

**An anhydrobiotic cell line expressing odorant receptors shows odorant
responses after dry storage
(Supplementary material)**

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1. Results

1.1. Screening for functional ORs in Pv11 cells

D. melanogaster ORs have already been characterized with identified ligands, listed in the DoOR Database of Odorant Responses¹. For a first screening in Pv11 cells, we selected ORs that were already reported as functional in other cultured cell lines, such as DmOr13a^{2,3}, DmOr22a⁴, and DmOr47a^{2,5,6}.

First, we transiently expressed GCaMP6f into Pv11 cells and showed a significant fluorescent response to stimulation with ionomycin (Fig. S1A), in comparison with the control DMSO solution. GCaMP6f-transfected cells did not show significant response to DmOrco's agonist VUAA1, confirming the absence of intrinsic VUAA1-sensitive receptors in Pv11 cells (Fig. S1A). Next, Pv11 cells were co-transfected with both GCaMP6f and co-receptor DmOrco. They showed significant responses to both ionomycin and VUAA1, confirming that DmOrco was functional in Pv11 cells (Fig. S1B).

Then, different ORs were co-transfected with DmOrco and GCaMP6f into Pv11 cells. Pv11 cells co-transfected with DmOr13a did not have a significant response to its ligand, 1-octen-3-ol³, at a concentration of 1 mM (Fig. S1C), but they still had significant responses to VUAA1 and ionomycin, showing that the transfection of DmOrco and GCaMP6f was successful (Fig. S1C). Similarly, Pv11 cells co-transfected with DmOr22a did not respond significantly to its ligand ethyl hexanoate⁷ (Fig. S1D). On the other hand, Pv11 cells co-transfected with DmOr47a did have a significant response to the ligand, pentyl acetate⁷, at a concentration of 1 mM (Fig. S1E). Note that the co-transfection with DmOr47a increased the response of DmOrco to VUAA1, compared to co-transfection with DmOr13a or DmOr22a (average $\Delta F/F_0 = 1.11$, vs 0.53 and 0.49 for DmOr13a and DmOr22a, respectively). The genes that were shown functional in Pv11 cells by transient expression screening, namely GCaMP6f, DmOrco, and DmOr47a, were selected to generate a stable expression Pv11 cell line (see main text results section 2.1).

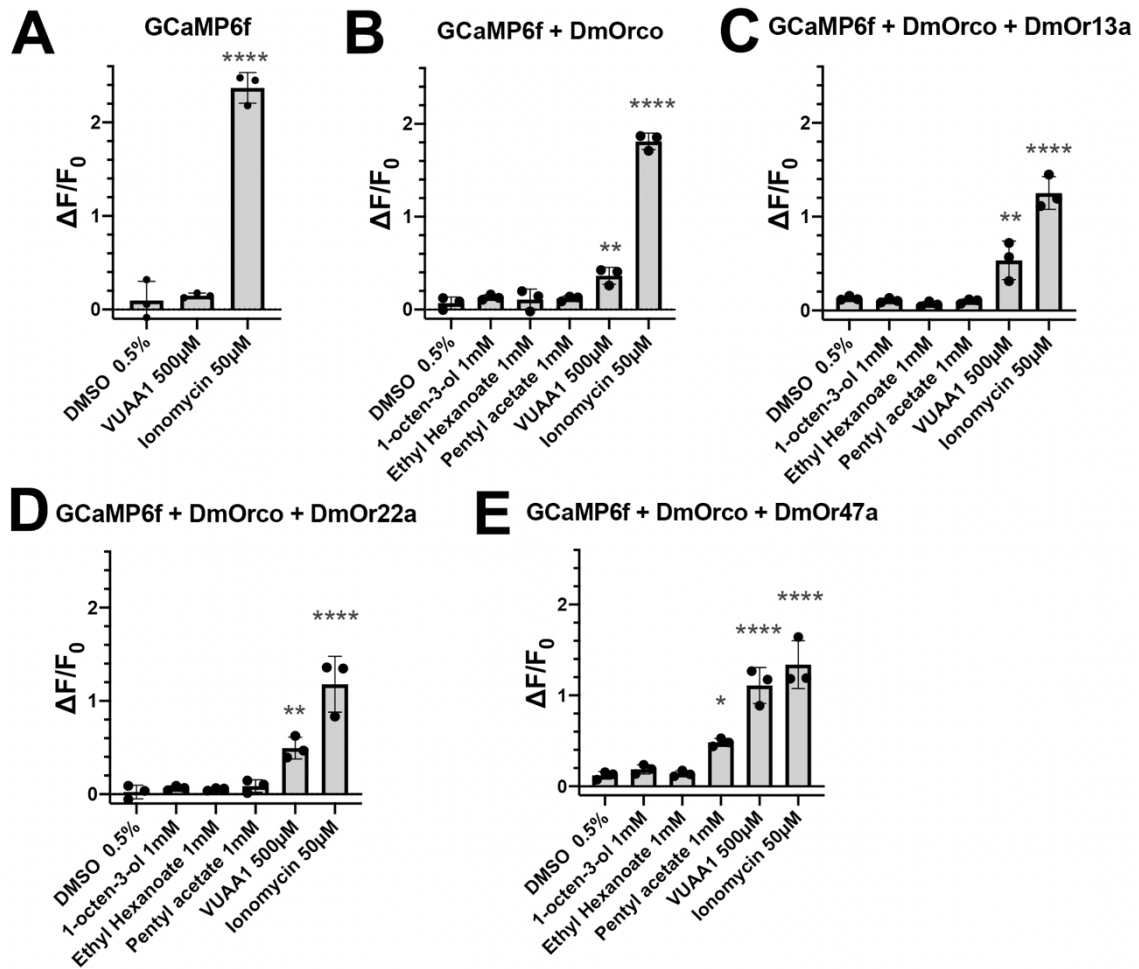


Figure S1: Ligand response expressed as a change in fluorescence intensity ($\Delta F/F_0$) for Pv11 cells with transient expression of calcium fluorescent marker GCaMP6f only (A), GCaMP6f and the co-receptor DmOrco (B), or co-expression of GCaMP6f and DmOrco together with DmOr13a (ligand 1-octen-3-ol) (C), DmOr22a (ligand ethyl hexanoate) (D), or DmOr47a (ligand pentyl acetate) (E). Points represent results from each trial (filled circles). Error bars show the mean of the 3 replicates $\pm$ one SD. Significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as * (p-value < 0.05); ** (p-value < 0.01); and **** (p-value < 0.0001).

1.2. OR protein expression in Pv11 cells

To verify if the odorant receptor proteins were correctly localized at the surface of Pv11 cells membrane after transient expression, we used Nano-Glo[®] HiBiT Extracellular Detection System (Promega, Madison, WI, USA). Concerning DmOr13a, no significant protein expression was detected at the surface of Pv11 cells neither when transfected alone, nor when co-transfected with DmOrco (Fig. S2, left). In contrast, DmOr22a protein was correctly expressed at the surface of Pv11 cells with detected protein levels significantly higher than the control with empty vector transfection (Fig. S2, middle). Note that when we co-transfected DmOr22a with the co-receptor

DmOrco, the protein level was increased at the surface of Pv11 cells. DmOr47a protein was also significantly detected on the Pv11 cell surface, when DmOr47a was co-transfected with DmOrco (Fig. S2, right). Intriguingly, in spite of correct protein expression, DmOr22a was apparently not functional with transient expression (Fig. S1D), suggesting that other factors may affect the function of the receptor under these conditions. Nevertheless, DmOr13a and DmOr22a have been shown to be functional in other types of cell culture, such as S2, Sf21, and HEK293²⁻⁴. Hence, we hope to get significant response to the ligands of these ORs when stably expressed in Pv11 cells.

