Patent

UNITED STATES PATENT · ANNOTATED READING EDITION US 7,344,859 B2 · granted 18 March 2008

Recombinant methods for expressing a functional sweet (T1R2/T1R3) taste receptor

Zoller, Li, Staszewski, O'Connell, Zozulya, Adler, Xu & Echeverri, for Senomyx, Inc. The original text, with every acronym linked to its database record or primary reference, and all 41 claims annotated.

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Front page

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ASSIGNEE	Senomyx, Inc. (San Diego) — now listed as Firmenich Inc
ATTORNEY	Hunton & Williams LLP
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HOW TO READ THIS PAGE

Dotted-underlined terms link out to the authoritative record — NCBI Gene, UniProt, Cellosaurus, MeSH, or the cited paper. Quoted blocks with a coloured rule are the patent's own words; everything else is annotation.

Background of the invention

The specification opens from sensory physiology, then narrows to a molecular problem: the T1R receptors had been cloned, but expressed singly they did not respond to tastants.

> The taste system provides sensory information about the chemical composition of the external world. Mammals are believed to have at least five basic taste modalities: sweet, bitter, sour, salty, and umami.
>
> Each taste modality is thought to be mediated by a distinct protein receptor or receptors that are expressed in taste receptor cells found on the surface of the tongue. The taste receptors that recognize bitter, sweet, and umami taste stimuli belong to the G-protein-coupled receptor superfamily. Other taste modalities are believed to be mediated by ion channels.
>
> US 7,344,859 B2, Background — citing Kawamura 1987, Kinnamon 1992, Lindemann 1996, Stewart 1997, Hoon 1999, Adler 2000

The mechanism the patent then recites is the canonical one. On ligand binding, the receptor changes conformation and activates a heterotrimeric G protein by promoting displacement of bound GDP by GTP on the G ? subunit and subsequent dissociation of G? from G? and G?. The free subunits then activate downstream effectors. The specification credits US 5,691,188 for this description.

The three receptors at issue — T1R1, T1R2 and T1R3 — are class C GPCRs, structurally related to the metabotropic glutamate receptors and the calcium-sensing receptor, both of which the patent uses as alignment references in Example 2.

The invention

> The present invention in part relates to the discovery that the T1R receptors assemble to form functional taste receptors. Particularly, it has been discovered that co-expression of T1R1 and T1R3 results in a taste receptor that responds to umami taste stimuli, including monosodium glutamate. Also, it has been discovered that co-expression of the T1R2 and T1R3 receptors results in a taste receptor that responds to sweet taste stimuli including naturally occurring and artificial sweeteners.
>
> The invention relates to the construction of cell lines that stably or transiently co-express a combination of T1R1 and T1R3; or T1R2 and T1R3; under constitutive or inducible conditions.
>
> US 7,344,859 B2, Summary

What the expression system has to solve

Obligate heteromerization. Neither subunit alone gives a tastant response. Both must be present in one cell and trafficked together to the plasma membrane. That single fact is the whole basis of claim 1.

A readable output. Native sweet transduction runs through gustducin and a PLC?2 / IP? cascade that is hard to read in a heterologous cell. The patent instead supplies G?15 or G?16, promiscuous Gq-class ? subunits that couple almost any GPCR to PLC?, turning receptor activation into a cytosolic Ca²? transient a fluorescent dye can report.

A host that tolerates it. The preferred host is a HEK-293 derivative already stably expressing G?15, obtained from Chandrashekar et al. (2000). Claims also reach COS, CHO, yeast, insect and Xenopus oocytes, and native taste cells.

Stability. Constitutive stable lines gave a greater than ten-fold activity gain over transient transfection, but lost activity during propagation. Example 12 answers that with the mifepristone-inducible GeneSwitch system, which claim 22 covers as "an inducible promoter".

Worked examples

Examples 2 to 13 of the specification. Figure numbers refer to the patent's 16 drawing sheets.

