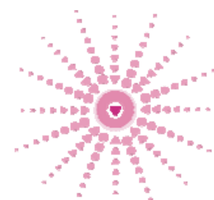
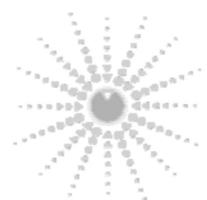




DATASHEET

PrecisION™

hERG-HEK

Recombinant Cell Line

CATALOG # CYL3039
REVISION # M04

ORDERING INFORMATION
AND TECHNICAL SERVICES
Millipore (UK) Ltd
6-7 Technopark
Cambridge CB5 8PB
UK
Tel: +44 (0) 1223 508191
Fax: +44 (0) 1223 508198

CUSTOMER SERVICES
UK: 0800 0190 333
US: 800 437 7500

Product description:
Recombinant HEK293 cell line expressing the human ERG (ether-a-go-go related gene) potassium channel (accession number U04270).

Format:
2 x 1 ml aliquots containing 1.68 x 10^6 cells/ml in 10% DMSO at passage 11 (lot 2).

Mycoplasma Testing:
The cell line has been screened using the PCR VenorGem kit (Minerva Biolabs) to confirm the absence of Mycoplasma species.

Functional Validation:
HEK293 cells expressing hERG were characterized in terms of their pharmacological and biophysical properties using whole-cell patch clamp techniques.

The cell-line was shown to be selectively expressing hERG current since the isochronal and fully activated I/V plots were the same as those reported in the literature. Furthermore, tail currents were significantly blocked by low nM concentrations of astemizole and cisapride.

Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 30 passages: 92% of cells expressed hERG currents at passage 30 giving a mean tail current amplitude at –50 mV of 880 ± 20 pA (n=315).

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

Licensing Statement

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:

This product enables the user which purchased/licensed it to use the cDNA construct for research purposes only, and additional restrictions apply to commercial uses of the cell line. Such commercial uses, include uses such as resale, bulk preparation of proteins encoding the cDNA, application of the product with transgenic animals or human experiments, or use in screening or drug development. All commercial uses require a separate written license from Millipore Corporation and may require additional licenses from RCT, whose contact is Dr. Jennifer Tonzello, phone, +001 520-748-4441.

IonWorks™ HT is a trademark of Molecular Devices Corporation

Electrophysiological Properties of the hERG Current.

Conventional Whole-Cell Patch Clamp Electrophysiology.

Current/Voltage Relationship:

Figure 1 shows hERG potassium currents, evoked by 4 s voltage steps from –60 mV to +40 mV in 10 mV increments, applied from a holding potential of –80 mV. Activation of the current occurred at potentials more positive to –50 mV and the amplitude increased progressively up to around -10/0 mV. At even more positive voltages the current amplitudes became progressively smaller due to the onset of fast inactivation. The isochronal current/voltage relationship (measured at arrow) is plotted in **Figure 2**. This bell-shaped relationship is typical for hERG currents expressed in mammalian cell lines (Zhou *et al.,* 1998).

Figure 1. hERG currents evoked by depolarizing voltage steps. Membrane currents (upper panel) elicited by 4 s depolarising voltage pulses (lower panel), stepped in 10 mV increments from –60 mV to + 40 mV from a holding potential of –80 mV every 15 s. The red dotted line indicates zero current level and the arrow denotes where membrane current was measured for the current voltage relationship in **Figure 2**.

