US Patent 7,344,859 B2

FRONT PAGE BACKGROUND INVENTION EXAMPLES CLAIMS 1–41 FAMILY GLOSSARY BIBLIOGRAPHY LICENCE

US Patent 7,344,859 B2 — Annotated Reading Edition

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

Zoller, Li, Staszewski, O’Connell, Zozulya, Adler, Xu & Echeverri — for Senomyx, Inc. Granted 18 March 2008.

The patent’s own text, hyperlinked throughout, with an individual annotation for every one of the 41 claims and a single combined bibliography.

© 2026 Computational Classnotes. Published 8 November 2026. Expanded edition 9 November 2026.

Licensed under Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0).

This notice covers the annotations, commentary, glossary, selection and arrangement in this edition. The quoted claim text and example text are from a United States patent published by the USPTO and are not themselves subject to this licence. See the full licence statement at the end.

How to read this edition

Three conventions run through the document.

Cite keys. Every source is numbered once in the combined bibliography and referred to by its key everywhere else — [P11] for primary literature, [D8] for patent documents, [G3] for database records, [M5] for methods and tutorials, [L2] for legal and standards material, [C1] for context. Nothing is cited twice with two different links.

Claim annotations. Each claim has its own note in four parts: *what it covers*, *why it is there*, *where the specification supports it*, and *to read further*. Claims that look like filler in a block usually are not; the annotations say what each one does that its neighbours do not.

Quotation. Block quotations and the numbered claim text are the patent's own words, reproduced exactly including the typographical errors as granted. Everything else is editorial.

Front page

FIELD	VALUE
Patent number	US 7,344,859 B2 [D1] — kind code B2, a grant that had a prior pre-grant publication [L1]
Application	Ser. No. 10/725,472, filed 3 December 2003
Priority date	7 March 2001
Pre-grant publication	US 2004/0191862 A1, 30 September 2004 [D2]
Parent application	Division of Ser. No. 10/179,373 (filed 26 June 2002), now US 7,368,285 [D3], under 35 U.S.C. §121 [L3]
Priority chain	US 09/799,629 → 7,244,835 [D4] · US 09/897,427 → 6,955,887 [D5] · US 10/035,045 → 7,241,880 [D6], plus seven provisionals filed 2001–2002 under 35 U.S.C. §119(e) [L2]

FIELD	VALUE
Assignee	Senomyx, Inc. (San Diego) — now listed as Firmenich Inc
Attorney	Hunton & Williams LLP
Claims / drawings	41 claims (1 independent, 40 dependent) · 16 drawing sheets
Classification	CPC G01N 33/5041, C07K 14/705, G01N 2333/726, G01N 2333/4719, C12Q 1/6876 [L5] · US class 435/69.1
Status	Expired — lifetime; adjusted expiration 2 December 2022
International family	PCT publication WO 03/001876 A2 [D7]; EP family member EP 2327985 [D8]
File history	USPTO Patent Center [L6] · Espacenet [L7] · PDF facsimile [D1a]

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 [G1] superfamily. Other taste modalities are believed to be mediated by ion channels.
>
> — *US 7,344,859 B2, Background*, citing [P8] [P12] [P6] [P1]

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 Gα subunit [G9], and subsequent dissociation of Gα from Gβ and Gγ. The free subunits then activate downstream effectors. The specification credits US 5,691,188 [D9]

for this description.

The three receptors at issue — T1R1 [G2], T1R2 [G3] and T1R3 [G4] — are class C GPCRs, structurally related to the metabotropic glutamate receptors [G5] and the calcium-sensing receptor [G6], both of which the patent uses as alignment references in Example 2.

> **For the general reader.** Class C GPCRs differ from the more familiar class A receptors (rhodopsin, adrenergic) in carrying a large bilobed extracellular ligand-binding module — the Venus flytrap domain — and in functioning as obligate dimers. That architectural fact is what makes this patent's central discovery plausible in hindsight and is the best single entry point to the biology. See the IUPHAR/BPS Guide to Pharmacology family pages [M1] and GPCRdb [M2].

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*

The four problems the expression system solves

1 — 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.

2 — A readable output. Native sweet transduction runs through gustducin [G7] and a PLCβ2 [G8] / IP_3 cascade [G12] that is hard to read in a heterologous cell. The patent instead supplies Gα15 or Gα16 [G9], promiscuous Gq-class α subunits that couple almost any GPCR to PLCβ,

turning receptor activation into a cytosolic Ca^{2+} transient a fluorescent dye can report. The approach was established for olfactory receptors by Krautwurst et al. [P10].

3 — A host that tolerates it. The preferred host is a HEK-293 [G16] derivative already stably expressing Gα15, obtained from Chandrashekar et al. [P22]. Claims also reach COS [G18], CHO [G17], yeast, insect and *Xenopus* oocytes, and native taste cells.

4 — 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 [G19], 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 [G24], rat T1R2 [G25], rat mGluR1 [G5a] and the human calcium-sensing receptor [G6a]. Seven transmembrane segments boxed; residues contacting the glutamate side-chain carboxylate and α-amino moiety in the mGluR1 crystal structure marked separately. *To read further:* multiple sequence alignment methods and interpretation, EMBL-EBI training [M3].

Example 3 — Expression in taste tissue (FIG. 2). RT-PCR [G20] detects both hT1R2 and hT1R3 in resected human circumvallate papillae. *To read further:* RT-PCR principles and controls [M4].

Example 4 — Heterologous expression method. HEK-293/Gα15 cells in DMEM [P23] 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 [G21] 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. *To read further:* transfection method selection [M5]; calcium indicator chemistry and loading [M6].

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 β_2-adrenergic response to 20 µM isoproterenol — the specificity control that rules out a general failure of the signalling pathway. 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. The rat pair also works; the results suggest T1R2 controls ligand specificity. This is the experiment that gives claim 2 its meaning.

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 — the argument that the recombinant receptor is a valid proxy for perception.

