

Eldgenössisches Institut für Geistiges Eigentum Institut Fédéral de la Propriété Intellectuelle Istituto Federale della Proprietà Intellettuale Swiss Federal Institute of Intellectual Property

Case 3:

A transgenic plant and the method for producing the same EpiPlanta Biotech Ltd.

Claims:

1. A transgenic plant in which a nucleic acid molecule encoding an m6A demethylase is introduced, wherein said m6A demethylase has the following two domains:

- i) N-terminal domain (NTD) having the function of AlkB oxidation demethylase; and
- ii) C-terminal domain (CTD).

2. The transgenic plant of claim 1, wherein said m6A demethylase is FTO (fatmass and obesity-associated) protein.

3. The transgenic plant of claim 2, wherein said FTO protein is from vertebrates or marine algae.



4. The transgenic plant of claim 3, wherein said FTO protein has at least 40% , ..., most preferably 100% identity to any of the SEQ ID NOs: 1-4

5. The transgenic plant of any of the claims 1-4, wherein said nucleic acid molecule encoding the m6A demethylase has at least 90%, ..., most preferably 100% identity to any one of SEQ ID NOs: 5-12 ...

8. A tissue, an organ, a pollen, a seed, a grain or a fruit of the plant of any of the claims 1-7

9. A plant cell...

...

15. A method for producing a transgenic plant...

SEQ ID NOs 1-4 (protein) and 5-12 (nucleic acids) are retrieved from lens.org: https://www.lens.org/lens/patent/196-196-347-571-28X/sequences



Questions:

- 1) What is the technical problem and does the first claim solve it?
- 2) What is the difference between m6A demethylase, AlkB oxidation demethylase and FTO?
- 3) Does 40% sequence ID in claim 4 makes sense?
- 4) How conserved are (the claimed) m6A demethylases?
- 5) What about the nucleic acid 90% identity in claim 5?
- 6) Are the sequence searches the right approach to find prior art?
- 7) Are there some claims which may fall under patentability exceptions, e.g. EPC Art 53 (b)?
- 8) Is the unity of the claims given?



1) What is the technical problem and does the first claim solve it?

Problem

Low yield of biomass of "agricultural plants" with state of the art breeding or transgenic approaches

Solution

Claim 1 features a transgenic plant having nucleic acid sequence coding for a m6A demethylase with an N-terminal being an AlkB oxidative demethylase and a C-terminal domain (not mentioning its function).

→ According to the description claim 1 solves the problem by introduction of a AlkB like m6A demethylase. This enzyme is known to remove the methyl group on adenosine in mRNA and has been termed fatmass obesity-associated (FTO) protein.

The applicant refers to Jia et al., Nat Chem Biol, 2011; <u>https://www.ncbi.nlm.nih.gov/pubmed/22002720</u> **"FTO belongs to the non-heme Fe^{II}/α-KG-dependent dioxygenase AlkB family proteins"**

Uniprot link to the human AlkB (check for prior art): <u>https://www.uniprot.org/uniprot/Q9C0B1</u>



2) What is the difference between m6A demethylase, AlkB oxidation demethylase and FTO?

M6A demethylases

https://enzyme.expasy.org/EC/1.14.11.51

N(6)-methyladenine in **DNA** + 2-oxoglutarate + O(2) <=> adenine in DNA + formaldehyde + succinate + CO(2)

https://enzyme.expasy.org/EC/1.14.11.53

N(6)-methyladenosine in **mRNA** + 2-oxoglutarate + O(2) <=> adenosine in mRNA + formaldehyde + succinate + CO(2)

It might be good to check further background citation on EC 1.14.11.53

•••



3) Does 40% sequence ID in claim 4 makes sense?