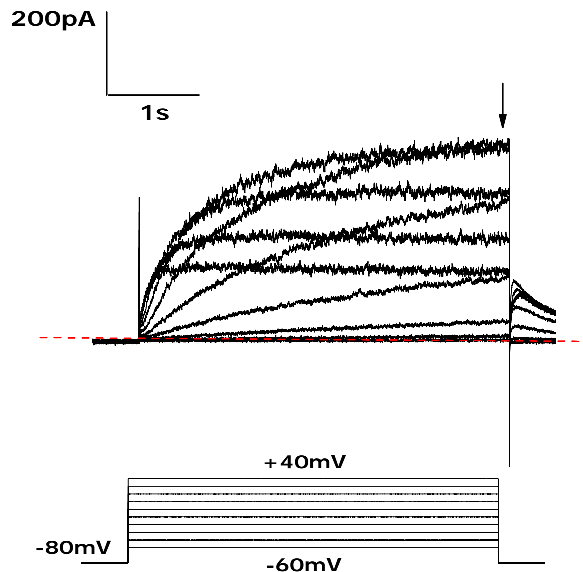
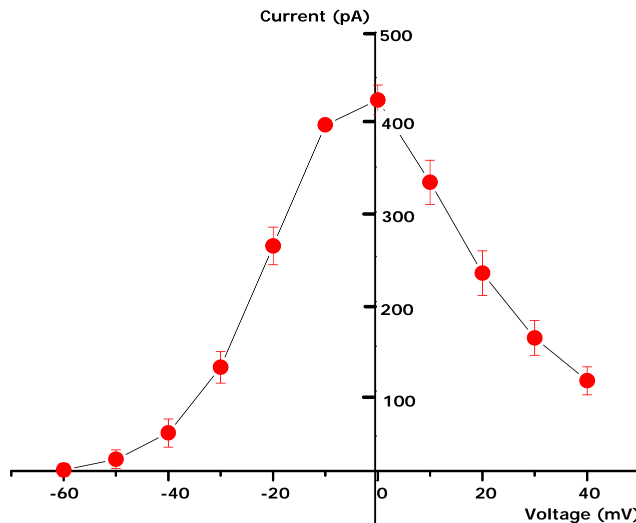


Figure 2. The isochronal I/V relationship of the hERG current. The current was measured at the end of the depolarizing steps as shown in **Figure 1** and plotted against voltage (mean ± S.E.M, n=4).



In order to obtain the fully activated current voltage relationship, cells were clamped at a holding potential of -80 mV and then voltage pulses were applied as described in **Figure 3.** At potentials negative to the reversal potential (e.g. -120 mV / -100 mV), large deactivating inward tail currents were recorded. The current reversed direction on stepping to -80 mV and stepping to more depolarized levels lead to progressively larger outward tails up to -40 mV and then lead to progressively smaller time-independent currents up to +40 mV as described by Zhou *et al.*, 1998.

Figure 3. hERG current evoked by repolarizing voltage steps. Membrane currents (upper panel) were elicited by the voltage protocol shown in the lower panel (membrane stepped to +40 mV for 4s followed by repolarizing steps from -120 mV to +40 mV in 20 mV increments). The red dotted line indicates zero current level and the arrow denotes where membrane current was measured for the current voltage relationship in **Figure 4**.

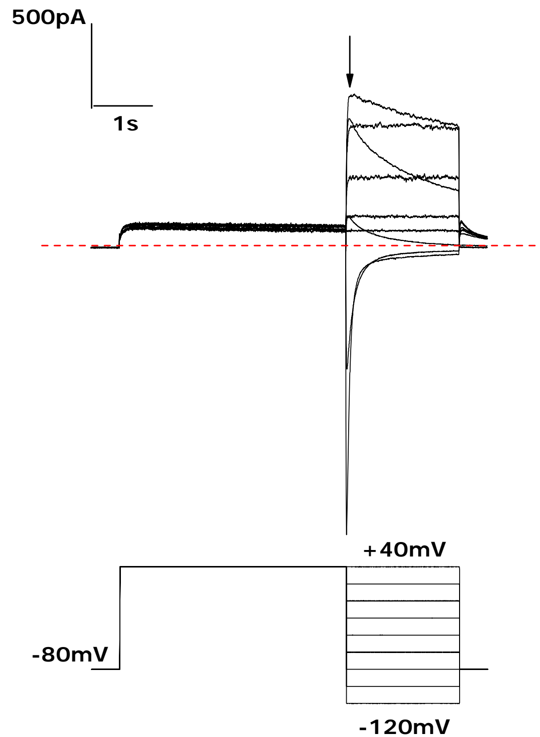
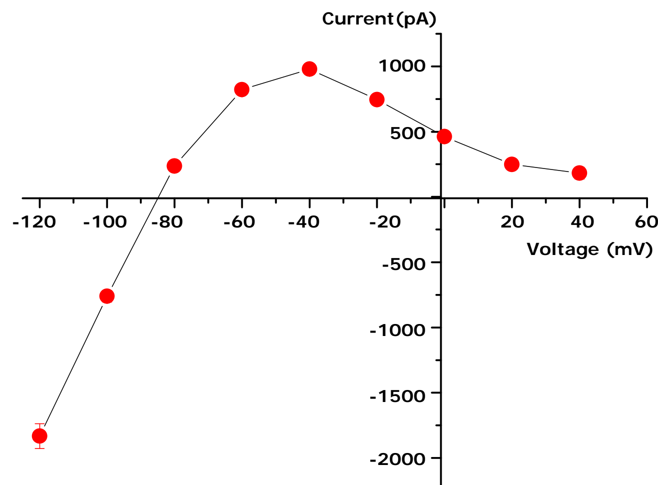


