

Ultra-high throughput functional enrichment of large monoamine oxidase (MAO-N) libraries by fluorescence activated cell sorting

Supporting Information

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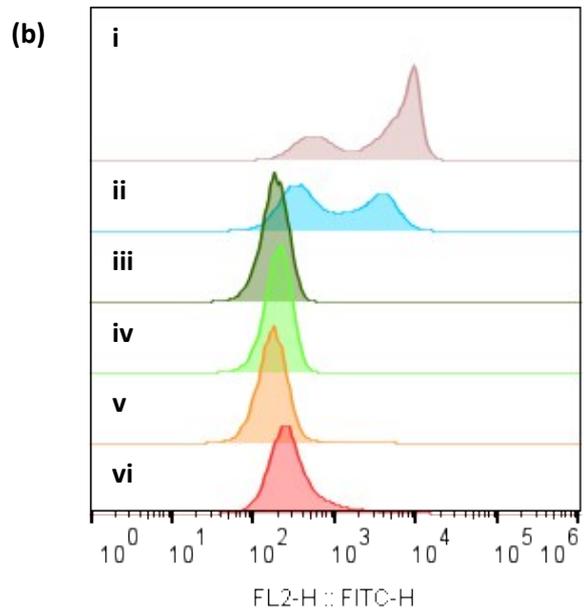
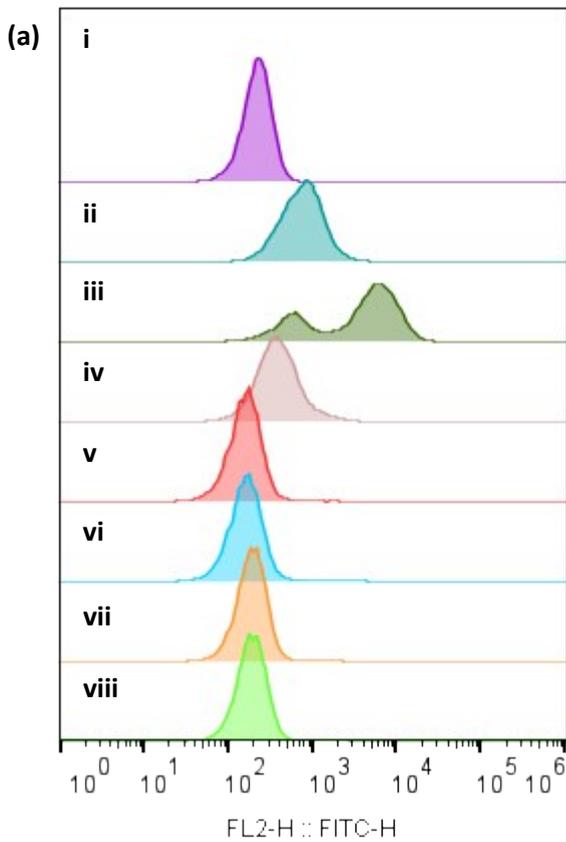
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Effect of freeze-thaw step on probe uptake