Example 2 — Sequence alignment (FIG. 1). Human T1R1, T1R2, T1R3 and rat T1R3 aligned against rat T1R1 (AAD18069), rat T1R2 (AAD18070), rat mGluR1 (P23385) and the human calcium-sensing receptor (P41180). Seven transmembrane segments boxed; residues contacting the glutamate side-chain carboxylate and ?-amino moiety in the mGluR1 crystal structure marked separately.

Example 3 — Expression in taste tissue (FIG. 2). RT-PCR detects both hT1R2 and hT1R3 in resected human circumvallate papillae.

Example 4 — Heterologous expression method. HEK-293/G?15 cells in DMEM with 10% FBS, non-essential amino acids and 3 µg/ml blasticidin; ~0.1 million cells per well on 24-well plates; lipofection with Mirus TransIT-293; switched to low-glucose DMEM/GlutaMAX at 24 h to limit glutamate- and glucose-induced desensitisation; loaded with 3 µM Fluo-4 in DPBS for 1.5 h. Responses were transient (rarely beyond 15 s) and asynchronous, so cells were counted manually at a fixed 30 s point.

Example 5 — hT1R2/hT1R3 is the sweet receptor (FIG. 3). Only the co-transfected cells respond to sucrose. Maximum responders ranged 10–30% depending on sweetener. Dose-response thresholds align with psychophysical detection thresholds from taste testing. Gurmarin (a 50-fold dilution of 10 g/l *Gymnema sylvestre* extract) blocked the response to 250 mM sucrose but not the endogenous ??-adrenergic response to 20 µM isoproterenol — the specificity control. Panel (c) covers sucrose, aspartame, D-tryptophan and saccharin.

Example 6 — Rat receptor and species swaps (FIG. 4). hT1R2/hT1R3, rT1R2/rT1R3 and both cross-species pairs tested against 350 mM sucrose, 25 mM tryptophan, 15 mM aspartame and 0.05% monellin. Rat pair also works; the results suggest T1R2 controls ligand specificity.

Example 7 — Plate-reader format (FIGS. 5–6). 12.5 mM cyclamate gives a response in hT1R2/hT1R3 cells and none in T1R3-only cells. Rank order and threshold values across commercial sweeteners mirror human sweet taste.

Example 8 — Conserved binding residues (FIG. 7). The mGluR1 glutamate-contact residues are conserved in T1R1.

Example 9 — hT1R1/hT1R3 is the umami receptor (FIG. 8). Glutamate dose-response with and without 0.2 mM IMP. Maximum responders ~5% for glutamate alone, ~10% with IMP.

Example 10 — PDZIP export sequence (FIGS. 9–10). The six-residue sequence SVSTW (SEQ ID NO: 1) fused to the hT1R2 C-terminus raises plasma-membrane expression, shown by immunofluorescence of myc-tagged receptor and by FACS. Sweetener pool no. 5 was saccharin, sodium cyclamate, acesulfame K and aspartame at 20 mM each.

Example 11 — Stable co-expressing lines (FIGS. 11–12). Linearised PEAK10- and pcDNA3.1/Zeo-derived vectors — plasmids SAV2485 (T1R1), SAV2486 (T1R2), SXV550 (T1R3) — co-transfected into the Aurora Biosciences HEK-293/G?15 line. Selection in 0.0005 mg/ml puromycin and 0.1 mg/ml zeocin. On VIPR-II instrumentation: 96-well plates at ~100,000 cells/well, 0.005 mM fluo-3-AM for 1 h, fluorescence at 480 nm excitation / 535 nm emission averaged 20–30 s after addition and normalised to 0.001 mM ionomycin. Active clones gave 80–100% responding cells against roughly 5% for transient transfection — a greater than ten-fold activity gain.

Example 12 — Inducible umami line (FIGS. 13–15). pGene-derived zeocin vectors SXV603 (T1R1) and SXV611 (T1R3) plus the puromycin-resistant pSwitch vector SXV628 carrying the GeneSwitch protein. Induction with 10?¹? M mifepristone; clone I-17 seeded at ~80,000 cells/well for 48 h, loaded with 3 µM fluo-4-AM, read on

FLIPR. Roughly 80% of induced cells responded to L-glutamate against ~10% transiently transfected, enough that activity could be summed over the field instead of counted cell by cell. Amino-acid screening showed selective activation by L-glutamate and L-aspartate; other L-amino acids responded only weakly and at much higher concentrations.