Figure 4. The fully activated I/V relationship of the hERG current. The voltage protocol described in **Figure 3** was applied every 15 s. Peak current (measured on stepping back to the various test voltages is plotted against membrane voltage (mean ± S.E.M, n=4).



Pharmacology - Cisapride:
In order to assess hERG potassium pharmacology, the gastrointestinal prokinetic cisapride was selected. This compound is a potent hERG channel blocker with a reported IC_{50} value in the low nanomolar range.

The reported IC_{50} values are highly dependent on the voltage protocols used. However when the voltage is stepped to depolarized levels greater than +20 mV, assessment of the reduction in tail current amplitudes on stepping back to negative potentials (-40/-50 mV) produces similar IC_{50} values; i.e. 7-44 nM (Rampe *et al.*, 1997), 7 nM (Mohammad *et al.*, 1997) and 20 nM (Walker *et al.*, 1999).

In this study bath application of cisapride (**Figure 5**, 3-300 nM) inhibited the tail current amplitude by 46 ± 3.7%, 82 ± 3.7% and 98 ± 2% (n=3) for 3, 30 and 300 nM concentrations respectively. Thus, the extent of block seen is similar to the published reports described above.

Figure 5. The effect of cisapride on the hERG tail current. Tail currents were evoked continuously (1 every 15 s). Peak tail currents are plotted against pulse number. The labelled red bars depict the presence of 3, 30 and 300 nM cisapride respectively.

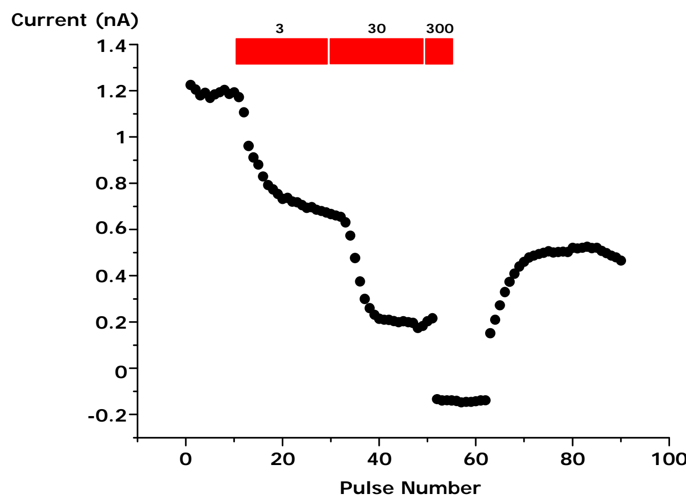
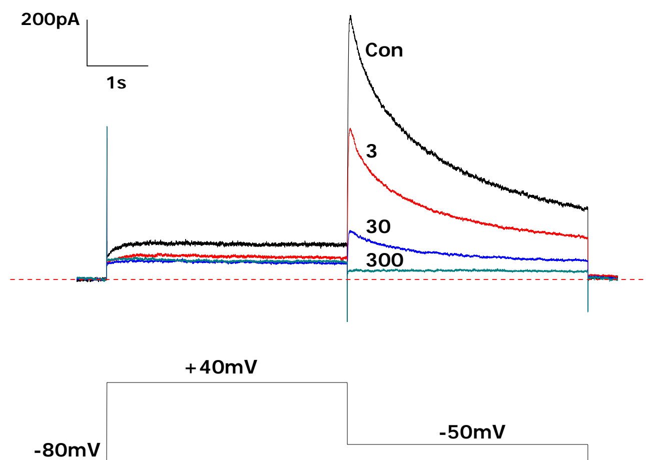


Figure 6. hERG current traces in the presence of cisapride. Typical currents recorded before ("Con", black trace) and after 2-5 min bath application of 3 nM ("3", red trace), 30 nM ("30", blue trace) and 300 nM ("300", green trace) cisapride. The voltage protocol is shown in the lower panel and applied every 15 s. The red dotted line indicates zero current level.