Get sequences (do not type it in the form, they are too long and you might introduce mistakes) US 2018/0340182 A1

https://www.lens.org/lens/patent/196-196-347-571-28X/sequences

To answer this question you have to ask:

4) How conserved are (the claimed) m6A demethylases?

https://www.ebi.ac.uk/Tools/msa/clustalo/

CLUSTAL O(1.2.4) multiple sequence alignment

Percent Identity Matrix - created by Clustal2.1 #

1: US_2018_0340182_A1_1	100.00	88.71	87.72
2: US_2018_0340182_A1_2	88.71	100.00	90.10
3: US_2018_0340182_A1_3	87.72	90.10	100.00

> 87% SEQ ID

human

pig cow



- 3) Does 40% sequence ID in claim 4 makes sense?
- 4) How conserved are (the claimed) m6A demethylases?

SEQ ID NO: 4 is not of vertebrate origin but marine algae

https://www.ebi.ac.uk/Tools/msa/clustalo/

US_2018_0340182_A1_4 ------MSPSSSVLEPEDGEPFARVHRAHYRGFVVDAPSVLPA 37 US_2018_0340182_A1_1 MKRTPTAEEREREAKKLRLLEELEDTWLPYLTPKDD-EFYQQWQLKYPKLILREASSVSE 59 US_2018_0340182_A1_2 MKRTPTAEERERGAKKLRLLEELEDTWLPYLTPKDD-EFYQQWQLKYPKLILREAGSVPE 59 US_2018_0340182_A1_3 MKRTPTAEEREREAKKLRLLEELEDTWLPYLTPKDD-EFYQQWQLKYPKLILREAASVPE 59 . * *:*. * : : : * ::: . :

1:	US_2018_	_0340182_	A1_4	100.00	31.33	30.60	30.60
2:	US_2018	0340182	A1_1	31.33	100.00	88.71	87.72
3:	US_2018_	0340182	A1_2	30.60	88.71	100.00	90.10
4:	US 2018	0340182	A1 3	30.60	87.72	90.10	100.00



- 3) Does 40% sequence ID in claim 4 makes sense?
- 4) How conserved are (the claimed) m6A demethylases?

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1:US_2018_0340182_A1_4100.0031.3330.6030.602:US_2018_0340182_A1_131.33100.0088.7187.723:US_2018_0340182_A1_230.6088.71100.0090.104:US_2018_0340182_A1_330.6087.7290.10100.00

... only 30-31% SEQ ID algae to human/pig/cow

→ Probably yes, proteins are sometimes quite diverse on the level of sequence identity. However, the function can be highly conserved in some domain.



5) What about the nucleic acid 90% identity in claim 5?

Difficult to predict... Get sequences from: <u>https://www.lens.org/lens/patent/196-196-347-571-28X/sequences</u>

There are per each protein two DNA sequences

- one of the natural (SEQ ID NOs: 5,7,9,11)
- one which has been codon optimised (SEQ ID NOs: 6,8,10,12) to enable or enhance expression in a plant (different codon usage).

→ The search using the **natural sequence is expected to give** documents describing the DNA sequence in its original... and might retrieve a high number of documents...

→ The search using the **codon optimised DNA** is likely to retrieve at **100% ID only the application**...

Does the sequence search really give you the necessary prior art?



6) Are the sequence searches the right approach to find prior art?

Some considerations:

- If you search with the sequence of one organism, you will probably find a lot of documents describing this sequence in its natural context (protein and DNA).
- The inventive concept in this application is not the sequence per se but rather the fact, that it is introduced in a foreign organism (transgenic plant)
- It might be better to start with a keyword search using the right enzyme names / functions and include the concept of the transgenic plant or patent classes and citations in literature...
- \rightarrow Keyword and patent classes (transgenic plants and demethylases...)
- \rightarrow Prior art in scientific journals
- \rightarrow Citation analysis (cited, citing, backward or forward citation)



