

P001 An integrated platform for high throughput expression of human proteases

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Parallel expression of large numbers of gene products is at present the major bottleneck in the characterisation of protein structure and function. We have applied our expertise in biochemical assay configuration for high throughput screening to develop an automated protein expression platform, by focusing our efforts on cloning, expression, purification and functional characterisation of the entire complement of human proteases, of one of the largest therapeutically relevant enzyme classes. A milestone in the process has been the construction of a non-redundant comprehensive database for all human proteases, by assembling and filtering public domain information and in-house generated data. This database comprises 571 unique annotated entries. We have in parallel set up the procedure for PCR amplification and cloning of the protease genes and we have already created a repository of 271 full length cDNAs encoding for proteases. Besides, parameters to transfect and culture the recipient host in the miniaturised conditions have been established. Experimental trials have defined a validated and reliable fully-automated robotic purification procedure to recover recombinantly expressed pure proteases in 96 MTP format. Finally, a fluorogenic readout strategy either with broad-specific or with class-specific substrates has been designed to detect the enzymatic activity of the recovered proteases in 384 MTP format.