



Report of Deliverable No: \_\_\_6.4\_\_\_

Grant agreement number 200800

Dissemination Level (please tick)

**PU** = Public

**PP** = Restricted to other programme participants (including the Commission Services).

**RE** = Restricted to a group specified by the consortium (including the Commission Services).

**CO** = Confidential, only for members of the consortium (including the Commission Services).

## Deliverable 6.4 plasmid expression constructs of chick homologues of human genes identified in WP1-4 due month 18

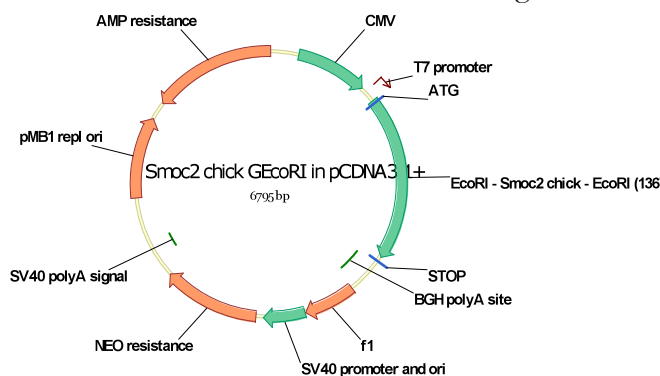
Chicken Smoc2 has been cloned by digital assembling of the available est sequences in the Genbank. Specific primers have been designed and the gen has been cloned using reverse transcription-pcr based strategy. Following cloning, sequencing analysis was carried out to confirm the gene identity.

Chick Smoc2- ATG in **bold**:

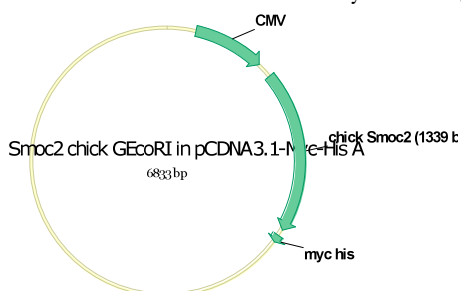
**ATGTGGGTGCCGCTGCTGCTGCTGCTGCCCGAGCTGCTCCCCGCCGTGCCGGCCCAGAAGCTCTCC**  
**GCGCTCACGT**TTTTGAGGGTGGATCAAGACAAAGACAGAGAATGCAGTCTGGACTGTGCAGGTTCC  
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 CAGTGAGTGAGAACTGGCCACAAAGAGAAGGCGGCTCGAGGGAAAAGCAGATGATGCCCTCAG  
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 GACTGAGCAAGTAAAGAGCAGACAAAACAAAACCAATAAAAGCTCAGCATCATCGTGTGATCAAGA  
 ACTGCAGTCAGCCCTGGAGGAAGCTAAACAACCCCAGAAAGATAATGTGTTCATTCAGAAATGTGC  
 TCAGGGTGGTCTTTACAAACCAGTGCAGTGCCATCCTTCCACAGGCTACTGCTGGTGTGTTTCTCGT  
 TGATACAGGACGTCTTATCCTGGTACATCAACAAGGTATGAACAACCTAAATGTGATAACAGTGC  
 CAGAGCTCACCCAACCAAAAACAAAGGATTTGTACAAAGGACGACAGCTTCAAGGTTGTCCTGGGGC  
 CAAGAAGAATGAGTTCCTGACAAGTGTTTTGGATGCACTGTCCACGGATATGGTTTCATGCAGTCTC  
 TGATCCATCGCTGTCTTCTAGCCGGTTTCAGAACCTGATCCAAATCACACTCTGGAAGAGAGAGT  
 AGTCCACTGGTACTTCAAACAGCTGGATAAAATTTCCAGTGGTGACATTTGGACAAGAAAAGAAATCAA  
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 AATACTGTGATGTGAACAATGACAAATCCTTGGTTCAGTTCAGGACATTAATGGGGATGCCTGGGA  
 GTAACAANAGAAGAAGGTAAAGCAGAAACAAAGAAACGCCATACAGGCCCTAAAACCAATACTGA  
 GAGCACTTCATCCAGCAGGCAGCAAGTTTCCAAGAAGCAAGGGTGA

The gene has been subsequently cloned into several expression vectors.