Search for transgenic plant

Search

 View section
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Symbol	Classification and description	
C12	BIOCHEMISTRY; BEER; SPIRITS; WINE; VINEGAR; MICROBIOLOGY; ENZYMOLOGY; MUTATION OR GENETIC ENGINEERING	i
C12N	MICROORGANISMS OR ENZYMES; COMPOSITIONS THEREOF (biocides, pest repellants or attractants, or plant growth regulators, containing microorganisms, viruses, microbial fungi, enzymes, fermentates or substances produced by or extracted from microorganisms or animal material <u>A01N 63/00</u> ; food compositions <u>A21</u> , <u>A23</u> ; medicinal preparations <u>A61K</u> ; chemical aspects of, or use of materials for, bandages, dressings, absorbent pads or surgical articles <u>A61L</u> ; fertilisers <u>C05</u>); PROPAGATING, PRESERVING OR MAINTAINING MICROORGANISMS (preservation of living parts of humans or animals <u>A01N 1/02</u>); MUTATION OR GENETIC ENGINEERING; CULTURE MEDIA (microbiological testing media <u>C12Q</u>)	s D i
▲ 🔽 C12N 15/00	Mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors, e.g. plasmids, or their isolation, preparation or purification; Use of hosts therefor (mutants or genetically engineered microorganisms, per se C12N 1/00, C12N 5/00, C12N 7/00; new plants per se A01H; plant reproduction by tissue culture techniques A01H 4/00; new animals per se A01K 67/00; use of medicinal preparations containing genetic material which is inserted into cells of the living body to treat genetic diseases, gene therapy A61K 48/00)	D
C12N 15/09	Recombinant DNA-technology	D
C12N 15/63	 Introduction of foreign genetic material using vectors; Vectors; Use of hosts therefor; Regulation of expression 	D
C12N 15/79	 Vectors or expression systems specially adapted for eukaryotic hosts 	D
C12N 15/82	●●●●for plant cells {, e.g. plant artificial chromosomes (PACs)}	D
C12N 15/8241	•••••{Phenotypically and genetically modified plants via recombinant DNA technology}	
C12N 15/8242	●●●●●{with non-agronomic quality (output) traits, e.g. for industrial processing; Value added, non-agronomic traits}	D
C12N 15/8243	●●●●●●{involving biosynthetic or metabolic pathways, i.e. metabolic engineering, e.g. nicotine, caffeine}	

IngridB.Mueller@ipi.ch



Search for transgeni	c plant	Search View section Index A B C D E F G H Y					
€ → 🗄 4	🕂 İ CPC 🏦 []	≥∞ 2000 « C12N13/00 »					
Symbol	Classification and descri	tion					
C12	Symbol	Classification and description					
		biosynthesis}					
	C12N 15/8253	•••••••{Methionine or cysteine}					
	C12N 15/8254	•••••••{Tryptophan or lysine}					
	C12N 15/8255	••••••{involving lignin biosynthesis}					
	C12N 15/8257	●●●●●● {for the production of primary gene products, e.g. pharmaceutical products, interferon}					
▲ 🔽 C12N 15/00	C12N 15/8258	••••••{for the production of oral vaccines (antigens) or immunoglobulins}					
	C12N 15/8259	●●●●●●{Phytoremediation}					
	C12N 15/8261	●●●●●{with agronomic (input) traits, e.g. crop yield}					
C12N 15/09		Definitions					
C12N 15/63		Glossary of terms					
C12N 15/79		In this place, the following terms or expressions are used with the meaning indicated:					
C12N 15/82	Input trait influences the input required for growth and dovelopment of the plan						
C12N 15/824		or its parts					
C12N 15/824							
C12N 15/8243	•••••{involving bi	synthetic or metabolic pathways, i.e. metabolic engineering, e.g.					

