



## DATASHEET

# PrecisIOn™

## hnAChR $\alpha 1/\beta 1/\delta/\epsilon$ -HEK

# Recombinant Cell Line

CATALOG # CYL3052  
REVISION # M04

### ORDERING INFORMATION AND TECHNICAL SERVICES

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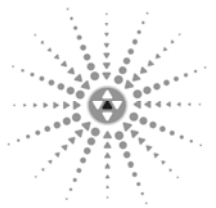
GPCR



Ion Channels



Kinase /  
Phosphatase



Safety & Toxicity  
Profiling

**Product description:**

Recombinant HEK293 cell line expressing the four subunits  $\alpha 1$ ,  $\beta 1$ ,  $\delta$  and  $\epsilon$  of the human nicotinic acetylcholine receptor type I.

**Format:**

2 x 1 ml aliquots containing  $3.34 \times 10^6$  cells/ml in 10% DMSO at passage 11.

**Mycoplasma Testing:**

The cell line has been screened using the MycoSensor™ PCR Assay Kit (Stratagene) to confirm the absence of Mycoplasma species.

**Functional Validation:**

HEK293 cells expressing hnAChR  $\alpha 1/\beta 1/\delta/\epsilon$  were characterised in terms of their pharmacological and biophysical properties using whole-cell patch clamp techniques and FLIPR calcium assay.

Using whole-cell patch clamp techniques, the mean peak current in response to 100  $\mu$ M ACh was 1418 pA. The mean pEC<sub>50</sub> value for the ACh-elicited current was 4.9. D-tubocurarine (10  $\mu$ M) blocked the 100  $\mu$ M ACh-elicited response by a mean of 94%.

In FLIPR calcium assays the agonists epibatidine, succinylcholine and nicotine had pEC<sub>50</sub> values of 6.3, 5.5 and 4.4 respectively. The antagonists pancuronium, D-tubocurarine and mecamylamine blocked the 60  $\mu$ M succinylcholine agonist response with a pIC<sub>50</sub> values of 6.4, 6.3 and 4.5 respectively.

Functional channel expression over time was monitored using FLIPR calcium assays. Channel expression is robust over at least 30 passages. At passage 30 the fluorescence signal window was >11,000 with a Z' value of approximately 0.7.

**Recommended Culture Conditions:**

Recommended culture conditions and standard operating procedure are provided with the product.

**Licensing Statements**

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242, USA.

The bovine growth hormone (bgh) polyadenylation signal and its use in the expression of recombinant proteins is covered by claims listed in U.S. Patent No. 5,122,458, EU Patent No. 0 173 552 and Japanese Patent No. 1955752 (collectively "CLAIMED DNA and/or CLAIMED CELLS") owned and licensed to Millipore (formerly Upstate Biotechnology Inc.) by Research Corporation Technologies, Inc., 101 North Wilmot Road, Suite 600, Tucson, AZ 85711-3335 ("RCT").

Use of this technology is restricted to research purposes only. The purchased/licensed cell line and all bacteria, phages and plasmids derived from this cell line, in whole or in part, and all proteins expressed from the cell line shall be used for research uses only. "Research purposes" means uses directed to the identification of useful recombinant proteins and the investigation of the recombinant expression of proteins. In no event shall research use include any of the following: any use in humans of a CLAIMED DNA or CLAIMED CELL; any use in human or protein expression or other substance expressed or made at any stage of its production that use the CLAIMED DNA or a CLAIMED CELL; or any use in which a CLAIMED DNA or CLAIMED CELL would be sold or transferred to a third party. No license, other than research use, is expressed or implied by the purchase/license of the cell line. By accepting or using Millipore's cell line product, you agree to be bound for the following use/license restrictions:

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**Electrophysiological Properties of the nAChR Current.****Conventional Whole-Cell Patch Clamp Electrophysiology.**

