

Actinomycete Superset

Product code: 18AS

Contents:

- 20 x 96-well microtitre plates containing semi-purified natural product samples derived from 80 selected actinomycete 'superproducer' strains
- 1 x 96-well microtitre plate containing crude extracts derived from 80 selected actinomycete 'superproducer' strains

Introduction

Actinomycetes are filamentous bacteria which are well known for producing many medicinally important secondary metabolites. MerLion Pharmaceuticals has used its proprietary UPLC/MS/UV/ELSD chemical fingerprinting technology to identify the most chemically diverse and productive samples from its comprehensive actinomycete extract library. To generate this superset 10,733 actinomycete extract fingerprints, each representing a single strain and growth medium combination, were analysed. Strains were ranked based on the numbers and diversity of compounds observed within each extract.

The extracts from the top 80 'superproducer' strains have been prefractionated by reverse phase HPLC into 20 fractions per extract (see Figure 1) and arrayed within microtitre plates for easy biological testing. The resulting set of 1600 enriched and semi-purified fractions represents a high quality focused screening library with excellent coverage of actinomycete secondary metabolite chemistry in comparatively few screening wells.

Each fraction has been analysed by UPLC/MS to determine the numbers and uniqueness of compounds within the set as a whole and contributed by each superproducer strain (see Tables 1 and 2). Only compounds showing significant peak intensities versus the background were included in this analysis therefore actual chemical diversity, including minor components, will be even higher.

Features:

- 80 actinomycete 'superproducer' strains specially selected because they yield a large number of chemically diverse compounds
- all strains have been characterised taxonomically using 16S rRNA sequencing
- over 100-fold enrichment of individual components in screening wells
 - individual components are concentrated approx. 9-fold compared to the crude extracts
 - total mass is reduced approx. 12.5 fold
- approximately 20 µg of enriched material present within each well (also this will vary according to the properties and distribution of compounds within each extract)
- UPLC/MS data available for all fractions

Actinomycete Superset Statistics

Table 1.

	Total number in superset	Average number per fraction	Maximum number per fraction
Compounds*	5,213	10.2	74
Unique Compounds**	3,632	2.3	37

* Compounds defined as individual mass and retention time combinations in UPLC/MS analysis with mass ion intensities 10 x over intensity threshold