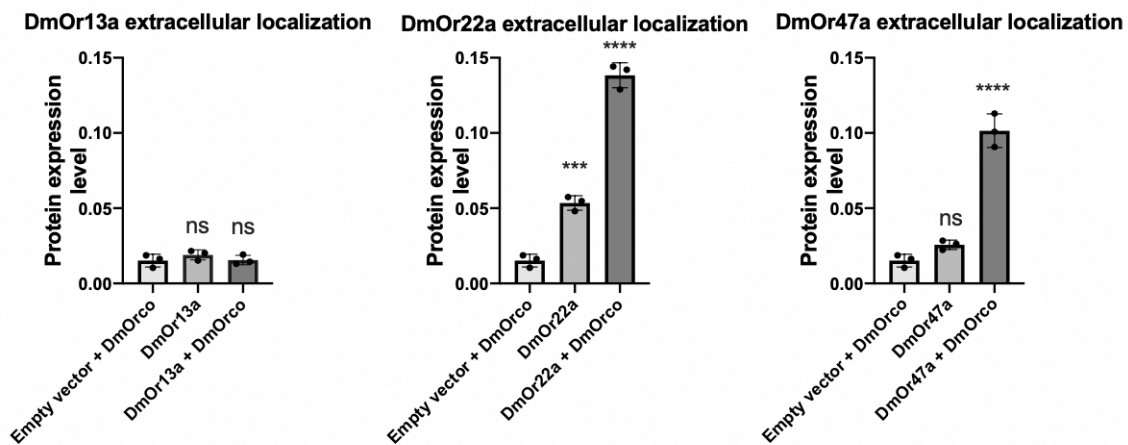
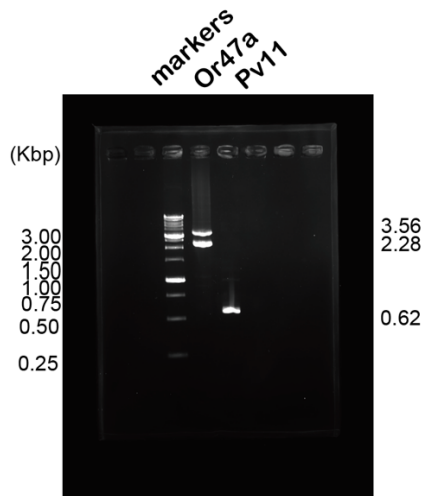


Figure S2: Extracellular localization of DmOr13a, DmOr22a, and DmOr47a proteins at the surface of Pv11 cells membranes, as detected with the Nano-Glo[®] HiBiT Extracellular Detection System. As a negative control, Pv11 cells were transfected with an empty expression vector and pPv121-Orco (Empty vector + DmOrco). Other samples represent cells transfected with OR-expression vectors only, or in combination with pPv121-Orco. The Y-axis represents the relative protein expression at the extracellular surface of transfected Pv11 cells as normalized to Luc2 luminescence as a transfection efficiency control (arbitrary units). Bars represent the mean of 3 replicates $\pm$ SD. Significant differences compared to the negative control (One-way ANOVA followed by Dunnett test) are expressed as *** (p-value < 0.001), **** (p-value < 0.0001) or ns (not significant).

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Figure S3: Original gel electrophoresis picture from Fig. 1B for the genomic PCR of Pv11 control cells (Pv11) and Pv11-00443-Or47a cell line (Or47a) showing specific bands for the 3'-end of the Pv.00443 gene (619bp), for the GCaMP6f-DmOrco insert (2,281bp), and for the zeocin resistance-DmOr47a insert (3,562bp).

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1.3. Stability of GCaMP6f fluorescence in Pv11-00443-Or47a cells

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Previous studies suggested that the function of cytosolic proteins is well preserved in Pv11 cells after dry storage and rehydration⁸. To verify if it was the case for GCaMP6f in Pv11-00443-Or47a cell line, we monitored the fluorescence of GCaMP6f from prior to desiccation to 48 h after rehydration (Fig S4). Monitoring GCaMP6f fluorescence of individual cells in the baseline preceding the addition of pentyl acetate showed significant variation after rehydration, compared to the fluorescence prior to desiccation (Fig. S4A). Whereas GCaMP6f raw fluorescence was 1372 arbitrary units (A.U.) prior to desiccation, the average fluorescence per cell dropped to 1038–1110 A.U. at 1 h and 6 h after rehydration, respectively (Fig. S4A). Subsequently, the average fluorescence per cell increased to a level significantly higher than that prior to desiccation, over 1500 A.U., except for 24 h after rehydration with a raw fluorescence of 1306 A.U., close to that observed prior to desiccation (Fig. S4A). After exposure to pentyl acetate 1 mM, the highest raw GCaMP6f fluorescence rose to 2000 A.U. prior to desiccation and recovered to this level 12 – 48 h after rehydration (Fig. S4B). Only cells at 1 – 6 h after rehydration showed a raw fluorescence response to 1 mM pentyl acetate limited to 1200 A.U. (Fig. S4B). Taken together, raw fluorescence data of individual cells before and after desiccation- rehydration suggested that GCaMP6f protein was only partially preserved during dry storage and recovered its original fluorescence 12 h after rehydration and later.

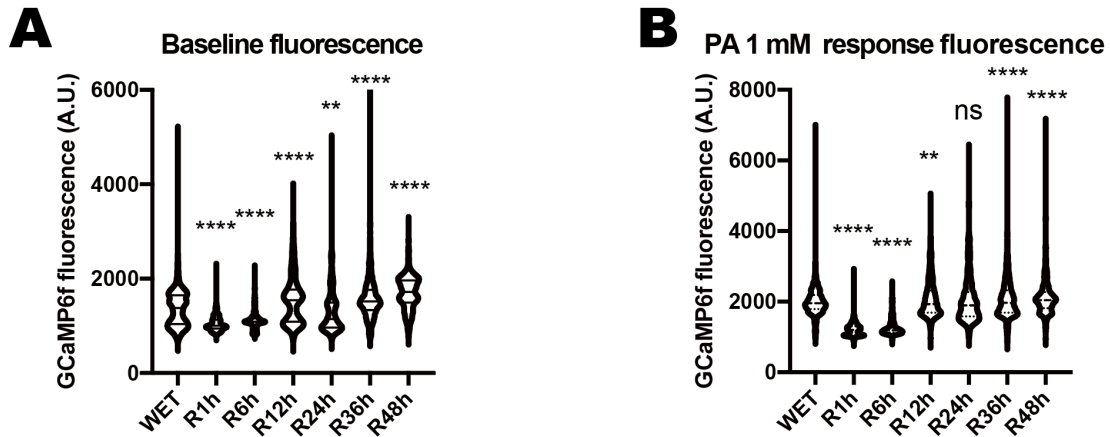


Figure S4: Monitoring the raw fluorescence of 522–1812 individual cells.

The raw fluorescence of individual cells expressed as arbitrary units (A.U.) was recorded prior to desiccation (WET) and 1 h, 6 h, 12 h, 24 h, 36 h, 48 h after rehydration (R1h, R6h, R12h, R24h, R36h, R48h, respectively). **(A)** Violin plots showing the raw fluorescence of cells in the baseline prior to the exposure to pentyl acetate. **(B)** Violin plots showing the highest raw fluorescence of cells after the exposure to 1 mM pentyl acetate. Significant differences compared to WET samples (one-way ANOVA followed by Dunnett test) are expressed as **** (p-value < 0.0001); ** (p-value < 0.01); ns (not significant).

1.4. Function retention under long-term storage, culture, and multiple dehydrations

Since a goal of the present work is a stable cell line that can be stored for long periods of time, we qualitatively evaluated the response of the Pv11-00443-DmOr47a cell line (cf. section 2.1 and Fig. 1 in the main text and Fig. S5) after 3 years of dry storage. The DmOr47a cells from NARO arrived at UMD in the dry state in July 2019 and were kept at room temperature until they were rehydrated in September 2022. The cells had been passaged five times over a period of three months when they were adhered to glass cover slips using collagen-BAM (Section 4.5, main text, or alternatively section 2.7 below). Fig. S5a shows, in brightfield, a layer of attached cells as well as some overlying unattached cells. Upon exposure to pentyl acetate (Fig. S5b-e), at least 20 cells became noticeably brighter compared to the fluorescence baseline in this field of view, with others becoming slightly brighter. Upon exposure to VUAA1, a large fraction of the cells showed increased fluorescence. Thus, the cells retain the ability to produce DmOR47a and Orco after 3 years of dry storage.

We also examined the response stability of the cell line after multiple rounds of desiccation

1 and long culture times. An example is shown in Fig. S6 of adhered cells that had been dehydrated
2 and rehydrated three times and passaged, from September 2020 through October 2024, a total of
3 92 times (at approximately 3-week intervals). The cells maintained some responsivity to pentyl
4 acetate, and of course also to VUAA1, the latter not shown. The sensitivity of the cell line to
5 pentyl acetate, however, decreased over this time.

