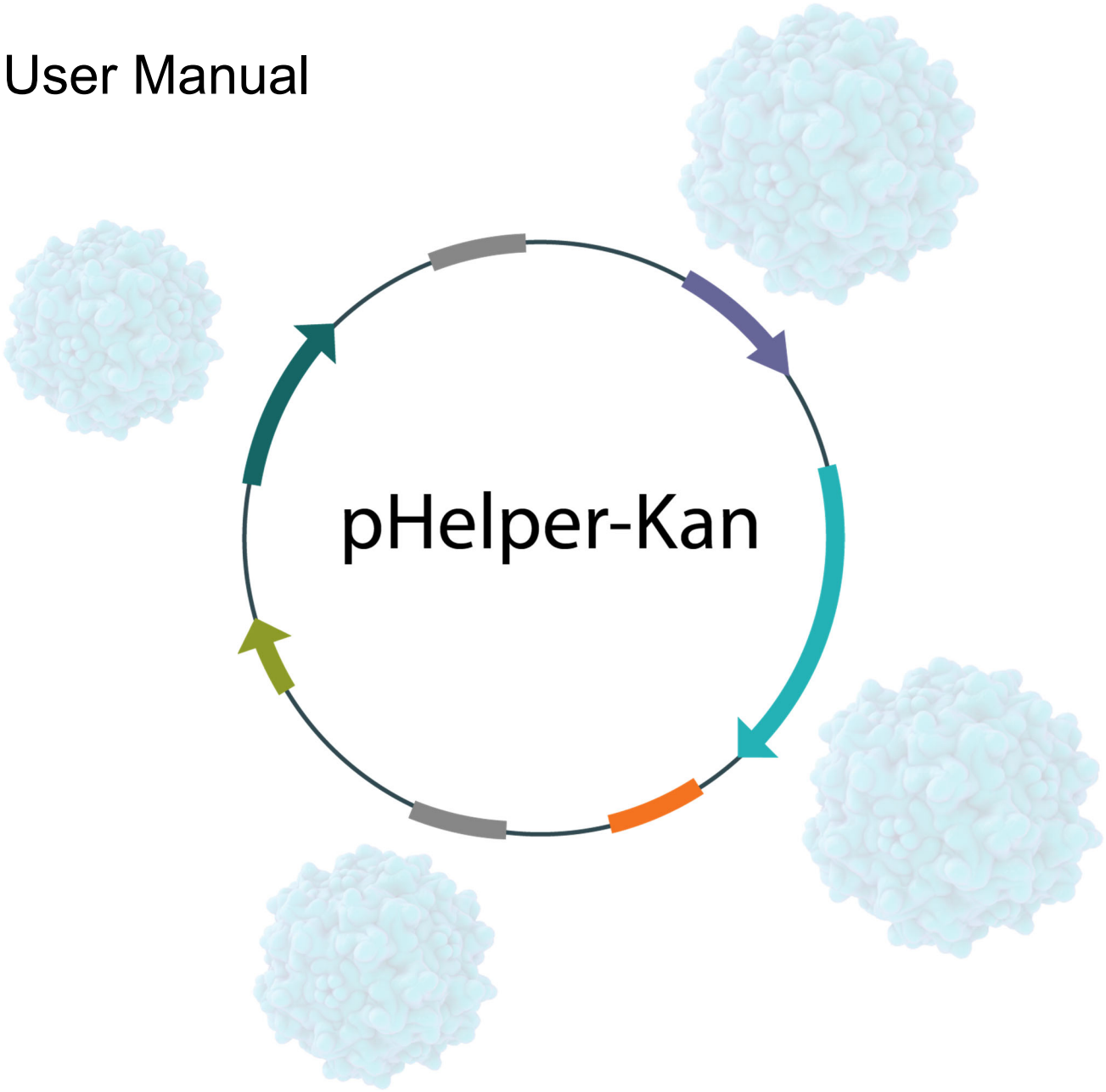
pHelper-Kan GMP-Ready™ Plasmid

User Manual



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Package Contents and Storage Conditions

Products	Catalog #	Shipping	Storage
pHelper-Kan, GMP-Ready™ helper plasmid, providing Ad helper function for AAV packaging	GR-PHELPER-KAN	Dry ice	-80°C

Related Products

AAV Custom Packaging Service – High quality, quick turnaround time
cGMP AAV Production – High-yield producer cells, experienced GMP team

Product Description

This plasmid -pHelper-Kan provides adenoviral helper genes, E2A, E4, VA for AAV packaging when combined with pRep/Cap and pGOI (gene of interest) in triple transfection HEK293 or 293T based AAV production. This ready-to-use GMP-Ready™ pHelper plasmid is meant to accelerate AAV cGMP manufacturing with a fully documented plasmid with IND filing information and a certificate of analysis (COA).