** Compounds which are unique to one extract (i.e. do not occur in any other extracts within the superset)

Example of Fractionation

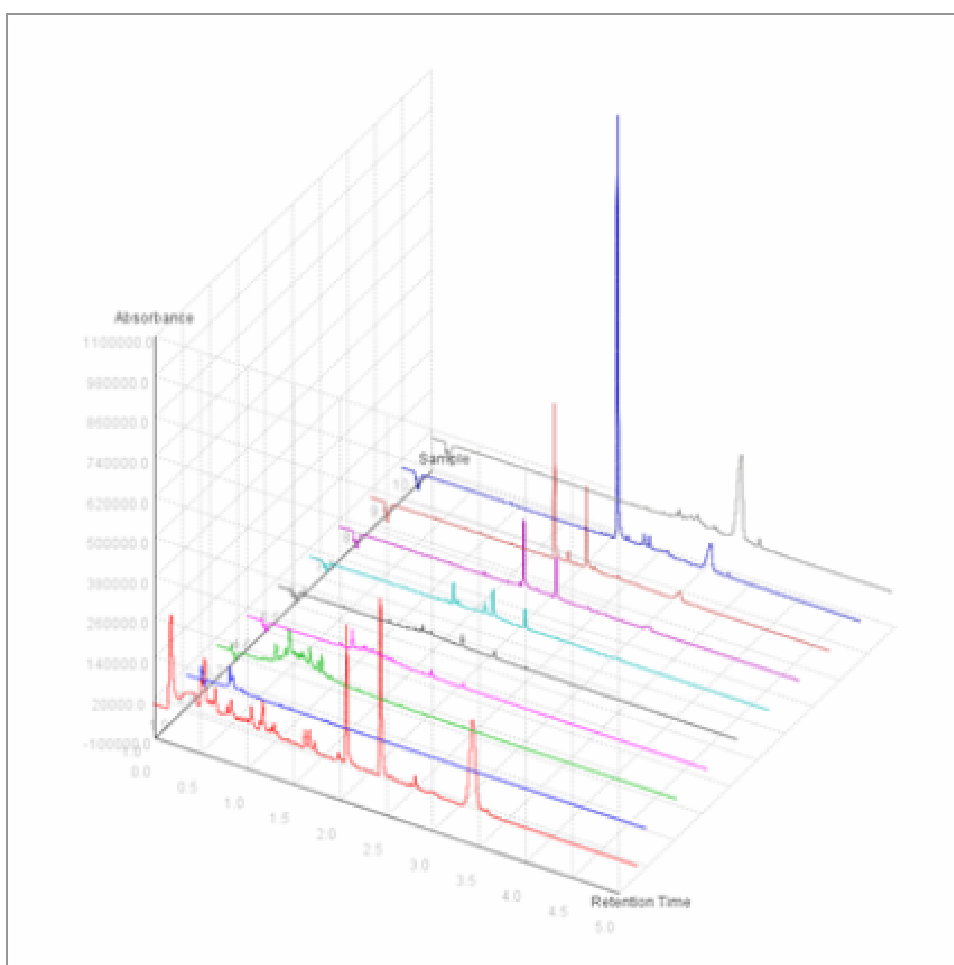


Figure 1. Overlaid UPLC/UV chromatograms of fractions from extract 18AS_25. Crude extract is shown in red with fractions 2, 4, 6, 8, 10, 12, 14, 16 & 18 behind.

Actinomycete Superproducer Strains

Table 2.

No.	Sample code	Strain taxonomy ¹	Plate no.	Rows ¹	Total Count in Each Extract		Average No. of Compounds per Fraction
					Compounds ²	Unique Compounds ³	
1	18AS_1	<i>Streptomyces hygrosopicus</i>	1	A,B	79	46	6.5
2	18AS_2	<i>Streptomyces malachitospinus</i>	1	C,D	46	20	3.2
3	18AS_3	<i>Streptomyces sp.</i>	1	E,F	59	14	3.4
4	18AS_4	<i>Streptomyces hiroshimensis</i>	1	G,H	52	19	4.4
5	18AS_5	<i>Streptomyces sp.</i>	2	A,B	66	22	5.9
6	18AS_6	<i>Streptomyces sp.</i>	2	C,D	25	9	1.8
7	18AS_7	<i>Streptomyces griseoaurantiacus</i>	2	E,F	58	33	3.7
8	18AS_8	<i>Streptomyces sp.</i>	2	G,H	43	19	2.7
9	18AS_9	<i>Streptomyces sp.</i>	3	A,B	38	18	2.6
10	18AS_10	<i>Streptomyces sp.</i>	3	C,D	26	2	1.6
11	18AS_11	<i>Streptomyces sp.</i>	3	E,F	22	9	1.9
12	18AS_12	<i>Streptomyces griseoflavus</i>	3	G,H	190	98	14.4
13	18AS_13	<i>Streptomyces sp.</i>	4	A,B	104	29	13.6
14	18AS_14	<i>Streptomyces hygrosopicus</i>	4	C,D	66	42	3.4
15	18AS_15	<i>Streptomyces sp.</i>	4	E,F	134	71	11.6
16	18AS_16	<i>Streptomyces viridocyaneus</i>	4	G,H	371	249	33.2
17	18AS_17	<i>Nocardopsis dassonvillei</i>	5	A,B	43	22	3.3
18	18AS_18	<i>Streptomyces cellulosa</i>	5	C,D	63	36	4.8
19	18AS_19	<i>Streptomyces champavatii</i>	5	E,F	72	18	4.9
20	18AS_20	<i>Achromobacter xylosoxidans</i>	5	G,H	74	27	8.4
21	18AS_21	<i>Streptomyces cavourensis</i>	6	A,B	361	208	41.2
22	18AS_22	<i>Streptomyces sp.</i>	6	C,D	122	55	14.5
23	18AS_23	<i>Streptomyces sp.</i>	6	E,F	72	22	4.8
24	18AS_24	<i>Streptomyces canus</i>	6	G,H	118	84	12.8
25	18AS_25	<i>Streptomyces variabilis</i>	7	A,B	105	74	14.0
26	18AS_26	<i>Streptomyces rameus</i>	7	C,D	86	29	7.4
27	18AS_27	<i>Nocardia vinacea</i>	7	E,F	142	31	18.3
28	18AS_28	<i>Streptomyces sp.</i>	7	G,H	92	43	10.3
29	18AS_29	<i>Streptomyces niger</i>	8	A,B	191	68	25.5
30	18AS_30	<i>Streptomyces sp.</i>	8	C,D	114	62	11.7
31	18AS_31	<i>Streptomyces sp.</i>	8	E,F	84	37	6.8
32	18AS_32	<i>Streptomyces sp.</i>	8	G,H	80	34	6.1
33	18AS_33	<i>Streptomyces sp.</i>	9	A,B	36	12	3.5
34	18AS_34	<i>Streptomyces albogriseolus</i>	9	C,D	39	12	3.4
35	18AS_35	<i>Kibdelosporangium aridum</i>	9	E,F	103	60	8.9
36	18AS_36	<i>Streptomyces sp.</i>	9	G,H	59	26	7.4
37	18AS_37	<i>Streptomyces flavogriseus</i>	10	A,B	123	50	11.1
38	18AS_38	<i>Streptomyces hygrosopicus</i>	10	C,D	274	142	23.6
39	18AS_39	<i>Actinosynnema mirum</i>	10	E,F	311	133	31.4
40	18AS_40	<i>Streptomyces albulus</i>	10	G,H	106	40	9.3
41	18AS_41	<i>Streptomyces flaveus</i>	11	A,B	228	97	15.6
42	18AS_42	<i>Streptomyces nashvillensis</i>	11	C,D	39	18	2.8
43	18AS_43	<i>Streptomyces platensis</i>	11	E,F	204	126	16.0
44	18AS_44	<i>Amycolatopsis halotolerans</i>	11	G,H	83	44	6.2
45	18AS_45	<i>Streptomyces griseoflavus</i>	12	A,B	157	50	10.5
46	18AS_46	<i>Streptomyces rutgersensis</i>	12	C,D	260	87	27.7
47	18AS_47	<i>Streptomyces iakyrus</i>	12	E,F	280	157	27.0
48	18AS_48	<i>Streptomyces iakyrus</i>	12	G,H	119	60	9.3
49	18AS_49	<i>Streptomyces krainskii</i>	13	A,B	87	17	6.5
50	18AS_50	<i>Streptomyces krainskii</i>	13	C,D	189	62	15.4
51	18AS_51	<i>Streptomyces cavourensis</i>	13	E,F	59	12	3.6
52	18AS_52	<i>Streptomyces exfoliates</i>	13	G,H	73	38	5.7

