

# Fungal Superset

Product code: 18FS

## Contents:

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

## Introduction

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The fermentation of fungal strains is well known for producing a huge diversity of secondary metabolites, including many which are medicinally important. 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 fungal extract library. To generate this superset 8,579 fungal 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 Fig. 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 fungal 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

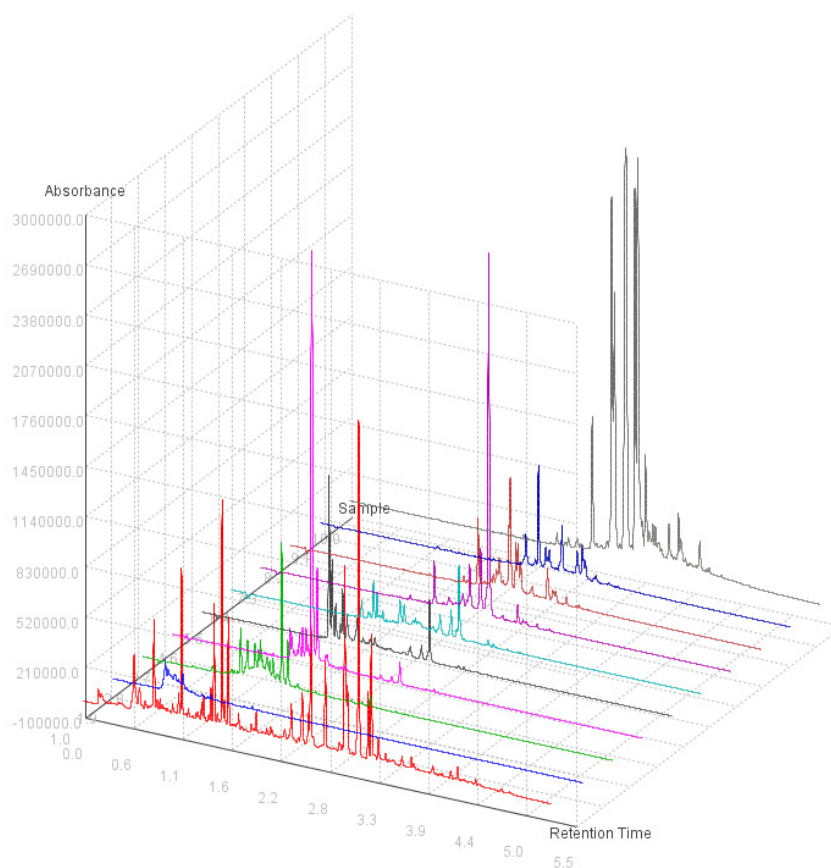
## Fungal Superset Statistics

	Total number in superset	Average number per fraction	Maximum number per fraction
Compounds*	7,987	23	163
Unique Compounds**	4,478	2.8	45

\* 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 strain 18FS-56. Crude extract is shown in red with fractions 2, 4, 6, 8, 10, 12, 14, 16 & 18 behind.