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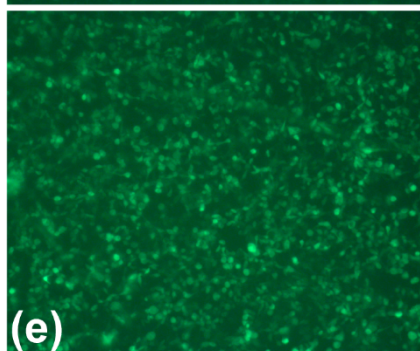
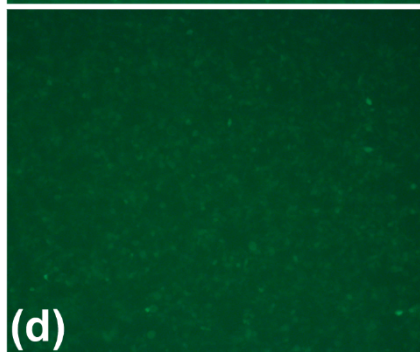
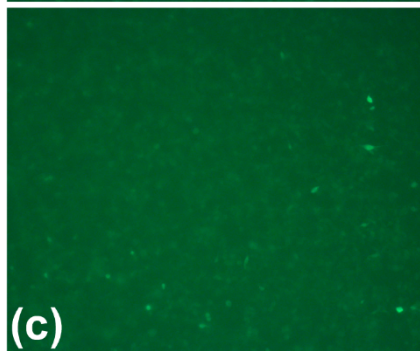
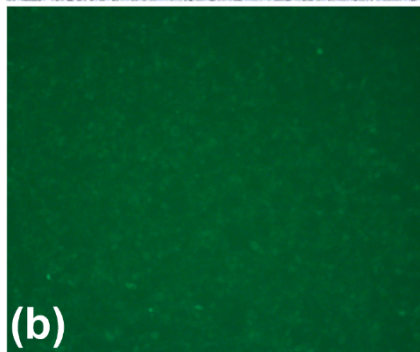
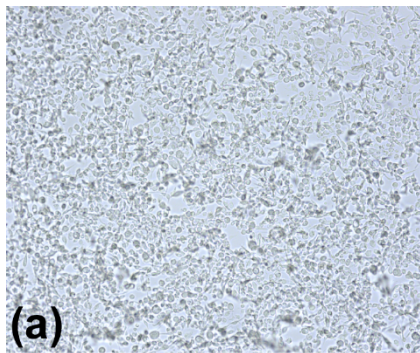


Figure S5: (a) Brightfield image of adhered Pv11-00443-Or47a cells after 3 years of dry storage and 3 months of cell culture. Fluorescence images (b) before any exposure to odors (baseline), (c) after exposure to 10^{-2} M pentyl acetate, (d) the new baseline 5 minutes later, and (e) after exposure to 10^{-3} M VUAA1. All images taken at the same position at 20x magnification. Image brightness and contrast were adjusted for viewability.

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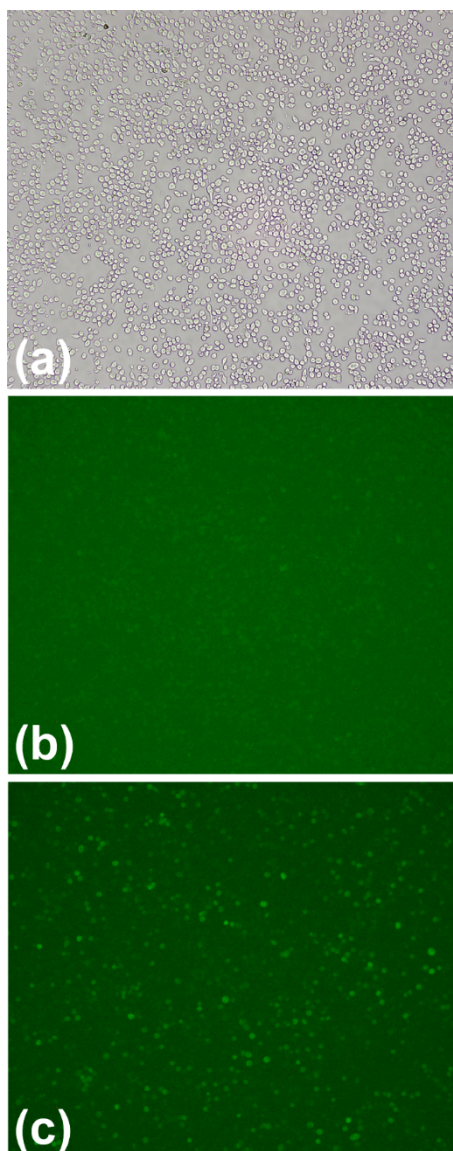


Figure S6: Image of adhered Pv11-00443-Or47a cells after dehydration at NARO, rehydration at UMD, 65 cell passages, dehydration for 1 month, rehydration, 11 cell passages, dehydration for 1 month, rehydration, and 16 additional cell passages. (a) Brightfield image. (b) Before exposure (baseline), and (c) after exposure to 10^{-1} M pentyl acetate. Images take at the same position at 20x magnification. Image brightness and contrast were adjusted for viewability.

A perfusion assay was performed to record the responses to successive ligand exposures of the Pv11-00443-DmOr47a cell line as detailed in section 3.9. The response to 10 cycles of 3-second exposures to 10 mM PA are shown in エラー! 参照元が見つかりません。, demonstrating that the cells continue to respond to successive exposures rather than just a single time. The curve in Fig. S7A for “all fluorescing regions” represents 1922 ROIs, while “most responsive 200” is the average of the most varying 200 cells. Fig. S7B shows the responses of 12 individual cells.

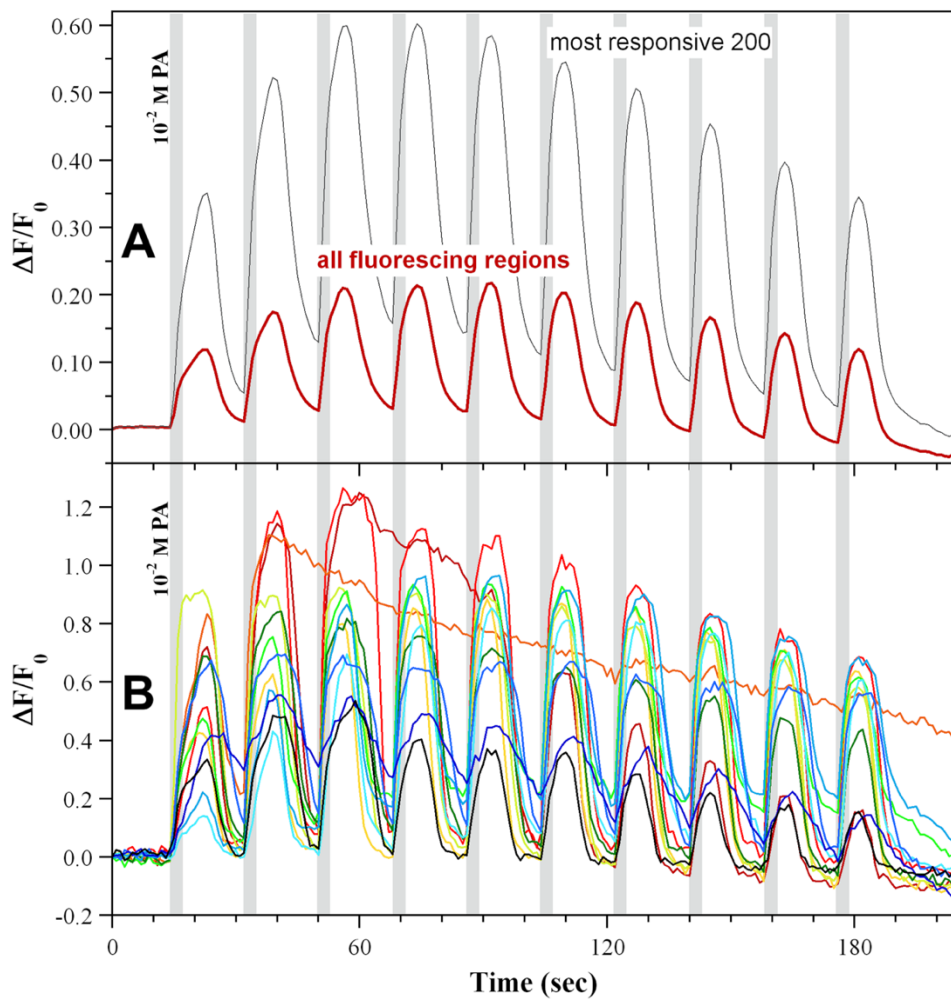


Figure S7: Change in fluorescence ($\Delta F/F_0$) of the stable cell line Pv11-00443-Or47a in response to 10 mM pentyl acetate (10^{-2} M PA) in HEPES-buffered saline (HBS), given in 3 second doses, indicated by the shaded regions, separated by 15 seconds of HBS. **(A)** The average response of 1922 individual cell ROIs (thick red line) and of the most responsive 200 cells (thin gray line). **(B)** The individual responses of 12 of the 200 most responsive cells (note that the vertical scales differ).

2. Discussion

2.1. Dry preservation of a transmembrane protein, DmOrco

Experiments with a translation inhibitor (cycloheximide) showed that DmOrco was functional just after rehydration and thus at least one fraction of DmOrco proteins should be preserved at the membrane surface of dried Pv11-00443-Or47a cells (Section 2.4 and Fig. 3A,B in the main text). To our knowledge, this is the first report of dry preservation of a membrane protein in a cell culture orthologous expression system. In the yeast *Saccharomyces cerevisiae*, the activity of the plasma membrane protein Agt1, an alpha-glucoside transporter, was investigated under reduced water content and its activity was reduced by 10–15% in a desiccation-sensitive strain, but not in a desiccation-tolerant strain⁹. That study demonstrated that a cryptobiotic organism can preserve the activity of a transmembrane transporter under low water content conditions, but the preservation of the activity of this transporter after dry storage and rehydration was not verified due to membrane leakage. Freeze-drying of red blood cells loaded with trehalose showed that cell membranes and associated proteins such as ankyrin or glycophorin A were preserved after dry storage and rehydration¹⁰. However, the integrity of these membrane proteins was only assessed by immunoblotting, which cannot assess whether those membrane proteins were functional. The freeze-drying method associated with trehalose was also effective in protecting the cell membranes and DNA of mammalian cells after dry storage and rehydration¹¹. From the above-cited studies, we believe that the protection of DmOrco activity in desiccated Pv11 cells is the first example of the preservation of an exogenous membrane protein retaining its activity after storage at room temperature and subsequent rehydration.