Table 2 continued...

No.	Sample code	Strain taxonomy	Plate no.	Rows ¹	Total Count in Each Extract		Average No. of Major Compounds per Fraction
					Major Compounds ²	Unique Major Compounds ³	
53	18AS_53	<i>Streptomyces carpaticus</i>	14	A,B	117	40	12.0
54	18AS_54	<i>Streptomyces spororaveus</i>	14	C,D	219	110	18.7
55	18AS_55	<i>Streptomyces parvus</i>	14	E,F	192	41	29.8
56	18AS_56	<i>Streptomyces subrutilus</i>	14	G,H	79	21	6.4
57	18AS_57	<i>Streptomyces anulatus</i>	15	A,B	154	41	19.2
58	18AS_58	<i>Streptomyces macrosporeus</i>	15	C,D	68	14	9.1
59	18AS_59	<i>Streptomyces albogriseolus</i>	15	E,F	91	6	11.3
60	18AS_60	<i>Streptomyces platensis</i>	15	G,H	130	74	14.2
61	18AS_61	<i>Streptomyces microflavus</i>	16	A,B	155	20	18.7
62	18AS_62	<i>Streptomyces virginiae</i>	16	C,D	74	13	9.6
63	18AS_63	<i>Streptomyces microflavus</i>	16	E,F	103	22	11.6
64	18AS_64	<i>Streptomyces violaceoruber</i>	16	G,H	119	4	12.8
65	18AS_65	<i>Streptomyces phaeofaciens</i>	17	A,B	147	81	13.0
66	18AS_66	<i>Streptomyces violaceoruber</i>	17	C,D	35	7	2.3
67	18AS_67	<i>Streptomyces cacaoi</i>	17	E,F	39	13	2.8
68	18AS_68	<i>Saccharopolyspora gloriosa</i>	17	G,H	32	19	2.8
69	18AS_69	<i>Rhodococcus wratislaviensis</i>	18	A,B	20	2	2.7
70	18AS_70	<i>Streptomyces microflavus</i>	18	C,D	75	20	4.9
71	18AS_71	<i>Streptomyces albidoflavus</i>	18	E,F	60	12	4.3
72	18AS_72	<i>Streptomyces krainskii</i>	18	G,H	44	11	3.3
73	18AS_73	<i>Streptomyces umbrinus</i>	19	A,B	187	121	20.9
74	18AS_74	<i>Streptomyces bacillaris</i>	19	C,D	19	0	2.3
75	18AS_75	<i>Streptomyces tendae</i>	19	E,F	81	50	6.4
76	18AS_76	<i>Streptomyces olivoverticillatus</i>	19	G,H	40	12	2.2
77	18AS_77	<i>Streptomyces galbus</i>	20	A,B	50	21	4.5
78	18AS_78	<i>Streptomyces tendae</i>	20	C,D	79	36	11.3
79	18AS_79	<i>Streptomyces bungoensis</i>	20	E,F	67	19	4.3
80	18AS_80	<i>Nocardioopsis dassonvillei</i>	20	G,H	46	9	2.7