## Fungal Superproducer Strains

No.	Sample code	Strain taxonomy	Plate no.	Rows <sup>1</sup>	Total Count in Each Extract		Average No. of Compounds per Fraction
					Compounds <sup>2</sup>	Unique Compounds <sup>3</sup>	
1	18FS_1	<i>Sarocladium sp.</i>	1	A,B	369	197	37.6
2	18FS_2	<i>Aspergillus sp.</i>	1	C,D	236	54	22.2
3	18FS_3	<i>Aspergillus sp.</i>	1	E,F	93	7	8.1
4	18FS_4	<i>Penicillium sp.</i>	1	G,H	139	27	12.4
5	18FS_5	<i>Penicillium sp.</i>	2	A,B	255	62	28.0
6	18FS_6	<i>Phomopsis sp.</i>	2	C,D	195	50	20.4
7	18FS_7	<i>Aspergillus sp.</i>	2	E,F	358	128	34.7
8	18FS_8	<i>Cladosporium sp.</i>	2	G,H	182	51	22.8
9	18FS_9	<i>Aspergillus sp.</i>	3	A,B	175	29	19.6
10	18FS_10	<i>Aspergillus sp.</i>	3	C,D	124	13	10.5
11	18FS_11	<i>Fusarium sp.</i>	3	E,F	195	45	17.0
12	18FS_12	<i>Aspergillus sp.</i>	3	G,H	180	8	13.3
13	18FS_13	<i>Aspergillus sp.</i>	4	A,B	259	58	23.4
14	18FS_14	<i>Phoma herbarum</i>	4	C,D	204	70	17.1
15	18FS_15	<i>Aspergillus melleus</i>	4	E,F	175	64	13.0
16	18FS_16	<i>Memmoniella echinata</i>	4	G,H	314	82	38.4
17	18FS_17	<i>Aspergillus sp.</i>	5	A,B	63	11	6.0
18	18FS_18	<i>Aspergillus sp.</i>	5	C,D	173	36	17.1
19	18FS_19	<i>Fusarium sp.</i>	5	E,F	129	36	13.4
20	18FS_20	<i>Aspergillus sp.</i>	5	G,H	220	68	20.8
21	18FS_21	<i>Penicillium sp.</i>	6	A,B	192	62	21.8
22	18FS_22	<i>Penicillium sp.</i>	6	C,D	134	40	13.1
23	18FS_23	<i>Acremonium sp.</i>	6	E,F	323	131	36.4
24	18FS_24	<i>Aspergillus sp.</i>	6	G,H	346	68	38.8
25	18FS_25	<i>Penicillium sp.</i>	7	A,B	386	136	37.0
26	18FS_26	<i>Fusarium tumidum</i>	7	C,D	75	23	6.8
27	18FS_27	<i>Aspergillus sp.</i>	7	E,F	152	35	13.5
28	18FS_28	<i>Penicillium sp.</i>	7	G,H	126	30	12.3
29	18FS_29	<i>Penicillium sp.</i>	8	A,B	562	143	73.1
30	18FS_30	<i>Acremonium sp.</i>	8	C,D	752	230	104.6
31	18FS_31	<i>Penicillium sp.</i>	8	E,F	239	26	26.5
32	18FS_32	<i>Aspergillus ochraceus</i>	8	G,H	166	54	17.7
33	18FS_33	<i>Periconia sp.</i>	9	A,B	49	6	4.3
34	18FS_34	<i>Emericella sp.</i>	9	C,D	33	7	2.7
35	18FS_35	<i>Aspergillus sp.</i>	9	E,F	140	38	15.4
36	18FS_36	<i>Pestalotiopsis sp.</i>	9	G,H	103	36	9.7
37	18FS_37	<i>Stachybotrys parvispora</i>	10	A,B	215	70	19.7
38	18FS_38	<i>Penicillium sp.</i>	10	C,D	32	5	3.0
39	18FS_39	<i>Penicillium sp.</i>	10	E,F	141	30	13.8
40	18FS_40	<i>Penicillium sp.</i>	10	G,H	207	60	20.4
41	18FS_41	<i>Chaunopychnis sp.</i>	11	A,B	218	78	26.0
42	18FS_42	<i>Chaetomium sp.</i>	11	C,D	58	12	4.7
43	18FS_43	<i>Penicillium sp.</i>	11	E,F	152	32	15.7
44	18FS_44	<i>Microsphaeropsis sp.</i>	11	G,H	66	8	7.3
45	18FS_45	<i>Plurophoma plurospora</i>	12	A,B	131	25	12.5
46	18FS_46	<i>Aspergillus sp.</i>	12	C,D	194	58	22.2
47	18FS_47	<i>Aspergillus sp.</i>	12	E,F	324	82	34.7
48	18FS_48	<i>Penicillium sp.</i>	12	G,H	119	23	11.5
49	18FS_49	<i>Aspergillus sp.</i>	13	A,B	180	12	16.6
50	18FS_50	<i>Trichoderma sp.</i>	13	C,D	226	54	21.9
51	18FS_51	<i>Penicillium sp.</i>	13	E,F	57	9	5.9
52	18FS_52	<i>Geomyces sp.</i>	13	G,H	48	13	3.3
53	18FS_53	<i>Aspergillus sp.</i>	14	A,B	392	71	44.7
54	18FS_54	<i>Eurotium echinulatum</i>	14	C,D	300	75	29.6
55	18FS_55	<i>Aureobasidium caulivorum</i>	14	E,F	56	13	3.8
56	18FS_56	<i>Eurotium cristatum</i>	14	G,H	262	87	28.0

Table continued...

No.	Sample code	Strain taxonomy	Plate no.	Rows <sup>1</sup>	Total Count in Each Extract		Average No. of Major Compounds per Fraction
					Major Compounds <sup>2</sup>	Unique Major Compounds <sup>3</sup>	
57	18FS_57	<i>Nectria cinnamomea</i>	15	A,B	169	42	20.6
58	18FS_58	<i>Trichothecium roseum</i>	15	C,D	423	104	54.3
59	18FS_59	<i>Emericella rugulosa</i>	15	E,F	174	48	17.5
60	18FS_60	<i>Alternaria targetica</i>	15	G,H	35	4	2.3
61	18FS_61	<i>Nigrospora sphaerica</i>	16	A,B	112	47	8.8
62	18FS_62	<i>Xylaria anisopleura</i>	16	C,D	213	45	24.7
63	18FS_63	<i>Arthrinium phaeospermum</i>	16	E,F	201	43	22.0
64	18FS_64	<i>Acremonium sp.</i>	16	G,H	438	140	48.7
65	18FS_65	Unidentified hyphomycete	17	A,B	322	147	37.0
66	18FS_66	<i>Torula sp.</i>	17	C,D	143	31	15.6
67	18FS_67	<i>Myrothecium sp.</i>	17	E,F	266	92	33.3
68	18FS_68	<i>Eupenicillium sp.</i>	17	G,H	278	64	27.9
69	18FS_69	<i>Penicillium sp.</i>	18	A,B	160	17	16.9
70	18FS_70	<i>Curvularia sp.</i>	18	C,D	182	41	23.1
71	18FS_71	<i>Penicillium sp.</i>	18	E,F	255	48	22.9
72	18FS_72	<i>Aspergillus sp.</i>	18	G,H	343	21	50.4
73	18FS_73	<i>Aspergillus sp.</i>	19	A,B	349	50	46.6
74	18FS_74	<i>Pseudallescheria boydii</i>	19	C,D	178	34	19.9
75	18FS_75	<i>Eupenicillium sp.</i>	19	E,F	339	78	28.2
76	18FS_76	<i>Emericellopsis sp.</i>	19	G,H	437	177	46.9
77	18FS_77	<i>Trichoderma longibrachiatum</i>	20	A,B	304	103	33.6
78	18FS_78	<i>Emericella sp.</i>	20	C,D	243	61	26.4
79	18FS_79	<i>Pestalotiopsis sp.</i>	20	E,F	134	7	14.8
80	18FS_80	<i>Penicillium sp.</i>	20	G,H	262	56	36.3

