IMPACT purification of native proteins

Brian Baltzar

Abstract

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Guidelines

IMPACT Column Buffer (1L)

Reagent	Volume
Tris-HCl 7-8 pH 1M	20 ml
NaCl 5M	100 ml
EDTA 0.5M	2 ml
Tween-20	2 ml
H_2O	-> 1000ml

IMPACT Cleavage Buffer (100ml)

Reagent	Volume
Tris-HCl 7-8 pH 1M	2 ml
NaCl 5M	10 ml
EDTA 0.5M	0.2 ml
DTT	0.2 ml
H_2O	-> 100ml

Before start

Make sure that the desired protein has the correct restriction sites and is in frame with the intein in the purification vector.

1. If that is not the case natively, design primers using the sequences in the following table.

RESTRICTION SITE	SEQUENCE (RESTRICTION SITE UNDERLINED)	CLONING VECTOR
NdeI	5′- GGT GGT CAT ATG NNN NNN... -3′ (forward primer)	pTXB1
SapI[1]	5′- GGT GGT TGC TCT TCC GCA NNN NNN...-3′ (reverse primer)	pTXB1
SapI[2]	5′- GGT GGT TGC TCT TCC AAC NNN NNN... -3′ (forward primer)	pTYB21
PstI[3]	5′- GGT GGT CTG CAG TCA NNN NNN... -3′ (reverse primer)	pTYB21

[1] *SapI digestion creates a 3-nt overhang (GCA) for ligation with the SapI-digested pTXB1 vector (containing a TGC overhang), resulting in an in-frame fusion to the N-terminus of an intein. The SapI site can be used to add one or more extra amino acid residue(s) to the target protein by including an appropriate sequence (e.g. add ACC in the reverse primer corresponding to a GGT codon for a glycine residue). The SapI site is not regenerated after cloning.*

[2] *SapI digestion creates a 3-nt overhang (AAC) compatible with the SapI digested pTYB21 (containing a GTT overhang). The SapI site is not regenerated after cloning.*

[3] *A stop codon should be included in the reverse primer when constructing a N-terminal fusion.*

2.
3. Then perform a Phusion PCR with the designed primers

Materials

- Chitin Resin - 100 ml S6651L by New England Biolabs
- EDTA (0.5 M), pH 8.0 AM9260G by Life Technologies
- Sodium Chloride PubChem CID: 5234 by Contributed by users
- Tween 20 170-6606-MSDS by Bio-rad Laboratories
- Econo-Pac Columns 7321010 by Bio-rad Laboratories

Protocol

Insert gene into IMPACT vector

Step 1.

1. Digest the pPCR product with NdeI & SapI in minimum 2 hours.
2. Digest the IMPACT vector (pTXB1) with NdeI & SapI in minimum 2 hours.
3. Purify the digest on colums
4. Ligate the purified digest products using the preferred ligation protocol

5. Make a TSB transformation into *E. Coli*:K-12 use your preferred transformation protocol

6. Inspect the ligation with a colonyPCR

7. Make ON of the verified transformation

8. Purify the plasmid from the ON from step 1.7 using Miniprep

Protein expression

Step 2.

1. Transform the minipreped plasmid from step 1.8 into *E. Coli*:ER2566

2. Inoculate 1L LB+Amp with a fresh colony from the transformation above.

3. Incubate at 37ºC till it reaches a OD_{600} at 0.5-0.8
4. Add IPTG for a end concentration of 0.4 mM
5. Incubate at 16-20ºC over night.
6. Centrifuge the 1L ON at 5000G in 15 min. at 4ºC - remove the supernatant.
7. Resuspend the cellpellet in 100 ml cold column buffer - (See recipe in description)
8. Centrifuge at 8000G in 20 min at 2ºC - remove the supernatant.
9. Resuspend the cellpellet in a small volume of cold column buffer (2-4 mL)
10. Lyse the cells using french press
11. Centrifuge the lysant at 15000G in 30 min. at 4ºC

Binding to Chitin beads

Step 3.

1. Take 10 ml Chitin beads put into a 50ml falcon tube

2. Centrifuge at 200G in 5 min. - remove the supernantant
3. Wash with 50 ml Column Buffer
4. Centrifuge at 200G in 5 min. - remove the supernantant
5. Load the supernantant from step 2.11
6. Attach on a rotor with slow spin for 2 hours.
7. Add to a collum, open valve just enough that it slowly drips out.
8. Add 200 ml of column Buffer.
9. When the column buffer has run trough, close the valve and add Cleavage Buffer (just enough that i covers the beads + 1ml.) (See recipe in description)
10. leave at room temperature over night (minimum 16 hours)

Eluate the protein

Step 4.

1. Open valve and drip eluate into tubes in 0.5 ml aliquots
2. Measure protein concentration

3. The aliquots with the highest concentrations is mixed and used for further work.