Example 13 — Lactisole (FIG. 16). Lactisole inhibits hT1R2/hT1R3 with an IC?? of 24 μM and hT1R1/hT1R3 with an IC?? of 165 μM. Muscarinic acetylcholine receptor responses were unaffected, ruling out non-specific inhibition of G?15 signalling. In human taste tests following Schiffman et al. (1999), millimolar lactisole raised detection thresholds for sweet and umami but not for sodium chloride. The inventors conclude the two receptors may share a lactisole-binding domain.

Claims 1–41

One independent claim, forty dependent. Claim text is verbatim; each group carries an annotation explaining what the limitation does and where to read more. Highlighted phrases mark the operative limitation.

Claim 1

The independent method claim

1.

A method of producing a heteromeric taste receptor comprising: expressing at least one T1R2 nucleic acid sequence and at least one T1R3 nucleic acid sequence in a recombinant host cell under conditions which result in a heteromeric taste receptor comprising at least one T1R2 and T1R3 polypeptide, wherein said T1R2 polypeptide is (i) encoded by a nucleic acid sequence comprising SEQ. ID. NO: 10, (ii) encoded by a nucleic acid sequence comprising a nucleic acid that hybridizes to SEQ. ID. NO: 10 under stringent hybridization conditions which are conducting the hybridization reaction at 42° C. in a solution comprising 50% formamide, 5×SSC, and 1% SDS and washing at 65° C. in a solution comprising 0.2×SSC and 0.1% SDS, or (iii) a T1R2 polypeptide possessing at least 90% sequence identity to the T1R2 polypeptide of SEQ. ID. NO: 6; and wherein said T1R3 polypeptide is (i) encoded by a nucleic acid sequence comprising SEQ. ID. NO: 9; (ii) encoded by a nucleic acid sequence that hybridizes to SEQ. ID. NO: 9 under stringent hybridization conditions which are conducting the hybridization reaction at 42° C. in a solution comprising 50% formamide, 5×SSC, 10% SDS; and washing at 65° C. in a solution comprising 0.2×SCC and 0.1% SDS, or (iii) a T1R3 polypeptide possessing at least 90% sequence identity to the T1R3 polypeptide of SEQ. ID. NO: 7.

ANNOTATION

Each subunit is defined three ways — literal sequence, hybridisation equivalents, or percent identity — so a competitor cannot design around by using a natural variant. The sequence identifiers are: **SEQ ID NO: 6** = human T1R2 protein (UniProt Q8TE23, 839 aa), **SEQ ID NO: 7** = human T1R3 protein (UniProt Q7RTX0, 852 aa), **SEQ ID NO: 9** = T1R3 nucleic acid, **SEQ ID NO: 10** = T1R2 nucleic acid. Sequence identifiers are governed by WIPO Standard ST.25 (since replaced by ST.26).

Reciting the stringency conditions inside the claim body is what makes branch (ii) definite: temperature, formamide, SSC and SDS concentrations are all specified rather than left to the art. For background on how stringency sets the identity cut-off, see Molecular Biology of the Cell, hybridisation.

Note the asymmetry. The T1R2 branch recites 1% SDS; the T1R3 branch recites 10% SDS, and spells SSC as "SCC". Both are almost certainly prosecution typographical errors, but they are the operative claim language as granted.

Claim 2

Same-species pairing

2.

The method of claim 1, wherein said T1R2 and T1R3 are of the same species origin.

ANNOTATION

Example 6 is the support: human and rat subunits were deliberately mixed (hT1R2/rT1R3 and rT1R2/hT1R3) and both chimeric pairs were tested, which is what makes the same-species restriction a meaningful narrowing rather than a formality. The same example led the inventors to suggest that T1R2 is the subunit that governs ligand specificity.