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 [G13]. 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 [G22]. Sweetener pool no. 5 was saccharin, sodium cyclamate, acesulfame K and aspartame at 20 mM each. *To read further:* epitope tagging strategy and placement [M7].

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/Gα15 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. *To read further:* stable cell line generation and clone selection [M8]; antibiotic selection marker choice [M9].

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^{-10} M mifepristone; clone I-17 seeded at ~80,000 cells/well for 48 h, loaded with 3 µM fluo-4-AM, read on FLIPR [G23]. 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. *To read further:* inducible expression systems compared [M10].

Example 13 — Lactisole (FIG. 16). Lactisole inhibits hT1R2/hT1R3 with an IC_{50} of 24 µM and hT1R1/hT1R3 with an IC_{50} of 165 µM. Muscarinic acetylcholine receptor responses were unaffected, ruling out non-specific inhibition of Gα15 signalling. In human taste tests following Schiffman et al. [P24], 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. *To read further:* IC_{50}, EC_{50} and dose-response curve fitting [M11].

Claims 1–41, annotated individually

One independent claim, forty dependent. Claim text is verbatim, including the typographical errors as granted.

> **Reading a patent claim.** A claim is a single sentence defining a legal boundary. An independent claim stands alone; a dependent claim incorporates everything in its parent and adds a limitation, so it is always *narrower*. Narrower claims are not weaker — they are harder to invalidate, which is why a well-drafted patent carries a ladder of them. For the examination standards behind these drafting choices, see the MPEP [L8].

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.

> **What it covers.** Any method of getting a T1R2 and a T1R3 subunit expressed together in one engineered cell such that they assemble into a heteromeric receptor. The method is the invention; the receptor itself and the screening assays are claimed in sibling patents [D10]

[D11] [D12].

Why it is drafted this way. Each subunit is defined three independent ways — literal sequence, hybridisation equivalents, percent identity — joined by “or”. A competitor using a natural variant, a codon-altered gene, or a primate orthologue still falls inside at least one branch. The sequence identifiers: **SEQ ID NO: 6** = human T1R2 protein [G3a], **SEQ ID NO: 7** = human T1R3 protein [G4a] (852 aa, ~93.4 kDa), **SEQ ID NO: 9** = T1R3 nucleic acid, **SEQ ID NO: 10** = T1R2 nucleic acid.

Support. Examples 5 and 7 for the sweet heteromer; Example 11 for the stable lines.

The typographical errors. The T1R2 branch recites 1% SDS; the T1R3 branch recites 10% SDS and spells SSC as “SCC”. Claims 11 and 20, which restate the same conditions for each subunit, *both* say 1% SDS — internal evidence that the 10% in claim 1 is a prosecution slip rather than a deliberate difference. It remains the operative language as granted.

To read further. Hybridisation stringency and how temperature, formamide and salt set the identity cut-off [M12]. Sequence identity calculation [M13]. Sequence listing format, governed by WIPO ST.25 and since replaced by ST.26 [L4].

Claims 2 — species pairing

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

What it covers. Pairs drawn from one organism — human with human, rat with rat — excluding chimeric pairings.

Why it is there. Because the inventors actually built the alternative and had to distinguish it. Example 6 deliberately mixed subunits across species (hT1R2/rT1R3 and rT1R2/hT1R3) and tested all four combinations. Without that experiment this claim would be an arbitrary restriction; with it, it is a tested boundary.

Support. Example 6, FIG. 4.

To read further. Species differences in sweet perception are large and practically important: rodents respond poorly or not at all to several sweeteners that humans detect readily, which is why a human receptor line was commercially necessary rather than merely convenient. Example 6’s finding that T1R2 appears to control ligand specificity is the mechanistic root of that difference. See Nelson et al. on mammalian sweet receptors [P15] and the amino-acid receptor paper [P16].

Claims 3–11 — defining the T1R2 subunit

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.

> **What it covers.** The literal human T1R2 protein, no variation permitted.
>
> **Why it is there.** This is the “picture claim” — the narrowest, most certainly valid statement of the invention. If every broader claim falls to prior art, this one survives, because it describes exactly what the inventors made and showed working.
>
> **Support.** Examples 5 and 7, which used human T1R2 throughout.
>
> **To read further.** The reference protein record [G3a].

4. The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide that exhibits at least 90% sequence identity to the polypeptide of SEQ. ID. NO: 6.

> **What it covers.** The widest identity band for T1R2. On a receptor of roughly 840 residues, 90% identity permits about 84 positions to differ.
>
> **Why it is there.** A budget of 84 substitutions is generous enough to reach non-human primate orthologues and engineered variants, not just human polymorphisms. This is the commercially broad position.
>
> **Caution for the reader.** Breadth of this kind is where identity-band claims are most often attacked, because a patent must enable what it claims. The question an examiner or a court asks is whether the specification teaches the reader to make and use the full 10% band, or only the single sequence actually built.
>
> **To read further.** Written description and enablement standards [L9]. The substitution-tolerance literature the specification relies on [P4] [P5].

5. The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide that exhibits at least 95% sequence identity to the polypeptide of SEQ. ID. NO: 6.

> **What it covers.** About 42 positions free to differ.
>
> **Why it is there.** 95% is the conventional first fallback. It comfortably contains the natural human allelic variation at *TAS1R2* while shedding the harder-to-defend outer band of claim 4.

6. The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide that exhibits at least 96% sequence identity to the polypeptide of SEQ. ID. NO: 6.

What it covers. About 34 positions. The first of four single-percentage-point steps.

Why it is there. Each step is a separate fallback. If prior art is found that happens to sit at 95.4% identity, claim 5 is anticipated and claim 6 is not. The granularity is cheap to draft and occasionally decisive.

7. The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide that exhibits at least 97% sequence identity to the polypeptide of SEQ. ID. NO: 6.

What it covers. About 25 positions free to differ.

Why it is there. Continuation of the ladder. At this tier the claim no longer plausibly reaches other species and is effectively a human-variant claim.

8. The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide that exhibits at least 98% sequence identity to the polypeptide of SEQ. ID. NO: 6.