Pharmacology - Astemizole:
Further assessment of hERG potassium pharmacology was achieved with the selective H_1 antagonist astemizole, another potent hERG channel blocker with a reported IC_{50} value again in the low nanomolar range (see below).

Similar to cisapride, the extent of block seen with astemizole is dependent on the voltage protocols used. The reported IC_{50} values are around 1 nM (0.5-2.4 nM (Yao *et al* 2005) and 0.9 nM (Zhou *et al* 1999)). Bath application of astemizole (4 nM) only slowly blocked the evoked hERG current so that even after 10 min the amplitude of the hERG current had not reached a reduced, steady-state amplitude (**Figure 7**). After a 10 min bath application of astemizole (4 nM) the current was inhibited by 56.7 ± 8.6% (n=3). Astemizole (400 nM) rapidly and completely blocked the hERG tail current (**Figure 7** and right hand traces in **Figure 8**). Thus astemizole blocks the hERG current within a concentration range similar to previous reports.

Figure 7. The effect of astemizole on the hERG tail current. Tail currents were evoked continuously (1 pulse every 15 s). Peak tail currents are plotted against pulse number. The red bars depict the presence of 4 and 400 nM astemizole respectively.

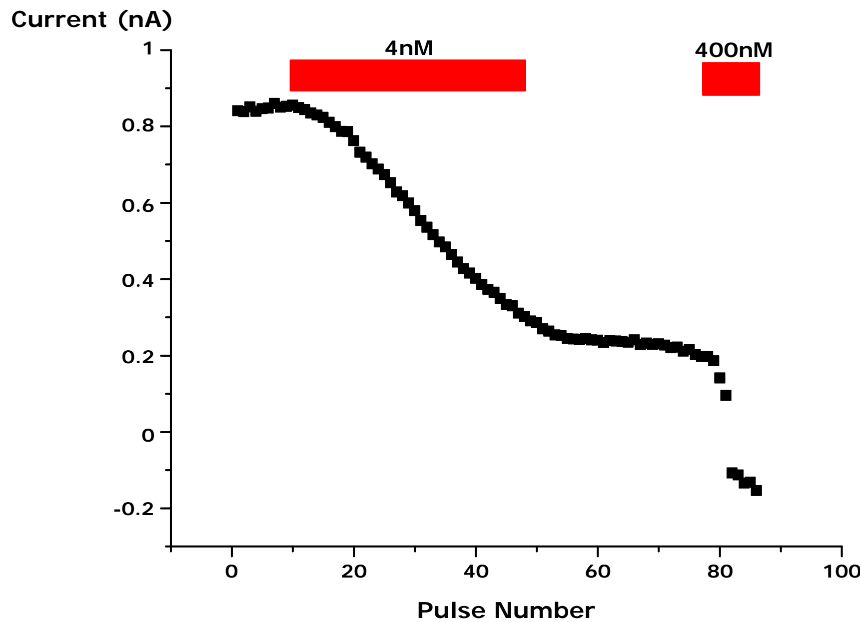
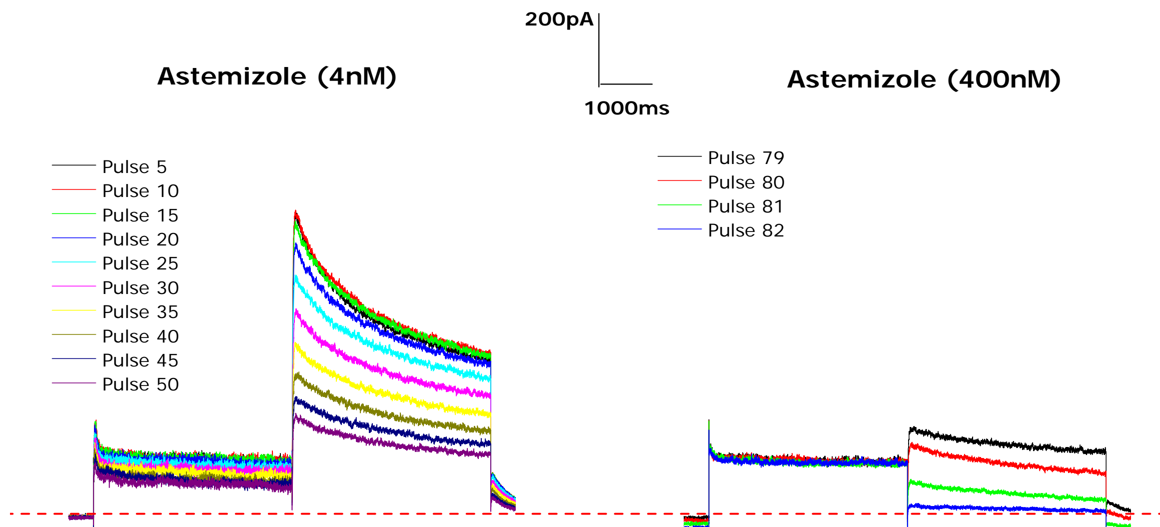


