

advances.sciencemag.org/cgi/content/full/6/21/eaay9320/DC

# Supplementary Materials for

# Characterization of imine reductases in reductive amination for the exploration of structure-activity relationships

Sarah L. Montgomery, Ahir Pushpanath, Rachel S. Heath, James R. Marshall, Ulrike Klemstein, James L. Galman, David Woodlock, Serena Bisagni, Christopher J. Taylor, J. Mangas-Sanchez, J. I. Ramsden, Beatriz Dominguez, Nicholas J. Turner\*

\*Corresponding author. Email: nicholas.turner@manchester.ac.uk

Published 22 May 2020, *Sci. Adv.* **6**, eaay9320 (2020) DOI: 10.1126/sciadv.aay9320

### This PDF file includes:

Figs. S1 to S6 Tables S1 to S4 Supplementary Materials and Methods



Figure S1. Putative IREDs in phylogenetic context with other families of dehydrogenases. The putative IREDs (green) are displayed alongside a number of previously described IREDs (blue) and characterised dehydrogenase enzymes (black).

# **1** Supplementary figures and tables



**Figure S2. Initial characterisation of putative IREDs.** Soluble expression in *E.coli* (green) and conversions to (*R*)-2 (blue) and (*S*)-2 (red, up to >99%) are shown, with stronger colours and larger bars corresponding to higher expression or conversion. Previously described IREDs are labelled in blue. Conversions were determined by GC-FID with comparison to chemically-synthesized standards.



Figure S3. Isopropylamine as a nucleophile. Soluble expression in *E. coli* (A) and conversion to 3 (up to >99%) with 10 mM cyclohexanone and 100 mM cyclopropylamine at 30°C (B) are shown, with brown bars showing conversion to 8 (up to 49%) with 10 mM cyclohexanone and 100 mM isopropylamine at 37°C.
Previously described IREDs are labelled in blue. Conversions were determined by GC-FID with comparison to chemically-synthesized standards, with darker colors and larger bars corresponding to higher conversions.



**Figure S4. Reductive aminations with benzylamine.** Soluble expression in *E. coli* (**A**) and conversion to **8** (up to 96%) with 10 mM cyclohexanone and 100 mM benzylamine at 30°C (**B**) are shown, with brown bars illustrating conversion to **14** (up to 74%) with methoxyacetone and benzylamine at 30°C.

Table S1. Conversions to 6 with a range of co-solvents. The total volumes of the biotransformations were 500 μL including any co-solvent, and cyclohexanone was added from a 250 mM aqueous stock solution to avoid any addition of DMSO to the transformations. <sup>a</sup>Conversions were determined by GC-FID with comparison to a chemically-synthesized standard.



Entry	Co-solvent	Conversion to Product (%) <sup>a</sup>
1	10% v/v EtOH	9
2	20% v/v EtOH	<1
3	50% v/v EtOH	<1
4	10% v/v cyclohexane	41
5	20% v/v cyclohexane	39
6	50% v/v cyclohexane	18
7	10% v/v DMSO	26
8	20% v/v DMSO	31
9	50% v/v DMSO	2
10	10% v/v MTBE	1
11	20% v/v MTBE	3
12	50% v/v MTBE	<1
13	none	7

 Table S2. Comparison of proposed catalytic residues between IREDs. A sequence alignment allows

 comparison of residues in the AspRedAm active site to analogous residues in SkIRED, p-IR23, p-IR48 and a

 number of novel IREDs. All gave >90% conversion to 3 in the rective amination of cyclohexanone with

 cyclopropylamine.

	93	169	177	210	239	240
AspRedAm	N	D	Y	W	М	Q
SkIRED	S	D	W	G	Т	н
p-IR08	Ν	D	Y	W	М	Q
p-IR09	Ν	D	Y	W	Μ	Q
p-IR13	Ν	D	Y	М	М	Q
p-IR14	Т	К	А	А	Н	F
p-IR16	Ν	D	Y	W	Μ	Q
p-IR19	Т	К	А	V	L	G
p-IR23	S	D	W	Т	А	н
p-IR28	Т	D	D	W	М	Q
p-IR48	S	D	W	F	А	н
p-IR83	Т	D	Y	W	М	Q

Table S3. Extended active sites of enzymes with varying conversions to 11. (A) Comparison of theoretical extended active site residues in AspRedAm to the analogous residues in the novel IREDs p-IR07, p-IR08, p-IR13 and p-IR28. (B) Comparison of theoretical extended active site residues which vary across the novel IREDs p-IR07, p-IR08, p-IR13 and p-IR28.

(A)	13	17	65	91	93	118	119	121	122	124	125	131	132	169
AspRedAm	М	L	С	L	N	Т	М	V	Р	М	I	V	L	D
p-IR07	М	L	С	L	Ν	1	М	V	Р	М	1	F	1	D
p-IR08	М	L	С	L	Ν	1	Μ	V	Р	М	1	F	1	D
p-IR13	М	L	С	L	Ν	L.	Μ	V	Р	М	1	F	1	D
p-IR28	М	L	С	L	Т	I	М	V	Р	М	I	F	I.	D

	170	172	173	176	177	179	180	183	210	214	217	218	221	232
AspRedAm	L	L	L	М	Y	L	F	F	W	М	Y	L	I	Т
p-IR07	- I	L	L	М	Y	Y	F	V	W	М	F	L	S	Т
p-IR08	I.	L	L	М	Y	Y	F	V	W	М	F	L	S	Т
p-IR13	1	L	L	М	Y	Ν	F	V	W	М	F	L	Т	R
p-IR28	I	L	L	L	D	I	L	Y	W	М	L	F	L	А

	235	236	237	239	240	244	246	247	261	264	268	280	281
AspRedAm	S	Ν	L	М	Q	V	Ν	I	I	I	I	D	L
p-IR07	S	Ν	I.	М	Q	Y	Ν	L	L	L	М	D	L
p-IR08	S	Ν	I.	М	Q	F	Ν	L	L	L	М	D	L
p-IR13	S	Ν	I.	М	Q	F	Ν	L	L	L	М	D	L
p-IR28	S	Ν	L	М	Q	М	Q	М	I.	М	1	D	S

(B)	93	176	177	179	180	183	217	218	221
p-IR07	Ν	М	Y	Y	F	V	F	L	S
p-IR08	Ν	М	Y	Y	F	V	F	L	S
p-IR13	Ν	М	Y	Ν	F	V	F	L	Т
p-IR28	Т	L	D	I	L	Y	L	F	L
	222	237	211	246	2/7	261	264	268	281

	232	237	244	246	247	261	264	268	281
p-IR07	Т	I	Y	Ν	L	L	L	М	L
p-IR08	Т	I.	F	Ν	L	L	L	Μ	L
p-IR13	R	I.	F	Ν	L	L	L	Μ	L
p-IR28	А	L	М	Q	М	I	М	I	S



**Figure S5. IRED quaternary structures. (A)** Crystal structure of AspRedAm with NADP<sup>+</sup> bound (PDB code: 5G6R) and **(B)** homology model of p-IR14 from *A. thaliana*, based on β-HADAt, PDB code: 3DOJ.



Figure S6. ArpDHII in phylogenetic context. Relationship of the ArpDHII enzyme (red) to previously described IREDs (blue), including several of those described in this work (green). Other dehydrogenase enzymes are shown in black.

**Table S4. Reaction conditions and conversions obtained with ArpDHII.** Conversions were determined by GC-FID with comparison to chemically-synthesized standards, and are compared to conversions obtained with purified AspRedAm (2). <sup>a</sup>Aldehyde and ketone substrates were diluted from 1 M stock solutions in DMSO.

 $H_2 N^{-R^3}$ D-glucose (25 mM)  $NADP^+$  (0.5 mM) CDX-901 GDH (0.5 g L<sup>-1</sup>) purified ArpDHII (1 g L<sup>-1</sup>) R<sup>1</sup> R<sup>2</sup> 0 || R<sup>2</sup>

5 mM<sup>a</sup> Tris-HCl buffer (100 mM, pH 8.0) 30°C, 18 hours

Entry	Carbonyl Substrate	Amine Nucleophile	[Amine Nucleophile] / mM	Enzyme	Product (%)
1	cyclohexanone	propargylamine	5	AspRedAm	94
2				ArpDHII	18
3		cyclopropylamine	5	AspRedAm	90
4				ArpDHII	93
5		allylamine	5	AspRedAm	73
6				ArpDHII	9
7		methylamine	10	AspRedAm	95
8				ArpDHII	15
9		benzylamine	5	AspRedAm	84
10				ArpDHII	2
11		isopropylamine	100	AspRedAm	94
12				ArpDHII	<1
13	hydrocinnamaldehyde	propargylamine	20	AspRedAm	97
14				ArpDHII	97
15		cyclopropylamine	20	AspRedAm	>97
16				ArpDHII	>97
17		allylamine	20	AspRedAm	>97
18				ArpDHII	89
19	1-indanone	propargylamine	250	AspRedAm	64
20				ArpDHII	<1

# **2** Supplementary materials and methods

### 2.1 General

Solvents used were of HPLC grade, and when necessary were further dried over molecular sieves. Analytical thin-layer chromatography (TLC) was performed using commercially available precoated plates (Macherey-Nagel Polygram SIL G/UV254) with visualisation by UV light (at 254 nm) and ninhydrin dip. Column chromatography was performed on silica gel (Fluka, Buchs, Switzerland, 220 - 440 mesh). In cases where a large number of biotransformations were run in parallel, workup was performed with the aid of a Hamilton Microlab STAR liquid handling system (Hamilton, Bonaduz, Switzerland), and where necessary, samples were derivatized using acetic anhydride with an excess of triethylamine at 25°C prior to analysis.

Spectra from <sup>1</sup>H and <sup>13</sup>C NMR runs were recorded on a Bruker Avance 400 instrument (400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C) in CDCl<sub>3</sub> or CD<sub>3</sub>OD using residual protic solvent as an internal standard. Reported chemical shifts ( $\delta$ ) in parts per million (ppm) are relative to the residual protic solvent signal (CHCl<sub>3</sub> in CDCl<sub>3</sub>: <sup>1</sup>H = 7.26; CDCl<sub>3</sub>, <sup>13</sup>C = 77.0; CH<sub>3</sub>OH in CD<sub>3</sub>OD: <sup>1</sup>H = 3.31, <sup>13</sup>C = 49.0). Coupling constants (J) are quoted in Hz and are rounded to the nearest 0.1 Hz. <sup>1</sup>H multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), or broad resonance (br), or a combination of these. High-resolution mass spectrometry (HRMS) was recorded using a Waters LCT time-of-flight mass spectrometer, connected to a Waters Alliance LC (Waters, Milford, MA, USA). Data were processed with Waters Masslynx software. GC analysis was performed on either an Agilent 7890B gas chromatograph with a flame ionization detector (FID) and autosampler equipped with a 30 m Phenomenex ZB WAXplus column with 0.25 mm inner diameter and 0.25 µm film thickness or an Agilent 6850 gas chromatograph with a flame ionization detector (FID) and autosampler equipped with a 25 m CP-Chirasil-DEX CB column with 0.25 mm inner diameter and 0.25 µm film thickness (Agilent, Santa Clara, CA, USA). GC-MS analyses were performed on an Agilent 7890B gas chromatograph coupled to an Agilent 5977B mass spectrometer with an electron ionization source (EI) operating at 70 eV and equipped with a 30 m HP-1 ms fused silica capillary column with 0.25 mm inner diameter and 0.25 µm film thickness.

## 2.2 IRED cloning



Plasmid map for cloned IREDs. The IRED gene cloned is into a pET-28b (+) vector between *Nde*l and *Xho*l restriction sites.

Enzyme	Organism	UniProt ID	Identity with AspRedAm
AspRedAm	Aspergillus oryzae	REDAM_ASPOR	100.0%
p-IR03 <sup>a</sup>	Aspergillus bombycis	A0A1F7ZK02_9EURO	84.4%
p-IR04	Aspergillus calidoustus	A0A0U5GEY4_9EURO	62.4%
p-IR09	Aspergillus lentulus	A0A0S7DXU4_9EURO	57.3%
p-IR12	Aspergillus nidulans	Q5AUX5_EMENI	57.5%
p-IR16	Aspergillus udagawae	A0A0K8L8S9_9EURO	58.3%
p-IR32	Emmonsia parva	A0A0H1B7G6_9EURO	43.3%
p-IR33	Emmonsia pasteuriana	A0A1J9QMV6_9EURO	50.0%
p-IR35	Exophiala aquamarina	A0A072NZT1_9EURO	30.7%
p-IR36	Fonsecaea multimorphosa	A0A178C791_9EURO	26.8%
p-IR37	Fusarium oxysporum	W9Z6K1_FUSOX	35.4%
p-IR47	Microdochium bolleyi	A0A136IJF0_9PEZI	27.6%
p-IR50	Madurella mycetomatis	A0A175WBH2_9PEZI	29.7%
p-IR58	Nectria haematococca	C7Z324_NECH7	32.8%
p-IR61	Penicillium arizonense	A0A1F5L0A3_9EURO	54.6%
p-IR62	Pochonia chlamydosporia	A0A179G8F8_METCM	31.7%
p-IR63 <sup>a</sup>	Penicillium camemberti	A0A0G4PFF8_PENCA	34.3%
p-IR64	Pseudogymnoascus sp. VKM F-4516	A0A094D824_9PEZI	35.5%
p-IR65	Purpureocillium lilacinum	A0A179GW01_9HYPO	39.2%
p-IR71	Rhinocladiella mackenziei	A0A0D2H0B2_9EURO	26.5%
p-IR78	Stachybotrys chartarum	A0A084RCS9_STACH	36.3%
p-IR88	Torrubiella hemipterigena	A0A0A1TF30_9HYPO	31.4%
p-IR89	Myceliophthora thermophila	G2QG78_MYCTT	31.3%
p-IR90	Myceliophthora thermophila	N/A	31.3%

**IREDs of fungal origin.** <sup>a</sup>p-IR03 and p-IR63 were previously characterized by Zawodny *et al.* (24).

Enzyme	Organism	UniProt ID	Identity with AspRedAm
p-IR-5	Araucaria cunninghamii	A0A0D6R2X8_ARACU	20.8%
p-IR10	Arabis alpina	A0A087GHW2_ARAAL	19.8%
p-IR11	Arabidopsis lyrata	D7MD48_ARALL	20.0%
p-IR14	Arabidopsis thaliana	3HID1_ARATH	18.3%
p-IR18	Brassica rapa subsp. pekinensis	M4DQA1_BRARP	19.1%
p-IR21	Corchorus capsularis	A0A1R3HV54_COCAP	20.0%
p-IR22	Citrus clementina	V4UJW6_9ROSI	19.0%
p-IR25	Citrus sinensis	A0A067FGQ9_CITSI	19.5%
p-IR26	Cucumis sativus	A0A0A0LIQ8_CUCSA	20.3%
p-IR27	Daucus carota subsp. sativus	A0A161WTE2_DAUCA	20.3%
p-IR31	Eucalyptus grandis	A0A059CN57_EUCGR	19.1%
p-IR34	Eutrema salsugineu	V4KLI0_EUTSA	20.6%
p-IR38	Gossypium hisutum	A0A1U8PU55_GOSHI	17.7%
p-IR40	Homo sapiens	GLYR1_HUMAN	10.8%
p-IR43	Jatropha curcas	A0A067KFP5_JATCU	18.7%
p-IR46	Lupinus angustifolius	A0A1J7FZG6_LUPAN	20.0%
p-IR51	Morus notabilis	W9T391_9ROSA	19.4%
p-IR52	Marchantia polymorpha subsp. polymorpha	A0A176VNX2_MARPO	17.4%
p-IR54	Medicago truncatula	G7L7F1_MEDTR	21.1%
p-IR56	Nicotiana attenuata	A0A1J6KVX5_NICAT	17.1%
p-IR57	Noccaea caerulescens	A0A1J3E499_NOCCA	20.9%
p-IR66	Picea sitchensis	A9NZ37_PICSI	18.9%
p-IR87	Theobroma cacao	A0A061GU76_THECC	20.4%

### IREDs of plant and mammalian origin.

IREDs of bacterial origin. <sup>a</sup>p-IR17, p-IR23, p-IR55, p-IR67, p-IR 81 and p-IR84 were previously characterized by Zawodny *et al.* (24). <sup>b</sup>p-IR48 was previously characterized by France *et al.* (8). <sup>c</sup>p-IR49 was previously characterized by Roiban *et al.* (9).

