

MARINE FUNGAL EXTRACTS: VALUABLE SOURCES OF ANTI-CANCER AND/OR ANTI-AGEING AGENTS

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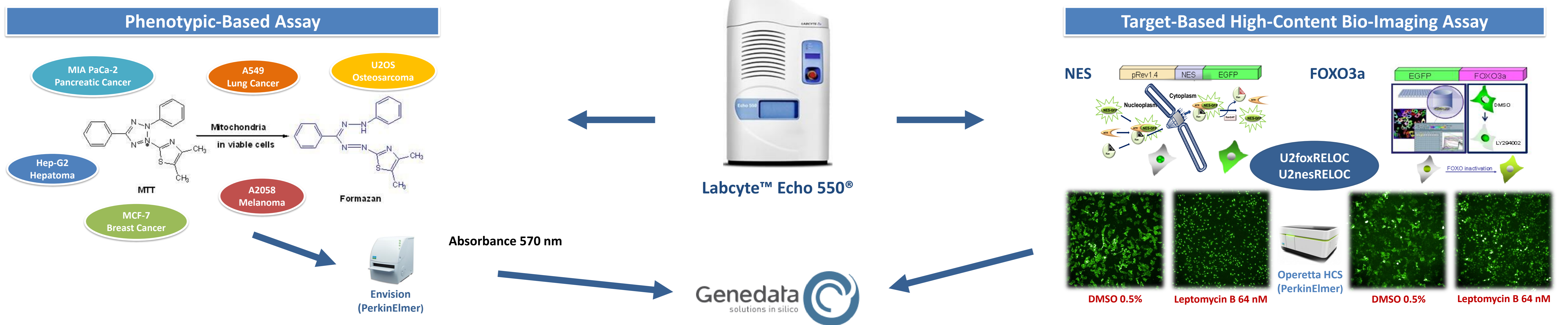
MEDINA & Marine Fungi

Filamentous fungi account for more than 42% of bioactive compounds developed as antibiotics and antifungal agents among other applications [1]. Numerous reports of marine fungi (*sensu strictu* or not) suggest that they are an excellent source of New Chemical Entities (NCEs), often associated with a variety of different biological activities [2]. Fundación MEDINA possesses one of the largest and most diverse Natural Product (NP) libraries in the world featuring more than 190,000 strains and 200,000 extracts. As of 2018, Fundación MEDINA has centred part of attention in expanding its existing library with marine extracts, 2,320 of these extracts are of marine fungal origin.