Features and Benefits

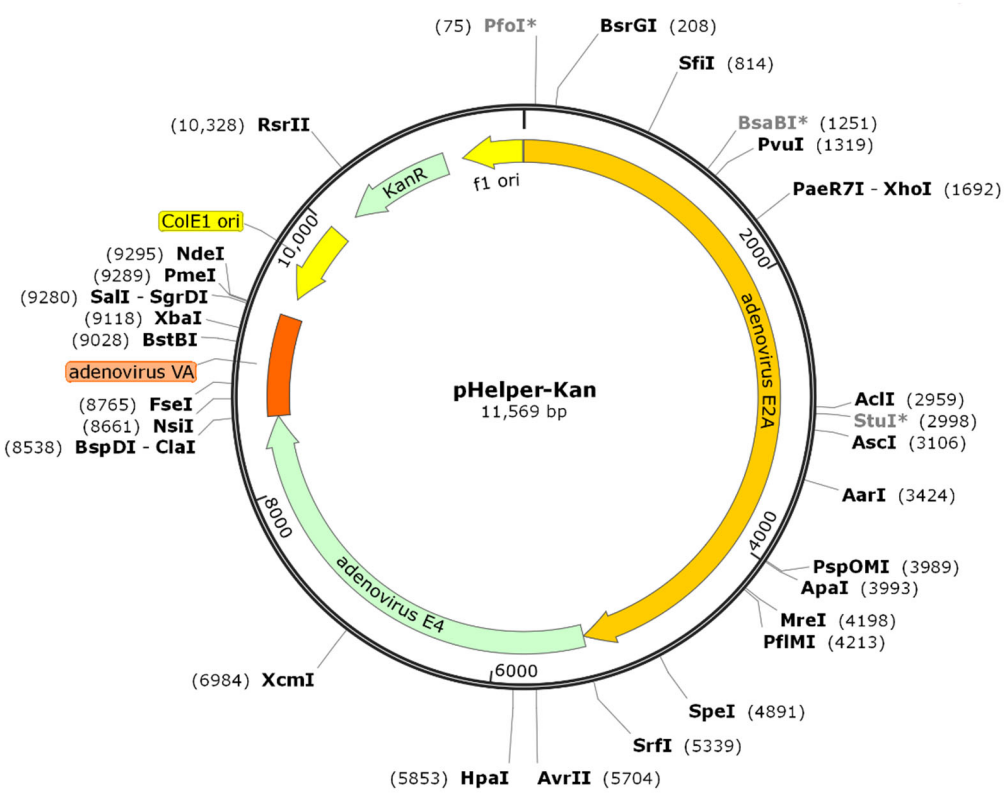
- **Save time** – In stock, immediately available
- **GMP-Ready™ grade –** production process control, material control, personnel control, and facility segregation from other productions.
- **GMP quality control (QC) testing**
- **Kanamycin resistance**
- **Animal component free production**
- **Proven for efficient AAV production**

GMP-Ready Plasmids Manufacturing

GMP-Ready™ plasmids may be used as critical raw materials for the production of clinical-grade viral vectors. GMP-Ready™ plasmid manufacturing is conducted using batch production records that outline the plasmid produced and release specifications (e.g., documentation, material segregation, traceability, etc.). Although GMP-Ready™ plasmids are not manufactured in a GMP suite, GMP-Ready plasmids are manufactured in dedicated clean-rooms during the entire production campaign with mandated cleaning changeovers before new production cycles.