Figure 8. hERG current traces in the presence of astemizole. Typical currents recorded during the addition of either 4 nM (left hand traces) or 400 nM (right hand traces) astemizole. The red dotted line indicates zero current level. Data is derived from **Figure 7**.



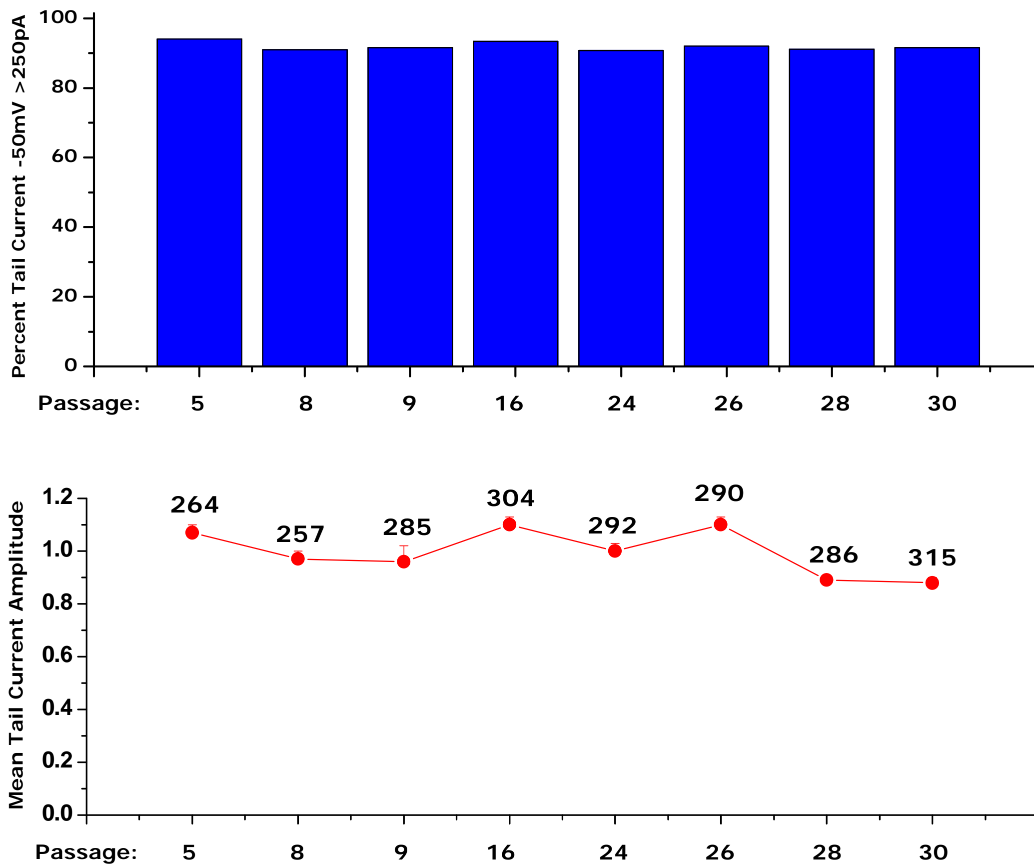
Stability of hERG Cell Line.

IonWorks™ HT Electrophysiology.

The hERG-HEK293 cell line has stable expression for >30 passages.

Functional channel expression, defined as cells expressing hERG tail current of ≥ 250 pA, was monitored using IonWorks™ HT. This data and the mean current amplitude is shown in **Figure 9**.