Enzyme	Organism	UniProt ID	Identity with AspRedAm
p-IR01	Allokutzneria albata	A0A1G9V2F1_9PSEU	37.2%
p-IR02	Allokutzneria albata	A0A1G9SYK8_9PSEU	38.9%
p-IR06	Actinoalloteichus hymeniacidonis	A0A1D8BXU6_9PSEU	40.3%
p-IR07	Amycolatopsis japonica	A0A075UU84_9PSEU	43.3%
p-IR08	Amycolatopsis keratiniphila	A0A1H2GBD9_9PSEU	41.4%
p-IR13	Amycolatopsis regifaucium	A0A154MR77_9PSEU	42.0%
p-IR15	Amycolatopsis thermoflava	WP_027931120.1	30.2%
p-IR17 <sup>a</sup>	Burkholderia lata	Q39NX6_BURL3	27.6%
p-IR19	Burkholderia vietnamiensis	A4JAJ9_BURVG	20.1%
p-IR20	Couchioplanes caeruleus	A0A1K0GAQ1_9ACTN	38.9%
p-IR23 <sup>a</sup>	Cystobacter ferrugineus	A0A1L9AVJ5_9DELT	29.0%
p-IR24	Chelatococcus sambhunathii	A0A0K6H4A1_9RHIZ	24.8%
p-IR28	Devosia geojensis	A0A0F5FDU7_9RHIZ	42.1%
p-IR29	Draconibacterium orientale	X5E3Y1_9BACT	17.2%
p-IR30	Desulfosporosinus orientis	G7W7U0_DESOD	21.9%
p-IR39	Granulicella mallensis	G8P071_GRAMM	21.2%
p-IR41	Isosphaera pallida	E8QYX7_ISOPI	19.0%
p-IR42	Jiangella alkaliphila	A0A1H2IPN0_9ACTN	37.6%
p-IR44	Kitasatospora cheerisanensis KCTC 2395	A0A066Z502_9ACTN	27.7%
p-IR45	Kibdelosporangium phytohabitans	A0A0N9HVM7_9PSEU	42.7%
p-IR48 <sup>b</sup>	Microvirga lupini	WP_051455530.1	31.9%
p-IR49 <sup>c</sup>	Mycobacterium magaritense	X5L9E3_9MYCO	30.5%
p-IR53	Minicystis rosea	A0A1L6L1L9_9DELT	27.9%
p-IR55 <sup>a</sup>	Micromonospora tulbaghiae	A0A1C4WQ45_9ACTN	37.5%
p-IR59	Pseudomonas aeruginosa	Q9I1R8_PSEAE	19.9%
p-IR60	Pseudomonas aeruginosa	A0A1F0IHK4_9PSED	19.3%
p-IR67 <sup>a</sup>	Paenibacillus tyrfis	A0A081P2I1_9BACL	40.7%
p-IR68	Rhizobium gallicum	A0A0B4XDG7_9RHIZ	25.6%
p-IR69	Rivularia sp. PCC 7116	K9RL01_9CYAN	17.2%
p-IR70	Rhodococcus jostii	Q0S7N9_RHOJR	19.5%
p-IR72	Rhodobacter sphaeroides	Q3IXE7_RHOS4	21.5%
p-IR73	Streptomyces albus	A0A0B5EJP8_STRA4	39.1%
p-IR74	Streptomyces albus	A0A0B5EWU4_STRA4	30.9%
p-IR75	Streptomyces avermitilis	N/A	38.1%
p-IR76	Streptomyces canus	N/A	41.6%
p-IR77	Streptomyces canus	N/A	41.6%
p-IR79	Streptomyces chattanoogensis	A0A0N0XYF8_9ACTN	40.8%
p-IR80	Synechococcus elongatus	A0A0H3K567_SYNP6	20.7%
p-IR81 <sup>a</sup>	Stackebrandtia nassauensis	D3Q3S0_STANL	39.5%
p-IR82	Streptomyces noursei ATCC 11455	A0A1B2GW12_STRNR	38.6%
p-IR83	Streptomyces purpeofuscus	WP_030392279.1	38.5%
p-IR84 <sup>a</sup>	Streptomyces resistomycificus	A0A0L8KUS9_9ACTN	38.9%
p-IR85	Streptomyces rimosus	N/A	38.3%
p-IR86	Thermobifida fusca	Q47QG6_THEFY	21.8%

### 2.3 Protein sequences

### 2.3.1 Glucose dehydrogenase sequence

> B. subtilis GDH (Bacillus subtilis, DHG\_BACSU) (9) MGSSHHHHHHSSGLVPRGSHMYPDLKGKVVAITGAASGLGKAMAIRFGKEQAKVVINYYS NKQDPNEVKEEVIKAGGEAVVVQGDVTKEEDVKNIVQTAIKEFGTLDIMINNAGLENPVP SHEMPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINMSSVHEVIPWPLFVHYAA SKGGIKLMTETLALEYAPKGIRVNNIGPGAINTPINAEKFADPKQKADVESMIPMGYIGE PEEIAAVAAWLASKEASYVTGITLFADGGMTQYPSFQAGRG\*

### 2.3.2 Characterized and putative imine reductase sequences

>AspRedAm (Aspergillus oryzae, REDAM\_ASPOR) (2) MGSSHHHHHHSSGLVPRGSHMSKHIGIFGLGAMGTALAAKYLEHGYKTSVWNRTTAKAIP LVEQGAKLASTISEGVNANDLIIICLLNNQVVEDALRDALQTLPSKTIVNLTNGTPNQAR KLADFVTSHGARYIHGGIMAVPTMIGSPHAVLLYSGESLELFQSIESHLSLLGMSKYLGT DAGSASLHDLALLSGMYGLFSGFLHAVALIKSGQDTSTTATGLLPLLTPWLSAMTGYLSS IAKQIDDGDYATQGSNLGMQLAGVENIIRAGEEQRVSSQMILPIKALIEQAVGEGHGGED LSALIEYFKVGKNVD\*

>AtRedAm (Aspergillus terreus, QOCCT3\_ASPTN) (2) MGSSHHHHHHSSGLVPRGSHMATTTTTKLTIFGLGAMGTAMATQFLKQGHTPTVWNRTA AKANPLVEQGAHLAATIPAAIAASPLLIFCLLDNAAVEQTLAAGPPSLAGKTILNLTNGT PSQARRLATLASARGARYFHGGIMATPDMIGAPHAVILYSGGGSAETYASVEGVLAVLGS GKYLGDDAGSASLHDLALLSGMYGLFAGFLHATALVRSEGEGVSATEFLGLLAPWLQAMT GYLGLLARQIDDGVYTAQTSNLEMQLVALENACAASREQGVSAEVMLPLKGLVERAVREG RGGHDISSLIDYFRNASV\*

>SkIRED (Streptomyces kanamyceticus, Q1EQE0\_STRKN) (30) MGSSHHHHHHSSGLVPRGSHMPDNPSTKGRMMRNQQAEHTPVTVIGLGLMGQALAGAFLG AGHPTTVWNRTAAKAEPLVARGAKSAGSVAEAVAASPLVVVCVSDYDAVHALLDPLDGTA LQGRTLVNLTSGTSAQARERAAWADGRGADYLDGAILAGPAAIGTADAVVLLSGPRSAFD PHASALGGLGAGTTYLGADHGLASLYDAAGLVMMWSILNGFLQGAALLGTAGVDATTFAP FITQGIGTVADWLPGYARQIDDGAYPADDAAIDTHLATMEHLIHESEFLGVNAELPRFIK ALADRAVADGHGGSGYPALIEQFRTHSGK\*

>R-IRED (Streptomyces sp. 3587, M4ZRJ3\_9ACTN) (12) MGSSHHHHHHSSGLVPRGSHMGDNRTPVTVIGLGLMGQALAAAFLEAGHTTTVWNRSAGK AEQLVSQGAVQAATPADAVAASELVVVCLSTYDNMHDVIGSLGESLRGKVIVNLTSGSSD QGRETAAWAEKQGVEYLDGAIMITPPGIGTETAVLFYAGTQSVFEKYEPALKLLGGGTTY LGTDHGMPALYDVSLLGLMWGTLNSFLHGVAVVETAGVGAQQFLPWAHMWLEAIKMFTAD YAAQIDAGDGKFPANDATLETHLAALKHLVHESEALGIDAELPKYSEALMERVISQGHAK NSYAAVLKAFRKPSE\*

>S-IRED (*Streptomyces* sp. 3546, M4ZS15\_9ACTN) (*13*) MGSSHHHHHHSSGLVPRGSHMSKQSVTVIGLGPMGQAMVNTFLDNGHEVTVWNRTASKAE ALVARGAVLAPTVEDALSANELIVLSLTDYDAVYAILEPVTGSLSGKVIANLSSDTPDKA REAAKWAAKHGAKHLTGGVQVPPPLIGKPESSTYYSGPKDVFDAHEDTLKVLTNADYRGE DAGLAAMYYQAQMTIFWTTMLSYYQTLALGQANGVSAKELLPYATMMTSMMPHFLELYAQ HVDSADYPGDVDRLAMGAASVDHVLHTHQDAGVSTVLPAAVAEIFKAGMEKGFAENSFSS LIEVLKKPAV\*

>ArpDHII (Streptomyces argillaceus, A0A1M4NDA0\_STRAA) (26)

MGSSHHHHHHSSGLVPRGSHMSNTNQGPVTVLGLGMMGAALATAFVKNGNPTTVWNRSAD KADALVEQGAVFAADIKQAIEASPVVVACVSTYEVLNDLFAGAADELKGKVVINLTSGTP EDARALAVWAEQNGVRYLDGAIMAVPQMIGLPQALIFYAGAQEVYAEHEELLKPLAGTNV YLGADTGVAMIYDLGLLSLLWSSLAGYFHAVALVNSAGVSAEAFTPFALTWIEHVITPSI PQSAKEIDSASFDTEISSLGVNKAAIEHLVATSKQLGINSDFSAAIQALIERRVEQGHAG HSLASLVEAFKQG\*

>p-IR01 (*Allokutzneria albata*, A0A1G9V2F1\_9PSEU) MGSSHHHHHHSSGLVPRGSHMSTKKVTVIGLGSLGSALAAALLRTGHDVTVWNRTPEKAE ALVAQGATRADTVAEAAAASPVVIVCVFDTEAARELLAPIQAGKAVVNLTSGSPDEAREL AAWAASRGVDYLDGAVMAVPAAIGTPDAFVMYSGSREVFDAHRAALDSFGASHFLGEDAG VAEFHDLGLLYAGYATLVGFLNSVAIVGTAGVTARELLPLVTTWLTGMVAYLADVAREVD ERDYTDGASSVAINQVALDKIIAASRAAGVSPDLLLPFKELLDRRAADGHARDSASSVIE LLRPGIHPE\*

>p-IR02 (*Allokutzneria albata*, A0A1G9SYK8\_9PSEU) MGSSHHHHHHSSGLVPRGSHMTDTSAKLTLLGLGAMGSALATAWLAADYDITVWNRTASR AEPLRTLGAQVADTAADAVAANDLVVACLLDDASVRSTLDGVDLTGKDLINLTTGTPGSG RELAACATARGARYVDGGIMAVPPMIGVPDSGAYVFYSGSAAAFEAHRDALAVGAGTKFV GEDPGFAALYDVALLSAMTGMFAGVSHAFALVRKENIDPREFAGLVSGWITAMSGYAHGI AEHLASGDYTTGVTSNLAMMVEGNATMLRTAHEQGVSPELLEPFMRLMRQRVDDGHGDED TTGVIDLLLSRR\*

>p-IR03, AbIRED (*Aspergillus bombycis*, A0A1F7ZK02\_9EURO) (24) MGSSHHHHHHSSGLVPRGSHMSKHISIFGLGAMGAALAARYLEHGYKTTVWNRTTAKATP LVEQGAKLASTISEGINASDLIIICLLNNEAAEDILRDVFHSLSSKTIVNLTNGTPYQAR NLADFVTSNGARYIHGGIMAVPAMIGSPHAVLLYSGGSFELFQSIESHLSLLGMSKFLGT DAGSASLHDLALLSGMYGLFSGFLHAVALIKSEQSKTATGLLPLLTPWLSAMMGYLSSIA SQIDEGDYATQGSNLEMQLVGVENILKAGKEQGISSQMILPMKALIEKAVGEGHGGEDLS VLIDYFKGEKSLE\*

>p-IR04 (*Aspergillus calidoustus*, A0A0U5GEY4\_9EURO) MGSSHHHHHHSSGLVPRGSHMSTITLFGLGAMGKALAAKYIEKGYTTTIWNRTPSKAAPL VEKGAKLANTVGEGLASADLIILCLLDNASVRQTLDQATAALNGKTVINLTNGTPSQARE TSEWVISHGAQYIHGGIMAVPDMIGSPHAVLLYSGESAETFSRVEAHLSHLGTSKFLGTD PGSASLHDLALLSGMYGLFSGFFHATALVKSQPGTTATGFVQLLTPWLSAMTHYLGALAK QIDEGDYATQGSNMAMQVTGVQNIVRASEEAGVTADLIMPILGRMTRAAEAGYADVDVSA VIEFMKE\*

>p-IR05 (*Araucaria cunninghamii*, A0A0D6R2X8\_ARACU) MGSSHHHHHHSSGLVPRGSHMADKPRVGWIGTGVMGQAMCGHILKDGYPVRVFNRTPSKA QTLIENGALMAENPKSVAEGSDVVFTIVGYPHDVSEVILGDKGVLQGLKPGGVIVDMTTS EPSLAQKIYKFAKERGCESVDAPVSGGDKGAKGGTLAIFAGGDEKVVETLKPLFDCMGKV TYMGGPGLGQCTKLGNQITIASTMVGLVEGMVFAHKAGLDVETYIRAISSGAAGSKSLEL YANRILKGDFEPGFFVNHFVKDLGISLRECQQMGLSLPGLALAQQLYVSLKAHGEGNLGT QALVLALERLNNVQLPRMQ\*

>p-IR06 (Actinoalloteichus hymeniacidonis, AOA1D8BXU6\_9PSEU) MGSSHHHHHHSSGLVPRGSHMPESTTPSTATPVTIIGLGAMGTALANAFLDAGHSTTVWN RTAARATALAARGAHHAETVTEAIAASPLVIACVLDYDAFHETLAPATDALAGRALVNLT NGTPKQARETASWAADHRIDYLDGGIMAIPPGIATPDSFILYSGPLGTFEAHRSTLEVLG AANHVGTDHGLASLHDLALLTGMYGMFAGILQAFALIDSEGIPAGDLAPMLTNWLTGMAH SVAHYAQQIDTGDYETGVVSNLAMQSAGFANLVQAGEDQGVDVGLLRPLFELMRHQVAAG YGNGDVASVIELIRREERRQPAKSPGADKITRARRP\*

>p-IR07 (*Amycolatopsis japonica*, A0A075UU84\_9PSEU) MGSSHHHHHHSSGLVPRGSHMSEHGKTPVTVLGLGAMGTALVEALLAAGHPVTAWNRTAS WAEAVAAQGASVASTISEALAANTIVIACLLDYDSVHEVLDPVAAELEGRQLINLTNGTP GQAREMSAWAEGLGAAYLDGGIMAVPPMIGTPAAFIFYSGSGTVFGHARTALDTFGGVNY LGADPGLAPLHDIALLSGMYGYFVGVIQAYALVGSAGVKAREFAPLLRGWLDAMGGFLER SAEQIDDGDYVTDVVSNIGMQAAAYPNLAQAAEDQGISAELLTPLQSLMDKRVARGHGAE DLIGVIELLKK\*

>p-IR08 (*Amycolatopsis keratiniphila*, A0A1H2GBD9\_9PSEU) MGSSHHHHHHSSGLVPRGSHMSEHGKTPVTVLGLGAMGTALVEALLTAGHPVTVWNRTAS RTEAVAAKGASVASTVSEALAADTNAIVLACLLDYDSVHEVLDPVAAELAGRQLINLTNG TPGQAREMNAWAEELGAAYLDGGIMAVPPMIGTPAAFIFYSGSGTVFGRARTALETFGGV NYLGADPGLAPLHDIALLSGMYGYFVGVIQAYALVGSAGVKAREFAPLLRGWLDAMGGFL ERSAEQIDDGDYMTDVVSNIGMQAAAFPNLAKAAADQGISAELLTPLQPLMDKRVADGHG AEDLIGVIELLKK\*