¹ Fractions are arranged sequentially in columns 1 to 10 in two consecutive rows. Columns 11 and 12 are left empty in all plates for the addition of controls. See plate layout in Figure 2.

² Compounds defined as individual mass and retention time combinations in UPLC/MS analysis with mass ion intensities 10 x over intensity threshold

³ Compounds which are unique to one extract (i.e. do not occur in any other extracts within the superset)

	1	2	3	4	5	6	7	8	9	10	11	12	
Extract 1	A	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10		
	B	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20		
Extract 2	C	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10		
	D	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20		
Extract 3	E	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10		
	F	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20		
Extract 4	G	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10		
	H	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20		

Figure 2. Plate layout of samples

Storage and Use

The superset libraries contain lyophilized pre-fractionated and crude extract material in sealed polypropylene 96-well microtitre plates. They should be stored at or below 4 °C until reconstituted.

Reconstitution should be performed either in neat DMSO or in a DMSO/water solution as appropriate.

- (i) Reconstitution in DMSO: Store below -20 °C. Use within 1 year. Warning: freeze-thawing may result in precipitation of some material and should be avoided as much as possible.
- (ii) Reconstitution in DMSO/water or DMSO/buffer: Store at 4 °C. Use within 2 weeks. (Note: It is best to dissolve in neat DMSO first and then dilute with water or buffer.)

It is recommended that screening be repeated at more than one concentration to identify the most appropriate screening concentration and to help identify fractions with the most potent activity.

Initially, it may be appropriate to screen samples at a Final Assay Concentration (FAC) of ~20 µg/mL.

Example: Each well (containing ~20 µg material) is reconstituted in 50 µL DMSO/water (8:1) to give a stock solution of 0.4 mg/mL. This is diluted twenty-fold in the assay to give FAC of ~20 µg/mL.

Hit Progression

Following testing of the Supersets within an assay system the customer should notify MerLion of hit fractions which can then be sub-fractionated and analysed in order to identify potential target compounds for isolation. The objective is to gain as much information as possible on the likely active compounds and to exclude known compounds (“dereplication”) before committing to large scale isolation, therefore saving considerable time and expense. These services are charged on a fee-for-service basis.

Hit progression is typically as follows (also see Figure 3):

1. Customer tests Superset fractions in assay
 - At appropriate concentration to identify specific hit fractions
 - Notifies MerLion of hit fractions
2. Sub-fractionation of hits
 - Sub-fractions supplied for testing
3. Customer tests sub-fractions in assay
 - Notifies MerLion of hit sub-fractions
4. Dereplication for known compounds
 - LCMS matching of compounds in active sub-fractions versus MerLion database
 - High resolution (HR)-MSMS analysis of non-dereplicated sub-fractions
 - DNP query for matching masses
 - Identification of potential target compounds
5. Estimate yield of target compounds
 - 30 min UPLC/ELSD/MS of crude extract to provide accurate quantification
 - Provide quote for compound isolation and structure elucidation
6. Select target compounds for isolation