As stated above, trehalose is a major protectant molecule for the preservation of biological molecules in the dry state, and it probably contributed to the protection of DmOrco in Pv11 cells. Actually, *P. vanderplanki* larvae produce large amounts of trehalose during the desiccation process, leading to the suggestion that anhydrobiosis and vitrification of trehalose physically protect the cells and biological molecules¹²⁻¹⁴.

Pv11 cells were desiccated into a concentrated trehalose mixture, and trehalose is taken up by the cells through specific transporters¹⁵⁻¹⁷. Thus, DmOrco was expected to be protected by trehalose both intracellularly and extracellularly. Specific chaperone proteins involved in the protection and stabilization of other proteins, called Late Embryogenesis Abundant (LEA) proteins were also identified in *P. vanderplanki* larvae^{18,19}. Especially LEA1 and LEA3 were associated with cell membranes^{20,21} and could potentially have participated in the protection of DmOrco in dry Pv11 cells. Similarly, other membrane-associated putative chaperones called Lea Island Located (LIL) proteins are expressed in Pv11 cells and are expected to be involved in the protection of cell membranes and transmembrane proteins²². Various other anhydroprotectants

were identified in *P. vanderplanki*^{14,23} and may play a role in the protection of membrane proteins such as DmOrco in Pv11 cells.

3. Methods

3.1. Expression vectors for transient expression

Total RNA was extracted from dissected heads of *Drosophila melanogaster* (Canton-S) flies with ReliaPrep™ RNA Tissue Miniprep System (Promega, Madison, WI). RNA was then reverse transcribed to cDNA with PrimeScript II 1st strand cDNA Synthesis Kit (Takara, Kusatsu, Japan) and this cDNA was used as a template to clone DmOrco, DmOr22a, and DmOr47a coding regions. Concerning the DmOr13a coding region, the sequence was obtained from flybase and artificially synthesized. The GCaMP6f coding sequence was cloned from the plasmid vector pGP-CMV-GCaMP6f (Addgene #40755). The Pv11 expression vector pPv121-MCS²⁴ was digested with BamHI and XhoI restriction enzymes and the sequences corresponding to DmOrco, DmOr13a, DmOr22a, DmOr47a, and GCaMP6f were respectively assembled in the expression vector using NEBuilder HiFi Assembly kit (New England BioLabs, Ipswich, MA). The obtained expression vectors were named pPv121-Orco, pPv121-Or13a, pPv121-Or22a, pPv121-Or47a, and pPv121-GCaMP6f, respectively (supplementary data 8–12). The primer oligonucleotides used to build these expression vectors are listed in table S1.

3.2. Transient expression of OR in Pv11 cells

Pv11 cells were passaged at a concentration of 3×10^5 cells/mL, and experiments were performed on the 5th day after passage. After cell counting, 1×10^7 cells were collected by centrifugation (300 g, 5 min) and washed with 500 μ L Opti-MEM (Thermo Fisher Scientific). After a second centrifugation step, cells were resuspended into 100 μ L Opti-MEM with 20 μ g of OR transient expression vector. Transfection of the cells with the expression vectors was performed with a NEPA21 electroporation system (NEPAGENE, Ichikawa, Japan) as described previously²⁵. Electroporation conditions were as follows: poring pulse: 250 V; pulse width 4 ms; pulse interval 50 ms; 6 pulses; decay rate 40%; polarity +. Transfer pulse: 20 V; pulse width 50 ms; pulse interval 50 ms; 5 pulses; decay rate 40%; polarity +/- . After transfection, the cells were transferred into 2 mL of supplemented IPL-41 medium in a 6 well plate (BMBio) and incubated for 3 days at 25 °C before performing a ligand binding assay.

3.3. Ligand binding assay after transient expression of OR in Pv11 cells

After transfection as described above, Pv11 cells were immobilized on 96-well plates

(EZview culture plate LB glass bottom; AGC Techno Glass, Shizuoka, Japan), following the protocol described in the method section 4.5 in the main text. Ligand binding assay was performed as described in the method section 4.6 in the main text. Ionomycin (> 95% purity; Fujifilm Wako, Osaka, Japan) was used as an ionophore to artificially increase intracellular calcium concentration and evaluate the GCaMP6f fluorescence response. VUAA1 (>98% purity; Sigma-Aldrich, Tokyo, Japan) was used as the DmOrco co-receptor agonist. The ligands 1-octen-3-ol (>98% purity; Tokyo Chemical Industry, Tokyo, Japan), ethyl hexanoate (>95% purity; Fujifilm Wako), and pentyl acetate (>97% purity; Fujifilm Wako) were used to induce the responses of DmOr13a, DmOr22a, and DmOr47a, respectively. These chemicals were first dissolved in dimethyl sulfoxide (DMSO; Fujifilm Wako) and then diluted in modified aCSF buffer at the following final concentrations: 1% DMSO, 100 μ M ionomycin, 1mM VUAA1, 2 mM 1-octen-3-ol, 2 mM ethyl hexanoate, and 2 mM pentyl acetate. Statistical analysis of the cells responses was performed as described in the methods section 4.9 in the main text.

3.4. Construction of expression vectors with HiBiT-tag

Transient expression vectors pPv121-Or13a, pPv121-Or22a and pPv121-Or47a were used as templates to insert with specific primers a linker and HiBiT-tag sequence (GSSGGSSGVSGWRLFKKIS) before the stop codon of each odorant receptor (OR) sequence. The Pv11 expression vector pPv121-MCS²⁴ was cut with BamHI and XhoI restriction enzymes and the PCR products of each OR with HiBiT-tag were respectively assembled in the expression vector using NEBuilder HiFi Assembly kit (New England BioLabs, Ipswich, MA). The obtained expression vectors were named pPv121-Or13a-HiBiT, pPv121-Or22a-HiBiT and pPv121-Or47a-HiBiT respectively. The primer oligonucleotides used to build these expression vectors are listed in table S3.

3.5. Transfection of expression vectors with HiBiT-tag to Pv11 cells

Pv11 cells were passaged at a concentration of 3×10^5 cells/mL, and experiments were performed on the 5th day after passage. After cell counting, 1×10^7 cells were collected by centrifugation (300 g, 5 min) and washed with 500 μ L Opti-MEM (Thermo Fisher Scientific, Tokyo, Japan). After a second centrifugation step, cells were resuspended into 100 μ L Opti-MEM containing 1 pmol of either control empty vector pPv121-MCS, pPv121-Or13a-HiBiT, pPv121-Or22a-HiBiT or pPv121-Or47a-HiBiT expression vector, together with 1 pmol of pPv121-Orco and 0.6 pmol of pPv632bp-luc2²⁶ as a transfection efficiency control. Transfection of the cells with the expression vectors was performed with a NEPA21 electroporation system (NEPAGENE, Ichikawa, Japan) as described previously²⁵. Electroporation conditions were as follows: poring pulse: 250 V; pulse width 4 ms; pulse interval 50 ms; 6 pulses; decay rate 40%; polarity +. transfer

pulse: 20 V; pulse width 50 ms; pulse interval 50 ms; 5 pulses; decay rate 40%; polarity +/- . After transfection, 12.5 μ L aliquots of cells were transferred into 250 μ L of supplemented IPL-41 medium in a 48 well plate (BMBio, Tokyo, Japan) and incubated for 2 days at 25 $^{\circ}$ C before performing a HiBiT assay.

3.6. Quantification of transmembrane proteins with HiBiT assay

For HiBiT assay, 50 μ L of the cells transfected as above were mixed to 50 μ L of HiBiT assay solution (Nano Glo HiBiT Extracellular Detection System, Promega, Madison, WI, USA) and transferred to a white 96-well immuno plate (ThermoFisher Scientific, Tokyo, Japan). After shaking at 600 rpm for 10 min with a Bioshaker M-BR-022 (Taitec, Koshigaya, Japan) for mixing samples, HiBiT luminescence was detected using an ARVO multi-label plate reader (PerkinElmer, Waltham, MA, USA). The same procedure was conducted to detect Luc2 luminescence using Luciferase Reporter Assay System (Promega, Madison, WI, USA). The amount of OR proteins present at the surface of Pv11 cells was expressed as the luciferase activity from HiBiT-tagged proteins, normalized to the Luc2 activity, which reflected gene transfer efficiency.