nicotine, caffeine}

IngridB.Mueller@ipi.ch



→ Can I start a new search using	Symbol	Classification and description	
 → Where can I view the description of a particular CPC class? → What is the meaning of the stars in front of the classifications found? → What does the text in brackets mean? 	C12N	MICROORGANISMS OR ENZYMES; COMPOSITIONS THEREOF (biocides, pest repellants or attractants, or plant growth regulators, containing microorganisms, viruses, microbial fungi, enzymes, fermentates or substances produced by or extracted from microorganisms or animal material A01N 63/00; food compositions A21, A23; medicinal preparations A61K; chemical aspects of, or use of materials for, bandages, dressings, absorbent pads or surgical articles A61L; fertilisers C05); PROPAGATING, PRESERVING OR MAINTAINING MICROORGANISMS (preservation of living parts of humans or animals A01N 1/02); MUTATION OR GENETIC ENGINEERING; CULTURE MEDIA (microbiological testing media C12Q)	s d i 😲
Selected electrifications			
C12N15/8261 /low X C12N9/0071 /low X Clear	▲	Enzymes; Proenzymes; Compositions thereof (preparations containing enzymes for cleaning teeth <u>A61K 8/66</u> , <u>A61Q 11/00</u> ; medicinal preparations containing enzymes or proenzymes A61K 38/43; enzyme containing detergent compositions <u>C11D</u> ; {enzymes with nucleic acid structure, e.g. ribozymes, <u>C12N 15/113</u> }); Processes for preparing, activating, inhibiting, separating or purifying enzymes (preparation of malt <u>C12C 1/00</u>)	Di
Find patents	C12N 9/0004	• {Oxidoreductases (1.)}	
Copy to search form	C12N 9/0071	 {acting on paired donors with incorporation of molecular oxygen (1.14)} 	
	C12N 9/0073	●●●{with NADH or NADPH as one donor, and incorporation of one atom of oxygen 1.14.13}	
	C12N 9/0075	●●●● {Nitric-oxide synthase (1.14.13.39)}	
	C12N 9/0077	●●●{with a reduced iron-sulfur protein as one donor (1.14.15)}	
m6A demethylases	C12N 9/0079	●●●● {Steroid 11 beta monooxygenase (P-450 protein)(1.14.15.4)}	
EC 1.14.11.53	C12N 9/0081	<l< td=""><td></td></l<>	
	C12N 9/0083	• • • {Miscellaneous (1.14.99)}	



2 results found in the Worldwide database for:

demethyl* or FTO or AlkB or M6A in the title or abstract AND C12N15/8261/low and C12N9/0071 as the Cooperative Patent Classification





2 results found in the Worldwide database for: demethyl* or FTO or AlkB or M6A in the title or abstract AND C12N15/8261/low and C12N9/0071 as the Cooperative Patent

Abstract of JP2015077134 (A)

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Patenttranslate powered by EPO and Google

PROBLEM TO BE SOLVED: To provide transgenic soybean plants exhibiting commercially acceptable tolerance to applications of dicamba herbicide.SOLUTION: The invention provides a transgenic soybean event MON87708 transformed to have dicamba monooxygenase (DMO), an enzyme cloned from Stenotrophomonas maltophilia commonly found in soil rhizosphere. Dicamba monooxygenase catalyzes deactivation of dicamba to a non-herbicidal compound, 3,5-dichlorosalicylic acid, via O-demethylation reaction. Also provided are polynucleotide specific for the event MON87708, as well as plants, plant cells, seeds, plant parts and commodity products comprising polynucleotides specific for event MON87708.

2. SOYBEAN TRANSGENIC EVENT MON87708 AND USE METHODS THEREOF

*	Inventor: RONALD J BRINKER WEN C BURNS (+5)	Applicant: MONSANTO TECHNOLOGY LLC	CPC: <u>A23D9/00</u> <u>A23L11/03</u> <u>C12N15/8274</u>	IPC: A01H1/00 C12N1/15 C12N1/21	Publication info: JP2015077134 (A) 2015-04-23 JP5985588 (B2)	Priority date: 2009-09-17
			(+3)	(+2)	2016-09-06` ´	