>p-IR09 (Aspergillus lentulus, A0A0S7DXU4\_9EURO) MGSSHHHHHHSSGLVPRGSHMSSVSIFGLGAMGTALASRFLEEKYKVAVWNRSPEKASPL LEKGATLSHTALDGINASDLIVICLLDNAAVQATLNSALEHLRGKTIINLTNGTPDQARK LSDLIVSHGAQYVHGGIMATPSMIGSPHALVLYSGSPDAFKTAEADLSVLAKCIFLGEDA GSASLHDLALLSGMYGLFSGFLHATALVRSSTPAVKFVDLLVPWLGAMTEYTKGMAKQID EGNYASEGSNLGMQLVAIQNIIDASAAQQVSADFIRPMKEFMEKAVVAGHGGDDISSLID FVKST\*

>p-IR10 (*Arabis alpina*, A0A087GHW2\_ARAAL) MGSSHHHHHHSSGLVPRGSHMPLLLRRYLSPSVVSSSFLRRSMAYSSISTDPINPSYSSI STDPINPSNTKIGWIGTGVMGRSMCGHLIRAGYSVTVFNRTISKAQTLLDMGAKLADSPS SVASQSDVVFSIVGYPSDVRNVLLDPNSGALSGLRKGGVLVDMTTSEPSLAEEIAKSASS VHCFSIDAPVSGGDLGAKNGKLSIFTGGDETTVKRLDPLFSLMGKVNFMGTSGKGQFAKL GNQITIASTMLGLVEGIIYAHKSGLDVKKFLEAISTGAAGSKSIDLYGDRIMKRDFDPGF YVNHFVKDLGICLNECQRMGLALPGLALAQQLYLSLKAHGQGDLGTQALILALERLNNVK IDFY\*

>p-IR11 (*Arabidopsis lyrata*, D7MD48\_ARALL) MGSSHHHHHHSSGLVPRGSHMPLLLRRFLSPSVVSSFFLRRSMASSSISSDPITPSNTKI GWIGTGVMGRSMCGHLIKAGYTVTVFNRTISKAQTLIDMGANLADSPNSVASQSDVVFTI VGYPSDVRHVLLDPKSGALSGLQQGGVLVDMTTSEPSLAEEIAKAASFENCFSIDAPVSG GDLGAKNGKLSIFAGGDETTVKRLDPLFSLMGKVNFMGTSGKGQFAKLANQITIASTMLG LVEGLTYAHKAGLDVKKFLEAISTGAARSKSIDLYGDRILKRDFDPGFYVNHFVKDLGIC LNECQRMGLALPGLALAQQLYLSLKAHGEGGLGTQALILALERLNNVSVQPSVS\*

>p-IR12 (Aspergillus nidulans, Q5AUX5\_EMENI) MGSSHHHHHHSSGLVPRGSHMSKISLFGLGSMGQALAHRYIDTGYTTTVWNRTPEKAQSS GLIQKGAHQALTVAEGLEAADMVILCLLDNASVRETLSKAVTSLSGKTIVNLTNGTPTQA RDLSEWAGAHGAEYIHGGIMAVPDMISSGSPHSILLYSGKSNEVFTRIEPDLAHLGAAKF LGTDPGSASLHDLALLSGMYGLFSGFFHATALVRSQSDTTSEGFLELLIPWLGAMTHYLG GLAKQIDSGDYTTQGSNIAMQVAGLENIIAASEEVGVTPAFILPIQRLMKRAADEGHGNT DISSLIQYLRTRE\*

>p-IR13 (*Amycolatopsis regifaucium*, A0A154MR77\_9PSEU) MGSSHHHHHHSSGLVPRGSHMTEHGKTPVTVLGLGAMGTALVEALLAAGHPVTAWNRTAS RAEGVAAKGASVASTVSEALAANKTVIACLLDYDSVHEVLDPVASGLEGRQLINLTNGTP GQAREMSAWAEELGAEYLDGGIMAVPPMIGTPGAFIFYSGSGTVFGQARTALDTFGGVNY LGADPGLAPLHDIALLSGMYGNFIGVIQAFALVGSAGVKAREFAPLLRGWMDAMSGFLER TAELIDDGDYERGVVSNIGMQAAAFPNLAKAAEEQGISAELLAPLQPLMDKRVAAGHGAE DLVGVIELLKK\*

>p-IR14 (Arabidopsis thaliana, 3HID1\_ARATH) MGSSHHHHHHSSGLVPRGSHMPLLLRRFPSPSVVSSFFLRRSMASSTISSDIITPSNTKI GWIGTGVMGRSMCGHLIKAGYTVTVFNRTISKAQTLIDMGANVADSPNSVAEQSDVVFTI VGYPSDVRHVLLDPKSGALSGLRQGGVLVDMTTSEPSLAEEIAKAASFKNCFSIDAPVSG GDLGAKNGKLSIFAGGDETTVKRLDPLFSLMGKVNFMGTSGKGQFAKLANQITIASTMLG LVEGLIYAHKAGLDVKKFLEAISTGAAGSKSIDLYGDRILKRDFDPGFYVNHFVKDLGIC LNECQRMGLALPGLALAQQLYLSLKAHGEGDLGTQALLLALERLNNVSVQSSDS\*

>p-IR15 (*Amycolatopsis thermoflava*, WP\_027931120.1) MGSSHHHHHHSSGLVPRGSHMTSATVIGLGPMGQAMVRVLLEHGTAVTVWNRTKSRADDV VARGAVLAGTPAEALKAAGLVILSLTDYQAMYDVLGDAGEALAGRVVVNLSSDTPQRTRE AAAWLAKRGATLVAGGIMVPAPLVGAEASYVFYSGPRDVFAEHEPVLRHIGRPEYLGEDH GLAQLFYQAELTVFLTSLSAYLQAFALLAAEGADPARLVPFAREVSGLAASYLDETVSQT RARAYPGDLSTATMMGATAEHILQACRDAGVDLALPEAVKSQYDRAIAAGHGGDNWTSLW EVVAKR\*

>p-IR16 (*Aspergillus udagawae*, A0A0K8L8S9\_9EURO) MGSSHHHHHHSSGLVPRGSHMSSVSIFGLGAMGKALASRFLAEKYKVAVWNRSPEKASPL LEKGATLSHTAVDGINASDLIIICLLDNAAVQATLDSALDRLHGKTIVNLTNGTPDQARK LSDLIVSHGAQYVHGGIMATPSMIGSPHALVLYSGSPDAFNAAEADLSVLANCVFLGEDA GSASLHDLALLSGMYGLFSGFLHATALVKSSTPAVKFLDLLVPWLGAMTEYTKGMAKQID EGQYASEGSNLAMQLVAVENIIDASAAQQVSADFIRPMKEFMEKAVAAGHGGDDISSLID FVKST\*

>p-IR17, BIIRED (*Burkholderia lata*, Q39NX6\_BURL3) (*24*) MGSSHHHHHHSSGLVPRGSHMANTVTAQAISVIGLGAMGAALAEALLAADHRVTVWNRTA SKSAALGEVGAQVAHSAAEAIDASQVVVVCVLDYGASDSLLRTPDVAARLKGKTIIQLTT GTPEDAREASEWAREHDVAYLDGTIGCYPKDIGTSDGSILYAGSRSTFEALRPTLANLSG HALFVGERFGNAAILDGAVVGSFSLGAALGFLYGAAVCDAEGISLDTYLSLALARRPFVE DTLQTCVQMIKKGNYSGSQATLDSWAAGIGQLVAYSQESGTDSSYPQEVLARLQQAVAKG HGQHELAAVFECFRKPLANRG\*

>p-IR18 (*Brassica rapa*, M4DQA1\_BRARP) MGSSHHHHHHSSGLVPRGSHMPLLLRRPLSPSVVSSFFLRRRSMSSKSDPISPSNTRIGW IGTGVMGRSMCGHLIKAGYSVTVFNRTISKAQPLLAMGANLAASPNSLASQSDVVFSIVG YPSDVRRVLLDPTSGALSGLSPGGVLVDMTTSEPSLAEEISKSASSAGCFSVDAPVSGGD LGAKNGKLSIFAGGDEATVKRLDPLFRLMGKVNFMGASGKGQFAKLANQITIASTMVGLV EGIVYAHKAGLDVRKFLEAISTGAAGSKSIDLYGERILKRDFDPGFYVDHFVKDLGICLN ECQRMGLALPGLALAQQLYLSLKAHGEGGLGTQALILALERLNNVSVQPSVS\*

>p-IR19 (*Burkholderia vietnamiensis*, A4JAJ9\_BURVG) MGSSHHHHHSSGLVPRGSHMDLGFIGLGEMGQAIATNLLKAGHTVRVWNRSRERAEPLA ALGAQIVATPADAFRGDAVFSMLADDAAARDVFDDALLAQAPRGLIHVNMATVSVALAES LAHAHASRGLDYVAAPVMGRPDVAAAARLTIMAGGPAEAIDRVQPLFDAIGQKTWRFGSL PQHANIAKIAANFTLASAIETLGEASALLGAHGVAMRDFLDVITSSVFPGPVYAGYGGMI

AERSYEPARFKARLGLKDVRLALQAGDAASVPLPVASVVRDSLLDALAHGGGDQDFAVLG EVALRRAGR\*

>p-IR20 (Couchioplanes caeruleus, A0A1K0GAQ1\_9ACTN) MGSSHHHHHHSSGLVPRGSHMAQNSVEKAPVTLLGLGAMGTALARTWLAGGHPLTVWNRT PARAAALSPEGARVADSAAAAVAANTLVVVCLLDDASVEEVLAGTDLADKDLVNLTTSTP AQARARAEWARERGARYLDGGIMAVPPMIGVPEIGGYVFYSGSRELFERHQQTLGVPAGT TYVGQDAGFAALHDVALLSAMYGMFAGAAHAFALIRKEDIDPASLAPLLADWLVAMAPTV HQTADQLRSGDYTKGVVSSLAMQVAGTPTFLDTAAQQGVSPELLSPYFTLMRRRLAEGSA EEDLTGVIDLLVRK\*

>p-IR21 (Corchorus capsularis, A0A1R3HV54\_COCAP) MGSSHHHHHHSSGLVPRGSHMETPYPTPISPTQTRIGWIGIGVMGAAMASHLLSAGYSVT IYARNPSKAASLQSQGAHLANSPQQLARQCDVVFTMVGNPQDVRQTVLESDGILSGLKPG AVIVDHTSSSPSLAREIYASARKKGCWSVDAPVSGGDIGAREGKLAIFAAGVCAVVEWLK PLFDLMGRVTYMGEAGCGQSCKIGNQIMVGANLMGLSEGLVFAEKAGLDLKKYMEAIRGG SAGSMAMELWGRRIIERDFKPGGFAEYMVKDMGMGVDVVKEEDDGKVMVLPGAALGKQMF SAMVANGDGKLGTQGLITVVERINGK\*

>p-IR22 (*Citrus clementina*, V4UJW6\_9ROSI) MGSSHHHHHHSSGLVPRGSHMPPLLPLLLVLRSRTAHSYSLSVSSLVTLLLRRRSMATVA STDPVCPTNTRVGWIGTGVMGRSMCAHLLNAGYTVTVFNRTLSKAQPLLDIGAHLADSPH SLASQSDVVFSIVGYPSDVRHVLLHPSSGALSGLRPGGIIVDMTTSEPSLASELSAAASS KNCSAIDAPVSGGDRGAKTGTLAIFAGGDESVVQKLNPLFALMGKVNYMGGSGKGQFAKL ANQITIASTMVGLVEGMVYAHKAGLNVELFLNAISTGAAGSKSLDLYGSRILKRDFEPGF YVNHFVKDLGICLKECQNMGLALPGVALAQQLYLSLKAHGEGNLGTQALILALERLNNVR LDNAVASKPSA\*

>p-IR23, CfIRED (*Cystobacter ferrugineus*, A0A1L9AVJ5\_9DELT) (*24*) MGSSHHHHHHSSGLVPRGSHMKPGISVLGTGRMGSALVGAFLKQGYNVAVWNRTKSKCAP LAALGARVATTVRDAVADAEVVVVNVNDYVTSEALLRQDDVTKGLRGKLIVQLTSGSPRQ AREMAAWARQHELQYLDGAIMGTPNFIGEPGGTILYSGPGALFEKYKPVLLVLGGNSLHV GSDVGHASALDSALLSFLWGSMFGVLQAVSVCEAEGLPLGAYMEYVQATKPMVDGAVTDF VKRIQTGRFAGDEKTLATVEAHHGALRHLIELCEEHGIHHAVPAAFGQLFQAALQAGHAQ DDFAVLNKFMK\*

>p-IR24 (Chelatococcus sambhunathii, A0A0K6H4A1\_9RHIZ) MGSSHHHHHHSSGLVPRGSHMTKTCVVGAGRMGSAFARALLAEGIETRVWNRSPEKVAPL VAAGAHAPESLAEAVAASDVVIVNVIDYAAADALLRMLAVERALAGKVVVQLTSGSPRQA REAGRWAAERDIAYLDGAIMATPNFIGGAETTILYSGMRQAFERHRDVLRVFGGNGVFVG EDAGHASALDTGLLTQMWGKLFGTLQALAVVRAEGFGLEAYARYMRDFQLVVDAATDDLI ARVGEGRWRGDAATLATIEAHYSAFHHLLAIGDEHGLDRALPAALDGLFKAALAAGHAAD DFAALMRFIERGGVRHAA\*

>p-IR25 (*Citrus sinensis*, A0A067FGQ9\_CITSI) MGSSHHHHHHSSGLVPRGSHMPPLLPLLLVLRSRTAHSYSLSVSSLVTLLLRRRSMATVA STDPVCPTNTRIGWIGTGVMGRSMCAHLLNAGYTVTVFNRTLSKAQPLLDIGAHLADSPH SLASQSDVVFSIVGYPSDVRHVLLHPSSGALSGLRPGGIIVDMTTSEPSLASELSAAASS KNCSAIDAPVSGGDRGAKTGTLAIFAGGDESVVQKLNPLFALMGKVNYMGGSGKGQFAKL ANQITIATTMVGLVEGMVYAHKAGLNVELFLNAISTGAAGSKSLDLHGSRILKRDFEPGF FVNHFVKDLGICLKECQNMGLALPGLALAQQLYLSLKAHGEGNLGTQALILALERLNNVR LDNAVASKPSA\*

>p-IR26 (*Cucumis sativus*, A0A0A0LIQ8\_CUCSA) MGSSHHHHHHSSGLVPRGSHMPIQFSLRSLYTQSHKLTPTLSLPFLSFLRRTMSTSGDIV SPSTTRVGWIGTGVMGCSMCSHIIKAGYKLTVFNRTISKAQPLLDLGANLATSPLAVAAQ SDVVFSIVGYPSDVRSVLLDPSSGALAGLRPGGVLIDMTTSEPSLASEIAASATAKGCGA IDAPVSGGDRGAKNATLAIFAGGDEDEVQRLSPLFSLLGKVNYMGESGKGQFAKLANQIT IASTMVGLVEGMIYAKKAGLDVGLFLNAISTGAAGSKSLDLYGSRILKRDFEPGFFVNHF VKDLGICLKECQNMGLALPGLALAQQLYVSLKAYGEGNLGTQALILSLERLNNVSLEGSG SS\*

>p-IR27 (*Daucus carota*, A0A161WTE2\_DAUCA) MGSSHHHHHHSSGLVPRGSHMSSITTAEPRSPSNTRLGWIGTGVMGLSMCSHLLKAGYTL TVFTRTQSKAQPLLDLGAKWAASPSSVASQSDVVFSIVGYPSDVRHVTLHPNSGALSGLN PGGILVDMTTSDPSLAVEIADAAAAKGCFSVDAPVSGGDRGARNGSLSIFAGGDKKVIDT LSPLFNLLGKVNYMGVSGKGQFAKLGNQITIASTMVGLIEGMMFAHKAGLDLSLFINAIS TGAAGSKSLELYGYRILKRDFEAGFFVNHFVKDLGICLRECQNMGIALPGLALAQQLYVS LQAHGEGDLGTQALVLALERLNNVSLQSVASSTEMA\*