Claims 3–11

The T1R2 identity ladder

3.
The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide comprising the amino acid sequence of SEQ. ID. NO: 6.

4.
…a human T1R2 polypeptide that exhibits at least 90% sequence identity to the polypeptide of SEQ. ID. NO: 6.

5.
…at least 95% sequence identity to SEQ. ID. NO: 6.

6.
…at least 96% sequence identity to SEQ. ID. NO: 6.

7.
…at least 97% sequence identity to SEQ. ID. NO: 6.

8.
…at least 98% sequence identity to SEQ. ID. NO: 6.

9.
…at least 99% sequence identity to SEQ. ID. NO: 6.

10.
The method of claim 1, wherein said T1R2 polypeptide is encoded by the nucleic acid sequence of SEQ. ID. NO: 10.

11.
The method of claim 1, wherein said T1R2 polypeptide is encoded by a nucleic acid sequence that hybridizes to SEQ. ID. NO: 10 under stringent hybridization conditions which are conducting the hybridization reaction at 42° C. in a solution comprising 50% formamide, 5×SSC, and 1% SDS and washing at 65° C. in a solution comprising 0.2×SSC and 0.1% SDS.

ANNOTATION

A standard defensive ladder. Each rung is a separate fallback position: if a prior-art reference later invalidates the 90% claim, the 95%, 96%, 97%, 98% and 99% claims survive independently. The metric is pairwise sequence identity as computed by an alignment algorithm — in practice BLAST or the Needleman–Wunsch global alignment the specification describes.

Why this works as prior art defence: the specification cites Bowie et al., *Science* 247:1306–1310 (1990) and Guo et al., *PNAS* 101:9205–9210 (2004) on protein tolerance to amino-acid substitution, the literature that justifies claiming a band of identity rather than one sequence.

Claims 12–20

The T1R3 identity ladder

12.
The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide comprising the amino acid sequence of SEQ. ID. NO: 7.

13.
…possesses at least 90% sequence identity to the polypeptide of SEQ. ID. NO: 7.

14.
…at least 95% sequence identity to SEQ. ID. NO: 7.

15.
…at least 96% sequence identity to SEQ. ID. NO: 7.

16.
…at least 97% sequence identity to SEQ. ID. NO: 7.

17.
…at least 98% sequence identity to SEQ. ID. NO: 7.

18.
…at least 99% sequence identity to SEQ. ID. NO: 7.

19.
The method of claim 1, wherein said T1R3 polypeptide is encoded by a nucleic acid sequence of SEQ. ID. NO: 9.

20.
The method of claim 1, wherein said T1R3 polypeptide is encoded by a nucleic acid sequence that hybridizes to SEQ. ID. NO: 9 or SEQ. ID. NO: 11 under stringent hybridization conditions which are conducting the hybridization reaction at 42° C. in a solution comprising 50% formamide, 5×SSC, and 1% SDS and washing at 65° C. in a solution comprising 0.2×SSC and 0.1% SDS.

ANNOTATION

The mirror ladder for the shared subunit. T1R3 matters more commercially than T1R2 because it is the subunit common to both the sweet and umami receptors — the reason Example 13's lactisole result (inhibition of both heteromers) was significant, and the reason the sibling patents claim T1R3-containing receptors so heavily.

Claim 20 is the one dependent claim that widens rather than narrows: it adds SEQ ID NO: 11 as an alternative hybridisation target alongside SEQ ID NO: 9. T1R3 is encoded at TAS1R3, chromosome 1p36.33; the mouse orthologue is the historic *Sac* saccharin-preference locus, mapped by Max et al. (2001).

Claims 21–22

Promoter control

21.
The method of claim 1, wherein said T1R2 and said T1R3 nucleic acid sequences are each operably linked to a constitutive promoter.

22.
The method of claim 1, wherein said T1R2 and said T1R3 nucleic acid sequences are each operably linked to an inducible promoter.