3.7. Immobilizing cells after long-term storage, culture and multiple dehydrations

For imaging of adhered cells on cover slips (Figs. S4 & S5), a modified protocol was also used. Type I collagen was dissolved in PBS to 0.1% w/v (C9791, collagen from calf skin, Millipore Sigma, Rockville, MD). BAM was dissolved in DMSO as described above, then further dissolved in PBS to form a 100 μ M solution. One part of the collagen solution was mixed with 4 parts of the BAM solution. After centrifuging the cells and replacing the insect medium with PBS, the cells were added directly 1:1 to the collagen-BAM solution. Droplets were immediately placed onto the cover slips and incubated for 30 minutes at 37 $^{\circ}$ C prior to rinsing with PBS to remove loose cells before addition of cell medium, PBS, or artificial cerebrospinal fluid buffer (section 4.8 main text).

3.8. Visualization of responses of cells adhered to cover slips

Image acquisition was performed with an Olympus reflected light microscope (BX50) using a 20x objective (LMPlanFl 20x/0.40, $\infty/0$). Brightfield illumination was with a halogen lamp (U-LS30-S 6V 30W). Fluorescence illumination was with an LED source (Excelitas X-Cite mini+), and a filter cube for GFP (EX: 480/30 nm, DM: 505 nm, EM: 535/40 nm) was used for detection. Images were recorded with a color CMOS camera (Teledyne Lumenera 5-5C 5.1 MP with software Infinity Analyze 7).

Cells were adhered to 22x22 mm² cover slips and kept in 35 mm petri dishes, covered with 1 mL of IPL-41 complete media. The slides were fixed in place within the petri dish with

1 magnets to prevent motion. Slides were positioned under the objective with a mechanical x-y
2 stage. Ligands were introduced by pipette directly over the area of observation while recording
3 video (1 frame/sec). A 10 second baseline was recorded before adding the ligand.

4 3.9. Recording cells ligand responses in perfusion assay

5 Cells: Pv11-00443-Or47a cells that had been passaged 65 times, dehydrated, rehydrated,
6 passaged a further 11 times, dehydrated, rehydrated, and then passaged 28 times were used. Cells
7 were kept in IPL complete medium after plating until use.

8 Perfusion System: An OctaFlow 8-channel system (ALA Scientific Instruments, Inc., New
9 York), was employed for the perfusion experiments. Cells were plated onto 22x22 mm² cover
10 slips using the 1-step collagen-BAM adhesion protocol described above. The cells were
11 positioned over a channel formed in PDMS of 100 µm depth, 2 mm width, and 14 mm length
12 with deeper inlet and outlet reservoirs into which the tip of the 8-channel manifold and outlet
13 tubing were connected. The cover slip was mechanically clamped over the PDMS channel. The
14 pressure applied to the perfusion reservoirs was 3 psi (200 mBar), resulting in a flow rate of 50
15 µL/minute. Taking into consideration the 230 µm ID tubing (60 cm) between the reservoirs and
16 the manifold and the 100 µm ID (5 cm) manifold tubing, the shear stress at the channel wall can
17 be estimated to be on the order of 1 dyn/cm². Cells remained adhered under these conditions.

18 Perfusion Conditions: Experiments were performed in HEPES-buffered saline (HBS: 125
19 mM NaCl, 2.5 mM KCl, 10 mM HEPES, and 4.5 mM CaCl₂), which is a phosphate-free version
20 of the aCSF used elsewhere in this work, to avoid the formation of precipitates in the fluidic
21 system. The cells were flushed continuously in HBS upon placement in the fluidic system, and
22 the carrier solution for the pentyl acetate was HBS. (No DMSO was used in the PA solutions.) To
23 minimize the formation of bubbles, the HBS underwent a freeze-thaw cycle after preparation, and
24 solutions were warmed to 37 °C before adding them to the perfusion system reservoirs. The fluidic
25 program consisted of a 15 second baseline of BSF followed by a loop, repeated 10 times, of 3
26 seconds of pentyl acetate 1 mM and 15 seconds of BSF.

27 Image Processing: A Matlab script was used to identify regions of interest (ROIs) in the
28 fluorescence videos that exceeded a threshold. Responses of all the 1922 ROIs were averaged, as
29 well as those of the 200 ROIs with the greatest intensity range.

3 References

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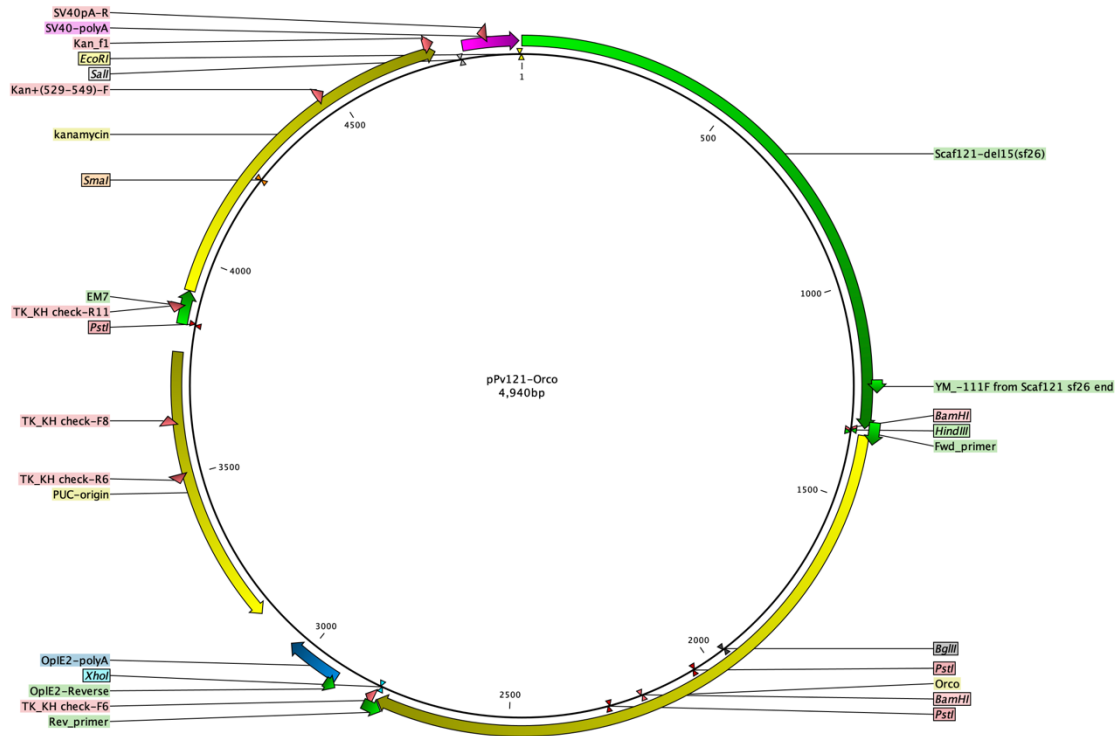
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2 desiccation-tolerant cell line, Pv11. *Extremophiles* **21**, 65-72,
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Supplementary data 8: pPv121-Orco vector map and sequence



> pPv121-Orco

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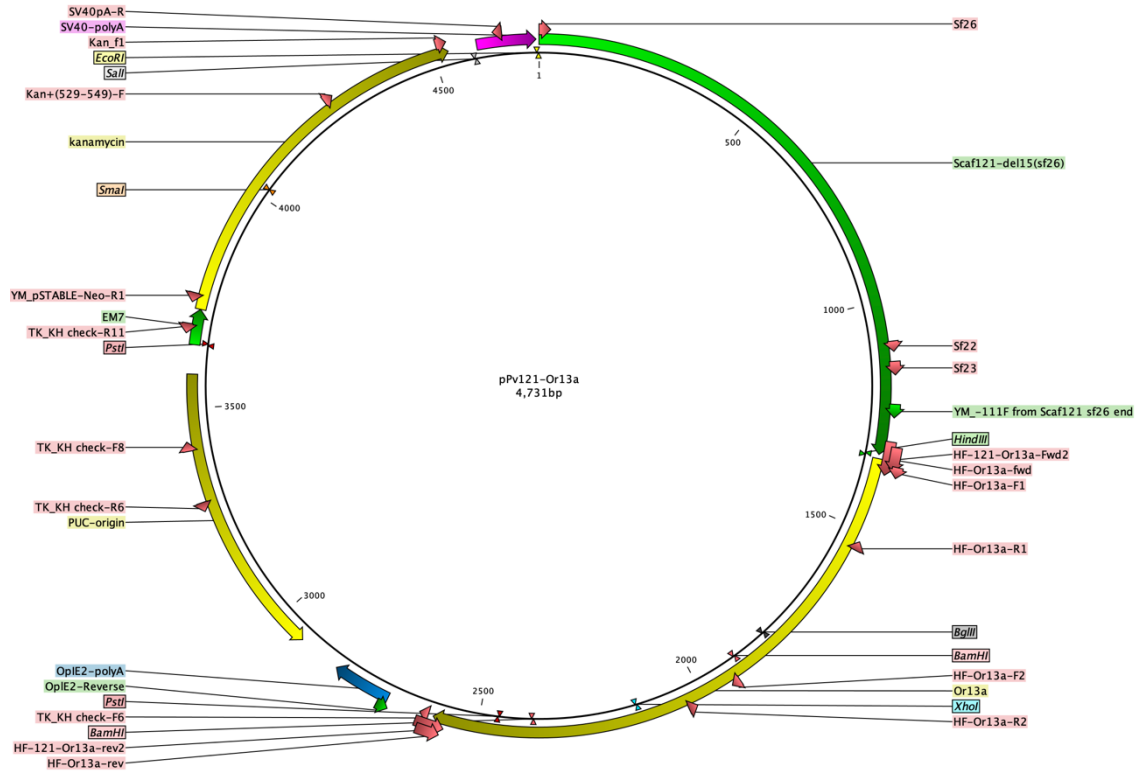
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10 2915..3044 bp: OpIE2-polyA
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13 3926..4741 bp: kanamycin resistance ORF
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Supplementary data 9: pPv121-Or13a vector map and sequence