What it covers. About 17 positions.

Why it is there. Near-literal. At 98% the claim covers the reference sequence plus common polymorphism and a handful of deliberate engineering changes — an epitope tag substitution, a trafficking motif.

9. The method of claim 1, wherein said T1R2 polypeptide is a human T1R2 polypeptide that exhibits at least 99% sequence identity to the polypeptide of SEQ. ID. NO: 6.

What it covers. About 8 positions — the tightest band before the literal claim 3.

Why it is there. The last rung. It captures natural human allelic variation only, and is the hardest of the band claims to invalidate because it is barely broader than what was built.

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

What it covers. A shift of frame: the subunit is now defined by its *coding sequence* rather than its protein sequence.

Why it is there. Protein-level and nucleic-acid-level claims catch different infringers. Because the genetic code is degenerate, many different DNA sequences encode the identical protein — so a protein claim is broader. The nucleic acid claim matters when the accused

product is a vector, a construct or a sequence file rather than an expressed receptor.

To read further. Codon degeneracy and why it makes nucleic-acid claims narrower than their protein counterparts [M14].

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.

What it covers. Any coding sequence that will physically anneal to the reference sequence under the stated conditions — a *functional* rather than a literal definition of the nucleic acid.

Why it is there. It reaches sequences nobody has sequenced. If an orthologue exists that would hybridise under these conditions, it is covered whether or not it was known in 2003.

Why the conditions are recited in full. A claim must be definite. “Stringent conditions” alone is a term whose meaning drifts between laboratories; reciting temperature, formamide percentage, SSC concentration and wash conditions fixes it. Note this claim says **1% SDS**, matching claim 1’s T1R2 branch.

To read further. Stringency, melting temperature and the salt/formamide relationship [M12].

Claims 12–20 — defining the T1R3 subunit

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.

What it covers. The literal human T1R3 protein — 852 residues, roughly 93.4 kDa, encoded at chromosome 1p36.33.

Why it matters more than its T1R2 counterpart. T1R3 is the subunit shared by *both* the sweet and the umami receptor. A competitor cannot build either heteromer without it. This is the commercially central sequence in the whole family, which is why sibling patents claim T1R3-containing receptors so heavily [D13] [D14].

To read further. The protein record [G4a]; the gene record [G4].

13. The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide that possesses at least 90% sequence identity to the polypeptide of SEQ. ID. NO: 7.

> **What it covers.** On 852 residues, about 85 positions free to differ — the broadest T1R3 band.
>
> **Why it is there.** Mirrors claim 4. Wide enough to reach primate orthologues and the mouse T1R3 variants that were already in the literature by 2001 [P13] [P14] [P9].

14. The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide that possesses at least 95% sequence identity to the polypeptide of SEQ. ID. NO: 7.

> **What it covers.** About 43 positions.
>
> **Why it is there.** The conventional fallback tier, and the one that matters most for T1R3 because the known human sequence variation at *TAS1R3* sits well inside it.

15. The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide that possesses at least 96% sequence identity to the polypeptide of SEQ. ID. NO: 7.

> **What it covers.** About 34 positions. First of the single-point steps on the T1R3 side.

16. The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide that possesses at least 97% sequence identity to the polypeptide of SEQ. ID. NO: 7.

> **What it covers.** About 26 positions.

17. The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide that possesses at least 98% sequence identity to the polypeptide of SEQ. ID. NO: 7.

> **What it covers.** About 17 positions — enough for tagging and trafficking modifications of the kind Example 10 describes, without leaving the claim.

18. The method of claim 1, wherein said T1R3 polypeptide is a human T1R3 polypeptide that possesses at least 99% sequence identity to the polypeptide of SEQ. ID. NO: 7.

> **What it covers.** About 9 positions. The tightest band.
>
> **A drafting observation.** Note the verb changes across the two ladders: the T1R2 claims say the polypeptide “exhibits” a given identity, the T1R3 claims say it “possesses”. Nothing turns on the difference — it is the signature of two passages drafted at different sittings and left

unharmonised.

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

> **What it covers.** The literal T1R3 coding sequence. The counterpart of claim 10.

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.

> **What it covers.** Hybridisation to *either* of two reference sequences.
>
> **Why it is unusual.** This is the only dependent claim in the set that widens rather than narrows relative to its siblings: it adds SEQ ID NO: 11 as a second hybridisation target alongside SEQ ID NO: 9. Every other dependent claim subtracts scope.
>
> **The SDS point.** Like claim 11, this claim recites **1% SDS**. Claim 1's T1R3 branch is the only place in the patent reciting 10%, which is the strongest internal argument that the 10% figure is an error.
>
> **To read further.** The mouse orthologue of this gene is the historic *Sac* saccharin-preference locus [G26], mapped to *Tas1r3* by Max et al. [P13] — one of three groups to reach the gene within weeks of each other in 2001 [P14] [P9].

Claims 21–22 — transcriptional control

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

> **What it covers.** Expression that runs continuously, at a rate set by the promoter rather than by the experimenter.
>
> **Support.** Example 11 used PEAK10-derived and pcDNA3.1/Zeo-derived vectors, whose promoters drive strong constitutive transcription in mammalian cells.
>
> **"Operably linked" is a term of art.** It means the promoter is positioned and oriented so that it actually drives transcription of the downstream coding sequence — not merely present on the same plasmid.

> **To read further.** Promoter selection and relative strengths in mammalian expression [M15].

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

> **What it covers.** Expression held off until the experimenter adds an inducing agent.
>
> **Why this is the most practically important dependent claim in the patent.** Example 12 states the problem plainly: constitutive stable lines "can rapidly lose activity during cell propagation". That is the predictable consequence of forcing a cell to continuously express a receptor that mobilises calcium — cells that silence the transgene outgrow those that do not. The inducible claim covers the fix, and the fix is what made the platform usable as a screening product rather than a demonstration.
>
> **Support.** Example 12: pGene/pSwitch GeneSwitch vectors, induced with 10^{-10} M mifepristone 48 hours before assay. GeneSwitch is a chimeric transcription factor built on a progesterone receptor ligand-binding domain [G19].
>
> **To read further.** Inducible mammalian expression systems compared — GeneSwitch, Tet-On/Tet-Off, ecdysone [M10].