Figure 9. Stability of expression over passage. The upper panel shows the percentage of cells expressing a mean peak tail current >250 pA at –50 mV at cell passages 5, 8, 9, 16, 24, 26, 28 and 30. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (maximum of 384 cells, numbers above red circles).



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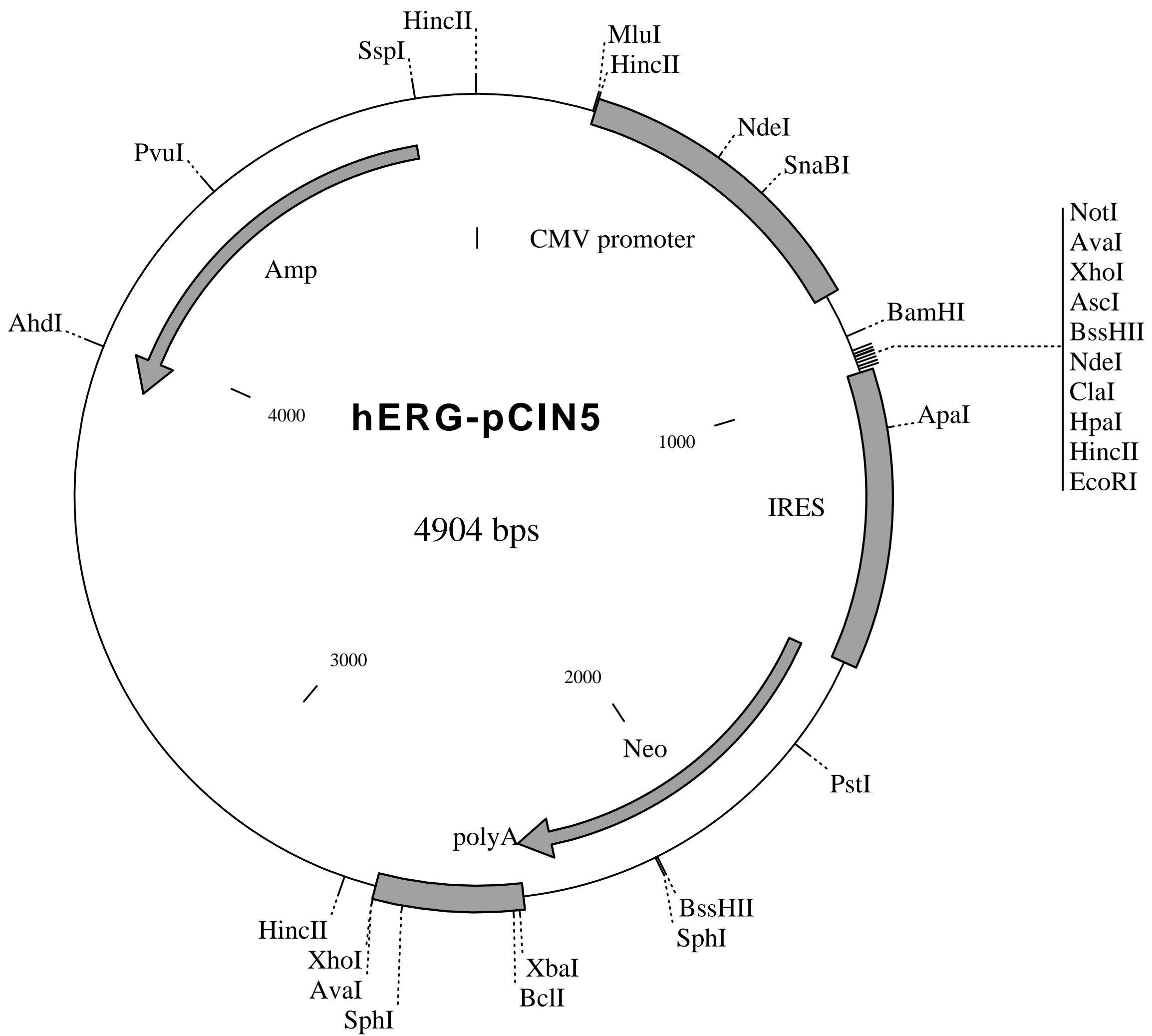

hERG Sequence (Accession Number U04270):

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