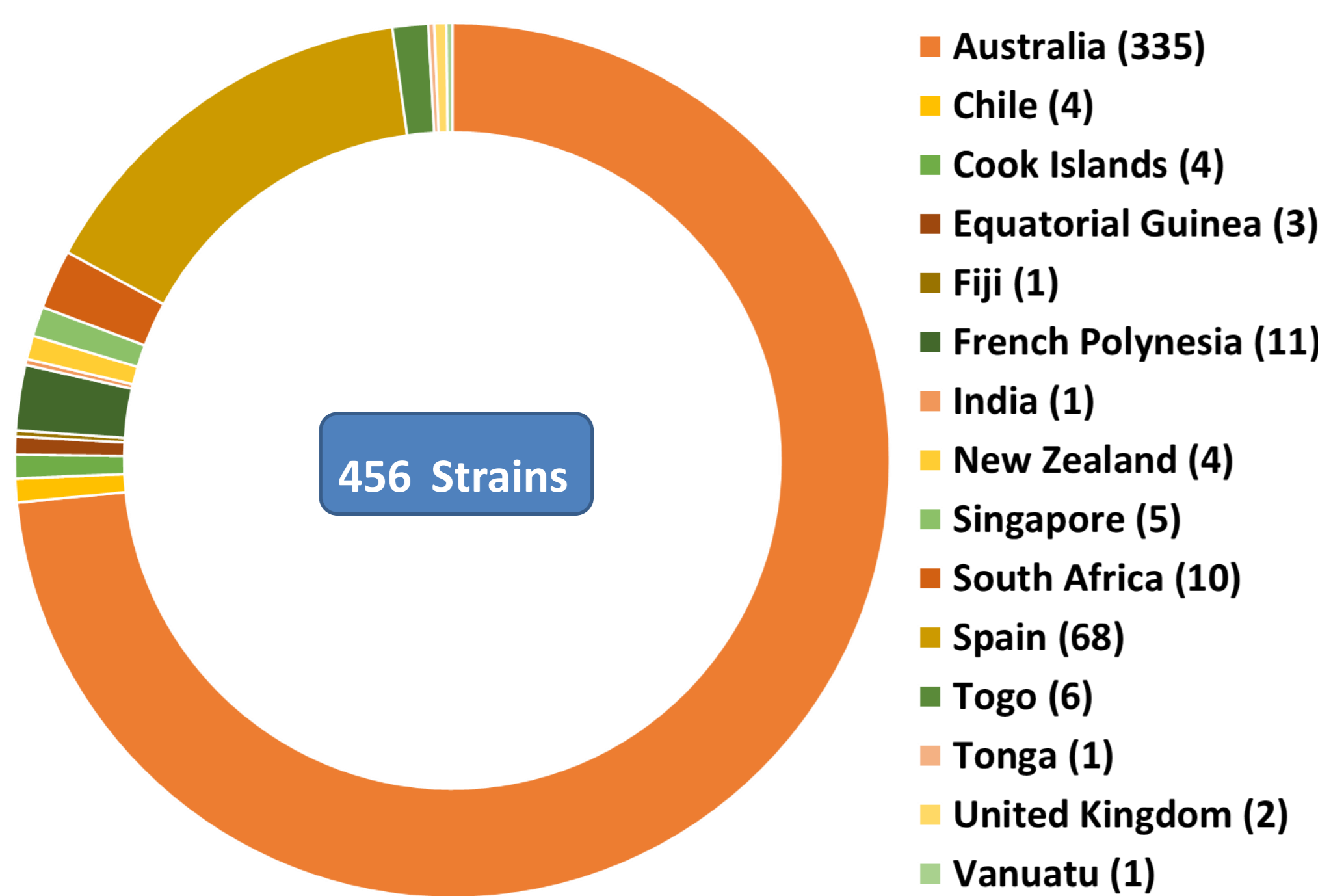


Phenotypic-Based & High-Content Bio-Imaging Assays

Recently, 2 types of assays have successfully been miniaturized and automated using the Labcyte™ (now Beckman) Echo® 550. It's Acoustic Droplet Ejection (ADE) technology has proven to be extremely precise when transferring samples in low percentages of DMSO for cell-based assays [3].