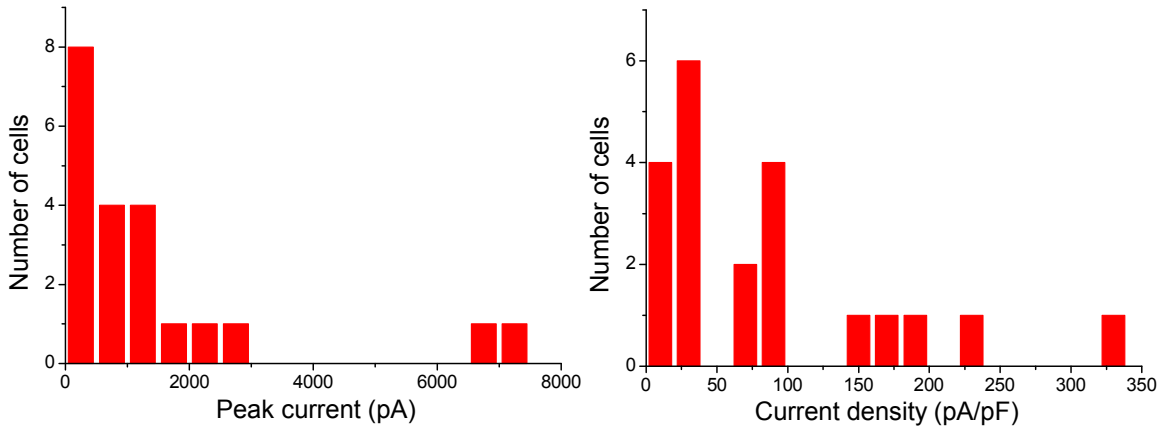
Membrane currents were recorded from cells using the conventional whole-cell recording. The intracellular solution contained (mM) 150 CsCl, 2 EGTA, and 10 HEPES, pH 7.3 (with CsOH). The external solution was (mM) 140 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 10 glucose, and 12 HEPES, pH 7.35 (with NaOH). Agonists were applied using fast perfusion systems. The holding potential was -60 mV throughout the experiment. Cells were stimulated with 1-2 s pulses of agonist delivered at intervals  $\geq 1$  minute.

**Peak Current Analysis:**

100  $\mu$ M ACh was used to activate the muscle nAChR mediated current. All cells (n=21) displayed a current in response to the acetylcholine (ACh) application. The mean peak current amplitude was  $1418 \pm 432$  pA (mean  $\pm$  SEM), the mean steady-state current amplitude was  $870 \pm 269$  pA (mean  $\pm$  SEM) and the mean peak current density  $86 \pm 18$  pA/pF (mean  $\pm$  SEM). The distribution of peak current amplitudes and densities are shown in **Figure 1**.

**Figure 1. Peak current amplitude and peak current density distributions.**

Data obtained from patch clamp recordings from hnACh  $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 cells (n=21, bin sizes 500pA and 20pA/pF respectively).



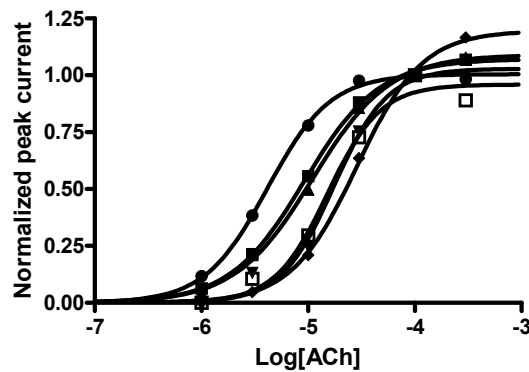
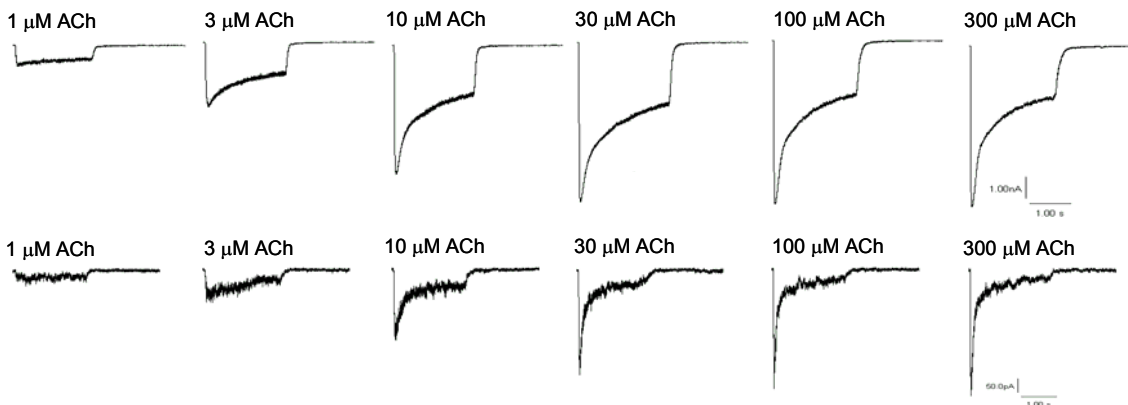
**Pharmacology – Acetylcholine and D-tubocurarine:**