>p-IR28 (*Devosia geojensis*, A0A0F5FDU7\_9RHIZ) MGSSHHHHHHSSGLVPRGSHMTTISILGLGAMGTALANALLDAGHAVTVWNRTPGKDEAL VSRGARRAQTPAEAIAASELVVICLVDYAGVETMLASAEKELSGRVVANLTTGTPKQARA LAAWVEAQGAAYVDGGIMAVPPMIGTPASFIFYSGNQPAFERHKAALEAFGDAVYVGPDA GAAPLNDIALLGILDGILSGYLHAAALIRASGGTAEGFTPLARRWAESMISLFPDLAKQI DSGDYRAEGGSNLAMQAAAMDQMVEAARDEGVDPVLIEPMRALIHQRVADGFGTDDSAGV IELLFLNGRGKR\*

>p-IR29 (*Draconibacterium orientale*, X5E3Y1\_9BACT) MGSSHHHHHHSSGLVPRGSHMKKDITIGWIGTGVMGTSMLGHLNKAGYECITYTRTKSKA ESLLANGVKWADSPAEVAAVSDVIFTIVGFPKDVREVYFGEKGILAKAKPGAVLVDMTTT EPSLAVEIYEAAKVKGIQSVDAPVSGGDVGAKNGTLSIMAGGEKEAFDKVYPLFESMGKQ IVYQGAAGSGQHTKMCNQITIAGTMIGVCEALLYGHKAGLDLPTMLSSISGGAAGCWTLD NLAPRIVNRNFDPGFFVEHFIKDMGIALKEAEAMGLSLPGLALVKQLYLAVQAQGHGKLG THALTLALEKLSGL\*

>p-IR30 (Desulfosporosinus orientis, G7W7U0\_DESOD) MGSSHHHHHHSSGLVPRGSHMKVGFIGIGAMGKPMAQNILRAGYSLYVNDVNEAALQELV AEGAKKAENPRELAREVDVVITMLPNGAVVEQVLLGEQGIFAGAKPGFTVIDMSSVGPTF TQKMAKLASDRQVGYMDAPVSGGVKGAEAGTLTIMVGGEKELVQRYHSLLEVMGKKIYHV GKTGAGDAVKIVNNLLLGINMAAAAEAFVLGTKLGLDPQVLLEIISVSSGSSYALTAKMP NFIFKGQFEAGFAIDLQYKDLELAVQTAKEAKIPMLLTNVAQQVFEQARAAGLGRDDISA VIKPLEDLMKIKVRA\*

>p-IR31 (*Eucalyptus grandis*, A0A059CN57\_EUCGR) MGSSHHHHHHSSGLVPRGSHMPMPSSLPLTGLLRSASPPSSSALYLLLRRAMSSAAAASD PIAPSNTRVGWIGTGVMGRSMCGHLIGAGFAVTVFNRTVSKAQPLLDMGARLADSPRSLA ALSDVVFSIVGYPSDVRSVLLDPSSGALASLRPGGVLVDMTTSDPSLAEEISAAASAKGC HAVDAPVSGGDRGARNAALAIFAGGDPATVRRLEPLFSVMGKVNYMGGPGKGQFAKLGNQ ITIASTMVGLVEGMVYAYKAGLDVGMFVSAISTGAAGSKSLDLYGNRILQRDFEAGFYVN HFVKDLGICLKECERMGLALPGLALAQQLYLSLKAHGEGNLGTQALILALERLNNVKLEN AGSSNV\*

>p-IR32 (*Emmonsia parva*, A0A0H1B7G6\_9EURO) MGSSHHHHHHSSGLVPRGSHMANSPVTVFGLGAMGTALATQFLQKGHKTTVRNRTAAKAQP LIAIGASHAPTIASAAAASSILIICQLDKTSVMQTLQQAPTAWATKTIVDLTNGTPAHARE TADWALAHGAQYIHGGIMAVPSMIGQPHAMILYSGPAEVGMYGLFSGFTHAVALVQSANVP AAGFVATQLIPWLTAMTQHLNLLATQVDEKDYGDGGSSLDMQAKAAPNILEASAAQGVSVE LIQPIFKLIERRVEEGGGSEGLAALVGMIMKAGTKDSV\*

>p-IR33 (*Emmonsia pasteuriana*, A0A1J9QMV6\_9EURO) MGSSHHHHHHSSGLVPRGSHMENSPVTVFGLGAMGTALATQFLRKHHRTTVWNRTAAKAKS LIASGASHAPTISSAASASSLLIICQSNKDSVLQTLQQEPAAWAGKTIVDLTNGTPAHARE TAEWALAHGAQYIHGGIMAVPSMIGQPHAVILYSGPGKVFEEVKETLDAVGSSTYVGEDVG LASLHDLALLSGMYGLFSGFTHAVAMVRSARVPAAGFVATQLIPWLTAMTQHLNMLAKQVD DEDFGDGGSSLEMQAHAVPNVLEASEAQGVSVELIRPIFMLIEKRVGEGGGKEGLSALVHM IMKGA\*

### >p-IR34 (*Eutrema salsugineu*, V4KLI0\_EUTSA) MGSSHHHHHHSSGLVPRGSHMASKSISSVPINPSNTRIGWIGTGVMGRSMCGHLIRAGYSV TVFNRTVSKAQTLIDMGAKLADSPNSLAAQSDVVFSIVGYPSDVRHVLLDPISGALSGLNP GGVLVDMTTSEPSLAEEISKAASSANCFSIDAPVSGGDLGAKNGKLAIFAGGDETTVKRLD PLFSLMGKVNFMGTSGKGQFAKLANQITIASTMLGLVEGMIYAHKSGLDVKKFLKAISTGA AGSKSIDLYGDRILKRDFDPGFYVNHFVKDLGICLKECQRMGLALPGLAIAQQLYLSLKAH GEGDLGTQALILSLERLNNVSVQPIM\*

>p-IR35 (*Exophiala aquamarina*, A0A072NZT1\_9EURO) MGSSHHHHHHSSGLVPRGSHMTSATSGITLVGLGNMGAALAHAFLKSGCLLTVWNRTSTR PTVVEAIKAGASFEPDLAKAISANSTIVFCVLEYKNIYQGLEPLVTSGVLKGKTMVNLTN GTPRDARDADKWFKDHGVASYFDGGIMVTPQNVGTPVSVVLISGEDESLLESRGVKDLIG AIGAPDYKGEDPGVAAGYDLALLAGMIGMFIGSLTALAMIQKQLDRAKSTAGANGAKETA TTGGLTEVVTTYFNPLLTALIPHNELLATAIDNNDQDNNHGNPMEMMRVAMHNIIRGCEE

#### EGVDAENLRHFAGLADQVVHLHGPDSGLAWVHSLLLK\*

>p-IR36 (Fonsecaea multimorphosa, A0A178C791\_9EURO) MGSSHHHHHHSSGLVPRGSHMAISSASQQAAEREVERSVSVLGLGQMGFAVAQAFTKKGY KTTVWNRTAAKARPLVAAGAIVATSVGDCIASNQLVLSCFIETQVLIEVLKTVDPQLCRG RVLVDFASGTLRETRQFQTIARELSFAYIRGSIASTPPYVGSSQIHAWYCGNETIFRSIQ PDLAALAQPGYIDNDPGTIALHECAGGNIFYAFAAGFVQAMAVVKASGKCHPGGAEDFVN KFMIPFLHTFPDTFRDWAHQIDNQNYDAHGKGARLGQSAKSINLMRRFNTELGLTSVILD PVLSLIKHRIAQGGSNEELSSLVETIADSKAGI\*

>p-IR37 (Fusarium oxysporum, W9Z6K1\_FUSOX) MGSSHHHHHHSSGLVPRGSHMTTSQSLTFLGLGNMGSALVQALLKASHKVTIWNRTTARP QVRAAVEAGAILEADVQTVISRNNIIVICLLDYSSINNVLDDIPTPAFKGKTIVNLTNGT PKQAREMANWATSHSAKQYFDGAVMVTPQMIGGPQSFFVVSGETPEAFKPIASFLEPLGR PEYLGTAIDAAARYDLAALSSMYGMFSGMFVAMALLKKGHAIADEKLEPVVSGSLNPFLG ALISYNGLIARSWDDKAWDDNLGNPIGMQAQALGNILEACRDDGMDDGFLKNLTTAMERV VKDRGENGGIAVIGEYLLNGRLSNE\*

>p-IR38 (Gossypium hisutum, AOA1U8PU55\_GOSHI) MGSSHHHHHHSSGLVPRGSHMRSLSYLLGSLSRYFPHSQSSSTPHLTFLLRSMATSAAAG PINPSNTRLGWIGTGVMGRSMCAHLINAGYSLTVFNRTLSKAQPLVDMGARLAQTPHDLA AQSDVVFSIVGYPSDVLQVLLDPVNGALSGLRPGGILVDMTTSEPSLAVEISTAASSKNC SSIDAPVSGGDRGAKNGTLAIFAGGDEAIVNRLNPIFTLMGKVNYMGPSGKGQFAKLANQ ITIASTMVGLVEGIIYAHKAGLNVASFLDAISTGAAGSKSLDLYGSRILKRDFDPGFFVN HFVKDLGICLKECQNMGLALPGLALAQQLYLSLKAHGEGNLGTQALILALERLNNATLGS \*

>p-IR39 (Granulicella mallensis, G8P071\_GRAMM) MGSSHHHHHHSSGLVPRGSHMRVAFLGLGKMGSALVPHLLNAGCEVTVWNRSSPAAKELA SKGATVALSAAEAVNSCEVVFSLLLNDAALEEVLFDGGVLDAMPKDAVHVSVGTISVALA KRLTLEHAKRGHRYVGAPVFGRPNVAAEGKLWTVVGGAKEAVQSVRSLLESYTRGMTVVS EEPWSAHAMKLAGNFSVAAMVATLSEAMMVAESMGVSPELFVEMANSATFRSPLYEVYGK TLTNPPKEVGASMELGEKDVRLFREAAAANGVKTPLAELMGKNFDAAIEAGMKDHDWATG YYELVRKRQVEEPVAL\*

### >p-IR40 (Homo sapiens, GLYR1\_HUMAN) MGSSHHHHHHSSGLVPRGSHMAAVSLRLGDLVWGKLGRYPPWPGKIVNPPKDLKKPRGKK CFFVKFFGTEDHAWIKVEQLKPYHAHKEEMIKINKGKRFQQAVDAVEEFLRRAKGKDQTS SHNSSDDKNRRNSSEERSRPNSGDEKRKLSLSEGKVKKNMGEGKKRVSSGSSERGSKSPL KRAQEQSPRKRGRPPKDEKDLTIPESSTVKGMMAGPMAAFKWQPTASEPVKDADPHFHHF LLSQTEKPAVCYQAITKKLKICEEETGSTSIQAADSTAVNGSITPTDKKIGFLGLGLMGS GIVSNLLKMGHTVTVWNRTAEKCDLFIQEGARLGRTPAEVVSTCDITFACVSDPKAAKDL VLGPSGVLQGIRPGKCYVDMSTVDADTVTELAQVIVSRGGRFLEAPVSGNQQLSNDGMLV ILAAGDRGLYEDCSSCFQAMGKTSFFLGEVGNAAKMMLIVNMVQGSFMATIAEGLTLAQV TGQSQQTLLDILNQGQLASIFLDQKCQNILQGNFKPDFYLKYIQKDLRLAIALGDAVNHP TPMAAAANEVYKRAKALDOSDNDMSAVYRAYIH\*

>p-IR41 (Isosphaera pallida, E8QYX7\_ISOPI) MGSSHHHHHHSSGLVPRGSHMTSSPSLPTVAPGRTRVGWIGTGVMGRWMCGHLMNAGYAA TVYNRTQSKARELIDRGATLATNPREVAEASDVIFTIVGFPSDVRQVILGSDGVLAGAKP GAIVVDMTTSEPELAREIHRVAAARGVHAIDAPVSGGDLGAREARLSIMVGGDPEPVAAV QPLFQIMGKTIVHQGPPGAGQHTKMVNQILIAGNMVGVCEALLYGYKAGLDLTTVLQSVS GGAAGSWSLSNLAPRILKGDMTPGFYVEHFIKDMGIALAEARRMKLALPGLALVEQLYQA LAAQGHARDGTQALTHALARLSGFEWKAVVTTTQGQS\* >p-IR42 (Jiangella alkaliphila, AOA1H2IPNO\_9ACTN) MGSSHHHHHHSSGLVPRGSHMTDHHPVTVIGLGAMGREIARVLLEAGHPTTVWNRNLERA VDLVSQGATRADTVVAAVGASPLVLVCVLDYAATRELLEPIAADLAGRTVVQLTTGTPKQ ARELAEWAAGHGIDYVDSGMMATPPMIGRPGSTFLYSGDEAAFERYRPALDLLGTSRWFG ADAGLASLYDLALLSGMYTMFAGFLHGAAMVGTAGVSAADFAESAGDWLRAMTVSLPYAA TFVDSGDYTTDVQDVEFNKSALDVIVQASIDQGVAPDLVAPIHELLARQLAEGHGKEAFD RIIESIRPAVTRPA\*

>p-IR43 (Jatropha curcas, A0A067KFP5\_JATCU) MGSSHHHHHHSSGLVPRGSHMILSLLRSRSHDFNTSRIHFTLPLSLFFRRSMATTTESAS PSNTRVGWIGTGVMGRSMCAHLIKAGYSLTVFTRTLSKAQPLVDMGANLAQSPLAVASQS DVVFSIVGFPSDVRQVLLDSTTGALQGLRPGGILVDMTTSDPSLASEISAAASAKNCHSI DAPVSGGDRGAKNGTLAIFAGGDEAVIDRLNPLFALMGKLNYMGAPGKGQFAKLANQITI ACTMVGLVEGIIYAYKAGLNVEHYLNAISTGAAGSKSLESYGARILKRDFEAGFYVNHFV KDLGICLKECQNMGLALPGLALAQQLYLSLKAHGEGNLGTQALILALERLNNVSLENLAS LQPAA\*

>p-IR44 (*Kitasatospora cheerisanensis* KCTC 2395, A0A066Z502\_9ACTN) MGSSHHHHHHSSGLVPRGSHMSRLPSAVSEYSTRGGTSSYETRCTMPSDSNSRRCEASTF SVTPGSSRRSSPNRRHGCSCSAHRISTFHLPPITSIAASIPQTCPSGVPGLFSVAMPPPL RPAGSPRGKHPPESAYLITTGPWTSLIGMAHTTVDPTAKTPVTLLGLGAMGTALARTWLA AGHPVTVWNRTPSRATALAADGASVAATAAEAVAANTLVVVCLLDDASIDQALAGTDLTG RDLVNLTTSTPEQARARAAWAHERGARYLDGGIMAVPPMIGVPQAGGYVFYSGSRELFDR HRPTLAVPVDTRWVGEDPGHAALHDVALLSAMYGMFAGAAHAFALIRKEDIDPTALAPLL ADWLTAMAPAVHQTADQLRTGDYTTNVVSNLAMQVAGTPTFLATAEQQGVSPELLTPYFD LMRRRLAEGNGNEDLTGVVDLLLR\*

>p-IR45 (*Kibdelosporangium phytohabitans*, A0A0N9HVM7\_9PSEU) MGSSHHHHHHSSGLVPRGSHMPDLSKPAVTVLGLGDMGSALARALLKAGYPTTVWNRTPE KAKPLVEAGATHAPTVADAVTASRLVIVCLLDYKSVTDVLDTARDALTGKVLVNLTNGTP GQARELATRYDVDYLDGGIMAIPPMIGAPPAFVFYSGSRSAFDTNKQVFEAFGGTNYVST DAGFAALYDIGLLSGMYGMFVGALHALSLVTSAGVPAQEFAPLLKQYVSAMLGSIDGMAE QIDKQDYAIDVVSNIGMQSEAYVNLTLSAEELGMSPELLAPLGPLMARRVADGHGHEDIA GVVEILKKGKK\*

>p-IR46 (Lupinus angustifolius, A0A1J7FZG6\_LUPAN) MGSSHHHHHHSSGLVPRGSHMASSTAAEPISPSNTRLGWIGTGVMGQSMCAHLIRAGYTL TVFNRTPSKAQPLLDLGAHQAHSPQALASQSDVVFSIVGYPSDVRSVLLDPTSGVLSGLL PGGILVDMTTSEPSLAVEIAAAAAEKNCHSVDAPVSGGDRGAKNGTLAIFAGGDKDTVHR LEPLFSLLGKVNYMGGSGKGQFAKLANQITIASTMVGLVEGMVYAHKAGLDVGLWLDAIS TGAAGSKSLDLYGQRILKRDLDPGFYVNHFVKDLGICLKECQNMGIALPGLALAQQLYVS LKAHGEGNLGTQALILVLERLNNVSLAPAASSS\*