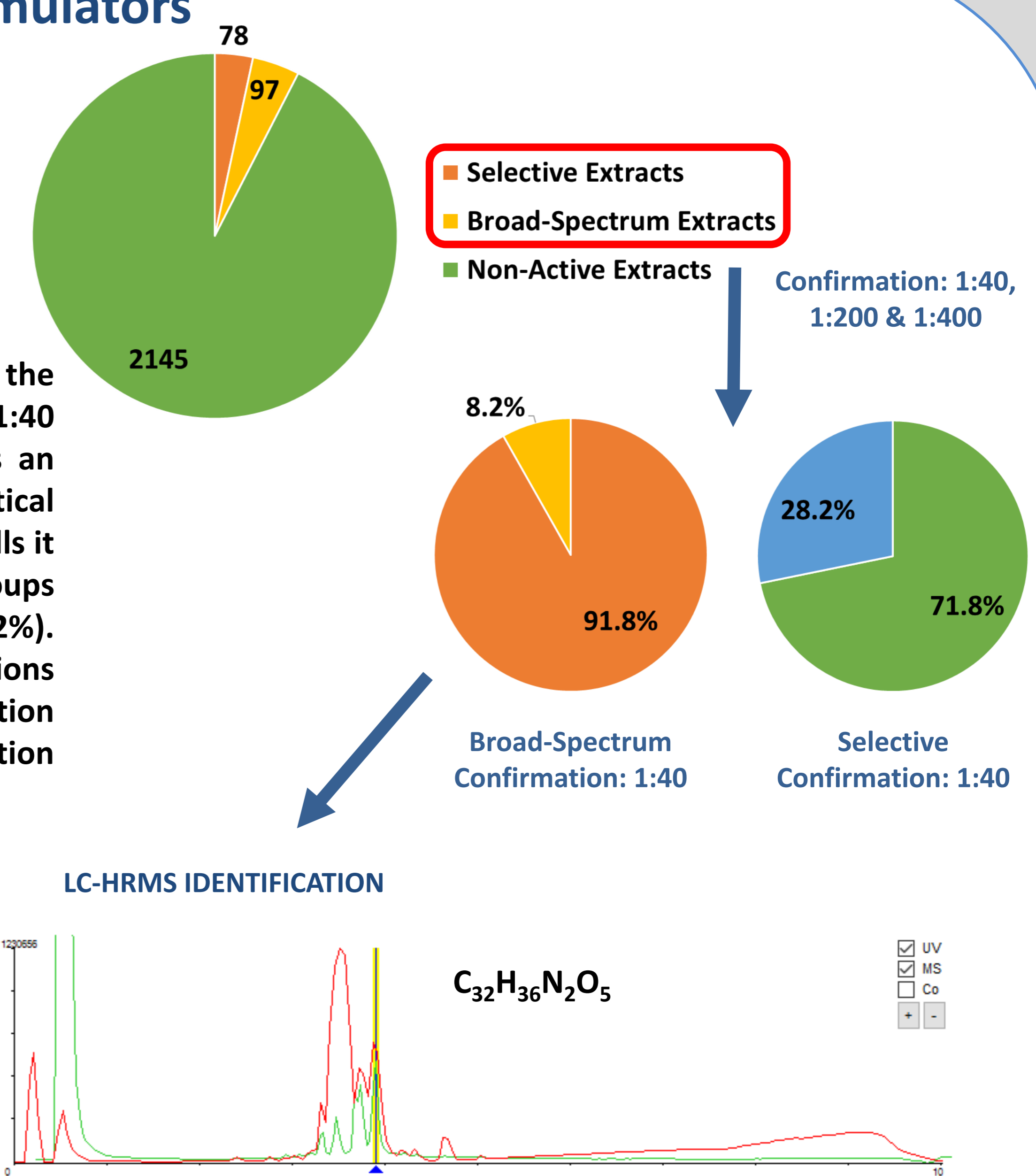
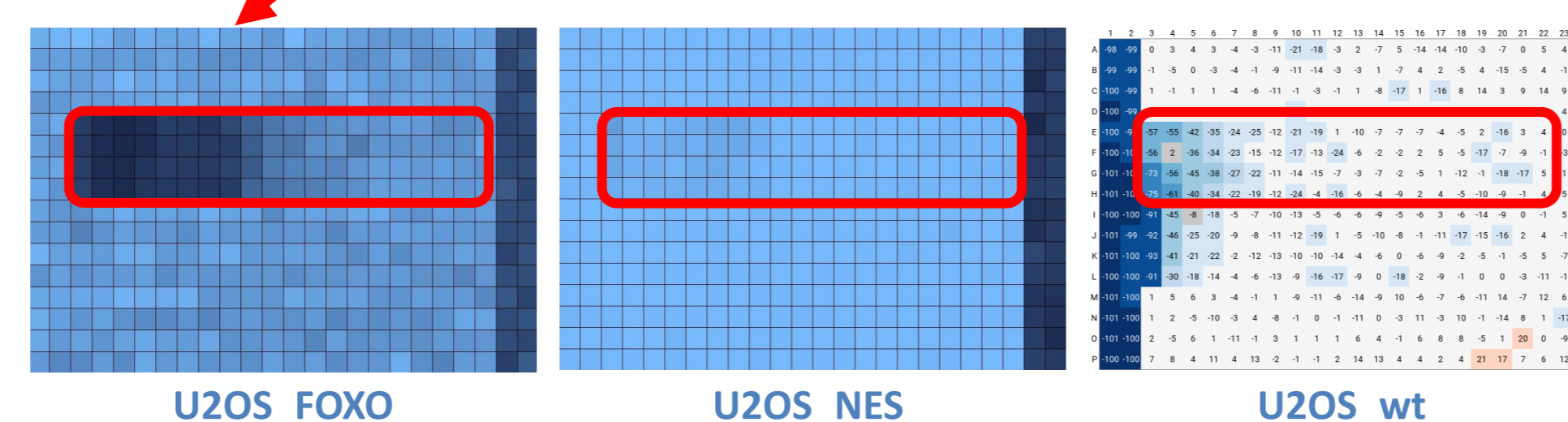
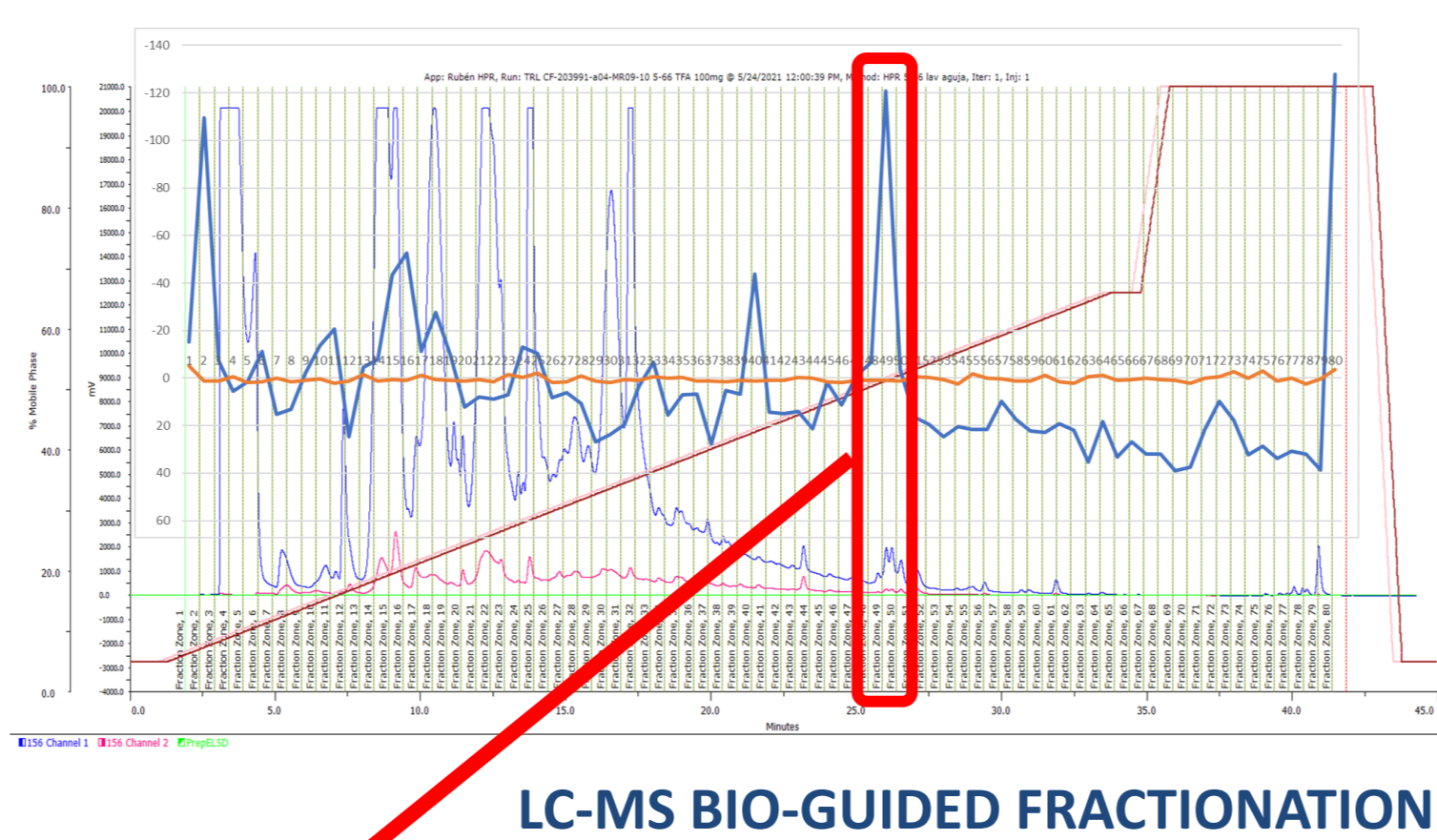
Marine Fungal Extracts as Nuclear Translocation Stimulators