Dose-response curves for acetylcholine (ACh) were obtained (**Figure 2**). Peak current responses to increasing concentrations of ACh were normalized to the current response elicited in the same cell by the application of 100  $\mu$ M ACh. The  $pEC_{50}$  values ranged from 5.4 to 4.5 with a mean value of  $4.92 \pm 0.12$  ( $n=6$ ). The nAChR  $\alpha 1/\beta 1/\delta/\epsilon$  current elicited by 100  $\mu$ M ACh was blocked by 10  $\mu$ M D-tubocurarine by  $94.2 \pm 3\%$  (mean  $\pm$  SEM,  $n=6$ , peak current measurements).

**Figure 2. Concentration-response curves and representative current records for activation of nAChR  $\alpha 1/\beta 1/\delta/\epsilon$  currents.**

**A.** Concentration-response curves for ACh-evoked currents. The abscissa shows the log concentration of ACh (M) and the ordinate the peak current amplitude normalized to the 100  $\mu$ M evoked current amplitude value. Each point represents the single value from each tested cells (6 cells tested). From the 3 parameter logistic equation the  $pEC_{50}$  values range from 5.4 to 4.5 (mean  $\pm$  SEM =  $4.92 \pm 0.12$ ).

**B.** ACh-induced currents recorded from two different cells, activation was by increasing concentrations of agonist applied for 2 s every 1 minute. The cells are representative of the different degrees of desensitization that was observed during the experiments.

**A****B**

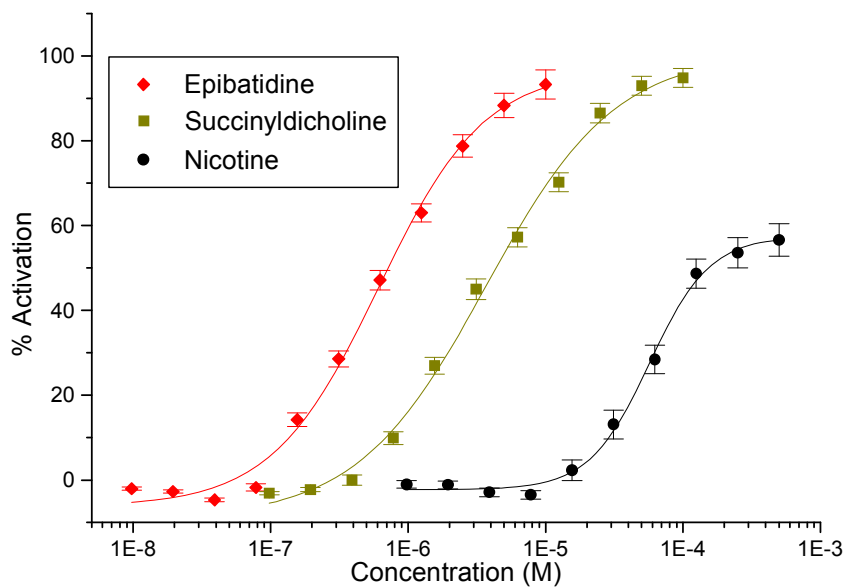
**FLIPR Calcium Assay.**

The suitability of the hnAChR  $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 cell line for plate-based assays was assessed using the Molecular Devices FLIPR3 instrument and the  $\text{Ca}^{2+}$  dye Fluo4. Cells were plated at a density of 15,000 cells/well and grown on the plates for 16-24 hrs at 37°C and 5%  $\text{CO}_2$  then moved to 30°C, 5%  $\text{CO}_2$  for 48 hours. The assay buffer contained (mM); 145 NaCl, 2.5 KCl, 1.2  $\text{MgCl}_2$ , 2  $\text{CaCl}_2$ , 10 Glucose, 10 HEPES (pH7.4 with NaOH) and 0.02% pluronic acid.

**Pharmacology – Epibatidine, Succinylcholine and Nicotine:**

To assess agonist pharmacology, three agonists were tested (**Figure 3**).  $\text{pEC}_{50}$  values are shown in **Table 1**.

