

Material suplementario

Diseño de un plásmido capaz de expresar la β -fructosidasa (invertasa) en una biofábrica de origen bacteriano

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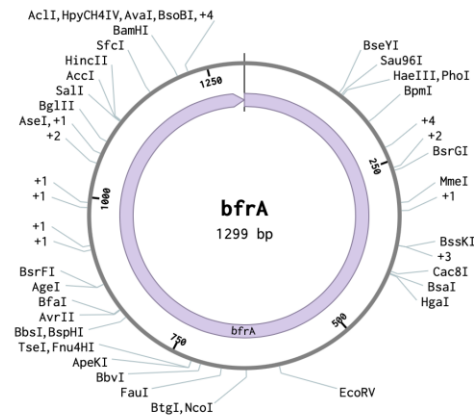
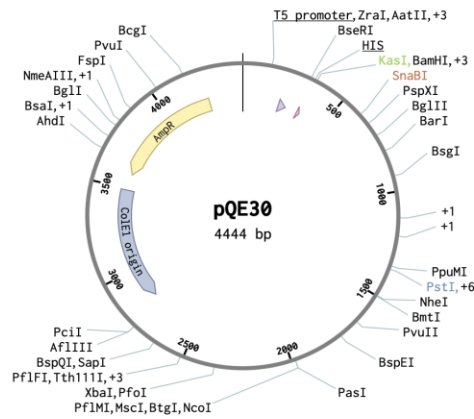
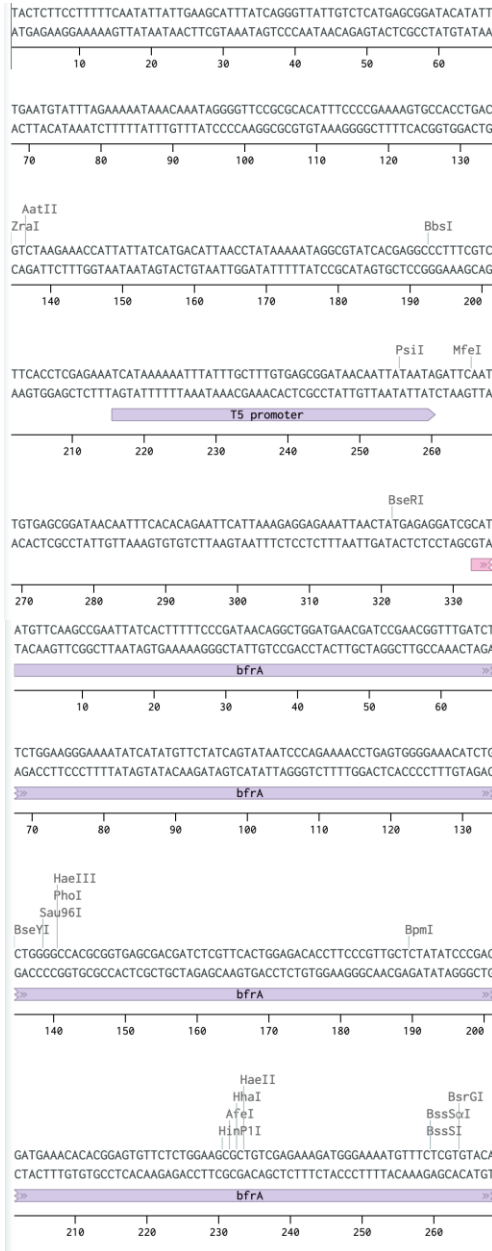
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Material suplementario 1

Descripción detallada del proceso de construcción del vector pQE30-hPolB + *bfrA* + GFP