>p-IR47 (*Microdochium bolleyi*, A0A136IJF0\_9PEZI) MGSSHHHHHHSSGLVPRGSHMRPCVGNMGTAAIQALRKASHSVTIWNRTKSRPQVQAAID AGATFEPDLAQALSRSGVIVLCLLDYATITKIFSSIGEPGAVLKGKSVINLTNGTPKEAR EMETYFTSLGTIVYLDGAIMVTPQMVGGPHAFLFLSGGQGSTETPDGATSRLVELVSPIG KPDYLGADVGAASRYDLASLAPMYGMFAGGFLGMALLKRGKPNAKLVPVVQEKIVPMIAA LVPYMTGIARAWDAEDWVENDGNPVGMMAVGVQNILAAAADEGVDGTVLEEFGGLMEKVE RDHGYDAGIAAVGTYLMK\*

>p-IR48, IR70 (*Microvirga lupini*, WP\_051455530.1) (8) MGSSHHHHHHSSGLVPRGSHMKSKVCVLGAGRMGSSIARCLVSHGYQTWVWNRTAAKCEP LAALGATVALSVEDGIRAADVVIINVLDYAASSASLRQDSVISGLAGKTVVQLTSGSPRL AREEALWVESHGASYLDGAIMATPDFLGRPEATLLYSGPREVFEVHKGLLSTLGGGTSYV GENPGQASALDTALLTQMWGGLFGALQGMAVVEGENLNLETFRHQLTAFKPVVDAALFDL IDRTIARRFAGDETTLASLGAHYSAFQHLLEACEEQGLDASLPNAMDRFFRQALSLGGPE SDFASLAPLFRANHTVAPFAELANA\* >p-IR49, IR-22 (*Mycobacterium mageritense*, X5L9E3\_9MYCO) (*9*) MGSSHHHHHHSSGLVPRGSHMKSKVCVLGAGRMGSSIARCLVSHGYQTWVWNRTAAKCEP LAALGATVALSVEDGIRAADVVIINVLDYAASSASLRQDSVISGLAGKTVVQLTSGSPRL AREEALWVESHGASYLDGAIMATPDFLGRPEATLLYSGPREVFEVHKGLLSTLGGGTSYV GENPGQASALDTALLTQMWGGLFGALQGMAVVEGENLNLETFRHQLTAFKPVVDAALFDL IDRTIARRFAGDETTLASLGAHYSAFQHLLEACEEQGLDASLPNAMDRFFRQALSLGGPE SDFASLAPLFRANHTVAPFAELANA\*

>p-IR50 (*Madurella mycetomatis*, A0A175WBH2\_9PEZI) MGSSHHHHHHSSGLVPRGSHMATITSIGIGNMGAALATALLKSSSPPMNVTIWNRTASRP QVQSLISAGAIFEPSLAAALASSEVILLCLLDYPAISSVFSQVDASAKPLAGKTILNLTN GTPKQARDMEAFFKSLGAAVYFDGGVMVTPQLVGTPAAFVVLSGETEQAYNERLANAGLL SPVGAVLYIAPDPGAASLVDCAALAAMYGMFIGAFTGIGLLKRQKHERDGEAAGAKAMVD KVMVPVLTALVPYVGLLAEQVDKEAWMDDLGNPLAMQAEGVRNIMQSCEDEGVDGTGLKF LSKLMEKGVKEGFGPGGVAVVAKYLMK\*

>p-IR51 (*Morus notabilis*, W9T391\_9ROSA) MGSSHHHHHHSSGLVPRGSHMPLSLPLRSLSSHHSSFNISSLSLRLLTHLHRSMATAAAA EPTICPSNTRVGWIGTGVMGRSMCAHLINAGYTLTVFNRTLSKAQPLLDMGAHLAHSPLA VASQSDVVFSIVGFPSDVRSVLLDPSSGTLAGLRPGGVLVDMTTSEPSLAAEISSAAASK GCSSIDAPVSGGDRGAKNGTLAIFAGGEESVIHRLNPLFSLLGKVNYMGGPGRGQFAKLA NQITIASTMVGLVEGMIYAHKAGLDVAMFLDAISTGAAGSKSLDLYGARILKRDFEPGFF VNHFVKDLGICLKECQNLGLGLPGLALAQQLYLSLKAHGEGNLGTQALILALERLNNVSL ESGGCSSSPAPAN\*

>p-IR52 (*Marchantia polymorpha*, A0A176VNX2\_MARPO) MGSSHHHHHHSSGLVPRGSHMMGGTPACPTAQLVLRVFNHGRDNGHLSQSSLITLYQVAQ SPTAPLLACTPIPAIVDSRCVGLNFCTVSMAQSEAVKEIVSDQTRVGWIGTGVMGQAMCG HILAAGFQVSVYNRTASKAQDLCSKGATMADSPLSVAQQSDVVFTIVGYPSDVREVMLGE KGILKGLRPGGIVVDMTTSQPSLARELSLAAKERSCESIDAPVSGGDKGAKSGTLAIMVG GEKNTFELLAPLFKCMGNATYMGPAGSGQSCKLANQVTIASTMIGLVEGMIYAQKAGLNV DTFLQAISGGAAGSKSLELYSGRIRNRDFDPGFYVNHFVKDLGIALEECRNMDLALPGLA LAQQLYVSLKAHGEGALGTQALILALERLNNIKQ\*

### >p-IR53 (*Minicystis rosea*, A0A1L6L1L9\_9DELT) MGSSHHHHHHSSGLVPRGSHMGAALARAYINAGHRVTVWNRSPSKAEPFQGQAKVAETAA QACSESDLTIVSVSNYQASDEVLRTPLAEQAAKGRTIVQLTSGTPGDARSGAAWAQEHGI EYLDGCILAYPSYIGGEQTTIFYSGPKALYDRHEATLRVIGGGTSHVGEPIGAAATLDCA LLESYYGATLAFLHGAAICRSEKFPLDAYFAGVQAIMPLISITADMCKRMLATGDFKGTD CTLDIHAGAIQHIVRLSRENNVDRRIPELILSYFDRALKLGHGSDEMAAVINAIQEPPRG \*

>p-IR54 (*Medicago truncatula*, G7L7F1\_MEDTR) MGSSHHHHHHSSGLVPRGSHMPLPFLRFRSLYRHSHTHLLTRHFMATSEPITPSNTRLGW IGTGVMGQSMCAHLIRSGYTLTVFNRTPSKAQPLLDIGATLATSPHAVASQSDVVFSIVG YPSDVRSVLLDPNTGALAGLKSGGILVDMTTSDPSLAVEIADAASVKSCHSIDAPVSGGD RGAKNGTLAIFAGGDESIVKKLSPLFSSLGKVNYMGTSGKGQFVKLANQITIASTMVGLV EGMVYAHKAGLDVGLYLDAISTGAAGSKSLELYGKRILKRDFEAGFYVNHFVKDLGICLK ECEKMGIALPGLALAQQLYVSLKAHGEGNLGTQALILVLERLNNVSLPPSVI\*

>p-IR55, MtuIRED (*Micromonospora tulbaghiae*, A0A1C4WQ45\_9ACTN) (*24*) MGSSHHHHHHSSGLVPRGSHMAPNTVDKTPVTLLGLGAMGTALARTWIAAGHPLTVWNRT PARAAAISDEGAGLADSAAAAVAANTLVVVCLLDDDSVGEVLAGTDLTGKDLVNLTTGTP AEARSRAEWARERGARYLDGGIMAVPPMIGLPEAGGYVFYSGSRELFERHRETLGVPAGT TYVGRDAGFAALHDVALLSAMYGMFAGAAHAFALIRKEDIDPASLAPLLADWLVTMAPAV HQTADQLRGGDYTKGVVSSLAMQVAGTPTFLSTAEQQGVSPELLSPYFELMRRRLAQGGG EEDLTGVIDLLVRGPDHVRTSARTARTSWR\*

>p-IR56 (Nicotiana attenuata, A0A1J6KVX5\_NICAT)

MGSSHHHHHHSSGLVPRGSHMRFMRLLRLLPNHQFHCYVPQAIFTTSAGTTTHLHHTMET ATTEAINPSNTRLGWIGTGVMGRSMCAHLINAGYTLTVFTRTPSKAQELISMGAHWVDSP KAVASQSDVVFSIVGYPSDVRHVILDSNSGALSGLRPGGVIIDMTTSEPSLAVEIYSAAS AAGCSAVDAPVSGGDLGARNAALSIFAGGDESIILRLNPLFNLLGRVYYMGAPGKGQFTK LGNQVVIASTMVGLCEGLIYAHKAGLDLNMFLNAISTGAAGSKSLDLYGSRILSRDFEPG FYVNHFVKDLGICLRECQNMGLALPGLALAQQLYLSLKAHGEGNFGTQALILALERINNL SLASSSSVGKP\*

>p-IR57 (*Noccaea caerulescens*, A0A1J3E499\_NOCCA) MGSSHHHHHHSSGLVPRGSHMPLLLRRSFSPSVVSSFFRRFSMASKSISSDPINPSNTRI GWIGTGVMGRSMCGHLIRAGYSVTVFNRTISKAQTLVDMGAKLAESPSSLAAQSDVVFSI VGYPSDVRHVLLDPNSGALSGLNPGGVLVDMTTSEPSLAEEISEAASSGNCFSIDAPVSG GDLGAKNGKLAIFAGGDETTVKRLDPLFSLMGKVNFMGTSGKGQFAKLANQITIASTMLG LVEGIIYAHKAGLDVRKFLEAISTGAAGSKSIDLYGDRILKRDFDPGFYVNHFVKDLGIC LKECERMGLALPGLALAQQLYLSLKAHGEGDLGTQALILALERLNNVSVS\*

>p-IR58 (Nectria haematococca, C7Z324\_NECH7) MGSSHHHHHHSSGLVPRGSHMATPQALTFLGLGNMGSALVQTLLKASHRVTIWNRTVDRP QVKAAVEAGAILEVDVQTAISRNNIIVICLLDYSSIKTALAGISASALDGKTIVNLTNGT PKQAREMAAWTASHSAKHYFDGAVMVTPQMIGGPQSFFVVSGQTSEAFKPIASLLEPIGR PEYLGTAIDAAARYDLAALSSMFGMFSGMFVAMALLKKGHTKTDEKLEPVVSGSLNPFLG ALIPYNGLLARSWDDKAWDDNLGNPIGMQVQALRNILEACRDDGMDDGFLKNLTTVMEGV VKDRGENGGIAVIGEYLLNGRLTKE\*

>p-IR59 (*Pseudomonas aeruginosa*, Q9I1R8\_PSEAE) MGSSHHHHHHSSGLVPRGSHMREHNDESFEFEVSVIGLGAMGTIMAQALLGQGRRVAIWN RSPGKAAALVAAGAHLCESAEAALAASPATIFVLLDNQATHEVLGMPGVMQALANRTIVD YTTNARDEGLALQSLVNRAGGHYVKGMIVAYPRNVGRRESHSIHTGDPEAFERHRALLEG LAGHTTFLPWDEALAFATVLHAHAFAAMVAFFEAVGAGVHFGLPPSKTARLMLDTSRFFV ADALEEAVRRLEAQDFAGDQARLDVHAQAFAHIAGALHAQGAWTPVFDALCQAVQRAAAM GYGDQDIVAVTRLFARECDTASAAGQ\*

>p-IR60 (*Pseudomonas aeruginosa*, A0A1F0IHK4\_9PSED) MGSSHHHHHHSSGLVPRGSHMSASLPSLAFAGLGLMGVPMCRRLLAAGYPLAVWNRSPGK RELLAAEGAKAVEVPAELAADAEILMLCLADTAAVREVVFGAGGIVENARPGQLLVDFSS AEPAATREMAAELEARCGVRWVDAPVSGGTPGAESGSLAIMAGGRAADIERLRPVLSRLG QRLTRMGEVGAGQVTKVCNQMIVACNALVIAEVVALAERAGVDASLVAPALAGGFADSKP LQILAPQMAESRYEPVKWHVRTLLKDLDTAVKLSREQGAATPMSGLAAQLMRLHGSQGYL ERDPATLVEQYRTAVE\*

>p-IR61 (*Penicillium arizonense*, A0A1F5L0A3\_9EURO) MGSSHHHHHHSSGLVPRGSHMSSVSILGLGAMGTALAARFLEKKYKVAVWNRSPEKASPL LDKGANIAQTAVDGINASDLIIICLLDNAAVQTTLAGALDQLQGKTIVNLTNGTPDQARK MSDLIVGHGARYVHGGIMATPSMIGSPHALVLYSGSQQAFKASEPDMLMLAKCVFVSEDA GAASLHDLALLSGMYGLFSGFLHATALVRSSTPAVKFMDLLVPWLGAMTEYTKRMAKQID EGNYASEGSNIAMQLVAIQNIIDTSAAQKVSADFIRPMKQFMEKAVAAGHSGDDISALIY FAKPS\*

>p-IR62 (Pochonia chlamydosporia, A0A179G8F8\_METCM) MGSSHHHHHHSSGLVPRGSHMSSEQTVSVLGLGLMGSALAKALVQNGWKTTVWNRSPEKA QPLVELGASASTTAADCVRASRVIITCVMDPAALQGILKTLTQEDCKGRILVDYTSGVPS EMRKCEDQALALSLKYFHGAILTMPSLIGMSTSTLLHSGADEITFESINPVLKAFGQSIY LGQDASWATLQEGILGCCFYGFAGGFVQAMALLKSSSFYVPGGAQTLMSQGILPLLTEQF PHIFADLARQIDEQAYDSEGNGIRLDTLKSSLEQLIRVNSEQGLVSNAFDPMLSLLKARI AQGGAAEEMSGLVEAISNPPKQT\*

>p-IR63, PcIRED (*Penicillium camemberti*, A0A0G4PFF8\_PENCA) (24) MGSSHHHHHHSSGLVPRGSHMSTSEPKSISILGLGQMGHAIASNFVSQGFKTFVWNRTPS KAADLVEQGAIQSPSSTECIRSSPLSILCVNTDDIVMDILAAAGDIPGHTIVNIVNGSPQ QVRKTAEKSISQYMAAGYLHGSVMASPGLVRSGGAMTIFAGSSETFKKWELTLQPLGVTL WLLDDVGAAPLYDCSLLSILSGIFSGFMQALAMIGAAGHSETEFARGFVVPLLGQMEEWL VRTAEEVQNKDYVAEKNGSPIAVQLDSTKHIFETAKELGVSSRLLQGFLDVVKEGVQRGQ GREEISGLVRLLREPK\*

>p-IR64 (*Pseudogymnoascus* sp. VKM F-4516, A0A094D824\_9PEZI) MGSSHHHHHHSSGLVPRGSHMTTTTEISIIGLGNMGTALAHTFLKANKTLTIWNRTPTAP SITALISAGATFVPSLPTALSASPLIILCILDYPAIDSLFATLPPGILKDKIIINLTNGT PRQARHLATWMQDNGVANYLDGGIMATPDMIGTPASSLFISGADEPAFASVKEEIAILGA PRYMGTDPGAAALYDLALLAGMYGMFAGSLTAMALMRRLGGEETLEARVEGLLNPWMAAL IPYQAAMAGSVDRGSFEALGNPVGMQREALKNILRACEEEGVDGGCLGYFEGLVGRVVED KGGDGGLAGVEGLLGSKE\*

>p-IR65 (*Purpureocillium lilacinum*, A0A179GW01\_9HYPO) MGSSHHHHHHSSGLVPRGSHMTTNTSLSGQDVSVIGLGNMGVAIANCLLRAGATVTVWNR TASKAEQLIAQGALLALSPSACISASPITIICLLSNATAEQALSDVQDLSRTIINLTNG SPGQARQMATLLQSQKGARYIHGAIMVPPLLLGQPTSVTLCSGPSDVFHACTSVLSALGT PRHVGEDISQASLLDNALLSLMGGIFEGWVQALAIVQRGGVDEVEFATGLAGPFVKAMAD WLPRIAESVRDEQYVGGSPLRMQLEALDNIAVTGEELGVGVLLGELRGVMERAVTLGKGE EGIAGLVPLLTGKK\*