**Figure 3. Representative agonist dose response curves (median  $\pm$ SEM).**



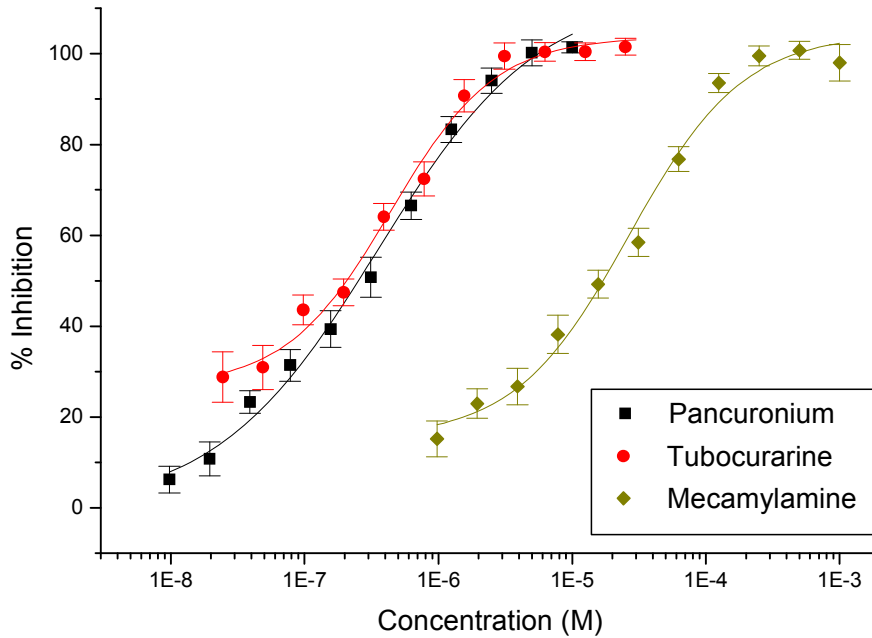
**Table 1. Agonist activity in the hnAChR  $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 cell line.**

Agonist	hnAChR $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 $\text{pEC}_{50} \pm \text{SEM}$
Succinylcholine	$5.49 \pm 0.04$
Epibatidine	$6.28 \pm 0.03$
Nicotine	$4.36 \pm 0.04$

**Pharmacology – Pancuronium, Tubocurarine and Mecamylamine:**

Several antagonists blocked the response to 60  $\mu$ M succinylcholine - **Figure 4** and **Table 2**.

**Figure 4. Representative antagonist dose response curves (median  $\pm$  SEM).**



**Table 2. Antagonist activity in the hnAChR  $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 recombinant cell line.**

Antagonist	hnAChR $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 $pIC_{50} \pm SEM$
Pancuronium	$6.38 \pm 0.07$
Mecamylamine	$4.50 \pm 0.05$
d-Tubocurarine	$6.29 \pm 0.07$
DMPP	$5.75 \pm 0.06$

**Stability of hnAChR Cell Line.**

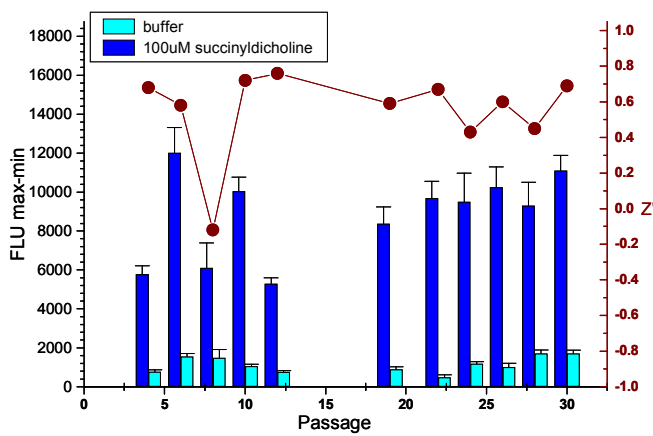
**FLIPR Calcium Assay.**

The hnAChR  $\alpha 1/\beta 1/\delta/\epsilon$ -HEK293 cell line has stable expression for >30 passages.

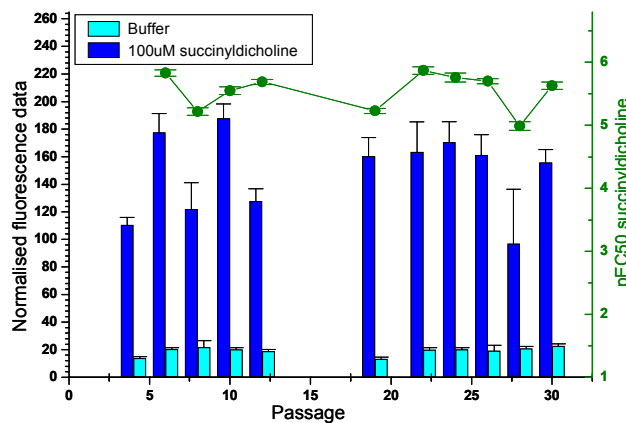
Functional channel expression was monitored using FLIPR - **Figures 5 and 6.**