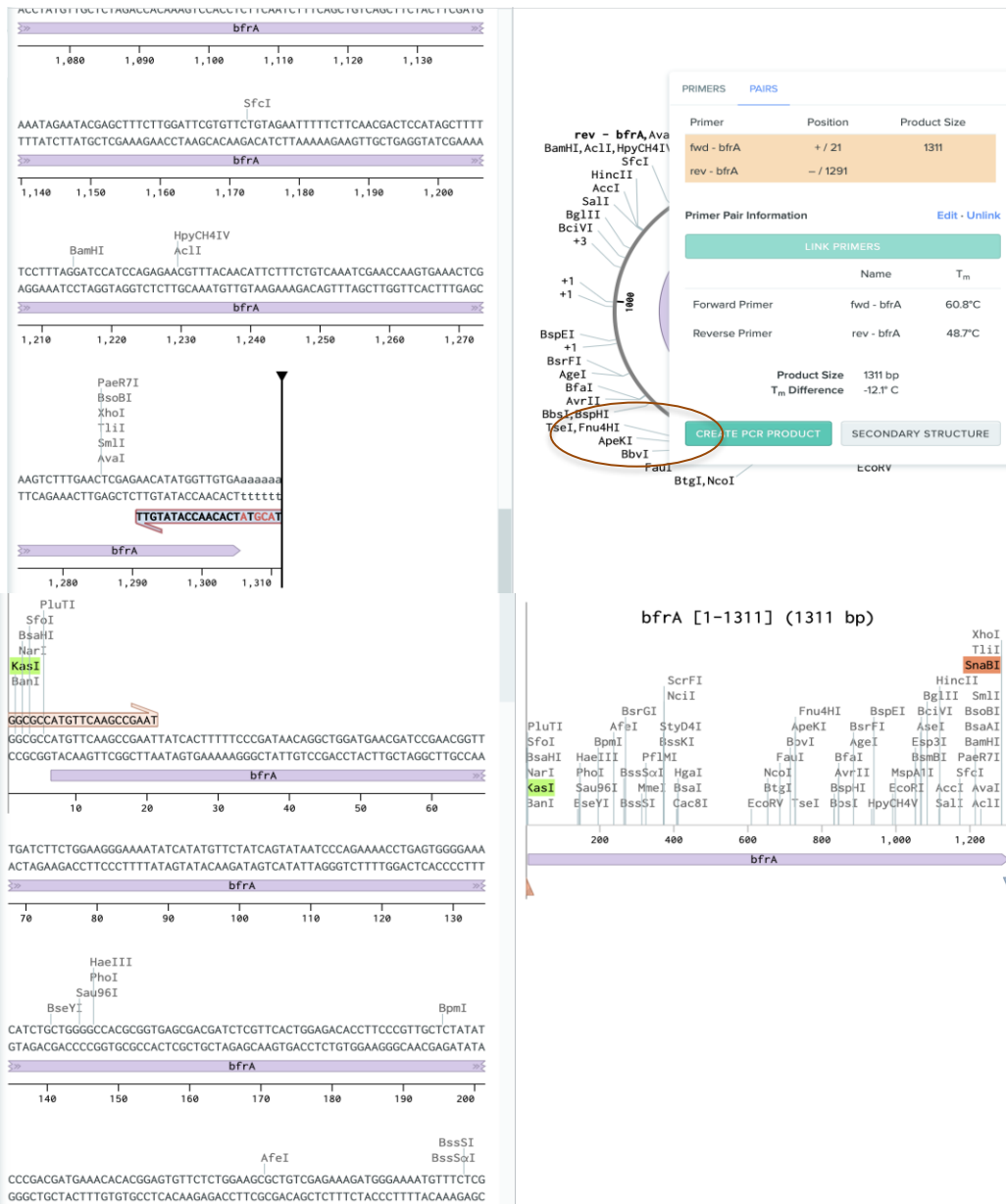
1. Dentro de la herramienta Benchling descargar la secuencia codificante del plásmido (pQE30) y gen de interés (*bfrA*)



3. Diseñar los oligonucleótidos con los que se amplificará el gen de interés, considerando características como el contenido de GC, Tm y los pares de bases que deben agregarse antes de la secuencia de la enzima de restricción (KasI, SnaBI) que se agregará cada oligonucleótido

The image shows a primer design software interface. On the left, a DNA sequence is displayed with a purple bar labeled 'bfrA' indicating the target region. The sequence is shown in three segments: positions 10-60, 70-130, and 140-200. Restriction sites are marked above the sequence: HaeIII, PhoI, Sau96I, BseVI, HaeII, HhaI, AfeI, HinP1I, BpmI, BssSqiI, and BssSI. On the right, the 'Design' panel shows the selected strand as 'Forward' and the primer sequence as '5' ATGTTCAAGCCGAAT 3''. The '3' Location' is set to 15, and the 'Overhang' is 0 bp. The 'Verify' panel shows a T_m of 43.3°C, GC Content of 40.00%, and Length of 15 bp. The 'Save' panel includes a name field and a 'Save To' dropdown set to 'Artículo', with a 'Save Primer' button.