Publicad.gov PubMed US National Library of Medicine Advanced	Search		
National Institutes of Health Advanced Format: Abstract → Send to → Nature, 2009 Apr 16;458(7240):894-8. doi: 10.1038/nature07848. Epub 2009 Feb 22. Inactivation of the Fto gene protects from obesity. Fischer J ¹ , Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, Rüther U. Imactivation Imactivation Author information	Full text links Full text links Nature Save items Add to Eavorites		
Abstract Several independent, genome-wide association studies have identified a strong correlation between body mass index and polymorphisms in the human FTO gene. Common variants in the first intron define a risk allele predisposing to obesity, with homozygotes for the risk allele weighing approximately 3 kilograms more than homozygotes for the low risk allele. Nevertheless, the functional role of FTO in energy homeostasis remains elusive. Here we show that the loss of Fto in mice leads to postnatal growth retardation and a significant reduction in adipose tissue and lean body mass. The leanness of Fto-deficient mice develops as a consequence of increased energy expenditure and systemic sympathetic activation, despite decreased spontaneous locomotor activity and relative hyperphagia. Taken together, these experiments provide, to our knowledge, the first direct demonstration that Fto is functionally involved in energy homeostasis by the control of energy expenditure.	Similar articles FTO effect on energy demand versus food intake. [Nature. 2010] Obesity-associated variants within FTO form long-range functional connections v [Nature. 2014]		
Comment in FTO effect on energy demand versus food intake. [Nature. 2010] PMID: 19234441 DOI: <u>10.1038/nature07848</u> [Indexed for MEDLINE]	Review The 'Fat Mass and Obesity Related' (FTO) gene: Mechanisms [Curr Obes Rep. 2015] Review FTO and obesity: mechanisms of association. [Curr Diab Rep. 2014]		
	See all		



Public ed.gov US National Library of Medicine National Institutes of Health	PubMed ~	Advanced			Search	Help
Format: Abstract → Mol Cell. 2018 Sep 20;71(6):97: Differential m ⁶ A, n Cytoplasm. Wei J ¹ , Liu F ¹ , Lu Z ² , Fei Q ¹ , • Author information	3-985.e5. doi: 10.1016/j.r 1 ⁶ A _m , and m ¹ A <u>Ai Y¹, He PC¹, Shi H¹, G</u>	nolcel.2018.08.011. Epub 2018 Sep 6 Demethylation Media Cui X ¹ , Su R ³ , Klungland A ⁴ , Jia G ⁵	t ed by FTO in the Cell Nucl , <u>Chen J³, He C⁶.</u>	Send to -	Full text links CellPress Save items ☆ Add to Favorites	•
Abstract FTO, the first RNA demethylase discovered, mediates the demethylation of internal N ⁶ -methyladenosine (m ⁶ A) and N ⁶ , 2-O-dimethyladenosine (m ⁶ A _m) at the +1 position from the 5' cap in mRNA. Here we demonstrate that the cellular distribution of FTO is distinct among different cell lines, affecting the access of FTO to different RNA substrates. We find that FTO binds multiple RNA species, including mRNA, snRNA, and tRNA, and can demethylate internal m ⁶ A and cap m ⁶ A _m in mRNA, internal m ⁶ A in U6 RNA, internal and cap m ⁶ A _m in snRNAs, and N ¹ - methyladenosine (m ¹ A) in tRNA. FTO-mediated demethylation has a greater effect on the transcript levels of mRNAs possessing internal m ⁶ A than the ones with cap m ⁶ A _m in the tested cells. We also show that FTO can directly repress translation by catalyzing m ¹ A tRNA demethylation. Collectively, FTO-mediated RNA demethylation occurs to m ⁶ A and m ⁶ A _m in mRNA and snRNA as well as m ¹ A in tRNA.				Similar articles FTO controls reversible m ⁶ Am RNA methylation during snRNA I [Nat Chem Biol. 2019] Structural insights into FTO's catalytic mechanism for t [Proc Natl Acad Sci U S A. 2019]		
				¹ A tRNA demethylation.	Reversible methylation of m ⁶ A _m ir [Nature. 2017]	
Copyright © 2018 Elsevier Inc	. All rights reserved.				Review FTO, m ^{6< and the hypothesis of reve}	/sup> A _m , ersible [FEBS Lett. 2018]
KEYWORDS: FTO; cap m(6), translation regulation	A(m); cytoplasmic deme	thylation; m(6)A; nuclear m(6)A der	nethylation; snRNA demethylation; tRNA m(1	1)A demethylation;	Review Novel positioning FTO, an m ^{[J Canc}	from obesity to cancer : er Res Clin Oncol. 2019]
PMID: 30197295 PMCID: PM	C6151148 [Available on 2	019-09-20] DOI: <u>10.1016/j.molcel.2</u>	2018.08.011			See reviews
[Indexed for MEDLINE]						See all