Claims 23–27 — the host cell

23. The method of claim 1, wherein said T1R2 and T1R3 nucleic acid sequences are expressed in a prokaryotic cell.

> **What it covers.** Bacterial expression.
>
> **Why it is almost certainly not practicable.** A bacterium has no endoplasmic reticulum, no N-glycosylation machinery, and none of the chaperones that fold a seven-transmembrane class C GPCR with a large glycosylated extracellular domain. T1R3 carries N-linked glycosylation sites at residues 84, 85 and 130 among others. Expression in *E. coli* would be expected to yield inclusion bodies, not a functional receptor.
>
> **Why it is claimed anyway.** It costs one sentence and forecloses an argument. A competitor who found some way to make it work in a prokaryote would otherwise fall outside every host claim.
>
> **To read further.** Why membrane protein expression is hard and how hosts differ [M16].

24. The method of claim 1, wherein said T1R2 and T1R3 nucleic acid sequences are expressed in a eukaryotic cell.

> **What it covers.** The complement of claim 23 — everything with a nucleus. Together, claims 23 and 24 exhaust the possibilities, which is deliberate: it guarantees a dependent claim is available whatever host an accused process uses.
>
> **Why it is the parent of claims 25 and 26.** Those two narrow progressively within the eukaryotic branch, so the useful host limitations all hang from here rather than from claim 1.

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

> **What it covers.** Four expression traditions, each with its own established GPCR practice: mammalian lines for native-like folding and signalling; yeast via the pheromone-response pathway; insect cells via baculovirus; amphibian oocytes for electrophysiological readout.
>
> **Why the list matters.** Each host implies a different readout. An oocyte is read by two-electrode voltage clamp rather than by calcium dye, so this claim quietly extends the patent beyond the fluorescence assays the examples describe.
>
> **To read further.** Comparison of heterologous expression hosts [M16].

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

> **What it covers.** Four named lines. (“Ooctye” is as printed.)
>
> **The lines.** HEK-293 [G16], derived from human embryonic kidney tissue and first described by Graham et al. in 1977 [P25]; COS-7 [G18], SV40-transformed African green monkey kidney; CHO-K1 [G17], the workhorse of recombinant protein manufacture; and *Xenopus laevis* oocytes.
>
> **To read further.** Cell line provenance and authentication — why the identity of a line is a reproducibility issue in its own right [M17].

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

> **What it covers.** The single host the inventors actually used.
>
> **Why HEK-293.** Two properties. It takes up and expresses transfected DNA with unusual efficiency, and it divides indefinitely, so a selected clone can be banked and distributed. Both were essential to building a screening line rather than running one-off experiments. The specific starting material was a derivative already stably expressing Gα15, from Chandrashekar et al. [P22].

> **Note on the public controversy.** HEK-293 originates from fetal kidney tissue, and this claim is the reason this patent circulates outside the scientific literature. See the note at the end of this document.

Claims 28–30 — G protein coupling

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

> **What it covers.** Any host cell carrying any G protein — read literally, almost every eukaryotic cell qualifies, since G proteins are ubiquitous.
>
> **Why it is there.** As a parent. Its breadth is the point: claims 29 and 30 narrow from it, and a broad parent gives them somewhere to hang.

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

> **What it covers.** G proteins that couple receptors regardless of the receptor's native preference.
>
> **"Promiscuous" is a genuine term of art, not loose drafting.** Most Gα subunits couple selectively. A promiscuous subunit couples broadly, which is what lets an experimenter redirect an unfamiliar receptor into a convenient readout. The strategy was established for olfactory receptors by Krautwurst et al. [P10], cited in the specification.
>
> **To read further.** G protein classes and selectivity [M1].

30. The method of claim 28, wherein said G protein is Gα15, Gα16 or gustducin.

> **What it covers.** Three named subunits. Gα15 (mouse) and Gα16 (human) are the same gene, *GNA15* [G9] — a Gq-class promiscuous subunit. Gustducin, gene *GNAT3* [G7], is the transducin-family subunit native to taste cells.
>
> **The asymmetry worth noticing.** Gα15/Gα16 route the receptor into calcium release, which the assays read. Gustducin routes it into the native pathway, which they do not. Including gustducin covers a competitor who builds a more physiologically faithful line, even though the patent's own examples never use it.
>
> **Related filings.** A sibling patent claims chimeric Gα15 variants [D15]; another covers coupling through Gi proteins instead [D16].

Claims 31–32 — trafficking and detection

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

> **What it covers.** Receptor that reaches the plasma membrane, as distinct from receptor that is merely synthesised.
>
> **Why this is a real limitation and not a formality.** Class C GPCRs traffic poorly in heterologous cells; a large fraction of expressed receptor can remain trapped in the endoplasmic reticulum, where it is invisible to extracellular tastants. A cell can be full of T1R2 protein and still be inert. This claim requires the version that works.
>
> **Support.** Example 10, which solved the problem by fusing the PDZ-interacting sequence SVSTW (SEQ ID NO: 1) — “PDZIP” — to the hT1R2 C-terminus, and demonstrated the increase by immunofluorescence and FACS.
>
> **To read further.** ER export signals and GPCR trafficking [M16].

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.

> **What it covers.** Constructs carrying a genetically encoded tag — an epitope, a fluorescent protein, an enzyme.
>
> **Why it is there.** It is how claim 31 is proved. Example 10 used a myc epitope tag read by antibody; the specification also lists GFP [G14], luciferase, β-galactosidase and chloramphenicol acetyltransferase.
>
> **A practical point for anyone building this.** Tag placement is consequential for a class C GPCR: an N-terminal tag sits in the Venus flytrap ligand-binding domain and can interfere with binding, while a C-terminal tag sits in the cytoplasmic tail near trafficking motifs and can interfere with export. The patent’s own PDZIP result is a demonstration of exactly how sensitive that C-terminus is.
>
> **To read further.** Epitope tag selection and placement [M7].

