



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

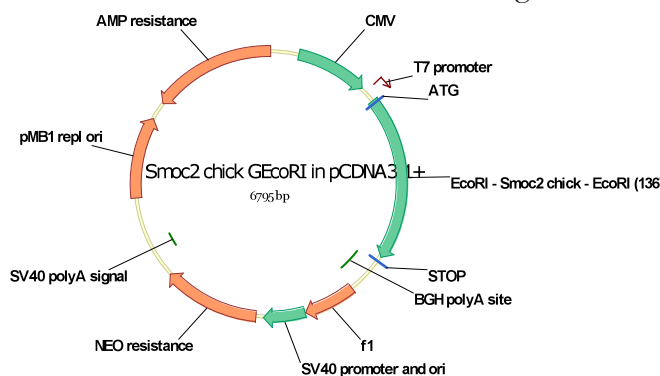
Chicken Smoc2 has been cloned by digital assembling of the available est sequences in the Genbank. Specific primers have been designed and the gen has been cloned using reverse transcription-pcr based strategy. Following cloning, sequencing analysis was carried out to confirm the gene identity.

Chick Smoc2- ATG in **bold**:

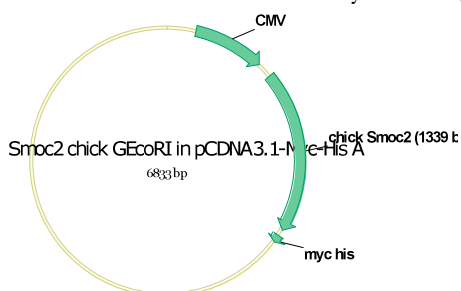
ATGTGGGTGCCGCTGCTGCTGCTGCTGCCCGAGCTGCTCCCCGCCGTGCCGGCCCAGAAGCTCTCC
GCGCTCACGTTTTTGAGGGTGGATCAAGACAAAGACAGAGAATGCAGTCTGGACTGTGCAGGTTCC
 TCCTCAGAAATCGCTTTGTGCATCTGATGGAAGAACTTTTTTGTCCCGGTGTGAATTTCAACGAGCA
 AAATGCAAAGATCCTCAGCTGGAAATTGCCTATCGGGGCAACTGCAAAGATGTTTCCAGATGTGTG
 GCTGAGAGGAAGTACACCCAAGAACAAGCTCGAACAAGAATTTCAACAAGTTTTTATTTCCAGAATG
 CAATGATGATGGCACATATAGTCAGGTTTCAGTGCCACAGTTATACTGGATACTGGCTGGTGTGTC
 ACTCCCAATGGTTCGTTCCAATCAGCGGTACTGGCTGTGGCCCACAAAAANTCCTCGGTGGCCAGGCT
 CAGTGAGTGAGAACTGGCCACAAAGAGAAGGCGGCTCGAGGGAAAAGCAGATGATGCCCTCAG
 CTCCTGCGCTGGAGACTCAGCCTCAAGGTGATGAAGAAGATAATTGCTTCTCGTTATCCTACATTATG
 GACTGAGCAAGTAAAGAGCAGACAAAACAAAACCAATAAAAGCTCAGCATCATCGTGTGATCAAGA
 ACTGCAGTCAGCCCTGGAGGAAGCTAAACAACCCCAGAAAGATAATGTGTTCATTCAGAAATGTGC
 TCAGGGTGGTCTTTACAAACCAGTGCAGTGCCATCCTTCCACAGGCTACTGCTGGTGTGTTTCTCGT
 TGATACAGGACGTCTTATCCTGGTACATCAACAAGGTATGAACAACCTAAATGTGATAACAGTGC
 CAGAGCTCACCCAACCAAAAACAAAGGATTTGTACAAAGGACGACAGCTTCAAGGTTGTCCTGGGGC
 CAAGAAGAATGAGTTCCTGACAAGTGTTTTGGATGCACTGTCCACGGATATGGTTTCATGCAGTCTC
 TGATCCATCGCTGTCTTCTAGCCGGGTTTCAGAACCTGATCCAAATCACACTCTGGAAGAGAGAGT
 AGTCCACTGGTACTTCAAACAGCTGGATAAAATTCAGTGGTGACATTTGGACAAGAAAAGAAATCAA
 GCCATTTAAACAGGTTCTTAAGAAAAGTCAAAGCCCCAAAAAATGTGTGNAAGAAATTTGTGGN
 AATACTGTGATGTGAACAATGACAAATCCTTGGTTCAGTTCAGGACATTAATGGGGATGCCTGGGA
 GTAACAANAGAAGAAGGTAAAGCAGAAACAAAGAAACGCCATACAGGCCCTAAAACCAATACTGA
 GAGCACTTCATCCAGCAGGCAGCAAGTTTCCAAGAAGCAAGGGTGA