> pPv121-Or13a

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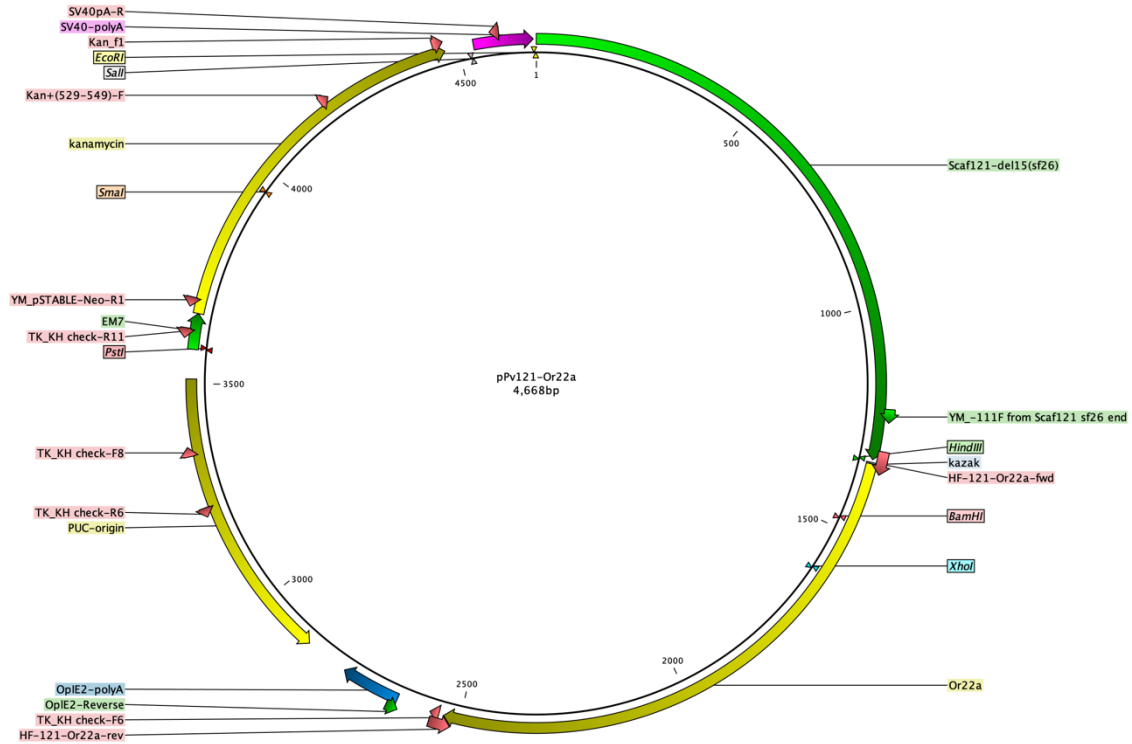
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11 3717..4532 bp: kanamycin resistance ORF
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Supplementary data 10: pPv121-Or22a vector map and sequence



> pPv121-Or22a

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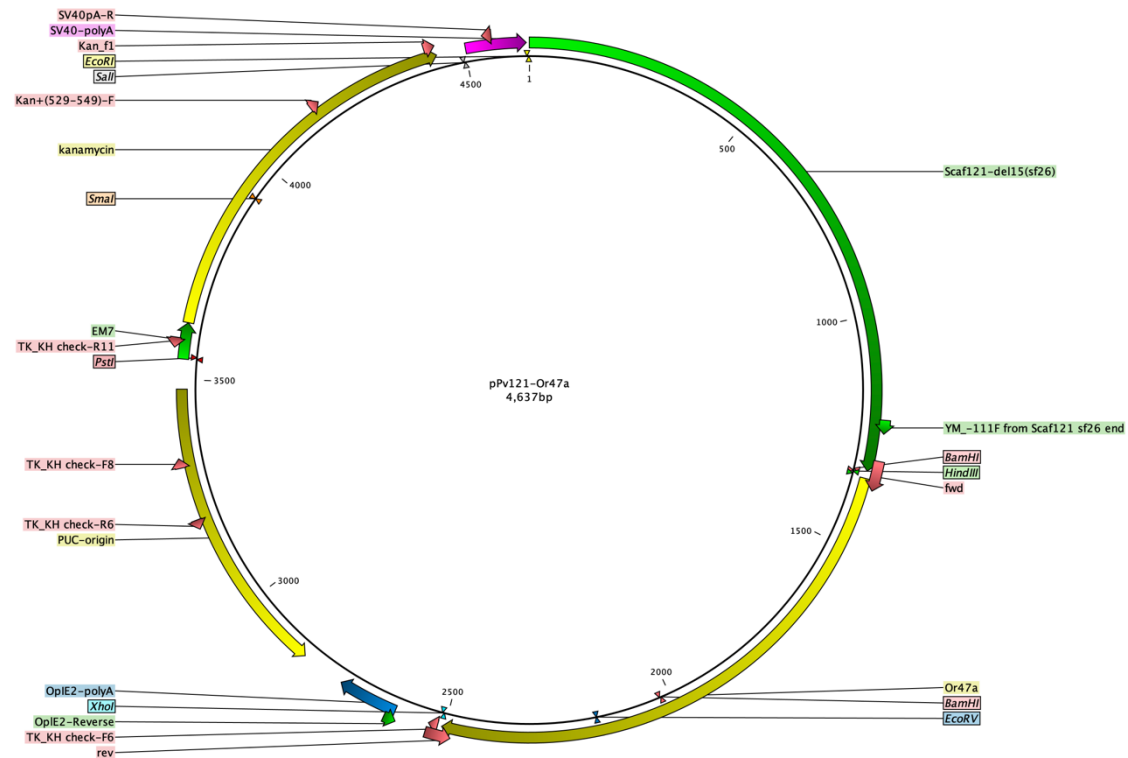
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7  1340..1342: kozak sequence
8  1343..2536 bp: DmOr22a ORF
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11 3576..3653 bp: EM7 promoter
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Supplementary data 11: pPv121-Or47a vector map and sequence



> pPv121-Or47a

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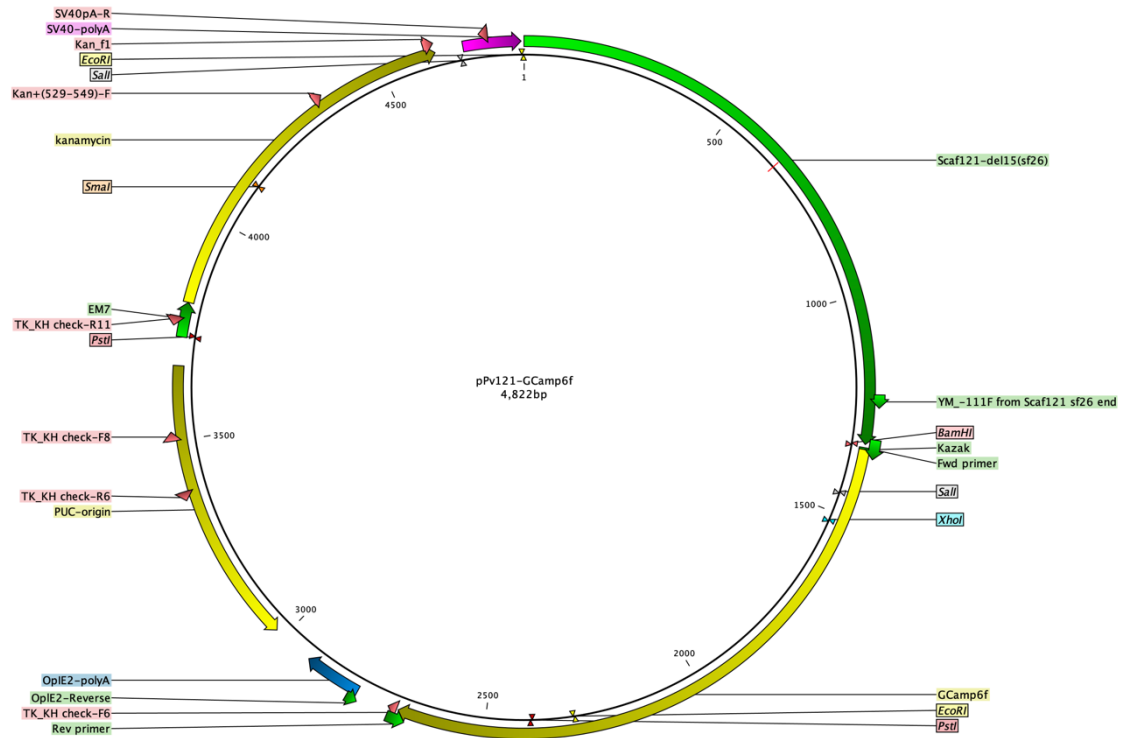
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Supplementary data 12: pPv121-GCaMP6f vector map and sequence