ANNOTATION

These two claims carry the practical heart of the patent. Example 11's constitutive stable lines gave 80–100% responding cells and a greater than ten-fold activity gain, but "can rapidly lose activity during cell propagation" — the predictable consequence of constitutively expressing a receptor that mobilises calcium.

Claim 22 covers the fix: the GeneSwitch system, a progesterone-receptor-derived chimeric transcription factor activated by 10?¹? M mifepristone. Expression stays off during propagation and is switched on 48 h before assay. "Operably linked" is a term of art: the promoter must be positioned so it actually drives transcription of the downstream coding sequence.

Claims 23–27

Host cell

23.
…expressed in a prokaryotic cell.

24.
…expressed in a eukaryotic cell.

25.
The method of claim 24, wherein said cell is a mammalian, yeast, insect or amphibian cell.

26.
The method of claim 24, wherein said cell is a HEK-293 cell, COS cell, CHO cell, or Xenopus ooctye.

27.
The method of claim 26, wherein the cell is a HEK-293 cell.

ANNOTATION

A funnel from the broadest host category down to the one actually used. Claim 23's prokaryotic option is aspirational — a bacterium has no machinery to fold and traffic a seven-transmembrane class C GPCR — but it costs nothing to claim and blocks a workaround.

Claim 27 is the commercially operative one. HEK-293 (ATCC CRL-1573) was derived by Frank Graham from human embryonic kidney tissue and first described in Graham et al., *J Gen Virol* 36:59–74 (1977). It is used because it takes up DNA efficiently and divides indefinitely. The other hosts: COS-7 (CRL-1651), CHO-K1 (CCL-61), and Xenopus oocytes, which are read by two-electrode voltage clamp rather than by calcium dye. "Ooctye" is as printed.

Claims 28–30

G protein coupling

28.
The method of claim 1, wherein said cell expresses a G protein.

29.
The method of claim 28, wherein said G protein is a promiscuous G protein.

30.
The method of claim 28, wherein said G protein is G?15, G?16 or gustducin.

ANNOTATION

"Promiscuous" is a real term of art, not loose drafting: it denotes a G? subunit that couples receptors regardless of their native G-protein preference. G?15 (mouse) and G?16 (human, gene *GNA15*) are the canonical examples; the approach was established for olfactory receptors by Krautwurst et al., *Cell* 95:917–926 (1998), which the patent cites.

Gustducin (gene *GNAT3*) is the native taste-cell G protein, included for completeness. A sibling patent, US 7,208,290, claims chimeric G?15 variants; another, US 7,022,488, covers coupling through Gi proteins instead.

`Claims 31–32`

Surface expression and labelling

31.
The method of claim 1, wherein said T1R2 and T1R3 polypeptides are expressed on the surface of said cell.

32.
The method of claim 1, wherein either of said T1R2 and T1R3 nucleic acid sequences are contained in a nucleic acid construct that comprises a nucleic acid sequence that encodes a detectable label.

ANNOTATION

Claim 31 is the functional requirement that distinguishes a working receptor from protein stuck in the endoplasmic reticulum. Class C GPCRs are notoriously hard to traffic, which is the whole point of Example 10: appending the PDZ-interacting **PDZIP** sequence SVSTW (SEQ ID NO: 1) to the C-terminus markedly increased hT1R2 at the plasma membrane.

Claim 32 covers the detection tags used to prove it: the myc epitope read by immunofluorescence and FACS in Example 10, and more generally GFP, luciferase, ?-galactosidase and CAT, all listed in the specification.

`Claims 33–37`

Stable versus transient expression

33.
The method of claims 1, wherein said cell stably expresses said T1R2 and T1R3 nucleic acid sequences.

34.
The method of claim 1, wherein said cell transiently expresses said T1R2 and T1R3 nucleic acid sequences.

35.
The method of claim 1, wherein said cell stably or transiently expresses a T1R2 sequence comprising SEQ ID NO: 6 and a T1R3 sequence comprising SEQ ID NO: 7.