The gene has been subsequently cloned into several expression vectors.

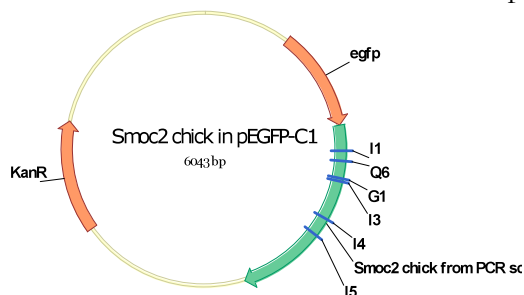
Deliverable 1: chick Smoc2 without a tag:



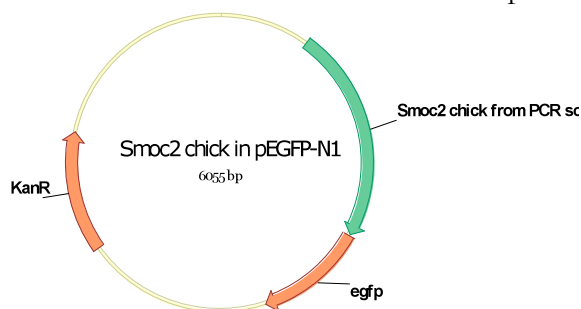
Deliverable 2: chick Smoc2 with Myc-His tag:



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

ATGACTGTCCAGTGGACGGCGGTTGCCGCCITCCTGTACGGCGAGGTGGGAGTGCTGCTCGTGCT
GIGCCTTCCGTTCATCTCCCCGCTGAGATGGCAGAAGATTTTATGATTCCTCTATGGAGCAAAATG
GCTGTCTTTTGGAAACAAAATGTTCCCTCACATAATAGTTCTACTGATCATCTTTGTTTCTTGATGCTGT
TAGAGAAGTTAAAAAGTACTCAGCCATTCAGCTGAACGAGAAGGTTGCAAATGTCAATGCCAATGC
CATTGATCATATTCAGATGAAACTCTTCCGGTCACAAAGAAACCTTTATATCTCAGGTTTTCCTTAT
TTCTGTGGCTTGTTTTGAGGCGTACTGTTACCCCTTCTTACTCAGTTGGCAAAGGCATGGCATCTCA
TGCAGCTCTGGAAACGCAAGTAAACGACGCGACTGAAGCAGCCAAAAAATACATGGCTGAGAATG
AAAGGCTACAGGAGGCTCTGAATGAAAAAGGAAGTGGCGAAAATAAAGAGATGGCGGAAACAAC
TAATGGAATGTTGAGGAAGGAAAATGGACAATTGAAAGCGGAGTTACAGAAGACATCAAATGCTC
TCCACAAGGCGAATAATGAAGTGGCAGCAGTTAAAAAGCAGTCTGAAGGCCCTTAGAAGAGAATAT
GACCATCTGATGAAAGAGTACGAACGACTTCAGAATGCTTCAAGTGAAGCAGAAGACAAGAAGGA
CCTGTAAGTGTGCCT