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.

What it covers. Constructs integrated into the host genome and maintained under selection, so the line can be banked, expanded and shipped. (“claims 1” is as printed.)

Support. Example 11: linearised vectors co-transfected into HEK-293/Gα15, selected in puromycin and zeocin on top of the blasticidin already selecting for Gα15 — three simultaneous selections.

To read further. Stable line generation, clonal selection and banking [M8].

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

What it covers. Plasmid delivered episomally and read within a day or two, before it is diluted out by division.

Why both are claimed. They are genuinely different processes with different economics. Transient expression is fast and needs no selection, which suits a few experiments; stable expression is slow to establish and far better for screening. Example 4 is the transient protocol; Example 11 is the stable one.

The quantitative gap. 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 overall. That number is the patent’s strongest technical disclosure.

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

What it covers. Both expression modes combined with both literal human sequences, in one claim.

Why it exists when claims 3, 12, 33 and 34 already cover the pieces. Convenience and certainty. A single claim reciting the exact commercial embodiment is easier to assert than an argument assembled from four separate dependent claims, and it removes any question about whether the combination was claimed.

36. The method of claim 34 wherein said cell is further expresses Gα15, Gα16 or gustducin.

What it covers. Transient expression *plus* a named G protein. (“is further expresses” is as printed.)

> **The dependency is worth noticing.** This claim hangs from claim 34 — the *transient* branch — not from claim 33. Its sibling claim 37 hangs from the stable branch. The drafter attached the G-protein limitation to transient expression and the host limitation to stable expression, producing two differently-shaped narrow claims rather than one.

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

> **What it covers.** Stable expression in HEK-293 — precisely the configuration of the commercial screening line.
>
> **Why this is the most defensible claim in the patent.** It is narrow, it is exactly what the examples demonstrate, and it is what any competitor building a practical sweet-receptor screen would most likely do. Narrow claims that read directly onto the commercially obvious implementation are the ones that matter in litigation.

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.

> **What it covers.** Expression in a cell that is already a taste receptor cell, rather than in an engineered line.
>
> **Why it is claimed.** It extends the method beyond cell culture to manipulation of native tissue, including transgenic animals — a direction sibling filings pursue [D17].

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

> **What it covers.** The three classical lingual papilla types that carry taste buds: fungiform on the anterior tongue, foliate in the lateral folds, circumvallate in the posterior V.
>
> **Support.** Example 3 resected human circumvallate papillae and detected both transcripts by RT-PCR — the experiment establishing that these receptors are expressed where the claim says they are.
>
> **To read further.** Taste bud anatomy and distribution [G15].

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

What it covers. Taste cells outside the lingual papillae.

On the vocabulary. *Geschmackstreifen* — literally “taste stripe” — is the German term for the band of taste tissue at the junction of the hard and soft palate, left untranslated in the English specification. Epiglottal taste cells are a known extra-lingual population. The oral cavity and gastrointestinal listings extend further still.

Why it is separate from claim 39. Claim 39 covers the textbook locations; claim 40 covers everywhere else taste cells are found. Keeping them apart means that if the extra-lingual populations were somehow disputed, claim 39 is unaffected.

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

What it covers. Sweet receptors expressed in the gut lining.

Why this is the most interesting claim in the patent. In 2003 it was a speculative narrowing — a dependent claim staking out a location the inventors had not worked on. It aged better than anything else in the set. Gut-expressed T1R2/T1R3 turned out to participate in glucose sensing, intestinal sugar absorption and incretin release, making it a target for metabolic rather than flavour applications.

The downstream evidence. Later filings citing this patent pursue exactly that: a method of controlling body weight through gut taste receptors [D18], and compositions for receptor-mediated glucose sensing [D19].

A lesson in claim drafting. The claim cost one sentence and no experiments. Dependent claims that name a plausible but unexplored application are cheap insurance against a field moving somewhere the inventors did not anticipate.

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	KEY

US 7,368,285	2002-06-26	Isolated cell expressing the umami T1R1/T1R3 heteromer — the parent of this application	[D3]
US 7,297,543	2003-12-02	Cell lines that stably or transiently express the sweet receptor	[D20]
US 7,297,772	2003-12-02	Isolated T1R2/T1R3 sweet receptor per se	[D10]
US 7,294,474	2003-12-03	Functional assays for sweet-taste modulators	[D11]
US 7,344,859	2003-12-03	**Method of producing the sweet heteromer — this patent**	[D1]
US 7,601,513	2003-12-03	Method of producing the umami heteromer — the direct counterpart	[D21]
US 7,303,886	2003-12-03	Functional assays for umami-taste modulators	[D22]
US 7,364,903	2003-12-03	Cell lines expressing the umami receptor	[D23]
US 8,071,719 · US 8,153,763	2008-11-13	Domain-defined heteromers (≥95% identical TM and extracellular domains)	[D13] [D14]
US 8,097,426 · US 8,323,912	2008–2012	Screening methods for sweet-taste modulators	[D12] [D24]
US 8,067,185 · US 7,927,825	2008-11-13	Quantifying taste of compounds for food or beverages	[D25] [D26]
US 10,670,584	2018-09-13	Detecting T1R2/T1R3-expressing cells — the last filing in the chain	[D27]

Acronym glossary

Receptors and genes

ACRONYM	EXPANSION	KEY
GPCR	G-protein-coupled receptor; seven-transmembrane receptor superfamily	[G1]
T1R1 / TAS1R1	Taste receptor type 1 member 1; umami subunit, SEQ ID NO: 5	[G2]
T1R2 / TAS1R2	Taste receptor type 1 member 2; sweet-specific subunit, SEQ ID NO: 6 / 10	[G3] [G3a]
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	[G4] [G4a]
mGluR	Metabotropic glutamate receptor; class C structural template for the Example 2 alignment	[G5] [G5a]
CaSR	Calcium-sensing receptor; second class C alignment reference	[G6] [G6a]
T2R / TAS2R	Taste receptor type 2 family; the bitter receptors, a separate GPCR family	[G10]
VFT	Venus flytrap domain; the bilobed extracellular ligand-binding module of class C receptors	[G11]
PDZIP	PDZ-interacting peptide SVSTW (SEQ ID NO: 1); C-terminal export tag raising surface expression	Example 10