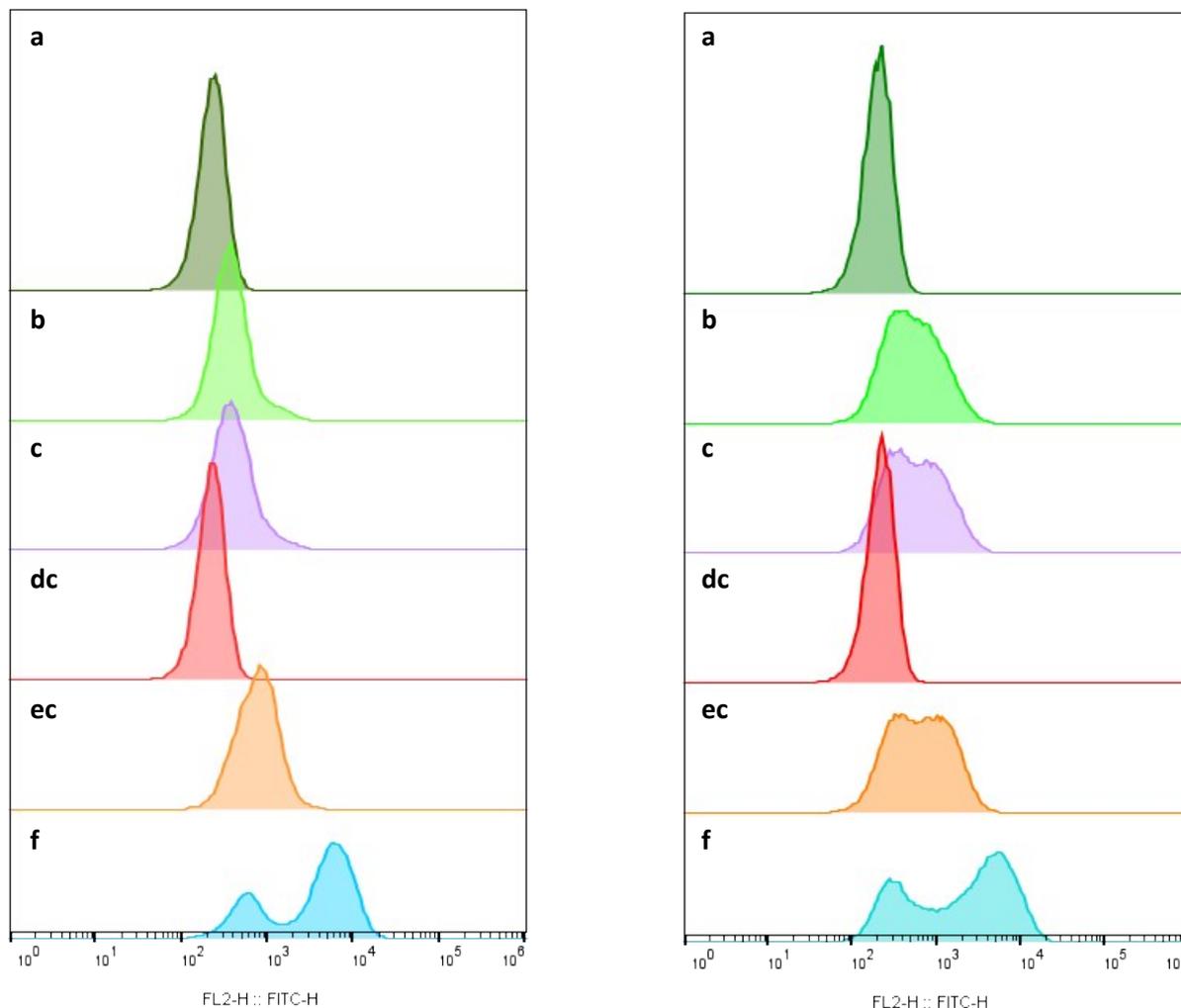


(a)	Sample	Population	Mean FITC-H	FITC-H CV
i	Induced freeze-thawed cells only	Total gated	232	36
ii	Induced freeze-thawed + probe	Total gated	861	56
iii	Induced freeze-thawed + probe + AMBA	Positive	6512	51
iv	Induced Non-induced freeze-thawed + probe + AMBA	Total gated	464	74
v	Induced + probe	Total gated	183	98
vi	Induced + probe + substrate	Total gated	202	155
vii	Non-Induced + probe	Total gated	217	81
viii	Non-Induced + probe + substrate	Total gated	222	94

(b)	Sample	Population	Mean FITC-H	FITC-H CV
i	Induced freeze-thawed + probe + H ₂ O ₂	Positive	7240	42
ii	Non-Induced freeze-thawed + probe + H ₂ O ₂	Total gated	3591	53
iii	Induced freeze-thawed + H ₂ O ₂	Positive	189	38
iv	Non-Induced freeze-thawed + H ₂ O ₂	Total gated	208	37
v	Induced + probe + H ₂ O ₂	Total gated	222	181
vi	Non-induced + probe + H ₂ O ₂	Total gated	492	223

Figure S1. (a) Comparison between the fluorescence of induced and non-induced *E. coli* cells expressing MAO-N before and after a freeze-thaw step. (b) Effect of freeze-thaw step on probe uptake of induced and non-induced cells determined by spiking with 10 μM H_2O_2 .