Hbp1

ATGGTGTGGGAAGTGAAGACGAATCAGATGCCTAACGCAGTTCAGAAGCTGCTCTTGGTAGTGGA
CAAGAGAACTTCAGGGATGAATGAGTCACTGGAGTTGCTGAAGTGAATGAAAACCTGCCCTCTTC
TCCTGGATATGCATCCTGTGATGAGCACATGGAGCCTGATGATCTTCCTGAACTACAGGCTGTGCA
GACAGACTCTGCCCCATCTGCGCTTTTTCAGCTTGGCACCGATGTTTCACATCAGGAACGTTCAAGG
CCTTCGTGGAACCAACATACCTCAAACAGCGATTCAGAAAGTGCTCATCTTGTGAGAATGGGGTTC
AACTGGCTGACAGAACTAGCAAACATCGCCACAAGTCTCAGAGCCCTCTGATGCAGTGCTCTTTT
ATAACAGATCATCTCCTGTTACATAATAGCTACAAGCAAAAGTTTACATTCCTATGCACGTCTCCA

CCGGGATCCTCAAAGAATGATCCCAACTTCTCCAAGAGTGATTTGGATGAAACACCGGTCAGACAC
GAAAGGACAAATAGCGAATCGGAGTCTGGTATTTCTGTCATGTCGTCACCTTCAGATGATGATGAT
CTGGGATGGTGCCATTTCTTGGCCCTCAACTGTTTGGCACTGTTTCTAAAAGGGTCTCGTTTGTGCT
TTCATAAAGGGTGCAATAAAGGAATGGCAGGACATCGAAGATTTTGCAAGATCTGAAGGCTGTGGA
AAAGAAGATAATCTCTCAGTGAGTGCTTACAAGGGCTATGGCTCCGATGGCTTGAAGCTCATTTCT
CATGAGGAAAGCATTTCCCTTTGGTGAGTCAGTGCTGAAGTTGACTTTTGATCCTGGCACAGTGGAG
GATGGCCTGCTCACAGTAGAGTGCAGACTTGATCACCTTTCTATGTTAAAAATAAAGGTTGGTCA
TCTTTTATCCAAGCTTGACTGTGGTACAGCATGGCATTCCATGCTGTGAAATGCATCTTGGAGATC
TGTGTCTACCTCCTGGACACCCTGATGCCATTAACCTTGATGATTCAGGTGTTTTTGATACATTTAA
AAGTTACGACTTCACACCGCTGGATTCCTCAGCAGTGTATGTGCTCAGCAGCATGGCTCGCCAGCG
CCGCGCTTCTCTGTCGTGCGGAGGATCAAACAATCAAGATGCCGAAAGATCAGAATGCAGTACTAA
AAACTGTGCAGCTACTGCGGCATCGCATCTTCCCTCCACTTCTTTGTACAGCAAAGCTGGCAAAGC
CACGGCTCAGGGACTGCAAGTACTGTGAGTGCCACTTCTCAAACAAGTGCAAAGACCAATGAAT
GCCTTCATGCTTTTTGCCAAAAAATACAGAGTTGAATATACTCAGATGTATCCAGGGAAAGACAAC
AGAGCCATAAGTGTGATACTTGGTGACAGGTGGAAGAAAATGAAGAATGAGGAGAGACGGATGT
ACACGCTAGAAGCCAAGGCCCTTGGCAGAAGAACAGAAACGTCTAAATCCTGATGTGCTGGAAGCGA
AAACGAACAAATTCTCTGACCAAGGATGACAAGTACTTCAAATAGGTGTTTGCTGCCATTAA