>p-IR66 (*Picea sitchensis*, A9NZ37\_PICSI) MGSSHHHHHHSSGLVPRGSHMAAEKPRVGWIGTGVMGQAMCGHILSAGYSVTVFNRTLSK AQTLCDRGAIMVDSPKSVAAESDIVFTIVGYPQDVREVIIGENGVLQGLKPGGTVVDMTT SEPSLAREIIDYAKQRGCESVDAPVSGGDKGAKAGALAIFAGGEETVVEGLKPLFDCMGK VTYMGGPGLGQCTKLGNQITIASTMVGLVEGMVYAHKAGLDVEMYIKAISAGAAGSRSLD LYADRILRRDFDPGFFVNHFVKDLGISLRECQQMGLSLPGLALAQQLYVSLKAHGEGNLG TQALVLALERLNNVQLPKIG\*

>p-IR67, PtyIRED (*Paenibacillus tyrfis*, A0A081P2I1\_9BACL) (*24*) MGSSHHHHHHSSGLVPRGSHMNSSPNEKENHENAGADSRTPVTVIGLGMMGTALAEAFLK AGHRTTVWNRSADKADALVAKGAIRAATAAEAVSASPLIVVCVLDYEAAHEILGSVGGLL TGRTLVNLTNGKPEQARKAAKWAAEQGADYLDGGIMAIPQMIAGPGALLLYSGSPGAFET YRGELDVLGASQYLGEDAGLAALYDLALLSAMYGMFGGFYHAVALVGTEKVEASAFTALV IPWLQAMMASLPLQAQAMDANVHATDVSSLYINKIGFVNLIKASQEQGISTELVAPLQAL VDRAVAEGYGADGLSRLAGLLKKPQ\*

>p-IR68 (*Rhizobium gallicum*, A0A0B4XDG7\_9RHIZ) MGSSHHHHHHSSGLVPRGSHMTSQILEFNSMTTIGFFGAGRMGASLVRTLAKSGHEVHVW NRTAAKAEALAPFGVQPRPTPEAAAAEAEIVFVNLLDYAASDAQLRKPEVTQALKGKLLV QLTSGSPKAARDTGAWATGHGIAYLDGAIMATPNVIGEPDTLILFAGSKSLYQKHERVFV ALGGKSAFLSEDFGAASALDSALLGQMWGTLFGTLQALAINRAENIDADAYSTYLKLVQP VIDGAERDLMQRVHDGRDRGDDETFATIAAHNVALQHLRHINAERGLNPVLADAFDSLFT TAIQNGHVGDDFAILARFMRAP\*

>p-IR69 (*Rivularia* sp. PCC 7116, K9RL01\_9CYAN) MGSSHHHHHHSSGLVPRGSHMQQIYCIHRQIKLSNMNKQKVTVLGLGAMGSRMGVNLLKA GYSVTVWNRSPKPTEALAAKGAVVATTPKLAVKEADVVISMVTDSDASRAVWLDSETGAL SAMRQSAIAIESSTLTVSWVKELATEFKHSGIAFLDAPVVGTRPQADSGNLIYLIGGEIE TLKQAENIFLSAGGGKINHAGEIGKGMAMKLAVNAMFGIQVAAISEIIGMLIKNGFGLEK AVQYLAELPVTSPAAKNAANLILKGNHAAMFPIDLVEKDFRYVMQTAKDVEAASPISEAI HRVYLDAVDKGYGGDNITGVAKLFV\*

>p-IR70 (*Rhodococcus jostii*, Q0S7N9\_RHOJR) MGSSHHHHHHSSGLVPRGSHMTTTEQVSVGYIGLGNMGAPMAKRLLDRPGGLIVCDTRPEA LEPFGKAGARVTSSPREVAENADVISVTVLNDEQVRAVVTQILDTARPGTVVAVHSTISDV TAVELADRCRAHGVDLIDAPVSGGAPGALEGKLAVMVGASDDAFEKVREPFGHWAELVVHA GDVGAGTRMKLARNLLHFISFTAAAEAQRLAEAAGLDITELGKVVRHTDAITGGAGAIMLR DTTDALDETDNWYPIMTHVRDLGEKDLSLAIGLGERLDVALPLARLALTGLGPGLGVDSTV DKKEQP\*

>p-IR71 (*Rhinocladiella mackenziei*, A0A0D2H0B2\_9EURO) MGSSHHHHHHSSGLVPRGSHMSSTTSHITLVGLGNMGTALAHALVKAGRRVTIWNRAGTRP SVSELVQVGAGYGPELPRAIAGSSTIIFCVLDYNSIHQVMDPVVTSQGTDAREMDRWMHKH GAARYFDGGIMVTPQLISTSTSFILCSGENEALFNDSDVIDLIIILGRPHYVASDPGAAAL YDLSLLAGMFGMFAGSMTAMALMQKQIHRDGNRPHSPSGAVKFTLEDRVSTLLNPLLNALV PYTLNIAQSITSGRMDENFGNPMAMVSMAMQNVLRACEEEGVDGGSLKYFADLMKAVVDER GGEVGIGWVSTLLTK\*

>p-IR72 (*Rhodobacter sphaeroides*, Q3IXE7\_RHOS4) MGSSHHHHHHSSGLVPRGSHMSDTVVGIAGTGRMGTAFARRLRETGTPVRVWNRSSDRTG AAVDAGAEAVALEALAEADLVLLSLTDAAAAEAVLAGMGAALAGRIVVEMSTLLPDQAEA LEAQATALGAQFLHCPVGGTVAPALKGQLLGFAGGPAETLEQARPVLERLCRRVEHLGSV GAAARMKLAVNLPLALYWQTLGESLLLLRGAGIPAEQAIGLMAESSGGPAVLKNRAQVVV ETLEGADQRGTFDIAGLAKDLHLALALAEREGAALPLSAAAEERYRAALEAGLGRFDGAS LARRTAEG\*

>p-IR73 (*Streptomyces albus*, A0A0B5EJP8\_STRA4) MGSSHHHHHHSSGLVPRGSHMAQNAVEKTPLTLLGLGAMGTALARAWLAAGHQVTVWNRT PGRAAPLVAEGAVLAESAAAAVAANSLVVLCLLDEASVEEVLAGTEPAGKDLVNLTTGTP AEARARAAWASARDARCLDGGIMAVPPMIGVPASGAYVFYSGSHELFTRHRETLAVPAGT TWTGTDPGHAALHDVALLSAMYGMFAGTAHAFALIRGEDIDPVAFAPLLADWLTAMAPAV HRTAERLKSGDLTGDVVSDLAMQTAGVPTFLGTAEQQGVSPELLSPYFALMRRRLAEGGG GEDLAGLIDLLRRAPGTAA\*

>p-IR74 (*Streptomyces albus*, A0A0B5EWU4\_STRA4) MGSSHHHHHHSSGLVPRGSHMAGTDRTPVTVIGLGLMGQALASAFVAAGHPTTVWNRSAD KAAAVVEQGTVLADTPAEAVAASELLVVCLLDNDAVHAVLDPVAGSLAGRVVANLTSGSS DQARENAAWAEEVGFRYLDAAIMTVPAAVGAPESVVFYSGPREAYDRHSDTLRLLGGGTT HLGEDHALSALYDVSLLGVMWGTLNSFLQGAALLSTAEVKAADFLPLALKWIDTVKLFAT DYAAQIDQKDGAFPANDATMEVHLGAVRHLLHESEAQGVNAELPRFVHALMERTVAKGFG QNSYASMSELFRTPEK\*

#### >p-IR75 (*Streptomyces avermitilis*)

MGSSHHHHHHSSGLVPRGSHMLITPEPVTSLIGMTQNAVEKTPVTMLGLGAMGTALARTW LAAGHRLTVWNRTPARAAALATEGARVADSAAEAVAANTLVIVCLLDDSSVDEVLAGIDL ADTDLVNLTTSTPAQARARAEWANERGARYLDGGIMAVPPMIGVPEAGSYVFCSGSRELF ERHQETLGIPAGTTYVGMDAGFAALHDMALLSAMYGMFAGVAHAFALIHKEDIDPTSLAP MLADWLVAMAPAVHQTADHLRSGDYTKGVVSTLAMQVAGTPTFLSTAEQQGVSPELLSPY FELMRRRLAEGSGEEDLTGVINLLVH\*

#### >p-IR76 (*Streptomyces canus*)

MGSSHHHHHHSSGLVPRGSHMTQNAVEKESVTLLGLGAMGTALARTWLAAGHPLTVWNRT PARAASLAADGAKVADSAAAAVAANALVVVCLLDDASVEDALTGTDLAGKDLVNLTTSTP AQARARAEWARERGARYLDGGIMAVPPMIGVPESGGYIFYSGSPELFQRHRETLVVPAGT VYVGHDAGFAALYDMALLSAMYGMFAGAAHAFALIRKEDIDPASLAPLLADWLVAMAPAV HRTADQLRSGDYTQGVVSNLAMQVAGTPAFVSAAEQQGVSPELLSPYFALMRRRLAEGSG EEDLTGVIDLLVR\*

### >p-IR77 (*Streptomyces canus*)

MGSSHHHHHHSSGLVPRGSHMTQNAVEKESVTLLGLGAMGTALARTWLAAGHPLTVWNRT PARAASLAADGAKVADSAAAAVAANALVVVCLLDDASVEDALAGTDLAGKDLVNLTTSTP AQARARAEWARERGARYLDGGIMAVPPMIGVPESGDYIFYSGSPELFQRHRETLVVPAGT VYVGHDAGFAALYDMALLSAMYGMFAGAAHAFALIRKEDIDPASLAPLLADWLVAMAPAV HRTADQLRSGDYTQGVVSNLAMQVAGTPAFVSAAEQQGVSPELLSPYFALVRRRLAEGSG EEDLTGVIDLLVR\*

>p-IR78 (Stachybotrys chartarum, A0A084RCS9\_STACH) MGSSHHHHHHSSGLVPRGSHMSSQITCIGIGNMGAALAQALLKAETPVTIWNRTVDRPQV KAVIEAGGILEPNIELAIQKSDIIVICVLNYETIFHALAPLKSSTSPLANKKIINLTNGT PRQAREAEEWMNARGASQYFDGAVMVTPSLVDSPHSFLVYSGESEDDFSSVATALKPLGQ ALYVAPDASAASAFDLAALAAMYGMFSGAFIGMSLLKRQFGKTVPGVDKVVLPVLTALVP YVGLIAKSVDEKSWMEDNLGNPLAMQLAGVGNILQACKDEGVHGAGLEFLAAMMERAVGE GAGDGGVAAVGDYYLVD\*

>p-IR79 (Streptomyces chattanoogensis, A0A0N0XYF8\_9ACTN) MGSSHHHHHHSSGLVPRGSHMTDNKVGSSHDTAGTRPLTLLGLGAMGAALGHAWLAAGYP LTVWNRTPGRAEPLAAQGAQVAGTAAEAVAANRLVVVCLLNDDTLGETLDGVDLAGKDLV NLTTSTPGQARVRAEWARERGARFLDGGIMAVPPMIGAPGSGASVFYSGSRALFDAHAEA LAVPAGTAYVGEDPGFAALHDVALLSAMYGMFAGVAHAFALIRKEEIPPKDFAPLLSGWL TAMTSSVHQAAAQLESGDYTKNVVSNLAMQVAGSETLLGTAEEQGVSAELLTPYLALMER RLAEGHGDEDTTGLIDLLVRRTGR\*

>p-IR80 (Synechococcus elongatus, A0A0H3K567\_SYNP6) MGSSHHHHHHSSGLVPRGSHMVGEQETRMRCGLIGTGLLGTAIAERLLTVGQLLTVWNRT AERSQPLVALGATIAPTPAALLADCEVCLLLLSDAEAIAATLLTEESRSQLVGKTIIQMG TISPAESRAIADQIAAAGGQYLEAPVLGSLPEARNGTLIVMVGAEPAVFEQWRSLLCHLS PEPEWIGPIGTAATLKLALNQLIGSLTSAFGGSLALLQRSGLAVEPFMAILRQSALYAPT FDKKLSRLLSHQYDNPNFPTTHLAKDLRLFRETAADLGITTDAVEGVESIVQKAIAQGWG DQDYSALYEAINPDSN\*

>p-IR81, SnIRED (*Stackebrandtia nassauensis*, D3Q3S0\_STANL) (*24*) MGSSHHHHHHSSGLVPRGSHMTKTPVSVIGLGRMGATIAEIYLREGHPTTVWNRTPAKAE PLLAKGARPAATVAEAVAASPVSVIMLADDTVVRASLESATSELDGRTIVNLTTGRPDEA RELGDWLAGHGAEYLDGGVLGVPQTLATPESVIIYSGSTAANQRHGEIVAALGTARYLGA DHGLASLNDMAILSGMYGLFSGYFHSVAMVDSEGFKAAEFTKDLLIPWLRSIVDVLPTLA DEIDSGDYPVNFSNLDVNVAGIENIMRTSRSQGVTDAPLVPLRDALAAQQEKGHGEASFS RAVEELKSSE\*

>p-IR82 (*Streptomyces noursei* ATCC 11455, A0A1B2GW12\_STRNR) MGSSHHHHHHSSGLVPRGSHMSVDDSVGNAAGNAVGQVEGNSAVNAAGNAAGAAARRNVA VLGLGDMGTALAAALLAAGHRVTVWNRTARKADGLVEQGARRAASPAEAIGASDVVIVCI LDYADVAPLLSDDGADAALKGKVVVNVTNGSPAEARELAERVAGLGADYLDGGIMAVPEI IGAPQAVVLYSGAKDAFEAHREVLDAFAGSVYLGADPGLAPLNDLALLSGMYGMFGGFLH AAGLVRSAGGNVTAFTTELLIPWLRAMADSQLPAMAAQVDSGDYGATGSNLAMQVSHDAI GDVSRAQGVSTELFAPLWELMKRRVADGHGAESVGGVVELVHPREARA\*

>p-IR83 (*Streptomyces purpeofuscus*, WP\_030392279.1) MGSSHHHHHHSSGLVPRGSHMTTNDTATRPGSTPSATTAPAPLTLLGLGAMGAALADAWL SAGHPLTVWNRTAARAEPFAAKGAITAATPAAAVAANRLVVVCLLDDESVGEALAGVDLT GKDIVDLVTGTPGTARARAAWAADRGARYLDGGIMAVPPMIGVPQAGPYVFYSGSRELFE EHREVLAVPAGTRYVGEDAGFAALHDVALLSAMYGMFAGVAHAFALVREESVDPVAFAPL LSSWLTAMTGSVATTARQLAAGDYTEGVVSDLAMQVAGVPTFLRTAEEQGVSAELLTPYF DLMKRRLVADARHGAEDLTGVVDLLRG\*

>p-IR84, SresIRED (*Streptomyces resistomycificus*, A0A0L8KUS9\_9ACTN) (*24*) MGSSHHHHHHSSGLVPRGSHMSNTSPVTVLGLGDMGRALAAAFLAEERATTVWNRTAAKA DELISLGAVRADSVAEAVTGSPLVVVCLLDYGVVRETLEPVAGQLRGRTVVNLTNGTPSQ AAEMAAWVTGHGGDYLDGGIMAVPTTVATPEAFILYSGEQRIFDTYRGVLGELGSARYLG DDIGLASLYDLALLSGMHLMFDGFHHAVAMATSQEGGSAFGFTELLVPWLTNMARLLPAF AAEVDADLTTAAEPRLTQGLDVQVSGLANIMDAARDAGVSTTPLESSMAALEALLAHGHK VWSAPASVRQLRTAP\*

>p-IR85 (Streptomyces rimosus)

MGSSHHHHHHHSSGLVPRGSHMRSEPAAVTVLGLGSMGTALAGALLKGGHATTVWNRSPHK AKPLAERGATVAATPEEAVAASPLLIACVLDYAALHTVLDPVADSLAGKTLVNLTSGSPE QAAEAAAWARAHGAHYLDGAIMTTPPGVGSPEMMFLYSGERTVFDTHRPVLASLGDPLHL GTDPGLASLYDAALLGLMWAAMTGWLHGTALVGAEGTPATAFTPVAIRWLSAVTGFLTTY APQVDAGHYPGDDATVDVQIAAIDHLIHAAAARGVDNALPELLKATMERTRAAGHGSSSY ASVIETLRKAAGTR\*