Signal transduction

ACRONYM	EXPANSION	KEY
Gα15 / Gα16	Promiscuous Gq-class G protein alpha subunit, gene *GNA15*	[G9]
gustducin	Native taste-cell G protein alpha subunit, gene *GNAT3*	[G7]
GDP / GTP	Guanosine di- and tri-phosphate; the exchange that activates a G protein	[G9a]
PLCβ2	Phospholipase C beta 2; cleaves PIP_2 to IP_3 and DAG	[G8]
IP_3 / DAG	Inositol 1,4,5-trisphosphate and diacylglycerol; the two PLC products	[G12]
MSG	Monosodium L-glutamate; the reference umami stimulus	[G13a]

ACRONYM	EXPANSION	KEY
IMP / GMP	Inosine / guanosine 5′-monophosphate; purine potentiators of the umami heteromer	[G13]

Cells, expression and assay

ACRONYM	EXPANSION	KEY
HEK-293	Human Embryonic Kidney 293; the preferred host, used as a Gα15-expressing derivative	[G16] [P25]
CHO	Chinese Hamster Ovary; alternative mammalian host in claim 26	[G17]
COS	CV-1 in Origin carrying SV40; SV40-transformed monkey kidney line	[G18]
GeneSwitch	Mifepristone-inducible expression system used in Example 12	[G19]
DMEM / DPBS / FBS	Dulbecco’s Modified Eagle Medium, Dulbecco’s Phosphate-Buffered Saline, fetal bovine serum	[P23]
FLIPR / VIPR-II	Fluorometric Imaging Plate Reader; Aurora Biosciences voltage/ion probe reader	[G23]
Fluo-3AM / Fluo-4	Acetoxymethyl-ester calcium indicator dyes	[G21]
EC_{50} / IC_{50}	Half-maximal effective / inhibitory concentration	[M11]
HTS	High-throughput screening	[G23]
FACS	Fluorescence-activated cell sorting	[G22]
RT-PCR	Reverse-transcription polymerase chain reaction	[G20]
GFP	Green fluorescent protein; one of the detectable labels of claim 32	[G14]

Sequence and legal

ACRONYM	EXPANSION	KEY
SSC	Saline-sodium citrate; 20× stock is 3 M NaCl + 0.3 M sodium citrate, pH 7.0	[M12]

ACRONYM	EXPANSION	KEY
SDS	Sodium dodecyl sulfate; 1% and (as granted) 10% in claim 1’s two branches	[M12]
SEQ ID NO	Sequence identifier, governed by WIPO ST.25, since replaced by ST.26	[L4]
BLAST	Basic Local Alignment Search Tool; how percent-identity limits are evaluated	[M13]
CPC / IPC	Cooperative / International Patent Classification	[L5]
MPEP	Manual of Patent Examining Procedure	[L8]
PCT / WO / EP	Patent Cooperation Treaty, its publications, and European patents	[D7] [D8]

Combined bibliography

Every source cited anywhere in this edition, numbered once. Keys are referenced throughout the preceding sections.

P — Primary literature

KEY	REFERENCE
P1	Adler E. et al., “A novel family of mammalian taste receptors”, *Cell* 100(6):693–702, 2000. doi:10.1016/S0092-8674(00)80705-9
P2	Alexander et al., “Altering the antigenicity of proteins”, *PNAS* 89:3352–3356, 1992. doi:10.1073/pnas.89.8.3352
P3	Bönigk W. et al., “The native rat olfactory cyclic nucleotide-gated channel is composed of three distinct subunits”, *J Neurosci* 19:5332–5347, 1999. doi:10.1523/JNEUROSCI.19-13-05332.1999

KEY	REFERENCE
P4	Bowie et al., “Deciphering the message in protein sequences: tolerance to amino acid substitutions”, *Science* 247:1306–1310, 1990. doi:10.1126/science.2315699
P5	Guo et al., “Protein tolerance to random amino acid change”, *PNAS* 101(25):9205–9210, 2004. doi:10.1073/pnas.0403255101
P6	Hoon M. et al., “Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity”, *Cell* 96:541–551, 1999. doi:10.1016/S0092-8674(00)80658-3
P7	Johnson C. et al., “The effect of the sweetness inhibitor Na-PMP on the taste of bitter-sweet stimuli”, *Chemical Senses* 19(4):349–358, 1994. doi:10.1093/chemse/19.4.349
P8	Kinnamon S. & Cummings T., “Chemosensory transduction mechanisms in taste”, *Annu Rev Physiol* 54:715–731, 1992. doi:10.1146/annurev.ph.54.030192.003435
P9	Kitagawa M. et al., “Molecular genetic identification of a candidate receptor gene for sweet taste”, *Biochem Biophys Res Commun* 283:236–242, 2001. doi:10.1006/bbrc.2001.4760
P10	Krautwurst D. et al., “Identification of ligands for olfactory receptors by functional expression of a receptor library”, *Cell* 95:917–926, 1998. doi:10.1016/S0092-8674(00)81716-X
P11	Li X. et al., “Human receptors for sweet and umami taste”, *PNAS* 99(7):4692–4696, 2002. PMC123709 — the inventors’ own publication of this work
P12	Lindemann B., “A taste for umami”, *Nature Neuroscience* 3(2):99–100, 2000. doi:10.1038/72153
P13	Max M. et al., “Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac”, *Nature Genetics* 28:58–63, 2001. doi:10.1038/88270
P14	Montmayeur J.-P. et al., “A candidate taste receptor gene near a sweet taste locus”, *Nature Neuroscience* 4(5):492–498, 2001. doi:10.1038/87440
P15	Nelson G. et al., “Mammalian sweet taste receptors”, *Cell* 106:381–390, 2001. doi:10.1016/S0092-8674(01)00451-2
P16	Nelson G. et al., “An amino-acid taste receptor”, *Nature* 416:199–202, 2002. doi:10.1038/nature726