Gpr22

ATGTGTTTCTCCCCATTCTGGAAGGCAACATGCAGTTTGAATCTAACTTCACAGTTTCGAGATGCCA
TTGATGACATCGACACCAACATGTACCGACCACTGTCATATGCATTAAGCTTCAAGTGTCTCTCAC
TGGATTTTGTATGTTAGAAATTTGTTTGGGACTTGGCAGCAACCTCACCGTGCTGGTACTTTACTGT
ATGAAATCCAACCTAATCAATTTCTGTCAGTAACATAATTACAATGAACCTTCACGTACTTGATGTAAT
AATTTGTGTGGGATGTATTCCTCTTACTATAGTTATCCTTCTGCTTTCACTGGAGAGTAACACTGCT
CTGATCTGCTGCTTCCACGAGGCTTGTGTCTCTTTTGAAGCGTTTCGACAGCAATCAATGTCTTTG
CTATCACTCTGGACCGATACGACATCTCTGTCAAGCCTGCCAACCGAATTTCTGACCATGGGAAGGG
CTGTGATACTAATGACATCAATATGGATCATTTTCGCTTTTGGCCTTCCCTGATTCCTTTTCAATTGAAGTC
AATTTCTTCAGTCTTCAAAGCGCAAGTACTTGGGAGAACAACCGCTGCTGTGTGTCAGCGCAAAC
GAGTACCACACCGAGCTAGGGATGTACTACCACCTCCTCGTCCAGATCCCAATCTTCTTCTTACCAG
TGGTAGTGATGCTAATCACGTACACCAAAAATACTCCAGGCTCTTAACATCCGAATCGGCACAAGATT
TACAACGGGACAGAAGAAGAAAGCTCGAAAAGAAAAAACTATTTCTCTCACCACGCAGCAGCAGAC
CACGGACATGTCGCAGAGCAGCGCAGGAAGAAACGTGGTCTTTGGCGTAAAGGACTTCCGTGTCTG
TGATCATTTGCCCTGCGCCGAGCCGTGAAGCGGCACCGGGAGCGACGAGAGCGGCAGAAGAGAGT
CTTCAGAAATGTCCCTCCTGATCATTTCCACCTTCTGCTCTGCTGGACACCCATCTCCGTTCTAAACA
CCACAATCTTGTGTTTGGGCCCAAGTGACCTTTTGGTAAAGTTGCGCTTATGTTTCTAGTCATGGC
ATACGGAACAACCTATTTTTCACCTTACTTTATGCATTCACGAGGCAAAAATTTTCAGAAAAGTTCTG
AAAAGTAAGATGAAGAAACGAGTTGTTTCAATCGTGGAAAGCAGATCCCATGCCAAATAACGCTGTC
ATACACAACCTCATGGATAGAGCCTAAACGGAACA AAAAGATCACCTTCGAAGACAACGAAGTAAGG
CAGAAATGTTTAGTACCTCAGGTTGTCACTGACTAGAC

Dus4L

ATGAGCGGGGACGTTCGCAGAACGCGAACAATGTCCGGGGAGGGACCCGGTGGGTCTGTTTCGGG
CTGGGCACGTTCGTGAAGATATGTGCCCGATGGTTTCGCTACTCCAAGCTGGCGTTCCGCACCCCTGG
TCCGGCGGTACGGCTGCGATCTGTGCTACACCCCATGGTTGTGGCGGCCGACTTCGTGAGGTCA
GCCAAAGCCAGGGACAGCGAGTTCACAACAAACAGAGGTGATAATCCATTGATCGTTCAGTTTGCT
GCTAAAGAAGCACAAGTTTTATGTGATGCTGCCCTTCTCGTCTGTCTTTCGCAGATGGAGTAGAC
CTAAACTGTGGCTGTCTCAGAGATGGGCAATGGCAGAAGGTTATGGTGCTTGCTTAATAAAATAAA
CCGGAGCTGGTTCGAGATATGGTGAGACATGTACGGAATCAGATCGACAATCCTCGGTTTTTCAGTA
TCTATTAATAAATAAGAATACATGAAGACTTAAAAAAAACAGTAGACCTGTGTAAAAAAGCTGAAGCA
ACTGGAGTTTCATGGATTACAGTACATGGGAGAAATATAGAAGAAAGACATCAGCCAGTACATTAT
GATGCAATTAAGTAATTAAGAAAGCATAACACATACCTGTTGTGGCTAATGGAGACATTAACA
TAAAAAGATGCTGAAAATGTTTCATCACCTGACAGGAGCAGATGGATACGAAGAAACACCTCTGAAG
TGCATCCAGGATTTGGGTTGACATGCTCTTGTAGCATGGAACCTCTTTTACATGTTTTACCACCACT
TAATGTACATGATGGAACGGATAACTTCAAACAAGAAAAGAAAGTTTTCAACATTTTGTCAAGTA
CCTCAGCAGTCTAGATTACCTGGGTCATCATTTATGGTGTGTGTAGCTGAAAGCATTTTCAGTTGTAT
GTAAACTCGTACTTCACAAT