> pPv121-GCaMP6f

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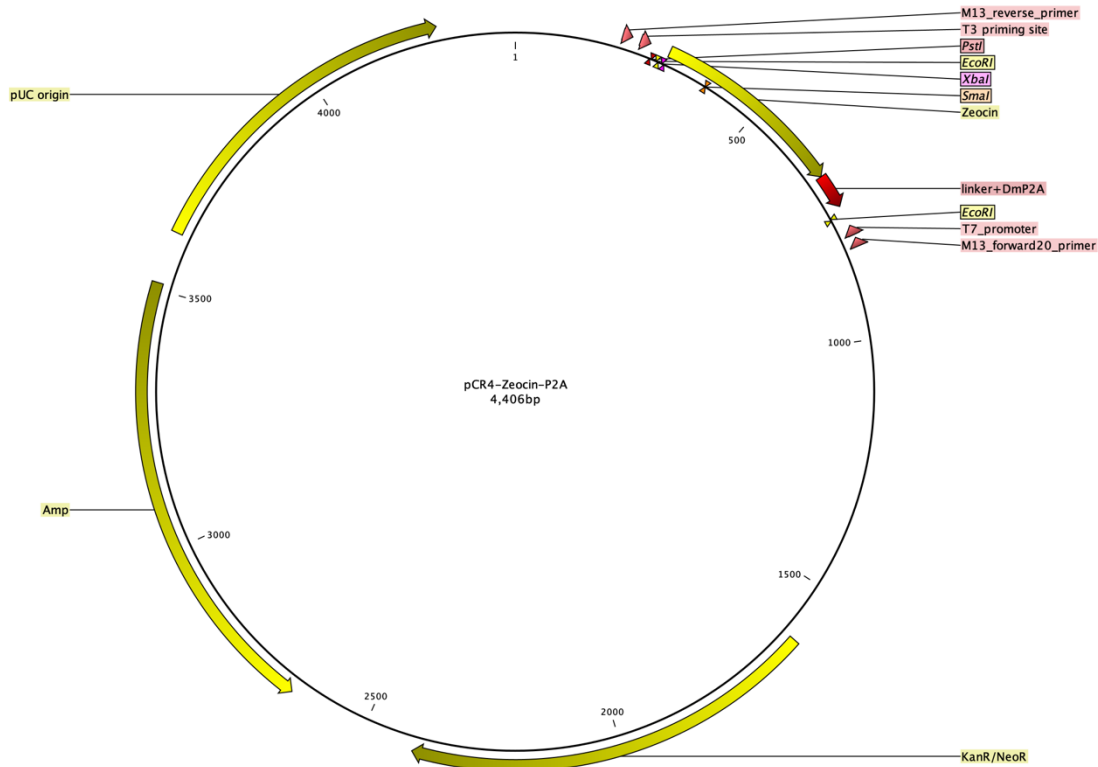
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Supplementary data 13: pCR4-Zeocin-P2A vector map and sequence



> pCR4-Zeocin-P2A

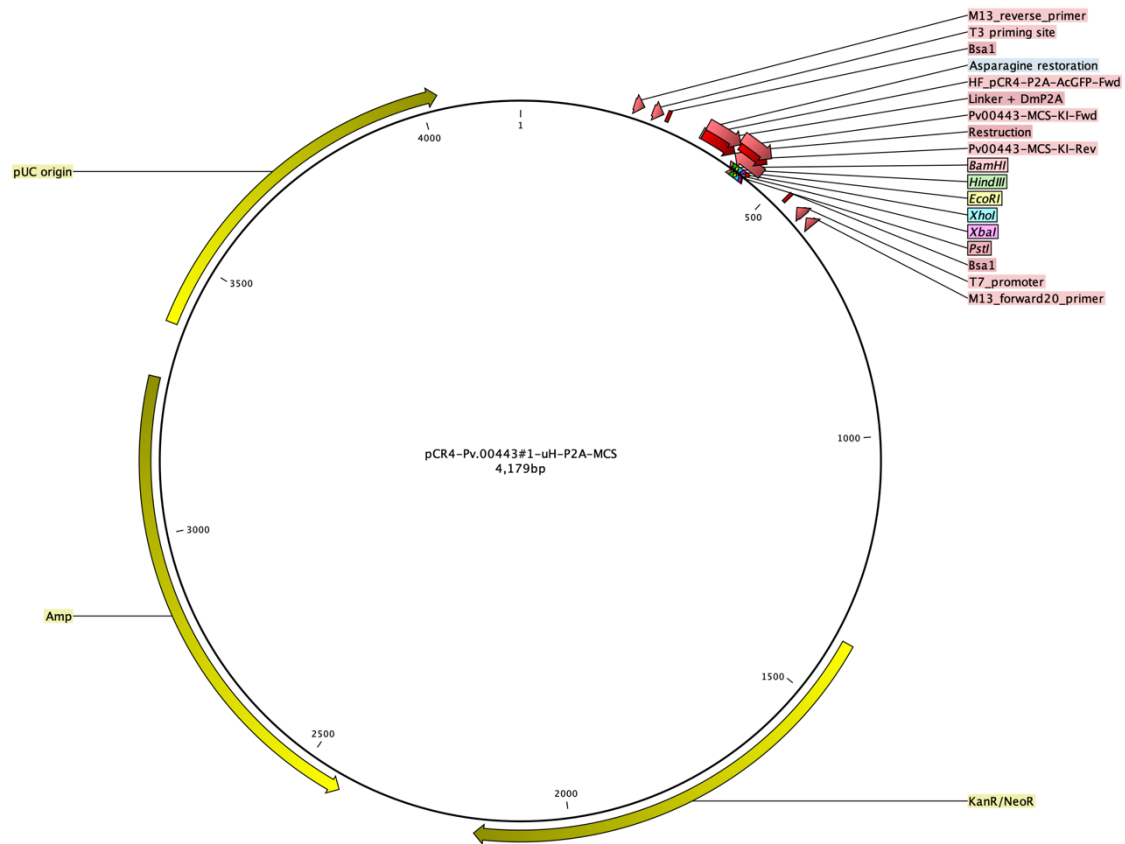
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1 CGGAAG
2
3
4 301..672 bp: Zeocin resistance ORF
5 673..738 bp: linker + DmP2A sequence
6 1612..2400 bp: Kanamycin/Neomycin resistance ORF
7 2653..3513 bp (complement): Ampicillin resistance ORF
8 3614..4257 bp: PUC origin
9
10
11