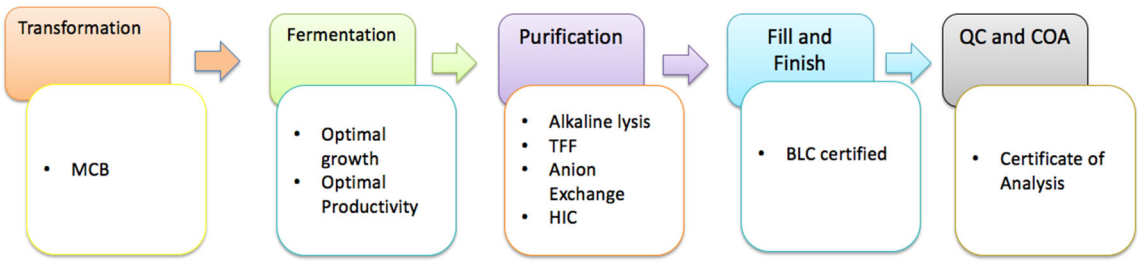
Plasmid Map of pHelper-Kan

Download sequence from web page: Vigenebio.com/phelper-kan-plasmid



Production Process & Release Testing

Production Process of pHelper-Kan GMP-R™ Plasmid



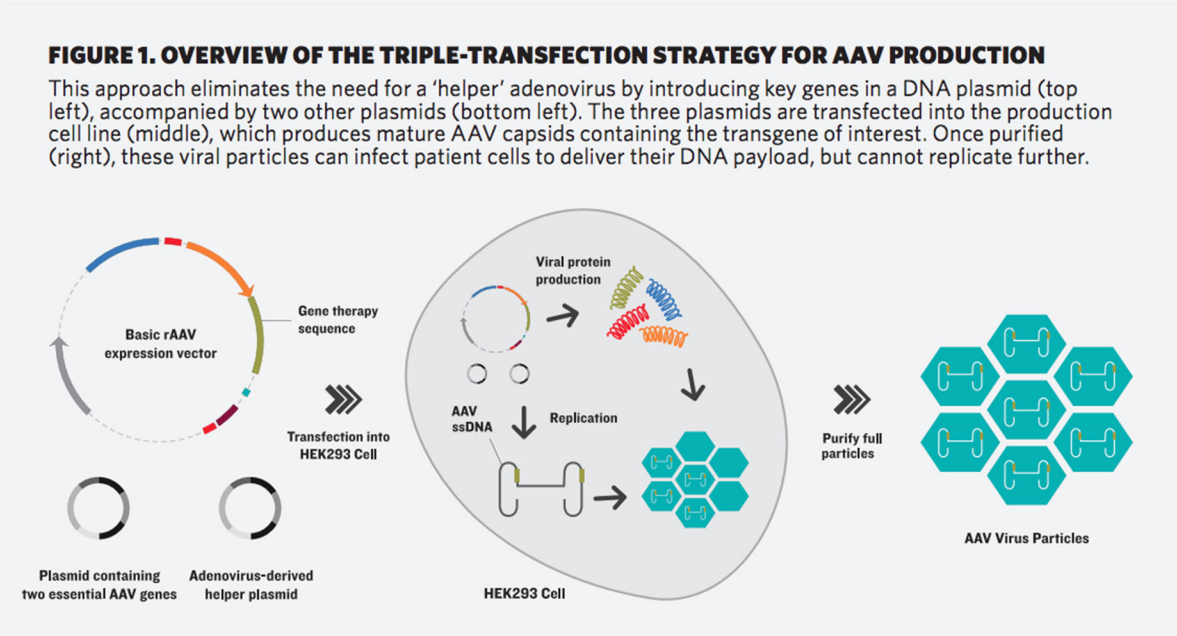
Controlled Aspects pHelper-Kan GMP-R Plasmid

- Establishment of E. coli master & working cell banks
- Full traceability of materials
- Full room changeover prior to each production
- Production in segregated and dedicated space
- Process & change control
- Aseptic fill/finish and vialing
- Document support for IND and IMPD filing
- Master batch records
- Product release tests

pHelper GMP-R Plasmid Release Testing

- **Safety**: Endotoxin, Sterility
- **Identity**: Restriction analysis & DNA sequencing
- **Purity**: UV spec, OD260/280
- **Purity**: Percentage of supercoiled
- **Purity**: Residual impurities, Host DNA, Host RNA, Host Protein
- **Purity**: Cross batch contamination test by Next-Generation Sequencing
- **Documentation**

Diagram of AAV Production Using Triple Plasmid Transfection Method



AAV Productivity using pHelper-Kan in Triple Transfection-based Production

The triple transfection based AAV production is depicted in Figure 1.