<sup>1</sup> 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 Figure 2).

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

<sup>3</sup> 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

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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.

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 an ~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 analysis

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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

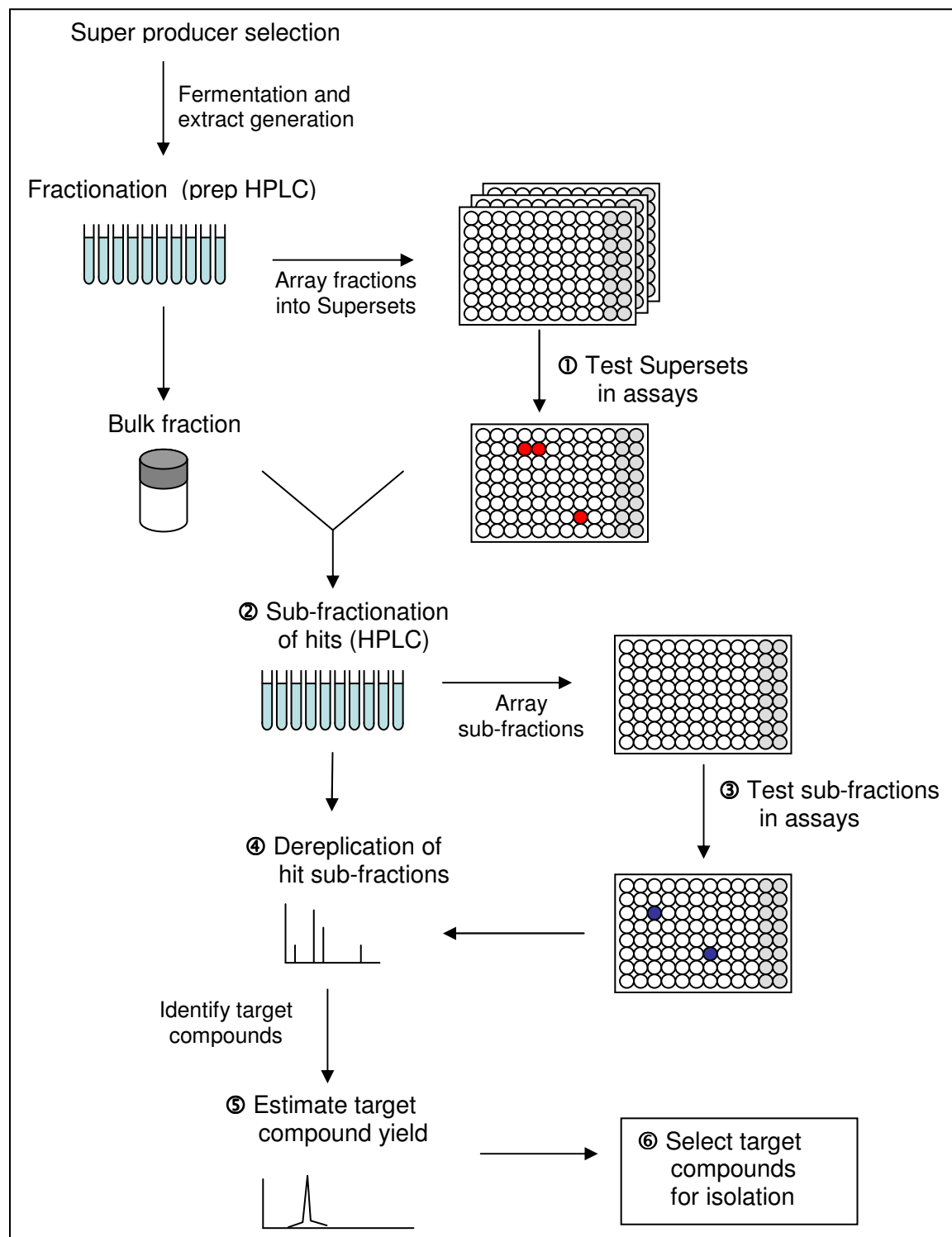


Figure 3. Schematic of Superset generation and hit progression

## **Safety**

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This product contains cell free extracts and extract fractions from fungal 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**

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1. Actinomycete superset (product code 18AS)
2. Pure natural product compound library (product code NP001)