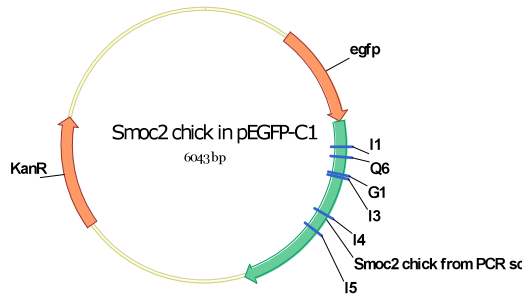
Deliverable 1: chick Smoc2 without a tag:



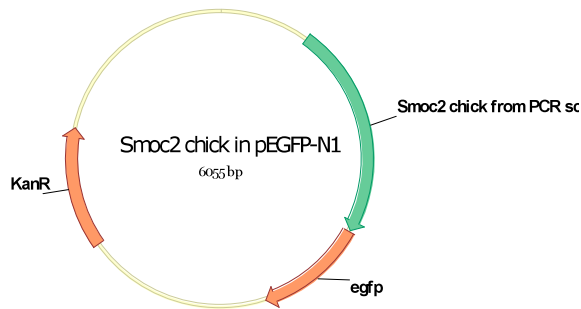
Deliverable 2: chick Smoc2 with Myc-His tag:



### Deliverable 3: chick GFP-Smoc2 fusion protein



### Deliverable 4: chick Smoc2-GFP fusion protein



The deliverable 6.2 **has been delivered on time, before month 18**. The consortium has identified however novel genes potentially associated with OA. Therefore the deliverable 6.4 has been modified and expanded to incorporate the newly discovered genes. Since that discovery took place later into the project, the expanded Deliverable 6.4 is not as complete yet as the original deliverable containing Smoc2. This is however an ongoing work and should the consortium identify more genes associated with OA, they will enter the same pipeline as described above.

The genes discovered within WP1-4 have been cloned by digital assembling of the available est sequences in the Genbank. Specific primers have been designed and the genes have been cloned using reverse transcription-pcr based strategy. Following cloning, sequencing analysis was carried out to confirm the identity of the gene. All the genes are currently cloned into the expression vectors following the strategy used for the original deliverable. The following sequences encoding open reading frames of chick homologues of OA associated genes have been cloned as a part of the **expanded** Deliverable 6.4:

Starting ATG is in **BOLD**

Bcap21

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ATGACTGTCCAGTGGACGGCGGTTGCCGCCITCCTGTACGGCGAGGTGGGAGTGCTGCTCGTGCT
GIGCCTTCCGTTCATCTCCCCGCTGAGATGGCAGAAGATTTTATGATTCCTCTATGGAGCAAAATG
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TCCACAAGGCGAATAATGAAGTGGCAGCAGTTAAAAAGCAGTCTGAAGGCCCTTAGAAGAGAATAT
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CCTGTAAGTGTGCCT
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Hbp1

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GACAGACTCTGCCCCATCTGCGCTTTTTCAGCTTGGCACCGATGTTTCACATCAGGAACGTTCAAGG
CCTTCGTGGAACCAACATACCTCAAACAGCGATTCAGAAAGTGCTCATCTTGTGAGAATGGGGTTC
AACTGGCTGACAGAACTAGCAAACATCGCCACAAGTCTCAGAGCCCTCTGATGCAGTGCTCTTTT
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CATGAGGAAAGCATTTCCCTTTGGTGAGTCAGTGCTGAAGTTGACTTTTGATCCTGGCACAGTGGAG  
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ACACGCTAGAAGCCAAGGCCTTGGCAGAAGAACAGAAACGCTCTAAATCCTGATGTGCTGGAAGCGA  
AAACGAACAAATTCTCTGACCAAGGATGACAAGTACTTCCAATAGGTGTTTGCTGCCATTAA

#### Gpr22

**ATG**TGTTTCTCCCCATTCTGGAAGGCAACATGCAGTTTGAATCTAACTTCACAGTTCGAGATGCCA  
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#### Dus4L

**ATG**AGCGGGGACGTGCGAGAACGCGAACAATGTCCGGGGAGGGACCCGGTGGGTCTGTTTCGGG  
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#### Prkar2B

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