Prkar2B

ATGAGCATCTCCATCCCGGCGGGGCTGACGGAGCTGCTGCAGGGCTTCACGGTGGAGGTGCTGC
GGAGCCAGCCCGCGGACCTGCTGGAGTTCGCCCTCCAGTACTTCGGGCGCCTCAAGGAGGAGGCG
GCGGCGGCCAAGGCGGCGGCGAAGGAGAAGGAGGCGAGCGGCCCGGGCGGGCACCCTCGC
CCCGGCCATGACCGGGGCAAGCGCCGCGCCGGGCCCCCCCCGACGCCCGCGGGGTCAACTTCGC
CGAGGAGCCCATGCAGACCGACTCGGAGAGCGGCGAGGACGAAGAGCAGCGGTTCCTGGTGATA
AATCGTTTCACAAGACGGGCGTCAGTGTGTGCAGAAGCTTACAATCCCGATGAAGAGGAGGACGA
TGCAGAAACCAGGATTATTCACCCTAAAACAGATGATCAAAGAAAACAGGCTGCAGGAGGCTTGCAA
GGACATTCTTCTTTTAAAGAACCTAGATCCGGAACAGATGTCTCAGGTGTTGGACGCGATGTTGA
AAAGCTGGTTGAAGGAGGGGAACATGTCATTGATCAAGGAGATGATGGTGACAACCTTCTATGTCA
TTGATAGAGGGACATATGATATTTATGTAAAAATGTGATGGTGTGGGAGGTGTGTTGGCACCTAT
GACAACCGAGGAAGTTTGGAGAGCTGGCCTTAATGTACAATACACCCAGAGCAGCTACAATTATA
GCTACCTCTCCTGGTGGCCATCTGGGGTTGGACAGGGTAACATTCGGAAGAATCATTGTAAAAAAT
AATGCCAAAAGAGAAGAATGTATGAAAATTTATTGAGTCGTTGCCATTCCTTAAATCTTTAGAAG
TATCCGAGCGTTTGAAAGTGGTTGATGTGATTTGGCACCAAAGTATACAAAGATGGTGAACAAATCA
TTGCTCAGGGCGACATGGCTGATTTCTTTCTTCATTTGTGGAATCTGGGGAAGTAAGGATTATAATGA
CAAGGAAGGGTAAACCAGATGTAGAGGTAGAAGAGAATGGAGCAGTTGAAATAGCTCGATGCTC
AAGAGGACAGTATTTGGAGAACTTGCTCTTGTAACAAACCACGAGCTGCTTCTGCATTTGCT
CTTGGCACTGTCAAGTGTTTAGTTATGGACGTGCAGGCATTTGAAAGGCTTTGGGACCTTGTATG
GAAATTCGAAAAGAAACATTTGCAAACCTATGAAGAGCAGCTAGTTGCTCTGTTTGGAAACAAACATG
GACATTGCCGACACCAGTGCATGA