>p-IR86 (*Thermobifida fusca*, Q47QG6\_THEFY) MGSSHHHHHHSSGLVPRGSHMARIAFIGLGIMGSPMAVHFHNAGHQVVGYNRTPAKTKAL VEAGGTAAASVSEAVADADVVALMVTDTPDVVEVLTGAGGVFETAKPGTLIIDFSTIRPD VTADLAAQARSRGLRMVDAPVSGGEVGAQNATLSIMVGGTEEDFAAARPFLEVVGKTIVH VGPSGSGQTVKAANQLIVAGHLAVLAEAIVFLEAYGVDVSAALDVLGGGLARSAIMEHKA ELMRRRSFTPGFRLDLHHKDLGIVTTAAREAGVVIPVAAAAAQLIAAARANGDGDLDHSA LFRTIARLSGRD\*

>p-IR87 (*Theobroma cacao*, A0A061GU76\_THECC) MGSSHHHHHHSSGLVPRGSHMRSLPFLLRTLSRHYLHSQTPSPPHLTFLHRSMATSATAE PISPSNTRLGWIGTGVMGRSMCAHLINAGYTLTVFNRTLSKAQPLVDMGARLAQTPHDLA AQSDVVFSIVGYPTDVRQVLLDPTNGALSGLSPGGVLVDMTTSEPSLAVEISAAASSKNC SSIDAPVSGGDRGAKNGTLAIFAGGDEAIVRRLNPLFTLMGKVNYMGPSGKGQFAKLANQ ITIASTMVGLVEGIVYAHKAGLNVASFLDAISTGAAGSKSLDLYGSRILKRDFEPGFFVN HFVKDLGICLKECQNMGLALPGLALAQQLYLSLKAHGEGNLGTQALILALERLNNVTLDS \*

>p-IR88 (*Torrubiella hemipterigena*, A0A0A1TF30\_9HYPO) MGSSHHHHHHSSGLVPRGSHMNMTTSRNVTAFGVGNMGAALAHALLKADTKVTIWNRTVD RPQVQSVLKAGATLEADVKAAISGSSDILLFCLIDYDAMYKTLEPIKGTSDGLAGKTIVN VTNGTPQQALEMRDWIKARGAARYFDGAVLVTPQMVATPQSLLVYSGESQETFDNIKTIL QPLGTPLYYGPQVDAAAAQDLAMLATMYGMFYGAFVGFGILKRSGQGQDVKVAPGTKQIT IPVMAALTEYLGLLADVIDSEDWASNGGNPLLMQVAGVANIIQAAKDANVNASGLEVLAE AMGKAVEDGWADGNVAAAAKFI\*

>p-IR89 (*Myceliophthora thermophila*, G2QG78\_MYCTT) MGSSHHHHHHSSGLVPRGSHMATITSIGIGNMGAALASALLKSVGLRQLTIWNRTADRPQ VKSLVEQGAHFEPSVAAAVARSDTILICLLDYQTVTAVFEAISPGGLAGKTVINVTNGTP RQAREAEARFKGLGAAAAYFDGGIMVTPQQIGTPEAFVILSGEDESAFRAAGGPAELLEP IGAIQYVASDAGAAALYDLAALAAMFGMFAGAFTGIALLKKQKRQRQDGGKEEGDRDNKA LAKPATDSVIVPVLNALVPYVGMIADEVDRENWMNDMGNPLKMQAIALHNILRACEEEGV DGEGLKFISRRMDRAVADGFGPGGVSAIARYMFK\*

### >p-IR90 (*Myceliophthora thermophila*) MGSSHHHHHHSSGLVPRGSHMATITSIGIGNMGAALASALLKSVGLRQLTIWNRTADRPQ VKSLVEQGAHFEPSVAAAVARSDTILICLLDYQTVIAVFEAISPGGLAGKTVINVTNGTP RQAREAEARFKGLGAAAAYFDGGIMVTPQQIGTPEAFVILSGEDESAFRAAGGPAELLEP IGAIQYVASDAGAAALYDLAALAAMFGMFAGAFTGIALLKKQKRQRQDGGKEEGDRDNKA LAKPATDSVIVPVLNALVPYVGMIADEVDRENWMNDMGNPLKMQAIALHNILRACEEEGV DGEGLKFISRRMDRAVADGFGPGGVSAIARYMFK\*

# 2.4 Sequence alignments of imine reductase variants

2.4.1	Putative avermitil	im lis	ine redu	ictases	Q82Q44_	STRAW	and	p-IR75	from	S.
Q82Q44	_STRAW	1	-MITPEPVT	SLIGMTQNA	VEKTPVTMLG	ILGAMGTALA	RTWLAA	GHRLTVW	49	
p-IR75		1	:        MLITPEPVT	 SLIGMTQNA	VEKTPVTMLG	 ILGAMGTALA	 RTWLAA	 GHRLTVW	50	
Q82Q44	_STRAW	50	NRTPARAAA	LAAEGARVA	DSAAEAVAAN	TLVIVCLLD	DSSVDE	/LAGIDL	99	
p-IR75		51	 NRTPARAAA	.       LATEGARVA	USAAEAVAAN	ILVIVCLLD	 DSSVDE	 /LAGIDL	100	
Q82Q44	_STRAW	100	ADTDLVNLT	TSTPAQARA	RAEWANERGA	RYLDGGIMA	VPPMIG	/PEAGSY	149	
p-IR75		101	ADTDLVNLT	 TSTPAQARA	RAEWANERGA	RYLDGGIMA	 VPPMIG	 /PEAGSY	150	
Q82Q44	_STRAW	150	VFCSGSREL	FERHQETLG	IPAGTTYVGM	IDAGFAALHD	MALLSA	MYGMFAG	199	
p-IR75		151	VFCSGSREL	FERHQETLG	IIIIIIIIII IPAGTTYVGM	IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	MALLSA	 MYGMFAG	200	
Q82Q44	_STRAW	200	VAHAFALIH	KEDIDPTSL	APMLADWLVA	MAPAVHQTA	DHLRSGI	DYTKGVV	249	
p-IR75		201	 VAHAFALIH	 KEDIDPTSL	.APMLADWLVA	MAPAVHQTA	 DHLRSGI	 DYTKGVV	250	
Q82Q44	_STRAW	250	STLAMQVAG	TPTFLSTAE	QQGVSPELLS	PYFELMRRR	LAEGSGI	EDLTGV	299	
p-IR75		251	STLAMQVAG	IIIIIIIII TPTFLSTAE	QQGVSPELLS	PYFELMRRR	LAEGSGI	EDLTGV	300	
Q82Q44	_STRAW	300	INLLVH	305						
p-IR75		301	IIIIII INLLVH	306						

# 2.4.2 Putative imine reductases A0A117QYA8\_9A and p-IR76

AØA117QYA8_9A	1	MTQNAVEKESVTLLGLGAMGTALARTWLAAGHPLTVWNRTPARAASLAAD	50
p-IR76	1	MTQNAVEKESVTLLGLGAMGTALARTWLAAGHPLTVWNRTPARAASLAAD	50
A0A117QYA8_9A	51	GAKVADSAAAAVAANALVVVCLLDDASVEDALAGTDLAGKDLVNLTTSTP	100
p-IR76	51	GAKVADSAAAAVAANALVVVCLLDDASVEDALTGTDLAGKDLVNLTTSTP	100
A0A117QYA8_9A	101	AQARARAEWARERGARYLDGGIMAVPPMIGVPESGGYIFYSGSPELFQRH	150
p-IR76	101	AQARARAEWARERGARYLDGGIMAVPPMIGVPESGGYIFYSGSPELFQRH	150
A0A117QYA8_9A	151	RETLVVPAGTVYVGHDAGFAALYDMALLSAMYGMFAGAAHAFALIRKEDI	200
p-IR76	151	IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	200
A0A117QYA8_9A	201	DPASLAPLLADWLVAMAPAVHRTADQLRSGDYTQGVVSNLAMQVAGTPAF	250
p-IR76	201		250
A0A117QYA8_9A	251	VSAAEQQGVSPELLSPYFALMRRRLAEGSGEEDLTGVIDLLVR 293	
p-IR76	251	VSAAEQQGVSPELLSPYFALMRRRLAEGSGEEDLTGVIDLLVR293	

# 2.4.3 Putative imine reductases A0A117QYA8\_9A and p-IR77

A0A117QYA8_9A	1	MTQNAVEKESVTLLGLGAMGTALARTWLAAGHPLTVWNRTPARAASLAAD	50
p-IR77	1	MTQNAVEKESVTLLGLGAMGTALARTWLAAGHPLTVWNRTPARAASLAAD	50
A0A117QYA8_9A	51	GAKVADSAAAAVAANALVVVCLLDDASVEDALAGTDLAGKDLVNLTTSTP	100
p-IR77	51	GAKVADSAAAAVAANALVVVCLLDDASVEDALAGTDLAGKDLVNLTTSTP	100
A0A117QYA8_9A	101	AQARARAEWARERGARYLDGGIMAVPPMIGVPESGGYIFYSGSPELFQRH	150
p-IR77	101	AQARARAEWARERGARYLDGGIMAVPPMIGVPESGDYIFYSGSPELFQRH	150
A0A117QYA8_9A	151	RETLVVPAGTVYVGHDAGFAALYDMALLSAMYGMFAGAAHAFALIRKEDI	200
p-IR77	151	IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	200
A0A117QYA8_9A	201	DPASLAPLLADWLVAMAPAVHRTADQLRSGDYTQGVVSNLAMQVAGTPAF	250
p-IR77	201	UIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	250
A0A117QYA8_9A	251	VSAAEQQGVSPELLSPYFALMRRRLAEGSGEEDLTGVIDLLVR 293	
p-IR77	251		

## 2.4.4 Putative imine reductases A0A0M9XDJ7\_ST and p-IR85

AØAØM9XDJ7_ST	1	MRSEPAAVTVLGLGSMGTALAGALLKGGHATTVWNRSPHKAKLLAERGAT	50
p-IR <b>8</b> 5	1	MRSEPAAVTVLGLGSMGTALAGALLKGGHATTVWNRSPHKAKPLAERGAT	50
A0A0M9XDJ7_ST	51	VAATPEEAVAASPLVIACVLDYAALYTVLDPVADSLAGKTLVNLTSGSPE	100
p-IR <b>85</b>	51	VAATPEEAVAASPLLIACVLDYAALHTVLDPVADSLAGKTLVNLTSGSPE	100
AØAØM9XDJ7_ST	101	QAAEAAAWARSHGAHYLDGAIMTTPPGVGSPEMMFLYSGERTVFDTHRPA	150
p-IR <b>85</b>	101	QAAEAAAWARAHGAHYLDGAIMTTPPGVGSPEMMFLYSGERTVFDTHRPV	150
AØAØM9XDJ7_ST	151	LASLGDPLYLGTDPGLASLYDAALLGLMWAAMTGWLHGTALVGAEGTPAT	200
p-IR <b>8</b> 5	151	LASLGDPLHLGTDPGLASLYDAALLGLMWAAMTGWLHGTALVGAEGTPAT	200
A0A0M9XDJ7_ST	201	AFTPVAIRWLSAMTGFLTTYAPQVDAGHYPGDDATVDVQIAAIDHLIHAA	250
p-IR <b>8</b> 5	201	AFTPVAIRWLSAVTGFLTTYAPQVDAGHYPGDDATVDVQIAAIDHLIHAA	25
A0A0M9XDJ7_ST	251	AARGVDNALPELLKATMERTRAAGHGSSSYASVIETLR-AAGTR 293	
p-IR85	251	AARGVDNALPELLKATMERTRAAGHGSSSYASVIETLRKAAGTR 294	

### 2.4.5 Putative imine reductases p-IR89 (G2QG78\_MYCTT) and p-IR90

G2QG78_MYCTT	1	MATITSIGIGNMGAALAS	ALLKSVGLRQLTIWNRTADRPQVKSLVEQGAH	50
p-IR90	1	MATITSIGIGNMGAALAS	GALLKSVGLRQLTIWNRTADRPQVKSLVEQGAH	5
G2QG78_MYCTT	51	FEPSVAAAVARSDTILIC	LLDYQTVTAVFEAISPGGLAGKTVINVTNGTP	100
p-IR90	51	FEPSVAAAVARSDTILIC	CLLDYQTVIAVFEAISPGGLAGKTVINVTNGTP	100
G2QG78_MYCTT	101	RQAREAEARFKGLGAAAA	YFDGGIMVTPQQIGTPEAFVILSGEDESAFRA	150
p-IR90	101	RQAREAEARFKGLGAAAA	YFDGGIMVTPQQIGTPEAFVILSGEDESAFRA	150
G2QG78_MYCTT	151	AGGPAELLEPIGAIQYVA	SDAGAAALYDLAALAAMFGMFAGAFTGIALLK	200
p-IR90	151	AGGPAELLEPIGAIQYVA	SDAGAAALYDLAALAAMFGMFAGAFTGIALLK	200
G2QG78_MYCTT	201	KQKRQRQDGGKEEGDRDN	IKALAKPATDSVIVPVLNALVPYVGMIADEVDR	250
p-IR90	201	KQKRQRQDGGKEEGDRDN	IKALAKPATDSVIVPVLNALVPYVGMIADEVDR	250
G2QG78_MYCTT	251	ENWMNDMGNPLKMQAIAL	.HNILRACEEEGVDGEGLKFISRRMDRAVADGF	300
p-IR90	251	ENWMNDMGNPLKMQAIAL	HNILRACEEEGVDGEGLKFISRRMDRAVADGF	300
G2QG78_MYCTT	301	GPGGVSAIARYMFK	314	
p-IR90	301	GPGGVSAIARYMFK	314	

### 2.5 Production of *B. subtilis* glucose dehydrogenase

The gene for the glucose dehydrogenases from *Bacillus subtilis* was subcloned into a pET-15b vector. For the transformation of plasmid DNA into *E. coli*, chemically competent *E. coli* cells were thawed on ice for ten minutes. The *B. subtilis* GDH/pET-15b plasmid DNA solution (1  $\mu$ L) was added to the cell suspension and gently mixed. The cells were incubated on ice for 30 min, after which the cells were heat shocked. DH5 $\alpha$  cells, for DNA isolation and sequencing, were heat shocked at 42°C for thirty seconds, while BL21 (DE3) cells for protein expression were heat shocked at 42°C for ten seconds. Following this, the cells were returned to ice for ten minutes. SOC media (300  $\mu$ L) was added and the cells incubated at 37°C with shaking at 250 rpm for one hour prior to plating onto LB agar supplemented with ampicillin (100 mg L<sup>-1</sup>).

Production of the *B. subtilis* GDH biocatalyst was achieved in *E. coli* BL21 (DE3) cells which had been transformed with the BsGDH/pET-15b plasmid. A single colony picked from an agar plate grown overnight was inoculated into 5 mL LB medium supplemented with ampicillin (100 mg L<sup>-1</sup>) and incubated overnight at 37°C in an orbital shaker at 250 rpm. This starter culture was used as the inoculum for a 500 mL culture in LB medium supplemented with ampicillin (100 mg L<sup>-1</sup>). The culture was then incubated at 30°C in an orbital shaker at 250 rpm until it reached an OD<sub>600nm</sub> of between 0.6 and 0.8. IPTG (0.5 mM) was added to induce protein expression, after which cell cultivation was continued at 20°C and 250 rpm for 16 hours.

Cells from a 500 mL culture were harvested by centrifugation (2,831 rcf, 20 min) and the pellet resuspended in 100 mL postassium phosphate buffer (100 mM, pH 7.0). Following cell disruption by sonication (4°C, 20 min, 30 sec pulse, 30 sec off, repeat x 20), the suspension was centrifuged at 39,191 rcf for 20 min. The resulting clarified lysate was either divided into aliquots, snap-frozen in liquid nitrogen and stored at -80°C until use, or snap-frozen in liquid nitrogen, lyophilized and then stored at -20°C until use.