The robust virus packaging productivity of pHelper-Kan plasmid has been tested in almost all AAV serotypes. pHelper-Kan is being used in Vigene's daily AAV production, both GMP grade and research grade. Please see the productivity data for AAV2, AAV5, AAV6, AAV8, and AAV9 serotypes.

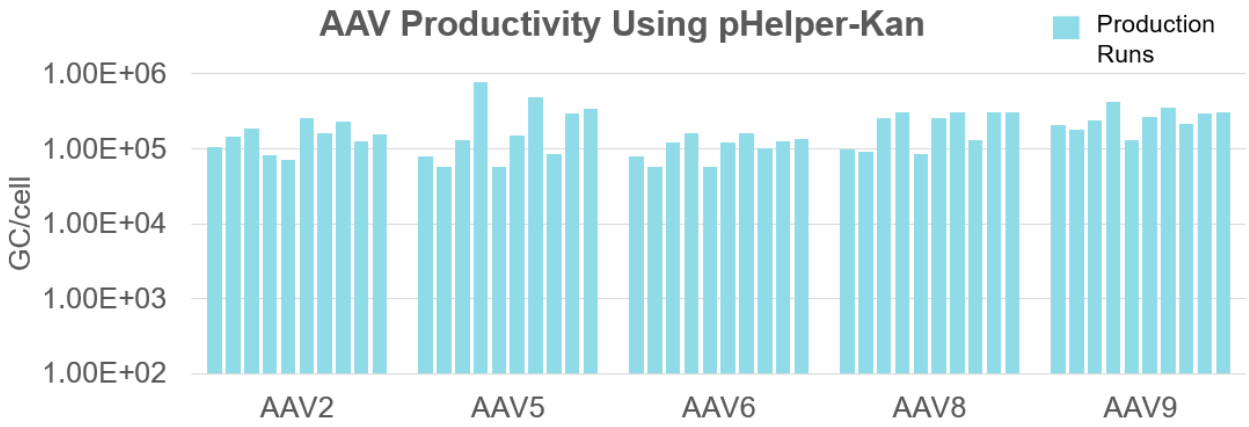


Fig. 2. pHelper-Kan was used in AAV production in adherent HEK293 cells. The productivity data for each AAV serotype is from 10 individual productions with different AAV transfer vectors containing unique genes of interest. pHelper-Kan was cotransfected with the AAV transfer vector and pRepCap for AAV packaging. Viral genome titer was measured using qPCR with primers targeting ITR.

Experimental Protocols

AAV Production With pHelper-Kan using Triple Plasmid Transfection Method

The protocol below may be used as a point of reference, in addition to, or in lieu of your own protocol. The sample protocol is for transfecting a 15 cm dish, which can be scaled up or down according to the corresponding culture surface area.

Day 1: Split and plate HEK293T cells

Split HEK293T cells 1:12 and seed cells in 15 cm dishes 72 hours before transfection. Cells should reach 80-90% of confluency in 72 hours. If transfection is performed 24 hrs or 48 hrs later, split cells with the ratios below. Grow cells in 5% CO_2 incubator at 37°C for 72 hours.

Split 1:3 if transfection in 24 hours.
Split 1:6 if transfection in 48 hours.

Note: For high packaging efficiency, the healthy status of HEK293T cells is critical. In addition, lower passage cells package virus more efficiently.

Day 4: Transfection

1. Change Media
   Before transfection, replace the culture media with 18 mL of fresh DMEM with antibiotics, no FBS.

2. Prepare transfection mix

   a) Add DNA to 2 mL Opti-MEM in a 5 mL sterile tube. The total DNA amount of transfer vector, pHelper-Kan, and pRepCap is 50 ug with the equal molar ratio of the 3 plasmids as 1:1:1, mix thoroughly.

   b) Add 150 uL PEI solution (1:3 ratio for DNA to PEI) to the diluted DNA.

   c) Mix the components and incubate at room temperature for 15 min.

   Note: For optimal transfection conditions, different amount of DNA and DNA:PEI ratio can be tested.

3. Add the transfection mixture to cells.
   Add the transfection mixture prepared in Step 2 dropwise to cells. Gently rock the plate back-and-forth and side-to-side to distribute the complex evenly.

4. Incubate the transfected cells for 72-96 hrs before harvesting the virus.

Day 7. Harvest virus

1. Scrape cells with cell scraper, transfer cells and media into a 50 mL conical tube.

2. Centrifuge at 1,000 g at 4°C to pellet cells.

   a) Transfer the supernatant into a new 50 mL tube. Keep the cell pellet for further processing.

   b) Precipitate virus from the supernatants in step a).