KEY	REFERENCE
P17	Perruccio et al., “Possible role for gustducin in taste transduction in *Hirudo medicinalis*”, *Soc Neurosci Abstr* 26, Abstract 66.15, 2000.
P18	Sainz E. et al., “Identification of a novel member of the T1R family of putative taste receptors”, *J Neurochem* 77:896–903, 2001. doi:10.1046/j.1471-4159.2001.00292.x
P19	Database EMBL, accession AC062024, 2000.
P20	GenBank accession AA907022, 1998.
P21	GenBank accession AL139287, clone RP5-89O03, 2000.
P22	Chandrashekar J. et al., “T2Rs function as bitter taste receptors”, *Cell* 100:703–711, 2000. doi:10.1016/S0092-8674(00)80706-0 — source of the HEK-293/Gα15 line
P23	Dulbecco R. & Freeman G., “Plaque production by the polyoma virus”, *Virology* 8:396–397, 1959. doi:10.1016/0042-6822(59)90043-1 — the origin of DMEM
P24	Schiffman S. et al., *Chemical Senses* 24:439–447, 1999. doi:10.1093/chemse/24.4.439 — the taste-threshold method used in Example 13
P25	Graham F., Smiley J., Russell W. & Nairn R., “Characteristics of a human cell line transformed by DNA from human adenovirus type 5”, *J Gen Virol* 36:59–74, 1977. doi:10.1099/0022-1317-36-1-59 — the derivation of HEK-293

D — Patent documents

KEY	DOCUMENT
D1	US 7,344,859 B2 — this patent
D1a	Official PDF facsimile
D2	US 2004/0191862 A1 — pre-grant publication
D3	US 7,368,285 — parent application
D4	US 7,244,835 — priority application
D5	US 6,955,887 — priority application
D6	US 7,241,880 — priority application

KEY	DOCUMENT
D7	WO 03/001876 A2 — PCT publication
D8	EP 2327985 — European family member
D9	US 5,691,188 — cited for the GPCR activation mechanism
D10	US 7,297,772 — isolated sweet receptor
D11	US 7,294,474 — sweet functional assays
D12	US 8,097,426 — sweet screening methods
D13	US 8,071,719 — domain-defined heteromers
D14	US 8,153,763 — heteromeric T1R2/T1R3 receptors
D15	US 7,208,290 — chimeric Gα15 variants
D16	US 7,022,488 — coupling through Gi proteins
D17	US 7,803,982 — T1R3 transgenic animals
D18	US 2009/0170949 — method to control body weight
D19	WO 2022/120143 — receptor-mediated glucose sensing
D20	US 7,297,543 — sweet-receptor cell lines
D21	US 7,601,513 — umami production method
D22	US 7,303,886 — umami functional assays
D23	US 7,364,903 — umami cell lines
D24	US 8,323,912 — sweet screening methods
D25	US 8,067,185 — quantifying taste
D26	US 7,927,825 — quantifying taste with heteromeric receptors
D27	US 10,670,584 — detecting T1R2/T1R3-expressing cells
D28	US 5,993,778 — Firestein, functional expression and assay
D29	US 6,383,778 — Univ. California, sensory transduction GPCR
D30	US 6,540,978 — Mount Sinai, bitter taste inhibitors
D31	WO 01/66563 — Senomyx, T1R receptors and genes

KEY	DOCUMENT
D32	US 2002/0151052 — Univ. Miami, umami receptor
D33	WO 01/83749 — Warner-Lambert, carbohydrate sensing
D34	US 2002/0160424 — Adler, hetero-oligomeric receptors
D35	US 2003/0040045 — Univ. California, mammalian sweet receptors
D36	US 2003/0166137 — Univ. California, heterodimeric receptors
D37	WO 03/025137 — IRM LLC, sweet taste receptors
D38	WO 2005/015158 — Senomyx, receptors, cell lines and taste compounds

G — Database records

KEY	RECORD
G1	GPCR — MeSH D011956
G2	TAS1R1 — NCBI Gene 80835 · UniProt Q7RTX1
G3	TAS1R2 — NCBI Gene 80834 · HGNC:14905
G3a	T1R2 protein — UniProt Q8TE23
G4	TAS1R3 — NCBI Gene 83756 · HGNC:15661
G4a	T1R3 protein — UniProt Q7RTX0
G5	GRM1 — NCBI Gene 2911
G5a	Rat mGluR1 — UniProt P23385
G6	CASR — NCBI Gene 846
G6a	Human CaSR — UniProt P41180
G7	GNAT3 (gustducin) — NCBI Gene 346562
G8	PLCB2 — NCBI Gene 5330
G9	GNA15 — NCBI Gene · UniProt P30679
G9a	GDP — MeSH D006153 · GTP — MeSH D006160

KEY	RECORD
G10	TAS2R family — NCBI Gene
G11	TAS1R2/TAS1R3 Venus flytrap structure — PDB 9NOU
G12	IP_3 — MeSH D015909 · DAG — MeSH D004075
G13	IMP — MeSH D007170 · GMP — MeSH D006157
G13a	Monosodium glutamate — MeSH D012970
G14	Green fluorescent proteins — MeSH D044967
G15	Taste buds — MeSH D013649
G16	HEK-293 — Cellosaurus CVCL_0045 · ATCC CRL-1573
G17	CHO-K1 — ATCC CCL-61
G18	COS-7 — ATCC CRL-1651
G19	Mifepristone — MeSH D015735
G20	RT-PCR — MeSH D020133
G21	Fluo-3 — MeSH C059069
G22	Flow cytometry — MeSH D005471
G23	High-throughput screening assays — MeSH D057166
G24	Rat T1R1 — GenBank AAD18069
G25	Rat T1R2 — GenBank AAD18070
G26	Mouse *Tas1r3* / *Sac* locus — MGI:1933547

M — Methods, protocols and tutorials

These are entry points into technique rather than citations to specific results. Each is a maintained reference resource.