4. Una vez que se diseñan los oligonucleótidos, se obtiene el producto de PCR



5. Realizar la ligación del vector con el producto de PCR del gen de interés (gen *bfrA*) usando la opción “assembly wizard” dentro de Benchling.

a) Para lograr de manera exitosa la ligación se debe seleccionar el vector desde la primera enzima de restricción (KasI), hasta la segunda enzima de restricción(SnaBI)

The image shows a screenshot of the Benchling software interface. On the left, a linear DNA sequence is displayed with various restriction enzyme sites highlighted. The sequence is as follows:

```
340 350 360 370 380 390 400
CACCATCACCATCACGGATCCTCTAAACGGAAAGCGCCGCGAGGAGACTCTCAACGGGGGAATCACC
GTGGTAGTGGTAGTGCCTAGGAGATTTGCCTTCGCGGCGTCCCTCTGAGAGTTGCCCCCTAGTGCC
370 380 390 400
ACATGCTCACAGAACTCGAAACTTTGAGAAGAAGCTGAGCCAAGCTATCCACAAGTACAATGCTTA
TGTACGAGTGTCTTGAGCGTTTGAACCTCTCTTGCACCTCGATTGATAGGTGTTTCATGTTACGAAT
410 420 430 440 450 460
CAGAAAAGCAGCATCTGTTATAGCAAAAATACCCACACAAAATAAAGAGTGGAGCTGAAGCTAAGAAA
GTCTTTTCGTCGTAGACAATATCGTTTTATGGGTGTTTATTTCTCACCTCGACTTCGATTTCTTT
470 480 490 500 510 520 530
TTGCCGGAGTAGGAACAAAAATTGCTGAAAAGATTGATGAGTTTTAGCAACTGGAAAATACGTA
AACGGACCTCATCCTTGTTTTAACGACTTTTCTAACTACTCAAAAATCGTTGACCTTTTAATGCAT
540 550 560 570 580 590 600
```

Restriction sites marked include BamHI, KasI, SnaBI, and PvuII. A pink arrow labeled 'HIS' is shown above the sequence between positions 340 and 350. A red arrow labeled 'SnaBI' is shown below the sequence at position 600.

On the right, a circular plasmid map for pQE30 (4444 bp) is shown. The map includes the following features and restriction sites:

- T5 promoter, ZraI, AatII, +3
- BseRI
- HIS
- KasI, BamHI, +3
- SnaBI
- PspXI
- BglII
- BarI
- BsgI
- +1
- +1
- PpuMI
- PstI, +6
- NheI
- BmtI
- PvuII
- BspEI
- PasI
- PciI
- AflIII
- BspQI, SapI
- Pf1FI, Tth111I, +3
- XbaI, PfoI
- Pf1MI, MscI, BtgI, NcoI
- ColEI origin
- amp^r
- BcgI
- PvuI
- FspI
- NmeAIII, +1
- BglI
- BsaI, +1
- AhdI

At the bottom, the 'PREVIEW' section shows the sequence: GTA AAG / CAT TTCCGCG. Below that, the 'pQE30' section shows '4.2 kb · SnaBI, KasI' and an 'Insert' button. A status bar at the bottom indicates '0 ERRORS AND 0 WARNINGS' and 'Looks like everything checks out'.

b) Después de seleccionar el sitio donde se ligará el gen de interés dentro del vector, se debe seleccionar el producto de PCR del gen de interés, nuevamente desde la primera enzima de restricción KasI hasta la segunda enzima de restricción SnaBI

The screenshot shows a bioinformatics tool interface for DNA sequence analysis. The main window displays the *bfrA* gene sequence (1-1311 bp) with various restriction enzyme sites marked. A specific fragment is highlighted in green, spanning from the *KasI* site to the *SnaBI* site. The sequence viewer shows the following DNA sequence:

```

    GCGGCCATGTTCAAGCCGAAT
    GCGGCCATGTTCAAGCCGAATTATCACTTTTCCCGATAACAGGCTGGATGAACGATCCGAACGGTT
    CCGCGGTACAAGTTCGGCTTAATAGTGAAAAAGGCTATTGTCGGACCTACTTGCTAGGCTTGGCAA
    TGATCTTCTGGAAGGAAAAATCATATGTTCTATCAGTATAATCCCAGAAAACTGAGTGGGAAAA
    ACTAGAAGACCTCCCTTTTATAGTATACAAGATAGTCATATTAGGGTCTTTTGGACTCACCCCTTT
    CATCTGCTGGGGCCACGCGGTGAGGACGATCTCGTTCAGTGGAGACACCTTCCCGTTGCTCTATAT
    GTAGACGACCCCGTGCACCCTCGCTGCTAGAGCAAGTGACCTCTGTGGAAGGGCAACGAGATATA
  