The stability of the hnAChR evoked calcium response to 100  $\mu$ M succinylcholine was assessed over 25 passages from cell revival (approximately 14 weeks). From passage 10 the signal window was consistently around 10,000 counts and this is reflected in the Z' value (0.43-0.76 (**Figure 5**)). The pEC<sub>50</sub> value for succinylcholine was 5.49  $\pm$  0.04 (mean  $\pm$  SEM, n=72, **Figure 6**).

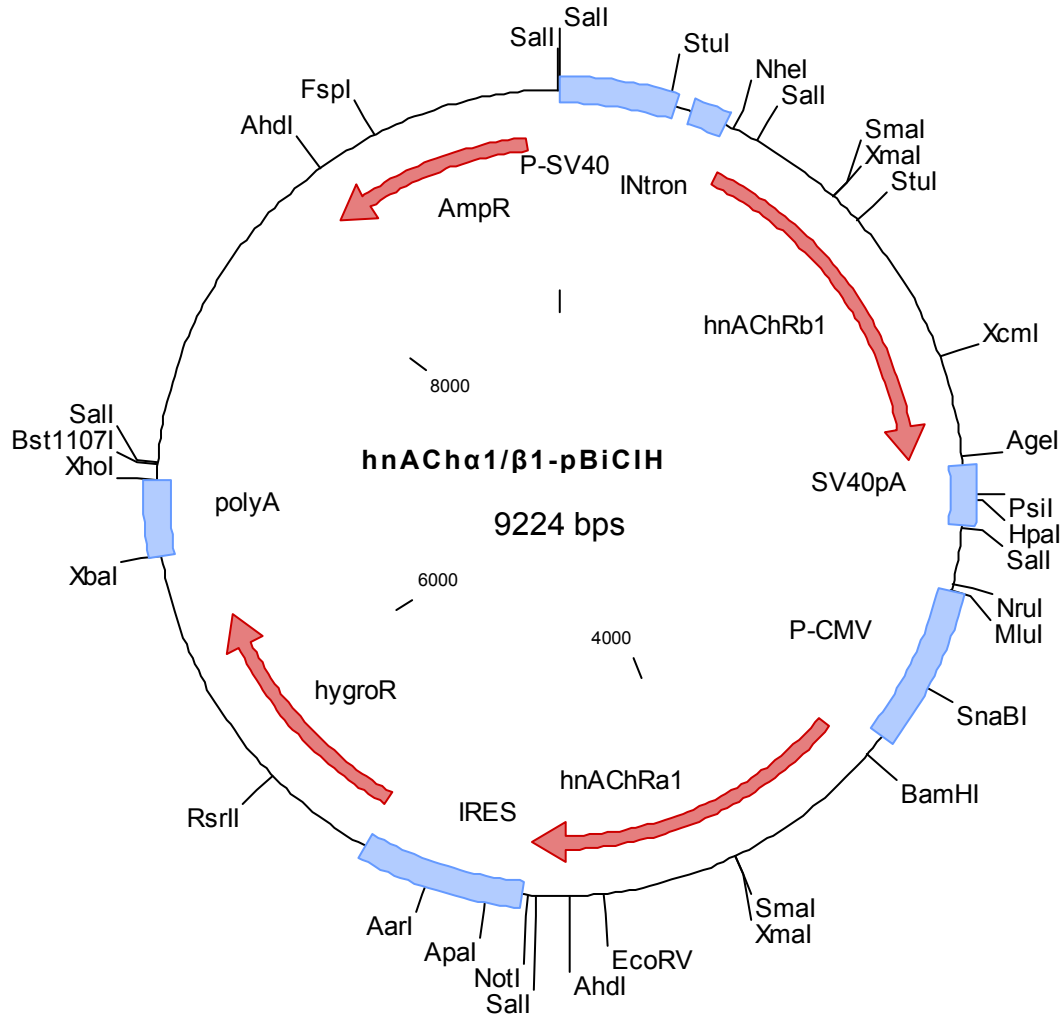
**Figure 5. Stability of the FLIPR fluorescence signal and Z-prime over cell passage.** The fluorescence signal window calculated for each well was the post-compound response minus the basal fluorescence for that well (n=16).