36.
The method of claim 34 wherein said cell is further expresses G?15, G?16 or gustducin.

37.
The method of claim 33 wherein said cell is a HEK-293 cell.

ANNOTATION

Stable expression means the constructs are integrated into the genome and maintained under antibiotic selection — in Example 11, puromycin plus zeocin (phleomycin D1) on top of blasticidin already selecting for G?15. Transient expression means episomal plasmid delivered by lipofection, read within 48 h.

The quantitative difference is the patent's strongest technical disclosure: roughly 5% of transiently transfected cells responded under optimal conditions, against 80–100% for a good stable clone, with larger per-cell responses — a greater than ten-fold gain. That is what made the receptor usable for high-throughput screening. Claim 37 (stable + HEK-293) is therefore the commercially narrowest and most defensible combination in the set. Claims 33 and 36 contain grammatical slips as printed ("claims 1", "is further expresses").

`Claims 38–41`

Native taste cells and the gut

38.
The method of claim 1, wherein said T1R2 and T1R3 nucleic acid sequences are expressed in an endogenous taste cell.

39.
The method of claim 38, wherein the cell is a taste cell present in foliate, circumvallate or fungiform papillae.

40.

The method of claim 38, wherein the cell is a taste cell present in geschmackstreifen, oral cavity, gastrointestinal epithelium or epiglottis.

41.

The method of claim 40, wherein the cell is a taste cell present in gastrointestinal epithelium.

ANNOTATION

The anatomy is real and specific. Taste buds sit in three papilla types: fungiform (anterior tongue), foliate (lateral folds) and circumvallate (the posterior V) — the last is the tissue Example 3 resected for RT-PCR. *Geschmackstreifen* is the German term for the taste stripe at the soft-palate junction, retained untranslated in the English specification; epiglottis taste cells are a known extra-lingual population.

Claim 41 is the forward-looking one. Narrowing all the way to gastrointestinal epithelium stakes out gut-expressed sweet receptors, which turned out to regulate glucose absorption and incretin release — a line of work developed later by others, as in Shirazi-Beechey's body-weight patent and the receptor-mediated glucose sensing filings that cite this patent. In 2003 it was a speculative dependent claim; it aged well.

References cited

The examiner considered 27 patent documents and 21 non-patent publications. These are the front-page citations as printed, with links resolved.

Non-patent literature

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21. GenBank accession AL139287, clone RP5-89O03, 13 Feb 2000.

Patent citations (selected from 27)

DOCUMENT	PRIORITY	ASSIGNEE	TITLE
US 5,993,778	1997	Firestein	Functional expression of, and assay for, functional cellular receptors in vivo
US 6,383,778	1998	Univ. California	Nucleic acids encoding a GPCR involved in sensory transduction
US 6,540,978	1998	Mount Sinai	Inhibitors of the bitter taste response
WO 01/66563	2000	Senomyx	T1R taste receptors and genes encoding same
US 2002/0151052	2000	Univ. Miami	Taste receptor for umami (monosodium glutamate) taste
WO 01/83749	2000	Warner-Lambert	Gene and sequence variation associated with sensing carbohydrate compounds and other sweeteners
US 2002/0160424	2001	Adler	T1R hetero-oligomeric taste receptors
WO 03/001876	2001	Senomyx	T1R hetero-oligomeric taste receptors and cell lines that express said receptors
US 2003/0040045	2001	Univ. California	Mammalian sweet taste receptors
US 2003/0166137	2001	Univ. California	Mammalian sweet and amino acid heterodimeric taste receptors
WO 03/025137	2001	IRM LLC	Sweet taste receptors
WO 2005/015158	2003	Senomyx	T1R hetero-oligomeric taste receptors, cell lines, and taste compounds

Full lists: 27 patent citations and 21 non-patent citations on Google Patents; the patent has been cited by 83 later documents.

The family

This patent is one of 28 US applications sharing the 7 March 2001 priority. The disclosure is single; the family splits it by claim type.