```

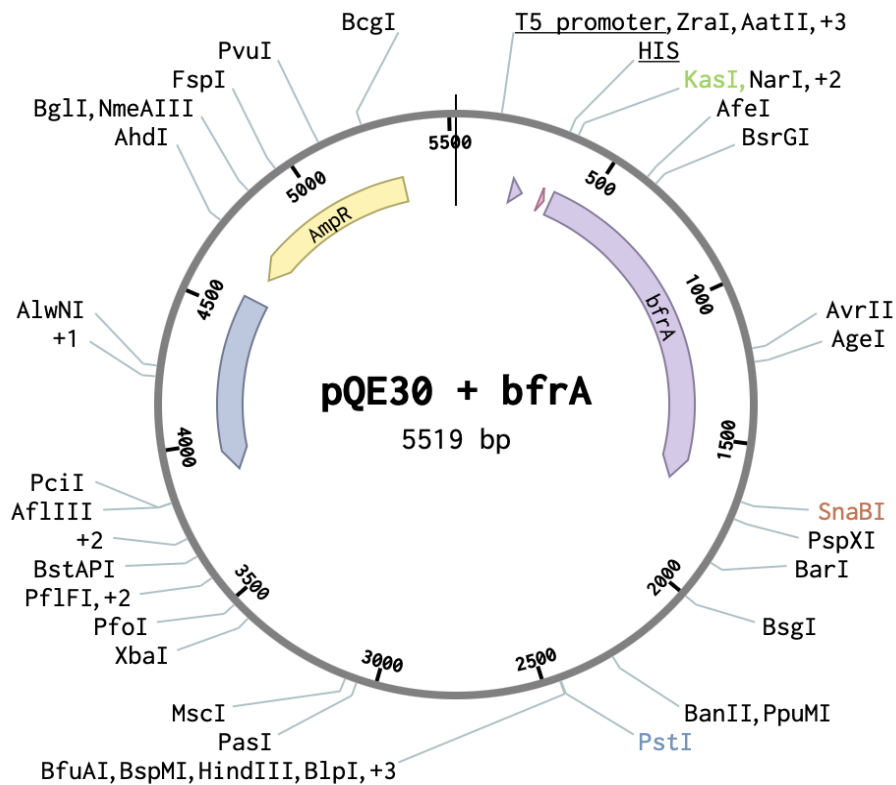
The interface also shows a preview of the PCR product:

```

    AAGCGCCAT TACGTA
    TTCCGGGTA ATGCAT
  
```

At the bottom, the tool indicates the selected fragment: *bfrA* [1-1311] (1.3 kb) with *KasI* and *SnaBI* sites. The overall status is "0 ERRORS AND 0 WARNINGS" and "Looks like everything checks out".

6. Finalmente, de selecciona la opción de ensamblar y se obtiene el vector recombinante con el gen de interés



Material suplementario 2

Secuencia completa del Vector recombinante “pQE30-hPolB + *brfA* + GFP”