Supplementary data 14: pCR4-Pv.00443#1 μ H-P2A-MCS vector map and sequence



>pCR4-Pv.00443#1 μ H-P2A-MCS

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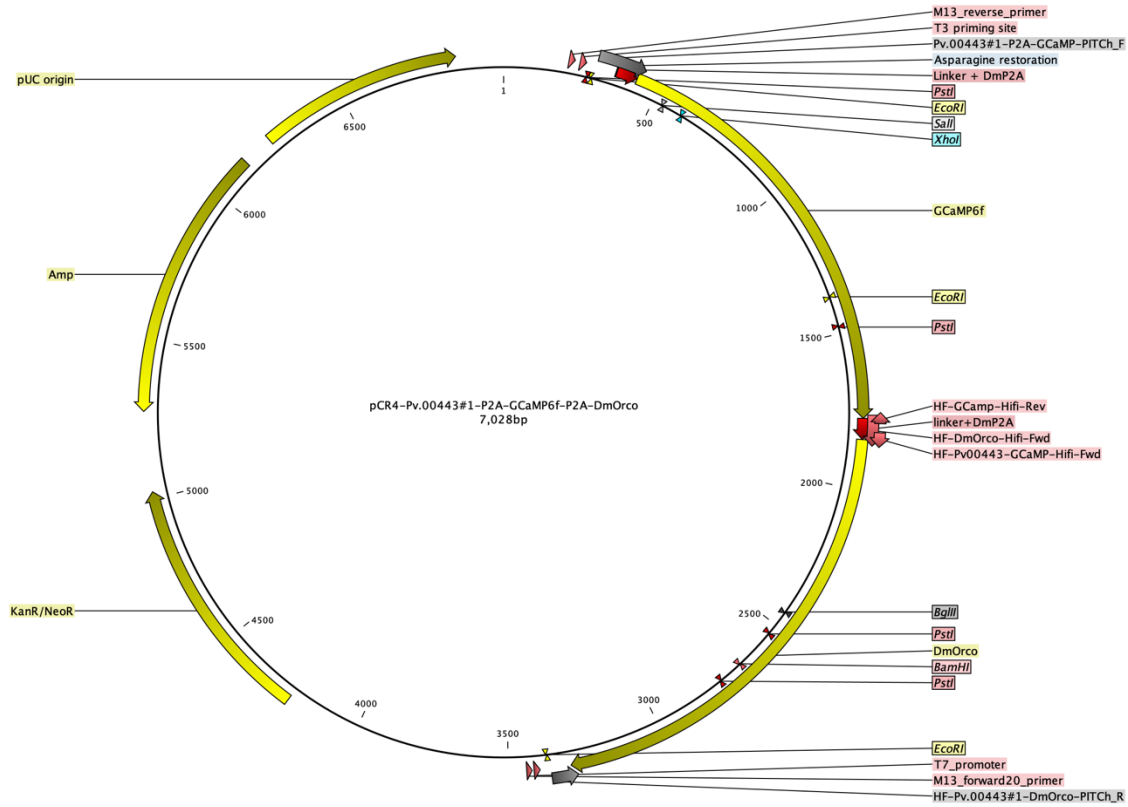
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1 274..296 bp: Pv.00443-gRNA#1
2 297..336 bp: Left microhomology sequence for Pv.00443
3 337..339 bp: Asparagine restoration site
4 340..405 bp: Linker + DmP2A sequence
5 462..501 bp: Right microhomology sequence for Pv.00443
6 502..524 bp (complement): Pv.00443-gRNA#1
7 1385..2173 bp: Kanamycin/Neomycin resistance ORF
8 2426..3286 bp (complement): Ampicillin resistance ORF
9 3387..4030 bp: pUC origin
10
11

Supplementary data 15: pCR4-Pv.00443#1 μ H-P2A-GCaMP6f-P2A-Orco vector map and sequence



> pCR4-Pv.00443#1 μ H-P2A-GCaMP6f-P2A-Orco

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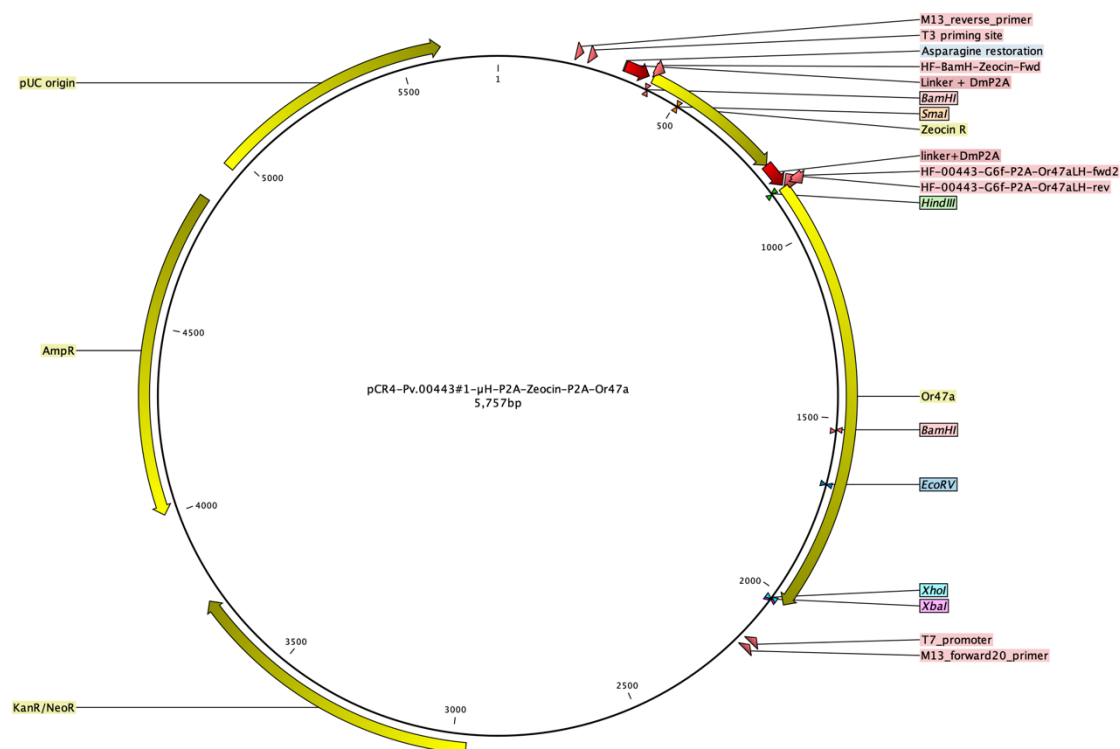
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32
33

34 **295..317 bp: Pv.00443-gRNA#1**
 35 **318..357 bp: Left microhomology sequence for Pv.00443**
 36 **358..360 bp: Asparagine restoration site**

1 361..426 bp: Linker + DmP2A sequence
2 327..1776 bp: GCaMP6f ORF
3 1777..1842 bp: Linker + DmP2A sequence
4 1843..3303 bp: DmOrco ORF
5 3304..3343 bp: Right microhomology sequence for Pv.00443
6 3344..3366 bp (complement): Pv.00443-gRNA#1
7 4234..5022 bp: Kanamycin/Neomycin resistance ORF
8 5275..6135 bp (complement): Ampicillin resistance ORF
9 6236..6879 bp: pUC origin
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11
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Supplementary data 16: pCR4-Pv.00443#1 μ H-P2A- Zeocin^R-P2A-Or47a vector map and sequence



> pCR4-Pv.00443#1 μ H-P2A- Zeocin^R-P2A-Or47a

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15 GTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTCGGGTCTCCGTTATCGGGGAAGAAGTGGCTGATC
16 TCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCATGAGATTATCAAAAAGGAT
17 CTTACCTAGATCCTTTTACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCTATCTGGACA
18 AGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGACGTGGGCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAA
19 GCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGCCGCCAAGGAT
20 CTGATGGCGCAGGGGATCAAGCTCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCT
21 CCGGCCGCTTGGGTGGAGAGGCTATTGCGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAG
22 CGCAGGGGCGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCT
23 GGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGG
24 CAGGATCTCTGTCTCTCACCTTGCTCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGG
25 CTACCTGCCCATTTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCT
26 GGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTTCGCCAGGCTCAAGGCGAGCATGCCGACGGCGAGGATCTCGTCGTGACC
27 CATGGCGATGCCTGCTTGCCGAATATCATGGTGGAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGACC
28 GCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGCGGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTAT
29 CGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAATTATTAACGCTTACAATTTCTGATCGCG
30 TATTTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTA
31 TTTTCTAAATACATTCAAATATGTATCCGCTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATAAAAATGAAGTT
32 TTAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCT
33 ATTTGTTTCATCCATAGTTGCCTGACTCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATG
34 ATACCGGAGACCCACGCTACCGGCTCCAGATTTATCAGCAATAAACCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAA
35 CTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGC
36 CATTGCTACAGGCATCGTGGTGTCAGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCACACGATCAAGGCGAGTTACATGA

1 TCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAAGTAAGTTGGCCGAGTGTTATCACTCATGG
 2 TTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG
 3 AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTC
 4 ATCATTGGAACAGTCTCTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCA
 5 ACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGC
 6 GACACGGAAATGTTGAATACTCATACTCTTCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGACCAAAATCCCT
 7 TAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCT
 8 GCTGCTTGCAAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGG
 9 CTTGAGCAGAGCGCAGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACA
 10 TACCTCGCTCTGCTAATCTGTATTACAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATAGTTAC
 11 CGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT
 12 ACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAG
 13 CGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGT
 14 GATGCTCGTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA
 15 CATGTTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACG
 16 ACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAG

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19 **274..296 bp: Pv.00443-gRNA#1**
 20 **297..336 bp: Left microhomology sequence for Pv.00443**
 21 **337..339 bp: Asparagine restoration site**
 22 **340..405 bp: Linker + DmP2A sequence**
 23 **418..789 bp: Zeocin resistance ORF**
 24 **790..855 bp: Linker + DmP2A sequence**
 25 **862..2019 bp: DmOr47a ORF**
 26 **2040..2079 bp: Right microhomology sequence for Pv.00443**
 27 **2080..2102 bp (complement): Pv.00443-gRNA#1**
 28 **2963..3751 bp: Kanamycin/Neomycin resistance ORF**
 29 **4004..4864 bp (complement): Ampicillin resistance ORF**
 30 **4965..5608 bp: pUC origin**

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