Comparison between K12 and B strains of *E. coli*

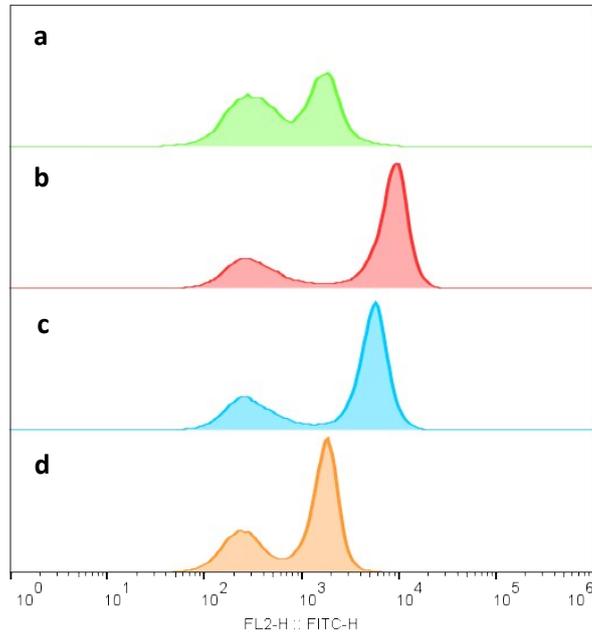


	Sample	Population	Mean FITC-H	FITC-H CV
a	T7 Non-Induced	Total gated	237	36
b	T7 Non-Induced + probe	Total gated	445	69
c	T7 Non-Induced + probe + AMBA	Positive	464	74
d	T7 Induced	Total gated	232	36
e	T7 Induced + probe	Total gated	861	56
f	T7 Induced cells + probe + AMBA	Positive	6512	51

	Sample	Population	Mean FITC-H	FITC-H CV
a	5-alpha Non-Induced	Total gated	211	40
b	5-alpha Non-Induced + probe	Total gated	694	84
c	5-alpha Non-Induced + probe + AMBA	Positive	758	86
d	5-alpha Induced	Total gated	227	38
e	5-alpha Induced + probe	Total gated	859	84
f	5-alpha Induced cells + probe + AMBA	Positive	5340	68

Figure S2. Comparison between (a) T7 express (B strain) and (b) 5-alpha cells (K12 strain) as hosts for the oxidase activity assay. For detection of B strain cells, it was necessary to reduce the FSC threshold to 1.5%.

Identification of an assay quenching agent



	Sample	Population	Mean FITC-H	FITC-H CV
a	No quench, t=5	Positive	1721	40
b	No quench; t=30	Positive	8716	38
c	10 mM sodium pyruvate at t=5 ^a	Positive	5589	38
d	10 mM L-ascorbic acid at t=5 ^a	Positive	1739	36

Figure S3. Fluorescence of *E. coli* cells under the optimised assay conditions after (a) 5 minutes incubation with AMBA; (b) 30 minutes incubation with AMBA; (c) quenching with 10 mM sodium pyruvate after 5 minutes incubation with AMBA; (d) quenching with 10 mM ascorbic acid after 5 minutes incubation with AMBA. ^aCells were analysed 30 minutes after quenching.

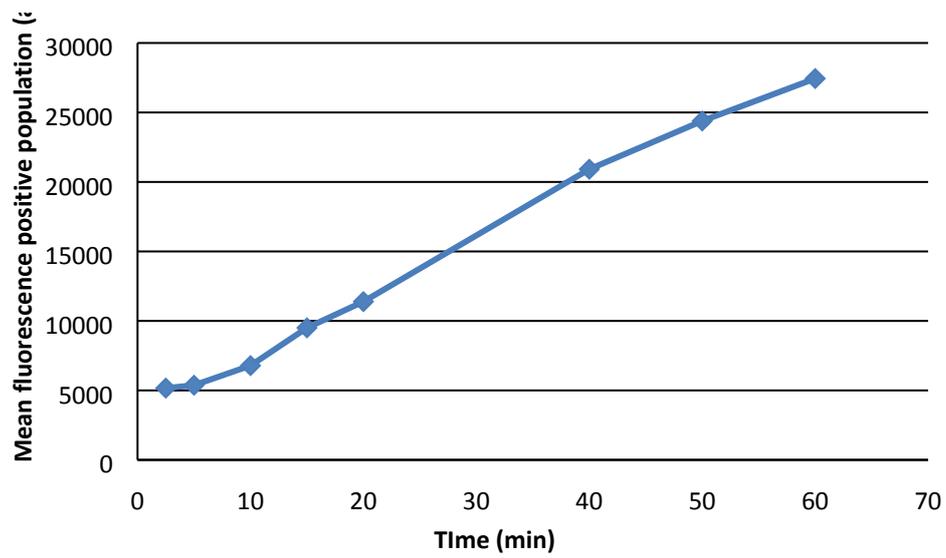


Figure S4. Mean fluorescence of *E. coli* cells under the optimised assay conditions quenched with 10 mM ascorbic acid at different timepoints after addition of AMBA.