      1) Add 2.5 mL of 40% PEG stock solution (containing 40% PEG, 2.4% NaCl) to each 10 mL of supernatants. Mix and incubate it at 4°C for 2 hours to allow virus precipitation. Precipitation of the viruses can be proceeded overnight at 4°C if needed.
      2) Centrifuge at 3,000 g for 30 min, AAV viruses are spun down in the pellet. Discard the supernatants and resuspend the virus (small pellet) in 2 mL PBS + 0.01% pluronic F68. Pipette up and down to resuspend the virus completely.

   c) Process the cell pellet in step a).

      1) Resuspend the pellet in 2 mL PBS + 0.01% pluronic F68 + 200 mM NaCl.
      2) Freeze at -80°C for 2 hrs.
      3) Thaw the cell pellet at 37°C.
      4) Sonicate the mixture.

         Sonicate the mixture on ice, 2 pulses per second for 1 minute and 12 second until the lysates flow freely in tube. Avoid overheating the mixture during the sonication.

5) Centrifuge at 3,000g for 45 min. Combine the supernatants with the precipitated virus in step b). Pipette to mix.

3. Purify virus via iodixanol ultracentrifugation or other purification methods. The virus solution can be stored at -80°C for purification at a later time.

Vigene Biosciences Terms and Conditions of Sale

The Terms and Conditions apply to catalog GMP plasmids and GMP-Ready™ plasmids.

1. Orders and Delivery

All orders are subject to our acceptance and availability of the products as listed on the website. We reserve the right to make delivery in installments and separately invoice each shipment. Delay in delivery of any installment shall not relieve Customer of Customer's obligation to accepting the remaining deliveries. Any changes that you may propose to the quantities, specifications, method of delivery and shipment must be provided to us in writing and may be accepted by us at our discretion. With further notice, we reserve the right to change product specifications that do not materially affect the quality or performance of the products.

Vigene Biosciences is committed to deliver your order as quickly, efficiently, and cost-effectively as possible. Delivery terms shall be FOB Vigene origin; freight cost(s) will be determined upon departure of our facility and will be invoiced. Vigene shall not be liable for any delay, loss or damaged to the Product during the course of shipment caused by acts beyond our reasonable control, including but not limited to accidents, theft, vandalism and nature disasters. You will receive a separate Sales Order Confirmation indicating estimate delivery time(s) within 24 business hours of submitting your order. However, due to unforeseen difficulties inherent to the nature of cloning work, the delivery time provide are only estimates and subject to change.

Buyer(s) may be responsible for the shipping charge(s) and may be added to customer's invoice. Shipping cost(s) may vary depending on the shipping requirements. Our Customer Service Representatives will confirm the shipping method, cost and schedule at the time of order and shipment of the goods. Please contact us at support@Vigenebio.com for support.

2. Product Pricing and Payment Terms

Despite our diligent efforts to maintain the same price for each product and display it correctly, Vigene reserves all rights to correct any errors in pricing prior to shipping and billing. Costumers are obligated to pay the price and other charges as set forth in the order confirmation. Please contact us if you suspect an error in pricing. You will typically receive an order confirmation within 24 business hours of placing the order, price of the product and service will be given to you. Final unit price will be based on the price listed in the confirmation email.

Please note that price we quote for you will not include any appropriate taxes, duties, levies or other government fees, standard delivery and handling charges that may apply to your order. It is Customer's responsibility to incur all these expenses. If the above expenses were to front by Vigene, they will be included in the final invoice.

Unless a purchasing account and a credit limit have been established, a credit card to be charged upon shipment is required for all orders. For open account shipments, payment terms are net 30 days from date of invoice in US dollars. Each order is a separate transaction, and you may not set-off payments from one order against another. If you fail to pay any invoice when due, Vigene reserves the rights to charge you a late payment charge equal to the lesser of one and one-half percent (1½%), or the maximum permissible rate under Maryland law, per month on the past-due balance, without prejudice to our other lawful remedies. If the account or any part thereof is referred for collection, you agree to pay all collection costs and attorney's fees. Vigene also reserves the rights to suspend delivery or cancel the order or Contract, reject Customer's future orders if a bad debt account were to be caused by the Customer.