**Figure 6. Stability of the FLIPR normalized fluorescence signal and succinylcholine pEC<sub>50</sub> over cell passage.**

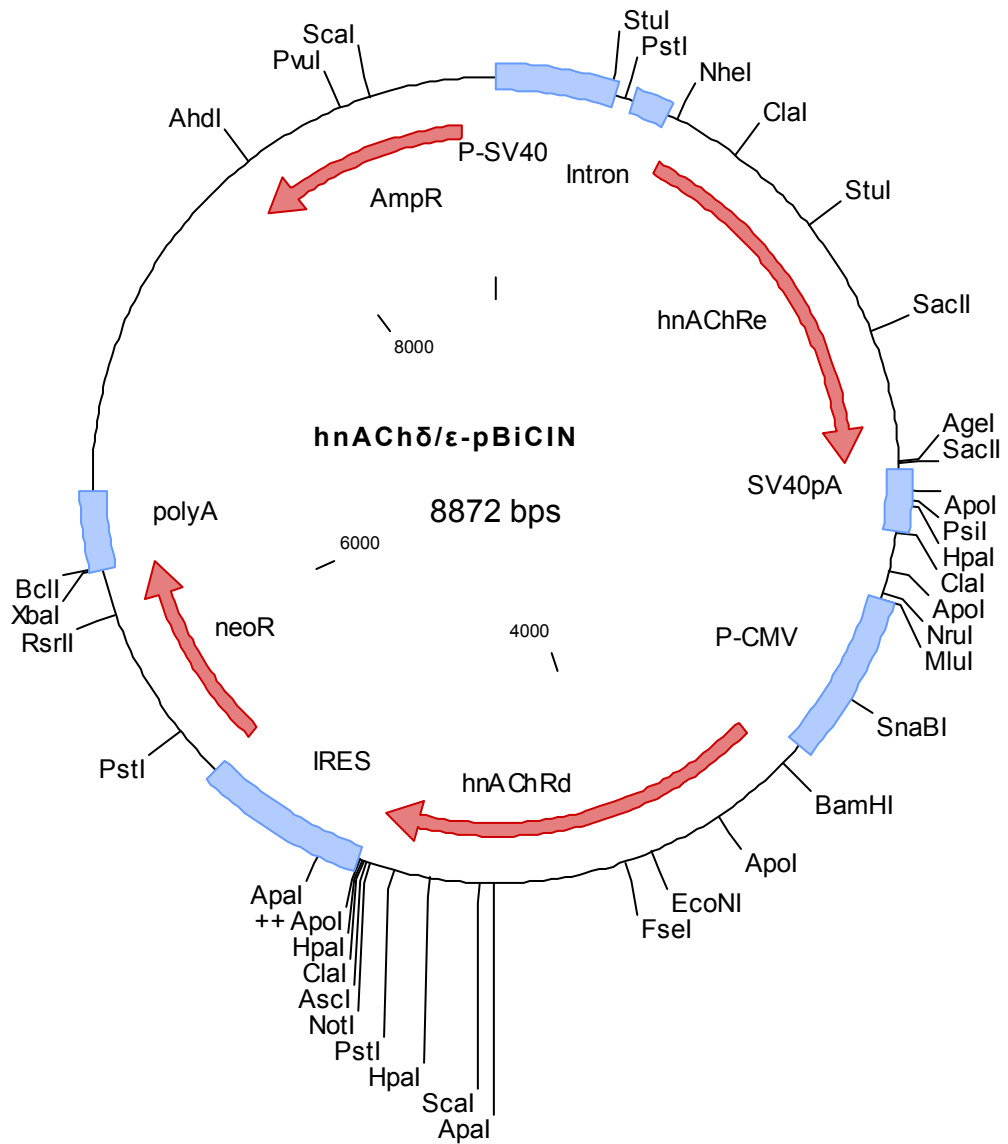


**Vector:**



Polylinkers: SV40-NheI- $\beta 1$ -AgeI-CMV-BamHI- $\alpha 1$ -NotI-IRES-*hygro*

**Vector:**



Polylinkers: SV40-NheI- $\epsilon$ -AgeI-CMV-BamHI- $\delta$ -NotI-IRES-*neo*

**hnACh  $\alpha$ 1 Sequence (Accession Number NM\_000079).**

**hnACh  $\beta$ 1 Sequence (Accession Number NM\_000747).**

**hnACh  $\delta$  Sequence (Accession Number NM\_000751).**

**hnACh  $\epsilon$  Sequence (Accession Number NM\_000080).**