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Filters 💿	Θ	Patent Summary	Cites 17 Works	Cites 11 patents	Family Info	Sequences	Legal Info	Notes 👩			
Date Range	\checkmark	(Add to Collection	share Patent								
Jurisdictions	~										
Inventors	~	In this Lens relea	n this Lens release, EP 3406726 A1 cites 17 scholarly works								
Owners (US)	\checkmark										
Applicants	\sim	⊗ N(6)-Methyl	adenosine RNA M	odification Regulate	es Shoot Stem (Cell Fate in Arab	oidopsis.	IO I 028-449-4	53-963-958		
Cited Works	\checkmark	Lisha Shen, Zh	ne Liang, Xiaofeng Gu,	Ying Chen, Zhi Wei Nor	man Teo, Xinglian	g Hou, Weiling Ma	ggie Cai, Peter	View this a	article via PubMed		
Document Families	\checkmark	C. Dedon, Lu L	iu, Hao Yu					View this a	article via Microsoft Academic		
Classifications	\checkmark	Developmenta	Developmental Cell, Issue: 2, Volume: 38, Pages: 186-200. Jul 7, 2016								
Document Types	\checkmark							54 Scholar 55 Find full-te	l-text at your institution		
Biologicals	\sim										
Your Workspace	\checkmark	🛛 Mammaliar	WTAP is a regulat	ory subunit of the R	NA N6-methyla	denosine metł	nyltransferase	IO I 066-078-4	3-489-133-41X		
Query Tools	\checkmark	Xiao-Li Ping, B	Xiao-Li Ping, Bao Fa Sun, Lu Wang, Wen Shao Xiao, Xin Yang, Wen-Jia Wang, Samir Adhikari, Yue Shi, Ying Ly.						article via PubMed		
New Structured Search	Ð	 Yu-Sheng Chen, Xu Zhao, Ang Li, Ying Yang, Ujwal Dahal, Xiao-Min Lou, Xi Liu, Jun Huang, Weiping Yuan, Xiaofan Zhu, Tao Cheng, Yong-Liang Zhao, Xinquan Wang, Jannie M. Rendtlew Danielsen, Feng Liu, Yun-Gui Yang Cell Research, Issue: 2, Volume: 24, Pages: 177-189. Jan 10, 2014 							article via Crossref article via Microsoft Academic cite this article arly Works cite this article ext at your institution		
		➢ A METTL3-M Jianzhao Liu, Qing Dai, Weiz Nature Chemi	1ETTL14 complex Yanan Yue, Dali Han, X chong Chen, Chuan He cal Biology, Issue: 2,	mediates mammali (iao Wang, Ye Fu, Liang : e Volume: 10, Pages: 93-9	an nuclear RNA Zhang, Guifang Jia 95. Dec 6, 2013	N6-adenosine n, Miao Yu, Zhike L	methylation u, Xin Deng,	 IOI 015-800-42 IOI View this a Solve View this a Solve View this a I Patents a 	33-225-097 article via PubMed article via Crossref article via Microsoft Academic cite this articl Feedback		





EUROPEAN SEARCH REPORT

Application Number EP 18 17 1487

	DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	GUAN-ZHENG LUO ET AL: "Unique features of the m6A methylome in Arabidopsis thaliana", NATURE COMMUNICATIONS, vol. 5, no. 1, 28 November 2014 (2014-11-28), XP55486590, DOI: 10.1038/ncomms6630 * abstract * * page 2, column 1, paragraph 3 * * page 4, column 2, paragraph 1 * * page 4, column 2, paragraph 3 * * page 4, column 2, paragraph 3 * * page 5, column 2 * * page 7, column 1, paragraph 2 * 	1-16	INV. C12N15/82 TECHNICAL FIELDS SEARCHED (IPC) C12N
A	SCHRAGA SCHWARTZ ET AL: "Perturbation of m6A Writers Reveals Two Distinct Classes	1-6	

IngridB.Mueller@ipi.ch



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