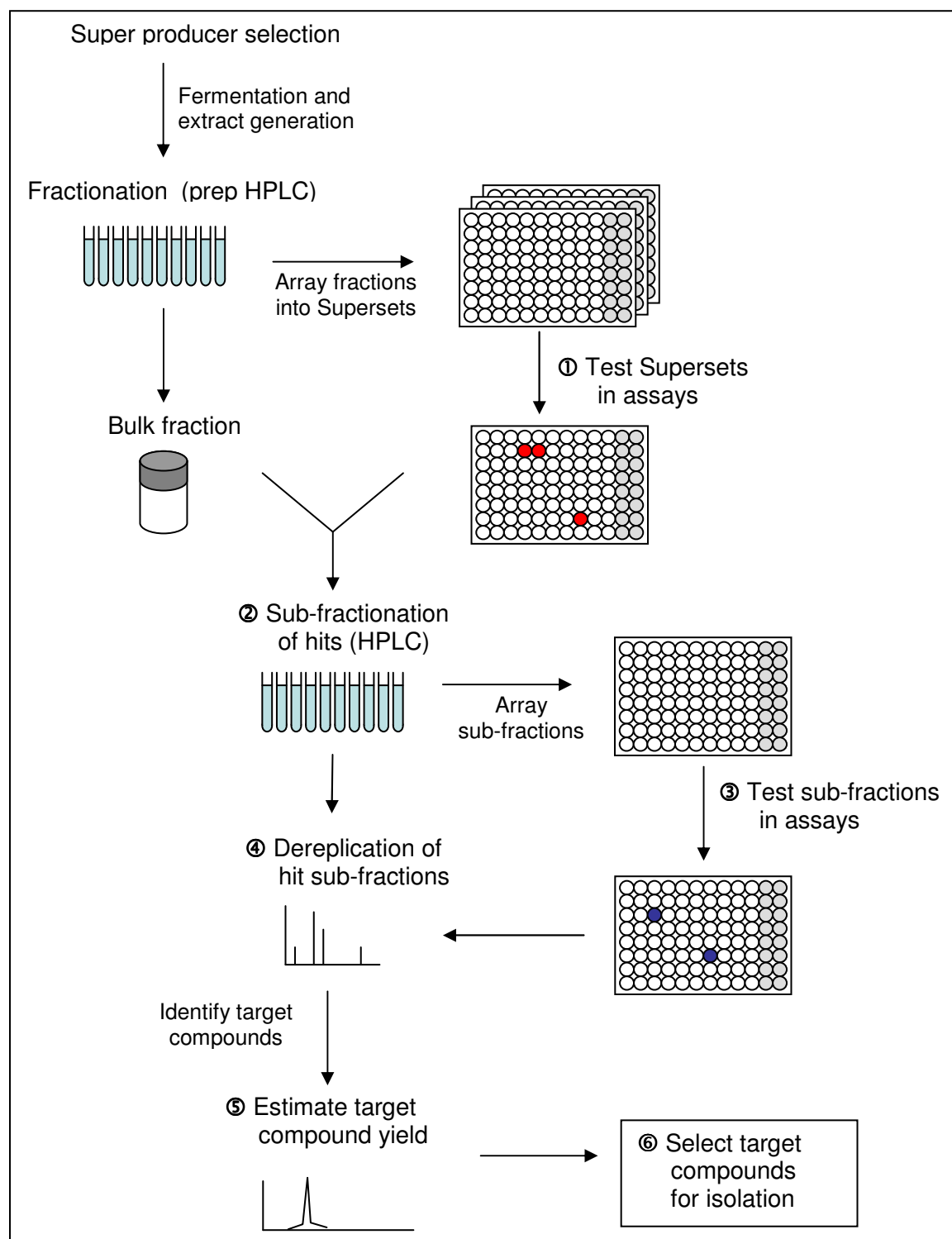


Figure 3. Schematic of Superset generation and hit progression

Safety

This product contains cell free extracts and extract fractions from actinomycete strains isolated from diverse environments and localities. The samples contain uncharacterised chemicals and therefore should be handled as if potentially hazardous.

Always wear personal protective equipment (laboratory gowns, safety spectacles and gloves) and dispose of as laboratory waste.

Other Products

1. Fungal superset (product code 18FS)
2. Pure natural product compound library (product code NP001)