3. Inspection

Upon receipt of the Products, it is Customer's responsibility to inspect the goods. Customer may only return products that are damaged or defective on delivery, or any shortages or delivery errors within 10 days of receiving the shipment. It is Customer's sole responsibility to use our products in accordance with our instructions. Vigene will not be responsible for any damages caused by improper use of the Product. Products claimed to be damaged on delivery must be returned to our facility in the conditions shipped, the case shall be investigated by Vigene first before a replacement unit will be issued.

If Customer fails to notify Vigene within 10 days of receiving the goods, it is deemed that Customer has conformed to these Terms and Conditions and accepted the goods. Authorization for all product returns, exchanges or related activities must be approved by Vigene and a case authorization number will be given to the Customer prior to the return, exchange or related activities of goods. Due to nature of the Product, temperature and packing requirements, not all items are eligible for return, exchange or related activities. Vigene reserves all rights to determine the return, exchange or related activities' eligibility.

Custom products and service that we make in accordance upon Customer's request and specifications cannot be returned, unless it fails to meet the given specifications stated in the order confirmation upon substantial evidence. Vigene reserves the rights to determine the case and approve the case for exchange or adjustments based our sole discretion.

All authorized returns must arrive at our facility in a satisfactory state, in order to qualify for a valid case. No shipping charges, including ongoing and forwarding, will be credited. A restocking charge of 25% may be applied on returns not caused by Vigene's manufacturing and packaging mistakes.

4. Credits and Refund Policies

For catalog GMP plasmids and GMP-ready plasmids, products cannot be returned after they are shipped due to GMP regulations. Pursuant to Section 3, customer agrees to pay the full amount for the product post inspection period.

5. Limitation of Liability

Disclaimer of Consequential Damages. NEITHER PARTY SHALL BE LIABLE FOR ANY SPECIAL, INDIRECT, COLLATERAL, INCIDENTAL, PUNITIVE OR CONSEQUENTIAL LOSS OR DAMAGES OR FOR ANY LOST PROFITS OR LOSS OF OPPORTUNITY IN CONNECTION WITH OR ARISING OUT OF THIS AGREEMENT REGARDLESS OF ANY NOTICE OF THE POSSIBILITY OF SUCH DAMAGES OR EVEN IF THE OTHER PARTY IS NEGLIGENT INCLUDING BUT NOT LIMITED TO GMP VIRAL VECTOR PRODUCTIONS USING THE PLASMIDS . Any amounts payable to a third party pursuant to Section 6 shall be considered direct damages for purposes of this Section.

The foregoing limitations on liability shall not apply to any liabilities resulting from fraud, intentional misconduct, or gross negligence by either party or breach of applicable laws by either party.

6. Indemnification

Indemnification by Vigene. Vigene will indemnify, defend and hold harmless Client, its Affiliates, and its and their respective officers, directors, employees and agents (collectively, the "Client Indemnitees") against any third party claims, including reasonable attorneys' fees for defending those claims, to the extent such claims arise out of or relate to (a) performance of Services (except to the extent such claims result from Client's breach of this Agreement or a Client Indemnitee's negligence or willful misconduct); (b) any Vigene Indemnitee's negligence or willful misconduct in performing obligations under this Agreement; or (c) Vigene's breach of this Agreement.

Indemnification by Client. Client will indemnify, defend and hold harmless Vigene, its Affiliates, and its and their respective officers, directors, employees and agents (collectively, the "Vigene Indemnitees") against any third party claims, including reasonable attorneys' fees for defending those claims, to the extent such claims arise out of or relate to (a) the use of the Deliverables by Client or its Affiliates (except to the extent such claims result from Vigene's breach of this Agreement or a Vigene Indemnitee's negligence or willful misconduct); (b) any use of the Client Materials by Vigene to perform the Services (c) any Client Indemnitee's negligence or willful misconduct in performing obligations under this Agreement; or (d) Client's breach of this Agreement; or (e) Client's breach of applicable laws and regulations.

Indemnification Procedures. Each party must notify the other party within thirty (30) days after receipt of any claims made for which the other party might be liable under this Section, as applicable. The indemnifying party will have the sole right to defend, negotiate, and settle such claims. The indemnified party will be entitled to participate in the defense of such matter and to employ counsel at its expense to assist in such defense; provided, however, that the indemnifying party will have final decision-making authority regarding all aspects of the defense of the claim. The indemnified party will provide the indemnifying party with such information and assistance as the indemnifying party may reasonably request, at the expense of the indemnifying party. Neither party will be responsible for or be bound by any settlement of any

claim/suit made without its prior written consent; provided, however, that the indemnified party will not unreasonably withhold or delay such consent.

