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|  | <b>One tube PCR Cloning</b> | Réf : MO_ONETUBeCLO<br>Version 1<br>date :27/11/2009<br>revised 23 07 2018<br>page 1/2 |
|                                                                                   |                             |                                                                                        |

### 1. Application field :

Cloning of PCR fragment.

### 2. Reference

### 3. Referent person

Colette Tournaire

### 4. Method principle

Cutting of plasmid and ligation of insert at the same time

### 5. Réactives

pBSKII or pBKSII or similar plasmid (50 ng)

blunt end cutting restriction enzyme

Insert : PCR fragment (Molar ratio insert / plasmid =3/1)

### 6. Operating mode

|                                                                    |           |
|--------------------------------------------------------------------|-----------|
| Ligase Buffer (T4 DNA ligase) 10 X                                 | 2 µl      |
| dNTP 2.5 mM (30 µM final)                                          | 0.25 µl   |
| T4 DNA ligase                                                      | 1 µl      |
| T4 DNA polymérase 2.5 U<br>(unnecessary if amplification with Pfu) | 0.3 µl    |
| ECORV (or other blunt end cutting enz)                             | 0.5 µl    |
| H <sub>2</sub> O                                                   | QSP 20 µl |

Ligation 16h to 24h at 16°C

10 à 80% of white colonies contains the insert.

|       | Rédacteur   | Vérificateur | Approbateur |
|-------|-------------|--------------|-------------|
| Nom : | C Tournaire |              |             |

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