PATENT	FILED	CLAIM TYPE
US 7,368,285	2002-06-26	Isolated cell expressing the umami T1R1/T1R3 heteromer — the parent of this application
US 7,297,543	2003-12-02	Cell lines that stably or transiently express the sweet receptor
US 7,297,772	2003-12-02	Isolated T1R2/T1R3 sweet receptor per se
US 7,294,474	2003-12-03	Functional assays for sweet-taste modulators
US 7,344,859	2003-12-03	**Method of producing the sweet heteromer — this patent**
US 7,601,513	2003-12-03	Method of producing the umami heteromer — the direct counterpart
US 7,303,886	2003-12-03	Functional assays for umami-taste modulators
US 7,364,903	2003-12-03	Cell lines expressing the umami receptor
US 8,071,719 · US 8,153,763	2008-11-13	Domain-defined heteromers (?95% identical TM and extracellular domains)
US 8,097,426 · US 8,323,912	2008–2012	Screening methods for sweet-taste modulators
US 8,067,185 · US 7,927,825	2008-11-13	Quantifying taste of compounds for food or beverages
US 10,670,584	2018-09-13	Detecting T1R2/T1R3-expressing cells — the last filing in the chain

Acronym glossary

Every abbreviation used in the patent text and in these annotations, with the authoritative record for each.

Receptors and genes

GPCR G-protein-coupled receptor; seven-transmembrane receptor superfamily MeSH D011956

T1R1 / TAS1R1 Taste receptor type 1 member 1; umami subunit, SEQ ID NO: 5 Gene 80835 · Q7RTX1

T1R2 / TAS1R2 Taste receptor type 1 member 2; sweet-specific subunit, SEQ ID NO: 6 / 10 Gene 80834 · Q8TE23 · HGNC:14905

T1R3 / TAS1R3 Taste receptor type 1 member 3; shared subunit of both heteromers, SEQ ID NO: 7 / 9. 852 aa, ~93.4 kDa, chromosome 1p36.33 Gene 83756 · Q7RTX0 · HGNC:15661

mGluR Metabotropic glutamate receptor; class C structural template used for the Example 2 alignment GRM1, Gene 2911 · rat P23385

CaSR Calcium-sensing receptor; second class C alignment reference Gene 846 · P41180

T2R / TAS2R Taste receptor type 2 family; the bitter receptors, a separate GPCR family NCBI Gene · Adler 2000

VFT Venus flytrap domain; the bilobed extracellular ligand-binding module of class C receptors PDB 9NOU (TAS1R2/TAS1R3 VFT)

PDZIP PDZ-interacting peptide SVSTW (SEQ ID NO: 1); C-terminal export tag that raises surface expression Example 10, FIGS. 9–10

Signal transduction

Gα15 / Gα16 Promiscuous Gq-class G protein alpha subunit (gene GNA15); couples T1R heteromers to PLC? in a heterologous cell NCBI GNA15 · P30679

gustducin Native taste-cell G protein alpha subunit, gene GNAT3 Gene 346562

GDP / GTP Guanosine di- and tri-phosphate; the exchange that activates a G protein MeSH D006153 / D006160

GTPγS Non-hydrolysable GTP analogue; alternative biochemical readout of receptor activation MeSH D015880

PLCβ2 Phospholipase C beta 2; cleaves PIP? to IP? and DAG downstream of Gq-class ? subunits Gene 5330

IP_3 Inositol 1,4,5-trisphosphate; releases sequestered intracellular calcium MeSH D015909

DAG Diacylglycerol; the second PLC product MeSH D004075

cAMP / cGMP Cyclic adenosine / guanosine monophosphate; alternative second-messenger readouts listed in the specification MeSH D000242 / D006152

MSG Monosodium L-glutamate; the reference umami stimulus MeSH D012970

IMP / GMP Inosine / guanosine 5?-monophosphate; purine nucleotides that potentiate the umami heteromer MeSH D007170 / D006157

Cells and expression

HEK-293 Human Embryonic Kidney 293; the preferred host, used as a derivative stably expressing G?15 CVCL_0045 · ATCC CRL-1573 · Graham 1977