KEY	RESOURCE	USE
M1	IUPHAR/BPS Guide to Pharmacology	GPCR classification, G protein coupling selectivity, receptor families including the taste receptors
M2	GPCRdb	Structure-based GPCR reference: sequence alignments, structures, residue numbering schemes

KEY	RESOURCE	USE
M3	EMBL-EBI Training	Sequence alignment, database searching, bioinformatics fundamentals
M4	NCBI Bookshelf	Full-text molecular biology and methods texts, including PCR and hybridisation chapters
M5	Addgene Protocols	Transfection, plasmid preparation, cell culture working protocols
M6	Thermo Fisher Molecular Probes Handbook	Calcium indicator chemistry, AM-ester loading, spectral properties of Fluo-3 and Fluo-4
M7	Addgene Molecular Biology Reference	Epitope tags, fluorescent proteins, selection markers, promoters — a single reference covering most of the constructs in Examples 10–12
M8	protocols.io	Community-deposited step-by-step protocols, including stable line generation and clonal selection
M9	Addgene Molecular Biology Reference — selection markers	Puromycin, zeocin, blasticidin: resistance mechanisms and working concentrations
M10	Addgene — inducible expression systems	GeneSwitch, Tet-On/Tet-Off and related systems compared
M11	NCBI Bookshelf — pharmacology texts	Dose-response curve fitting, EC_{50} and IC_{50} definitions, Hill slope
M12	Addgene Molecular Biology Reference — nucleic acid methods	Hybridisation stringency, melting temperature, the salt and formamide relationship underlying claim 1
M13	NCBI BLAST Help	How percent identity is computed, and why alignment parameters change the answer
M14	NCBI — the genetic code	Codon degeneracy: why many nucleic acid sequences encode one protein
M15	Addgene — promoters	Constitutive promoter strengths in mammalian cells (CMV, EF-1α, SV40)
M16	JoVE	Video protocols for membrane protein expression, trafficking assays and calcium imaging
M17	Cellosaurus	Cell line provenance, authentication status and known misidentification — essential before relying on any named line

KEY	RESOURCE	USE
M18	USPTO Patent Public Search	Searching the full text of US patents, including this family

L — Legal and standards

KEY	SOURCE
L1	USPTO kind codes — what B1, B2 and A1 mean
L2	35 U.S.C. §119 — provisional priority
L3	35 U.S.C. §121 — divisional applications
L4	WIPO Standards ST.25 / ST.26 — sequence listing format
L5	Cooperative Patent Classification · IPC
L6	USPTO Patent Center — the file wrapper for application 10/725,472
L7	Espacenet record
L8	Manual of Patent Examining Procedure (MPEP) — claim interpretation, dependency, restriction practice
L9	MPEP Chapter 2100 — patentability — written description and enablement, the standards against which identity-band claims are judged
L10	Creative Commons BY-NC-SA 4.0 · legal code

C — Context and fact-checks

KEY	SOURCE
C1	USA TODAY fact check: fetal tissue not in food
C2	Associated Press: no, food products don’t contain fetal tissue
C3	Reuters: food products do not contain human fetal cells
C4	US Food and Drug Administration
C5	Google Patents record — 27 patent citations, 21 non-patent citations, 83 citing documents

Notes and licence

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. HEK-293 was derived from fetal kidney tissue in the early 1970s and described by Graham et al. in 1977 [P25]; the line has been propagated continuously ever since and no original tissue is involved in its use. An FDA official has stated there are no conditions under which human fetal tissue would be considered safe or legal in food [C4]. Fact checks by USA TODAY [C1], the Associated Press [C2] and Reuters [C3] set out the full position.

Accuracy and verification

Claim text and example text are verbatim from the granted patent, including its typographical errors: 10% SDS in the T1R3 branch of claim 1, “0.2×SCC” in the same branch, “ooctye” in claim 26, “claims 1” in claim 33, and “is further expresses” in claim 36. Annotations, groupings and all cite keys are editorial.

Two specific cautions. The UniProt accession P30679 for human Gα15 [G9] should be verified independently before formal citation. The resources in section M are maintained reference sites linked at section level rather than deep permalinks, chosen so that they continue to resolve; the specific page within each that addresses a given technique may move.

Verify anything load-bearing against the official PDF facsimile [D1a] or the Patent Center file wrapper [L6].

Copyright and licence

© 2026 Computational Classnotes. All rights reserved in the original material. First published 8 November 2026; expanded edition 9 November 2026.

This work — the annotations, commentary, glossary, bibliography, editorial selection and arrangement — is licensed under the **Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (CC BY-NC-SA 4.0)** [L10].

You are free to:

- **Share** — copy and redistribute the material in any medium or format
- **Adapt** — remix, transform, and build upon the material

Under the following terms:

- **Attribution** — You must give appropriate credit to Computational Classnotes, provide a link to the licence, and indicate if changes were made.
- **NonCommercial** — You may not use the material for commercial purposes.
- **ShareAlike** — If you remix, transform, or build upon the material, you must distribute your contributions under the same licence as the original.

Full licence text: https://creativecommons.org/licenses/by-nc-sa/4.0/ Legal code:

https://creativecommons.org/licenses/by-nc-sa/4.0/legalcode

Scope of this licence. The licence applies to the editorial content of this document. It does not apply to the quoted text of US Patent 7,344,859 B2, which is a United States patent document published by the USPTO and is not subject to this licence, nor to the third-party works cited, which remain under their respective publishers' copyright.

Suggested citation: Computational Classnotes, *US Patent 7,344,859 B2 — Annotated Reading Edition*, expanded edition, 9 November 2026. CC BY-NC-SA 4.0.

Revision #5
Created 2026-11-09 00:35:55 UTC by Dara
Updated 2026-11-09 01:03:53 UTC by Dara