pENQUIS-T/A cloning vector

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Origin:

The pENQUIS-T/A cloning plasmid was designed and assembled by Sylvain Marcellini in April 2009, in the **LAboratory of Development and Evolution**, Department of Cell Biology, Faculty of Biological Sciences, University of Concepción, in Chile. In Chilean, the "Penquista" adjective is commonly used to refer to people from the Concepcion area.

There are no restrictions of use, and this material can be distributed freely to other laboratories without seeking my consent. However, when distributing the pENQUIS-T/A plasmid, please include this PDF file, so people can acknowledge its origin, and obtain the following technical information:

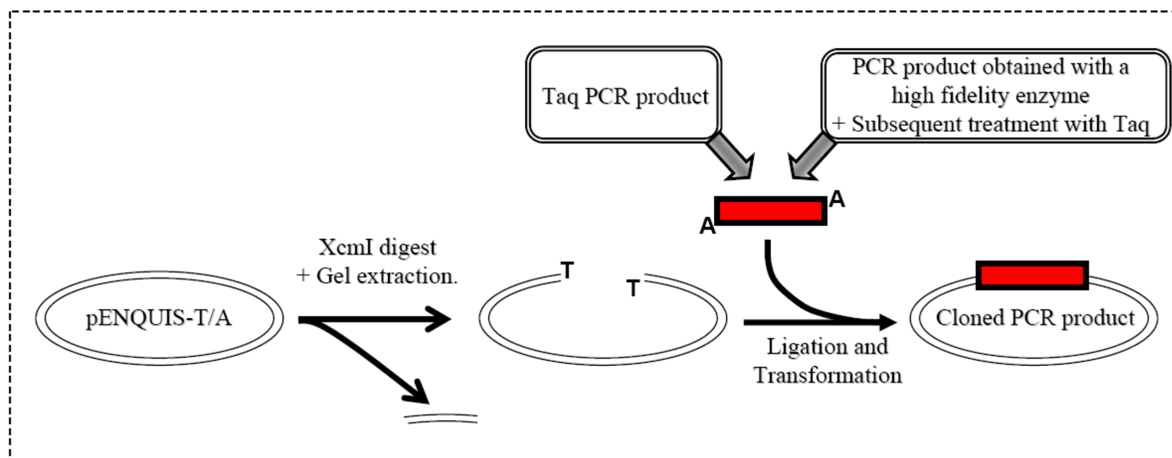
Purpose and description:

Cloning PCR products is routinely performed in many laboratories, often facing the following difficulties:
-Not enough PCR product is obtained, and, after purification, it is too diluted to allow efficient ligation.
-Several restriction enzymes are not able to cut restriction sites close to DNA ends. This makes the introduction into a first "shuttle" cloning vector an obligate requirement.
-Commercially available cloning vectors for PCR products are usually expensive.

The pENQUIS-T/A cloning vector is based on the backbone of pBluescript SK minus (from Stratagene). **It allows the easy, fast, and cheap cloning of any PCR products.** Because the insert is flanked by the universal T3 and T7 primers, pENQUIS-T/A is particularly well-suited to perform a one-step cloning of Taq PCR products that will be used to synthesize *in situ* hybridization RNA probes.

Rationale:

- The digested pENQUIS-T/A has two deoxythymidines with a 3' overhang, and will therefore easily ligate Taq PCR products (or PCR products obtained with a high fidelity enzyme and subsequently treated with Taq) because they have two deoxyadenosines with a 3' overhang.
- Self-ligation of the vector is virtually impossible, so the background is extremely low or absent.
- Note that if the deoxythymidines are lost by degradation, the *LacZ* ORF will be reconstituted, and the colonies bearing an empty vector should appear blue in a LB agar plate containing Xgal and IPTG. This white-blue control is possible, but it should, however, be unnecessary.



Protocol, step 1: Preparing the vector

-Digest 5 µg of pENQUIS-T/A with XcmI (purchased from NEB), for 2 hours at 37°C, in a total volume of reaction of approximately 60 µl (so it fits in a single well of an agarose gel).
-Add DNA loading buffer; load in a 0,8% agarose gel; run for 45 minutes at 80 Volts and purify with your favorite Gel Extraction kit.
-After elution, measure the DNA concentration and dilute to 50 ng/µl with TE pH8.
-Store at -80 °C for months.

Protocol, step 2: Preparing the insert

IF	the PCR gives **a single band** of the expected size.
THEN	estimate the concentration on an agarose gel and use in a ligation reaction without purifying.

IF	the PCR gives several **non-specific amplification products**.
THEN	run in an agarose gel and purify the insert. Measure its concentration (optional).

IF	the DNA polymerase is a **Taq**
THEN	the PCR product already contains two deoxyadenosines at its 3' ends.

IF	the DNA polymerase has a **proofreading activity** (high fidelity enzymes)
THEN	-purify the PCR product (column or gel extraction, as required), elute in nanopure water -incubate an appropriate amount of insert for 30 minutes at 72 °c in a 10µl reaction (1.5mM $MgCl_2$, 1X Taq buffer, 0.5 µl Taq enzyme and 0.2mM dATP)

Protocol, step 3: Ligation and transformation

Mix the insert and 25 to 50 ng of digested vector in a 10 µl ligation reaction (T4 DNA ligase). Incubate overnight at 4 °C (one hour at room temperature should work, although less efficiently). Transform approximately 5 µl with competent bacteria, select on AMPICILIN plates.

Structure of the digested pENQUIS-T/A polylinker

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   KpnI  ApaI     XhoI  HincII   ClaI  HindIII     EcoRI                    BamHI SpeI  XbaI  NotI        SacII SacI
5'-GGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCCATCAGT      TATATGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTC-3'
3'-CCATGGCCCGGGGGGGAGCTCCAGCTGCCATAGCTATTCGAACTATAGCTTAAGGGTAGTC      TTATATACCTAGGTGATCAAGATCTCGCCGGCGGTGGCGCCACCTCGAG-5'
                                         EcoRV
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pENQUIS-T/A sequence

(Note that this vector has been cloned and functionally tested, but that it has not been sequenced)

Green = pBluescript T7 primer

Yellow = pBluescript T3 primer (annealing site)

Grey = pBluescript Multiple Cloning Site

Dark blue = Specific nucleotides of the two XcmI restriction sites

Black = Nucleotides allowing the T/A cloning

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CCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTAATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGGTATC
GATAAGCTTGATATCGAATTCCCATCAGTTAATTGGCCCGGGCCAGACTATATATGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGC
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