Up to date, 7 strains with interesting activity as nuclear translocation stimulators have been through this process and several compounds have been identified, purified and assayed in dose-response curves against U2foxRELOC cell line (to test FOXO3a nuclear translocation), U2nesRELOC cell line (as a counter cell line, to discard non-specific nuclear translocation stimulators) and U2OS_wt (cell line as reference to test the toxicity of active compounds) [6]. Curves: 20-point curves, 1/2 dilutions starting at 100 μM in quadruplicate.

Compound $C_{32}H_{36}N_2O_5$ exhibited medium toxicity against U2OS_wt at high concentrations only, strong activity as a FOXO3a nuclear translocation stimulator and no activity as a NES nuclear translocation promoter, making it FOXO3a specific.

2,320 Marine fungal extracts were assayed in an MTT assay against the cell lines mentioned above and in a nuclear translocation assay at a 1:40 dilution. We study FOXO3a localization due to the fact that it is an evolutionary conserved tumour suppressor protein that plays a critical role in ageing and different metabolic processes [4] and in cancer cells it can present an aberrant localization [5]. Results generated two groups of active extracts: selective (78, 3.4%) and broad-spectrum (97, 4.2%). These 2 groups were then assayed again at 1:40, 1:200 & 1:400 dilutions as a confirmation screen and analysed by means of High-Resolution Mass Spectrometry (HRMS). Confirmation hit rates at 1:40 dilution were: selective 56 (71.8%) and cytotoxic 89 (91.8%).



22 Marine fungal strains have been prioritized for regrowth to then initiate a bioassay-guided fractionation coupled to Liquid Chromatography – Mass Spectrometry (LC-MS) to identify the molecules responsible for the activity. Molecules highly cytotoxic could be used to develop antibody-drug conjugates (ADCs) and, molecules with cell-specific activity could be good candidates as new anti-tumour drugs.

General Conclusions

- One compound was identified as a specific FOXO3a nuclear translocation stimulator, validating the target-based screening platform with NPs. Furthermore, marine fungal extracts represent a rich source of NP extracts with anti-cancer and/or anti-ageing properties by stimulating the nuclear translocation of FOXO3a.
- NP extracts continue to be one of the most important sources for drug discovery, which is why it is of great importance to promote new campaigns for the detection of NCEs and others to study molecules lacking in described biological activity.

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