

## HIGH QUALITY PROTEIN PRODUCTION - FILGRASTIM (HUMAN MET-GCSF)

A variety of expression plasmids with unique secretion leaders were screened in combination with an array of host strains to identify a *P. fluorescens* production strain expressing high titers of soluble, active protein. A deletion of the N-terminal methionine (des-Met), likely due to protease activity, was observed in some high expressing production strains. To identify an optimal production strain, samples from small scale culture lysates were screened using mass spectrometry to identify those strains with very low levels of des-Met. Host strains with specific protease gene deletions were identified that enable periplasmic expression of full length Met-GCSF in

a soluble form, obviating the need for costly refold processes.

Following production strain selection, optimized fermentation and purification processes were developed, monitoring G-CSF activity (binding to G-CSF receptor) and quality (%Des-Met) throughout the development. The soluble expression strategy allowed for the development of a scalable periplasmic release step, enriching the target prior to capture chromatography. The final purified target protein (Pfenex filgrastim) shows a high degree of similarity to the reference product Neupogen. Peptide mapping results show similar

peptide fragments identified in mirrored mass spectrometry results. Protein folding and functionality were also extensively analyzed. The binding of Pfenex filgrastim to the G-CSF receptor demonstrated similar KD and multiple lots of Pfenex filgrastim showed similar binding rates when compared to multiple lots of Neupogen when analyzed by biolayer interferometry (BLI). Enabled by the Pfenex platform, a high quality filgrastim molecule was produced which not only shows high similarity to Neupogen, but also shows high similarity to Neulasta upon pegylation of the filgrastim drug substance intermediate.

Strain Screening Results (0.5 mL scale)

Host	Yield (mg/L)	des-Met (%)
MID4697	247	0.1
DC0859	173	0.5
DC1020	281	1.0
DC1068	204	1.4
DC1021	146	1.6
DC469	321	1.7
DC518	335	2.7
DC1023	39	3.0
DC0977	191	3.2
DC1011	193	5.0
MID4707	299	6.0
DC0955	302	10.0
DC0956	208	15.0
DC1041	331	20.0
DC1026	350	25.0
DC1010	196	30.0
DC488	372	35.0
DC1033	164	40.0
DC696	192	45.0
DC1032	151	80.0
DC1034	132	100.0

Peptide Mapping Comparison