7. Proprietary Rights

Notwithstanding the foregoing, Vigene will retain full ownership rights in and to all templates, programs, methodologies, processes, technologies and other materials developed or licensed by Vigene and its Affiliates prior to or apart from performing its obligations under this Agreement and the modifications and improvements thereto (collectively, with all associated intellectual property rights, the "Vigene Property"), regardless of whether such Vigene Property is used in connection with Vigene's performance of its obligations under this Agreement.

8. Confidentiality

Definition. "Confidential Information" means any and all non-public scientific, technical, financial or business information, or data in whatever form (written, oral or visual) that is (a) furnished or made available by one party (the "Discloser") to the other (the "Recipient") or developed by Vigene in connection with Services; and (b) if Client is the Discloser, such information (i) if in tangible form, is labeled in writing as proprietary or confidential; or (ii) if in oral or visual form, is identified as proprietary or confidential at the time of disclosure or within fifteen (15) days after such disclosure. Confidential Information of Client includes (x) Client Materials, Deliverables and Records; (y) development and marketing plans, regulatory and business strategies, financial information, and forecasts of Client; and (z) all information of third parties that Client has an obligation to keep confidential.

Obligations. During the term of this Agreement and for a period of ten (10) years thereafter (and in the case of trade secrets, until such time as Discloser no longer treats such information as a trade secret), Recipient agrees to (a) hold in confidence all Discloser's Confidential Information, and not disclose Discloser's Confidential Information without the prior written consent of Discloser; (b) use Discloser's Confidential Information solely to carry out Recipient's rights or obligations under this Agreement; (c) treat Discloser's Confidential Information with the same degree of care Recipient uses to protect Recipient's own confidential information but in no event with less than a reasonable degree of care; and (d) reproduce Discloser's Confidential

Information solely to the extent necessary to carry out Recipient's rights or obligations under this Agreement, with all such reproductions being considered Discloser's Confidential Information.

Permitted Disclosures. Recipient may provide Discloser's Confidential Information solely to its employees or contractors (but if Recipient is Vigene, then solely to Vigene Personnel on a need-to-know basis and solely as necessary to carry out Recipient's rights or obligations under this Agreement; provided, that Recipient remains liable for the compliance of such employees or contractors (or if Vigene is Recipient, the compliance of such Vigene Personnel) with the terms of this Agreement.

Exceptions. Recipient's obligations of non-disclosure and non-use under this Agreement will not apply to any portion of Discloser's Confidential Information that Recipient can demonstrate, by competent proof:

I. is generally known to the public at the time of disclosure or becomes generally known through no wrongful act on the part of Recipient;
II. is in Recipient's possession at the time of disclosure other than as a result of Recipient's breach of any legal obligation;
III. becomes known to Recipient on a non-confidential basis through disclosure by sources other than Discloser having the legal right to disclose such Confidential Information; or
IV. is independently developed by Recipient without reference to or reliance upon Discloser's Confidential Information.

If Recipient is required by a governmental authority or by order of a court of competent jurisdiction to disclose any Confidential Information, Recipient will give Discloser prompt written notice of such requirement or order and Recipient will take all reasonable and lawful actions to avoid or minimize the degree of such disclosure. Recipient will cooperate reasonably with Discloser at Discloser's expense in any efforts to seek a protective order.

Each Party agrees that its obligations hereunder are necessary and reasonable in order to protect the other party and the other party's business, and expressly agrees that monetary damages would be inadequate to compensate the other party for any breach of the terms of this Agreement. Accordingly, each party agrees and acknowledges that any such violation or threatened violation may cause irreparable injury to the other party, and that, in addition to any

other remedies that may be available, in law, in equity or otherwise, the other Party shall be entitled to seek injunctive relief against the threatened breach of this Agreement or a Statement of Work or the continuation of any such breach, without the necessity of proving actual damages or posting bond.

9. MATERIAL TRANSFER AGREEMENT

By accepting the goods and services from VIGENE, CUSTOMER agrees that the materials received will not be propagated duplicated or reverse engineered in any way without written permission from VIGENE.