pOnebyOne™

Highly efficient lineal vector ready-to-clone your PCR gene and express your Protein of Interest

All vectors includes for 20 rxn:

- 20 µL Linearized vector (50 ng/µL)
- 40 µL Glue Enzyme (10 u/µL)
- 50 µL Glue-Enzyme Buffer (10x)
- 10 µL Insert Control (30 ng/µL)
- 5 µL pOnebyOne™ Control (50 ng/µL)

Description:

pOnebyOne™ are efficient, accurate and flexible Bicistronic Mammalian expression family vectors that contains an expression cassette based in 2A sequence breakthrough technology.

As Bicistronic vector, it allows simultaneous expression of two proteins from the same mRNA. Unlike the transfection with vectors with two different expression cassettes, cells transfected with bicistronic vectors ensure that if one of the proteins is present, the other one is present too.

Bicistronic expression vectors are supported on viral elements: the IRES or 2A sequence. IRES has been widely used. It is a relative short sequence, around 600-700 bp, although this length could be a disadvantage in viral vectors where packaging capacity is limited. IRES based expression vectors are characterized by a non-stoichiometric production of both proteins, generally there is a lower expression of the downstream gene.

Many 2A sequences from several families of viruses have been described for producing multiple polypeptides. 2A mediated cleavage is a universal phenomenon in all eukaryotic cells. With just 20 bp in length, the 2A sequence has been used succesfully to generate multiple proteins in some biological models: plants, zebrafish, transgenic mice or eukaryotic cell lines. Vectors based on 2A produce stoichiometric proportion of both proteins.

Canvax offers a ready-to-clone solution of your gene of interest onto a wide collection of bicistronic vectors based on 2A sequence. You can choose among different promoters, selection antibiotics or reporter genes.

Related Products:

- WideUse™ Plasmid Purification Kit (p.92)
- CVX5α™ Chemically Competent cells (p.18)
- FastCONTROL™ Dual Reporter Plasmid (p.27)
- FastPANGEA™ High Fidelity DNA Pol. (p.105)
- Ampicillin (p.126)
- CANFAST™ Transfection reagent (p.76)
- Nerve Growth Factor Receptor Antibody (p.123)
- pASSEMBLE™ Packaging Systems (p.33)
- pOnebyOne™ MCS1-2A-MCS2

Advantages & Features:

- ✔ **Complete solution:** a directional cloning vector to clone and produce a protein of interest.
- ✔ **Breakthrough technology:** based in 2A sequence simultaneous expression of two proteins in mammalian cells (a protein of interest and reporter).
- ✔ **Highly efficient:** cloning system tested with up to 4 kb inserts.
- ✔ **Time-saving cloning process:** linearized vector ready-to-mix with your PCR fragment.
- ✔ **Easy-to-use:** facilitates the selection of positive cells expressing the recombinant gene of interest.
- ✔ **Cost avoidance:** avoids the use of restriction enzymes.
- ✔ **Accurate:** proven performance for most common cell lines.
- ✔ **Flexible:** allows transfection in difficult cell lines.
- ✔ **Really low experimental background:** < 1%.
- ✔ **Convenient:** available with different resistance marker cassettes.
- ✔ **Directional cloning:** of PCR with the gene of interest.
- ✔ **Reporter checking:** stoichiometric amount of your protein of interest and a reporter protein.

Applications:

pOnebyOne™ Non-viral Mammalian Expression vectors

- ✔ Protein Expression of intracellular, extracellular or transmembrane in higher cells in an equimolecular ratio with a surface marker that allows quantification.

pOnebyOne™ Retroviral Expression vectors

- ✔ Introduction of DNA in refractile transfection cell lines.
- ✔ Co-expression of your gene of interest and a reporter gene.

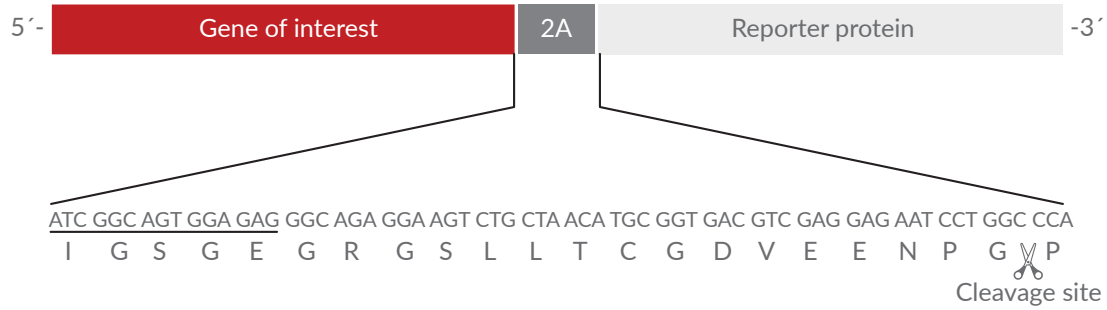
pOnebyOne™ Lentiviral Expression vectors

- ✔ Highest generation Lentiviral transfer vector to transform replicating and non-replicating cells, including stem and neuronal cells.
- ✔ Vector of choice for short-interfering RNA (siRNA) delivery and for gene therapy.

Quality control of all pOnebyOne™:

- ✔ Cloning of SEAP open reading frame and testing phosphatase alkaline activity in mammalian cells.

Figure 2.1.: 2A sequence based vector.



Both genes must be in frame and the nascent peptide is cleaving between the glycine and proline. After the cleavage, the short peptide IGSGEGRGSLLTCGDAEENPG (21 aminoacids) remains fused to the C-terminus of the protein of interest while the proline is added to the N-terminus of the reporter protein. 2A sequence used has high cleavage efficiency in some biological systems. Essential reverse primer sequence for directional cloning is underlined.