# 2.6 Synthetic procedures and characterisation data for chemical standards

### 2.6.1 Reductive amination procedure A

To a stirred solution of 1-indanone (264 mg, 2.0 mmol) in dry THF (10 mL, 200 mM) under N<sub>2</sub> were added the amine nucleophile (2.2 mmol – 4.0 mmol), Na(OAc)<sub>3</sub>BH (0.636 g, 3.0 mmol) and glacial AcOH (154  $\mu$ L, 2.0 mmol). The reaction was stirred for 16 hours at 20°C under N<sub>2</sub> then quenched by addition of 5 M NaOH (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were then extracted with 1 M HCl (3 x 20 mL) and the aqueous phase basified (pH 12) by addition of 5 M NaOH. The product was extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (20 mL), dried over MgSO<sub>4</sub> and the solvent removed under reduced pressure to afford the corresponding secondary amine.

#### N-propargyl-1-aminoindan 10



Grey solid isolated, 49% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 7.80 – 7.71 (m, 1 H, C3-*H*), 7.39 – 7.21 (m, 3 H, C1-*H*, C2-*H*, C6-*H*), 4.95 – 4.88 (m, 1 H, C9-*H*), 3.67 – 3.61 (br s, 2 H, C11-*H*), 3.44 (dt, J = 8.2, 8.1 Hz, 1 H, C7-*H*<sub>a</sub>), 2.97 – 2.87 (m, 1 H, C7-*H*<sub>b</sub>), 2.59 (t, J = 2.6 Hz, 1 H, C13-*H*), 2.50 – 2.39 (m, 2 H, C8-*H*). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 145.4 (C5), 135.6 (C4), 130.1 (C6), 127.0 (C3), 126.4 (C1/C2), 125.6 (C1/C2), 78.2 (C12), 73.3 (C13), 60.7 (C9), 33.5 (C11), 30.5 (C7), 28.8 (C8). HRMS calcd. for C<sub>12</sub>H<sub>14</sub>N<sup>+</sup> 172.1126 [M]<sup>+</sup>, found 172.1120.

### 2.6.2 Reductive amination procedure B

To a stirred solution of the ketone (2.0 mmol) in dry MeOH (5 mL) over 4 Å molecular seives under  $N_2$  was added the amine nucleophile (2.2 - 4.0 mmol). The reaction was stirred at 20°C overnight, after which the solution was cooled to 0°C and NaBH<sub>4</sub> (0.151 g, 4.0 mmol) added over 10 minutes. The mixture was stirred for a further 2 hours, then the solvent removed under reduced pressure. The resulting slurry was resuspended in EtOAc (10 mL), filtered, and the solid washed with EtOAc (2 x 5 mL). The combined organic phases were extracted with 1 M HCl (3 x 10 mL). The combined aqueous phases were then basified (pH 12) with 5 M NaOH and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with brine (10 mL), dried over MgSO<sub>4</sub> and the solvent removed under reduced pressure to afford the corresponding secondary amine.

#### N-butylcyclohexylamine 4



Yellow oil isolated, 88% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 2.59 (t, J = 7.3 Hz, 2 H, C8-*H*), 3.44 – 3.33 (m, 1 H, C6-*H*), 1.90 – 1.80 (m, 2 H, C1-*H*, C5-*H*), 1.76 – 1.65 (m, 2 H, C2-*H*, C4-*H*), 1.64 – 1.52 (m, 1 H, C3-*H*), 1.50 – 1.38 (m, 2 H, C9-*H*), 1.37 – 0.97 (m, 7 H, C1-*H*, C2-*H*, C3-*H*, C4-*H*, C5-*H*, C10-*H*), 0.89 (t, J = 7.2 Hz, 3 H, C11-*H*). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 56.6 (*C*6), 46.4 (*C*8), 33.2

(C1, C5), 32.2 (C9), 25.9 (C2, C4), 24.8 (C3), 20.3 (C10), 13.7 (C11). **HRMS** calcd. for  $C_{10}H_{22}N^+$  156.1752 [M+H]<sup>+</sup>, found 156.1754.

### N-cyclohexylphenethylamine 5



Orange oil isolated, 80% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 7.41 – 7.20 (m, 5 H, C11-*H*, C12-*H*, C13-*H*, C14-*H*, C15-*H*), 3.06 – 2.91 (m, 2 H, C8-*H*), 2.90 – 2.77 (m, 2 H, C9-*H*), 2.57 – 2.46 (m, 1 H, C6-*H*), 2.36 (br s, 1 H, N7-*H*), 1.99 – 1.87 (m, 2 H, C1-*H*, C5-*H*), 1.83 – 1.71 (m, 2 H, C2-*H*, C4-*H*), 1.70 – 1.61 (m, 1 H, C3-*H*), 1.37 – 1.07 (m, 5 H, C1-*H*, C2-*H*, C3-*H*, C4-*H*, C5-*H*). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 139.5 (C10), 128.3 (C11, C15/C12, C14), 128.1 (C11, C15/C12, C14), 125.8 (C13), 56.3 (C6), 47.5 (C8), 35.7 (C9), 32.6 (C1, C5), 25.7 (C2, C4), 24.7 (C3). HRMS calcd. for C<sub>14</sub>H<sub>22</sub>N<sup>+</sup> 204.1752 [M+H]<sup>+</sup>, found 204.1749.

#### N-(1-phenylethyl)cyclopropylamine 11



Yellow oil isolated, 31% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 7.34 – 7.14 (m, 5 H, C1-*H*, C2-*H*, C3-*H*, C4-*H*, C6-*H*), 3.83 (q, *J* = 6.7 Hz, 1 H, C7-*H*), 3.01 (br s, 1 H, N9-*H*), 1.95 – 1.86 (m, 1 H, C10-*H*), 1.34 (d, *J* = 6.7 Hz, 3 H, C8-*H*), 0.39 – 0.25 (m, 4 H, C11-*H*, C12-*H*). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 144.8 (C5), 128.4 (C1, C3), 126.7 (C2, C4, C6), 58.4 (C7), 28.7 (C10), 23.0 (C8), 6.04 (C11, C12). HRMS calcd. for C<sub>11</sub>H<sub>16</sub>N<sup>+</sup> 162.1283 [M+H]<sup>+</sup>, found 162.1283.

### 4-(2-(cyclopropylamino)propyl)phenol 12



Orange oil isolated, 87% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 7.01 (d, J = 8.1 Hz, 2 H, C4-H, C6-H), 6.74 (d, J = 8.1 Hz, C1-H, C3-H), 3.12 – 3.02 (m, 1 H, C8-H), 2.79 – 2.68 (m, 1 H, C7- $H_a$ ), 2.65 – 2.56 (m, 1 H, C7- $H_b$ ), 2.16 – 2.09 (m, 1 H, C12-H), 1.17 (d, J = 6.2 Hz, 2 H, C9-H), 0.61 – 0.37 (m, 4 H, C13-H, C14-H). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 155.4 (C2), 130.2 (C4, C6), 129.24 (C5), 115.7 (C1, C3), 55.8 (C8), 41.6 (C7), 28.5 (C12), 19.2 (C9), 6.3 (C13, C14). HRMS calcd. for C<sub>12</sub>H<sub>18</sub>NO<sup>+</sup> 192.1388 [M+H]<sup>+</sup>, found 192.1378.

N-(1-(4-methoxyphenyl)propan-2-yl)cyclopropylamine 13



Yellow oil isolated, 81% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 7.12 (d, J = 8.6 Hz, 2 H, C4-*H*, C6-*H*), 6.85 (d, J = 8.6 Hz, 2 H, C1-*H*, C3-*H*), 3.80 (s, 3 H, C11-*H*), 3.06 – 2.95 (m, 1 H, C8-*H*), 2.71 (dd, J = 6.7, 6.5 Hz, 1 H, C7-*H*<sub>a</sub>), 2.56 (dd, J = 13.5, 6.5 Hz, 1 H, C7-*H*<sub>b</sub>), 2.06 (tt, J = 6.8, 7.2 Hz, 1 H, C12-*H*), 1.11 (d, J = 6.4 Hz, 3 H, C9-*H*), 0.54 – 0.26 (m, 4 H, C13-*H*, C14-*H*). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 157.8 (*C*2), 131.3 (*C*5), 130.0 (*C*4, *C*6), 113.6 (*C*1, *C*3), 55.4 (*C*11), 55.0 (*C*8), 42.4 (*C*7), 28.5 (*C*12), 20.2 (*C*9), 6.9 (*C*13, *C*14). HRMS calcd. for C<sub>13</sub>H<sub>20</sub>NO<sup>+</sup> 206.1545 [M+H]<sup>+</sup>, found 206.1547.

#### N-benzyl-1-methoxy-2-propylamine 14



Colourless oil isolated, 80% yield. <sup>1</sup>H NMR  $\delta_{H}$  (400 MHz, CDCl<sub>3</sub>): 7.34 – 7.03 (m, 5 H, C8-*H*, C9-*H*, C10-*H*, C11-*H*, C12-*H*), 3.81 (s, 2 H, C6-*H*), 3.67 (d, *J* = 6.6 Hz, 1 H, C1-*H<sub>a</sub>*), 3.50 (d, *J* = 6.6 Hz, 1 H, C1-*H<sub>b</sub>*), 3.27 (s, 3 H, C4-*H*), 2.93 – 2.84 (m, 1 H, C2-*H*), 2.73 (br s, 1 H, N5-*H*), 1.01 (d, *J* = 6.5 Hz, 3 H, C3-*H*). <sup>13</sup>C NMR  $\delta_{C}$  (100 MHz, CDCl<sub>3</sub>): 140.3 (C7), 128.3 (C8, C12/C9, C10), 128.1 (C8, C12/C9, C10), 127.4 (C10), 58.7 (C2), 51.7 (C4), 51.2 (C6), 45.5 (C1), 16.8 (C3). HRMS calcd. for C<sub>11</sub>H<sub>18</sub>NO<sup>+</sup> 180.1388 [M+H]<sup>+</sup>, found 180.1376.

## 2.7 Chromatographic analysis

### 2.7.1 Conditions and retention times

### 2.7.1.1 Analysis with Agilent 7890B gas chromatograph

Retention times for GC-FID analysis with an achiral stationary phase. Phenomenex ZB WAXplus column, inlet temperature: 250°C, detector temperature: 225°C, flow rate: 5.0 mL min<sup>-1</sup>, temperature program: 50°C (0.5 min), 50°C min<sup>-1</sup> (250°C), 250°C (0.5 min).

Compound	Retention Time / min
cyclohexanol	1.8
2-methylcyclohexanol	1.8
1-indanol	3.3
1-phenylethanol	2.7
1-(4-hydroxyphenyl)-2-propanol	3.8
1-(4-methoxyphenyl)-2-propanol	3.9
1-methoxy-2-propanol	1.3
cyclohexanone	1.4
2-methylcyclohexanone	1.5
1-indanone	3.2
acetophenone	2.4
1-(4-hydroxyphenyl)-2-propanone	3.4
1-(4-methoxyphenyl)-2-propanone	3.4
methoxyacetone	0.9
butylamine	0.8
phenethylamine	2.4
aniline	2.6
benzylamine	2.3
N-cyclopropylcyclohexylamine 3	1.6
N-butylcyclohexylamine 4	2.0
N-phenethylcyclohexylamine 5	3.7
N-cyclohexylaniline 6	3.5
N-benzylcyclohexylamine 7	3.3
N-isopropylcyclohexylamine 8	1.7
N-(2-methylcyclohexyl)acetamide	3.2 (A), 3.3 (B)
N-(1-phenylethyl)cyclopropylamine 10	2.4
N-propargyl-1-aminoindan <b>11</b>	3.5
N-(4-hydroxy)cyclopropylamphetamine 12	3.5
N-(4-methoxy)cyclopropylamphetamine 13	3.6
N-benzyl-1-methoxypropan-2-amine 14	2.8

### 2.7.1.2 Analysis with Agilent 6850 gas chromatograph

Retention times for GC-FID analysis under general conditions with a chiral stationary phase. CP-Chirasil-

Dex CB column, inlet temperature: 200°C; detector temperature: 250°C; flow rate: 1.2 mL min<sup>-1</sup>; temperature program: 50°C (0 min), 5°C min<sup>-1</sup> (200°C), 200°C (2 min).

Compound	Retention Time / min
cyclohexanol	12.9
1-indanol	19.3 (A), 19.4 (B)
cyclohexanone	10.3
2-methylcyclohexanone	9.7 (A), 9.8 (B)
1-indanone	18.0
acetophenone	11.9
1-(4-hydroxyphenyl)-2-propanone	26.2
1-(4-methoxyphenyl)-2-propanone	20.1
methoxyacetone	3.1
phenethylamine	13.6
aniline	13.1
benzylamine	13.0
N-cyclopropylcyclohexylamine 3	14.2
N-butylcyclohexylamine 4	14.3
N-phenethylcyclohexylamine 5	25.0
N-cyclohexylaniline 6	18.9
N-benzylcyclohexylamine 7	24.2
N-isopropylcyclohexylamine 8	11.2
2-methylcyclohexylamine 9	9.5 (A), 10.6 (B)
rac-N-propargyl-1-aminoindan 10	21.6 ( <i>S</i> ), 21.8 ( <i>R</i> )
rac-N-(1-phenylethyl)cyclopropylamine 11	15.8 (A), 15.9 (B)
rac-N-(4-hydroxy)cyclopropylamphetamine 12	25.5
rac-N-(4-methoxy)cyclopropylamphetamine 13	27.3
rac-N-benzyl-1-methoxypropan-2-amine 14	18.4

Retention times for GC-FID analysis of substrates and products under conditions optimized for faster analysis of higher-boiling compounds with a chiral stationary phase. CP-Chirasil-Dex CB column, inlet temperature: 200°C; detector temperature: 250°C; flow rate: 1.2 mL min<sup>-1</sup>; temperature program: 50°C (0 min), 10°C min<sup>-1</sup> (110°C), 5°C min<sup>-1</sup> (200°C), 200°C (2 min).

Compound	Retention Time / min
2-phenylpiperidine 1	14.9
2-phenylpiperideine 2	16.7

Retention times for GC-FID analysis of substrates and products under conditions optimized for separation of high-boiling enantiomers with a chiral stationary phase. CP-Chirasil-Dex CB column, inlet

# temperature: 200°C; detector temperature: 250°C; flow rate: 1.2 mL min<sup>-1</sup>; temperature program: 50°C (0 min), 10°C min<sup>-1</sup> (150°C), 3°C min<sup>-1</sup> (200°C), 200°C (2 min).

Compound	Retention Time / min
2-phenylpiperideine 1	16.7
N-acetyl-2-phenylpiperidine	20.7 (S), 21.0 (R)

Retention times for GC-FID analysis of substrates and products under conditions optimized for faster separation of low- and high-boiling compounds with a chiral stationary phase. CP-Chirasil-Dex CB column, inlet temperature: 200°C; detector temperature: 250°C; flow rate: 1.2 mL min<sup>-1</sup>; temperature program: 50°C (0 min), 10°C min<sup>-1</sup> (120°C), 5°C min<sup>-1</sup> (160°C), 10°C min<sup>-1</sup> (200°C), 200°C (2 min).

Compound	Retention Time / min	
cyclohexanone	6.0	
aniline	7.7	
N-cyclohexylaniline 6	17.9	

Retention times for GC-FID analysis of substrates and products under conditions optimized for faster separation of low-boiling compounds with a chiral stationary phase. CP-Chirasil-Dex CB column, inlet temperature: 200°C; detector temperature: 250°C; flow rate: 1.2 mL min<sup>-1</sup>; temperature program: 50°C (0 min), 10°C min<sup>-1</sup> (100°C), 5°C min<sup>-1</sup> (130°C), 10°C min<sup>-1</sup> (200°C), 200°C (2 min)

Compound	Retention Time / min
cyclohexanone	6.5
N-cyclopropylcyclohexylamine	9.5

### 2.7.2 Chromatograms



### 2.7.2.1 Example chromatograms for biocatalytic imine reduction

Typical GC-FID chromatogram for the biocatalytic reduction of imines. Reduction of 1 using p-IR84 and *B. subtilis* GDH.



**Typical GC-FID chromatogram for determination of enantiomeric excess.** Reduction of **1** using IRED p-IR84 and *B. subtilis* GDH.



2.7.2.2 Example chromatograms for reductive aminations with cell-free extract

Typical GC-FID chromatogram for biocatalytic reductive amination with IRED CFE. Reductive amination of methoxyacetone with benzylamine using IRED p-IR79 and *B. subtilis* GDH.



2.7.2.3 Example chromatograms for reductive aminations with purified enzyme

Typical GC-FID chromatogram for biocatalytic reductive amination with purified IRED. Reductive amination of hydrocinnamaldehyde with cyclopropylamine using ArpDHII and *B. subtilis* GDH.