CHO Chinese Hamster Ovary; alternative mammalian host in claim 26 ATCC CCL-61

COS CV-1 in Origin carrying SV40; SV40-transformed monkey kidney line ATCC CRL-1651

GeneSwitch Mifepristone-inducible expression system used in Example 12 (plasmid SXV628 carries the switch protein) MeSH D015735

DMEM Dulbecco's Modified Eagle Medium; low-glucose/GlutaMAX form used to limit desensitisation Dulbecco & Freeman 1959

D-PBS / DPBS Dulbecco's Phosphate-Buffered Saline; dye-loading and stimulation buffer MeSH D001899

FBS Fetal bovine serum, used dialysed at 10% in selection medium MeSH D044967

Assay technology

FLIPR Fluorometric Imaging Plate Reader (Molecular Devices); the 96/384-well calcium-flux instrument in Example 12 MeSH D057166

VIPR-II Aurora Biosciences voltage/ion probe reader used for the stable lines in Example 11 Example 11

Fluo-3AM / Fluo-4 Acetoxymethyl-ester calcium indicator dyes; 0.005 mM and 3 µM respectively in the patent's protocols MeSH C059069

EC_{50} / IC_{50} Half-maximal effective / inhibitory concentration. Lactisole: 24 µM on the sweet receptor, 165 µM on umami MeSH D020128

HTS High-throughput screening; the commercial purpose of the stable and inducible lines MeSH D057166

FRET / FP / SPA Fluorescence resonance energy transfer, fluorescence polarization, scintillation proximity assay — alternative readouts in the specification D017949 · D005454

FACS Fluorescence-activated cell sorting; used in Example 10 to quantify surface hT1R2 MeSH D005471

ELISA / RIA Enzyme-linked immunosorbent assay / radioimmunoassay; immunochemical detection formats D004797 / D011863

Molecular biology and sequence

SSC Saline-sodium citrate; 20× stock is 3 M NaCl + 0.3 M sodium citrate, pH 7.0. Claim 1 uses 5× for hybridisation and 0.2× for washing MeSH D009693

SDS Sodium dodecyl sulfate; 1% (T1R2 branch) and 10% (T1R3 branch) as granted MeSH D012967

cDNA Complementary DNA; source of the cloned T1R coding sequences MeSH D018076

RT-PCR Reverse-transcription polymerase chain reaction; used in Example 3 on circumvallate papillae MeSH D020133

LCR Ligase chain reaction; alternative amplification method listed MeSH D020725

GFP / CAT / β-gal Green fluorescent protein, chloramphenicol acetyltransferase, ?-galactosidase; the detectable labels of claim 32 D044967

SEQ ID NO Sequence identifier in the patent sequence listing, governed by WIPO Standard ST.25 (now ST.26) WIPO ST.25 / ST.26

BLAST Basic Local Alignment Search Tool; how the percent-identity limits in claims 3–18 are evaluated in practice NCBI BLAST

Patent and regulatory

USPTO United States Patent and Trademark Office; granting authority Patent Center file wrapper

B2 Kind code for a granted patent that had a prior pre-grant publication (B1 means none) USPTO kind codes

CPC / IPC Cooperative / International Patent Classification; the subject-matter schemes CPC · IPC

PCT / WO / EP Patent Cooperation Treaty, its published applications, and European patents WIPO PCT · EPO

§119(e) / §121 Statutory bases for claiming provisional priority and for filing a divisional application 35 U.S.C. §119 · §121

FDA US Food and Drug Administration; source of the statement that fetal tissue is not permissible in food under any condition fda.gov

On the HEK-293 question. This patent is frequently cited in claims that food products contain fetal cells. The cell line is a laboratory screening substrate; the claims here cover a method of producing a receptor in a host cell, not a food composition. Fact checks by USA TODAY, the Associated Press and Reuters set out the full position.

Claim text and example text are verbatim from the granted patent, including its typographical errors. Annotations, links and groupings are editorial. Verify anything load-bearing against the official PDF facsimile.

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