Promotor T5

His

brfA

GFP

Cloranfenicol

Ampicilina

TACTCTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTC
TCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAAT
AGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAA
GAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCA
CGAGGCCCTTTCGTCTTCACCTCGAGAAA**TCATAAAAAATTTATTG**
CTTTGTGAGCGGATAACAATTATAATAGATTCAATTGTGAGCGGAT
AACAAATTCACACAGAATTCATTAAAGAGGAGAAATTA ACTATGAG
AGGATCGCATCACCATCACCATCACGGATCCTCTAAACGGAAGGCG
CC**ATGTTCAAGCCGAATTATCACTTTTTCCCGATAACAGGCTGGATG**
AACGATCCGAACGGTTTGATCTTCTGGAAGGGAAAATATCATATGT
TCTATCAGTATAATCCCAGAAAACCTGAGTGGGGAAACATCTGCTG
GGGCCACGCGGTGAGCGACGATCTCGTTCCTGGAGACACCTTCCC
GTTGCTCTATATCCCGACGATGAAACACACGGAGTGTTCTCTGGAA
GCGCTGTCGAGAAAGATGGGAAAATGTTTCTCGTGTACACCTACTA
CCGCGATCCGACACACAACAAGGAGAAAAAGAAACCCAGTGTGT
GGCTATGAGTGAAAACGGATTGGATTTCGTAAAGTACGATGGAAAC
CCGGTCATATCTAAACCCCCAGAGGAAGGGACGCACGCCTTCAGAG
ACCCGAAGGTGAACAGAAGCAACGGTGAGTGGCGAATGGTACTGG
GATCTGGTAAAGATGAGAAGATTGGAAGAGTGCTTCTCTATACCTC
AGATGACCTTTTTCACTGGAAGTACGAGGGTGTGATCTTCGAAGAT
GAAACCACAAAAGAAATAGAGTGTCCCGATCTTGTGAGAATTGGA
GAGAAAGATATCCTCATATACTCGATAACGAGTACAAACAGCGTTC
TGTTTTCCATGGGAGAGTTAAAGGAAGGAAAACCTGAATGTCGAAA
AGCGGGGGCTTCTCGATCACGGAACGGATTTCTACGCTGCTCAAAC
TTTCTTTGGAACAGACAGAGTTGTAGTTATCGGATGGCTTCAAAGC
TGGTTGAGAACAGGGCTTTACCCGACAAAACGAGAAGGATGGAAC
GGTGTCATGAGTCTTCTAGGGAGCTGTATGTAGAAAACAACGAGT
TGAAGGTGAAACCGGTGGATGAACTCTTGGCTCTCAGAAAGAGAA
AGGTTTTCGAACTGCAAAGTCCGGAACATTTCTGCTGGATGTCAA
GGAAAACAGTTATGAAATTGTGTGTGAATTCAGCGGAGAAATCGA
ACTTCGAATGGGAAATGAATCTGAAGAAGTGGTGATAACGAAGAG
TCGAGACGAATTAATCGTGGATAACAACGAGATCTGGTGTTCAGGT
GGAGAAGTTAGAAAGTCGACAGTCGAAGATGAAGCTACAAATAGA
ATACGAGCTTCTTGGATTCGTGTTCTGTAGAATTTTTCTTCAACGA

CTCCATAGCTTTTTCTTTAGGATCCATCCAGAGAACGTTTACAACA
TTCTTTCTGTCAAATCGAACCAAGTGAAACTCGAAGTCTTTGAACTC
GAGAACATATGGTTGTGATACGTAATGTCTGAAGGGCGAGGAGCTGT
TCACCGGCGTCGTCCCGATCCTGGTTCGAGCTGGACGGTGACGTCAA
CGGCCACAAGTTCTCCGTCTCCGGCGAGGGTGAGGGGCGACGCCACC
TACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGTAAGCTGC
CGGTCCCGTGGCCGACCCTGGTCACCACCCTGACCTACGGCGTCCA
GTGCTTCTCCCGCTACCCGGACCACATGAAGCGCCACGACTTCTTC
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TCAAGGACGACGGTAACTACAAGACGCGTGCCGAGGTCAAGTTCG
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CAAGGAGGACGGTAACTCCTGGGCCACAAGCTGGAGTACAACCTA
CAACTCCACAACGTCTACATCACCGCGGACAAGCAGAAGAACGG
CATCAAGGCCAACTTCAAGACCCGCCACAACATCGAGGACGGTGG
CGTCCAGCTAGCCGACCACTACCAGCAGAACACCCCGATCGGGCGAC
GGCCCGGTCTGTGCTGCCGGACAACCACTACCTGTCCACCCAGTCCG
CCCTGTCCAAGGACCCGAACGAGAAGCGCGACCACATGGTCTGTCT
GGAGTTCGTACCCGCCCGCCGGCATCACCCACGGCATGGACGAGCTG
TACAAGTAG

CTGCAGCCAAGCTTAATTAGCTGAGCTTGGACTCCTGTTGATAGAT
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GTTGCCGCCGGGCGTTTTTTATTGGTGAGAATCCAAGCTAGCTTGGC
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CCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCG
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGC
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GTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAC
GCTGTAGGTATCTCAGTTCGGTGTAGGTTCGTTCCGCTCCAAGCTGGG
CTGTGTGCACGAACCCCCGTTACGCCCAGCGCTGCGCCTTATCC
GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGC
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