

S1 | The results of the analysis with several widely used tools. From the 10 tools tested, only 4 tools were able to retrieve all correct annotations while correctly avoiding the GO terms associated with the 'NOT' qualifier. Some tools failed by including the 'NOT' annotations in their analysis; others even failed to retrieve the correct annotations.

Tool	Input type	Results	Reference	Enrichment and/or depletion analysis
Onto-Express	Gene symbol	Did not include any NOT terms; all correct annotations found	Khatri <i>et al.</i> 2002, <i>Genomics</i> 79 , 266–270.	Both enrichment and depletion
GoMiner	Gene symbol	All NOT terms included in results	Zeeberg 2003, <i>Genome Biology</i> 4 , R28.	Only enrichment
GeneMerge	UniProt accession	Two NOT terms included in results	Castillo-Davis 2002, <i>Bioinformatics</i> , 19 , 891–892.	Described as performing only enrichment but could not run
FuncAssociate	Entrez Gene ID	Did not find any term, but did not find all valid annotations either	Berriz <i>et al.</i> 2003, <i>Bioinformatics</i> , 19 , 2502–2504	Only enrichment
GOTM	Gene symbol	Found most of the terms, not all. Could be due to outdated database.	Zhang <i>et al.</i> 2004, <i>BMC Bioinformatics</i> , 5 , 16.	Described as performing only enrichment but could not run
FatiGO	Gene symbol	Did not include any NOT terms; all correct annotations found	Al-Shahrour <i>et al.</i> 2004, <i>Bioinformatics</i> , 20 , 578–580.	Both enrichment and depletion
Gostat	Gene symbol	Did not include any NOT terms; all correct annotations found	Beissbarth <i>et al.</i> 2004, <i>Bioinformatics</i> , 20 , 1464–1465.	Both enrichment and depletion
GoToolBox	UniProt accession	Did not include any NOT terms; all correct annotations found	Martin <i>et al.</i> 2004, <i>Genome Biology</i> , 5 , R101.	Both enrichment and depletion
GoSurfer	Entrez Gene ID	Annotations are not up-to-date. Finds only three genes with any annotations, although all input genes are well-annotated in GO.	Zhong <i>et al.</i> 2004, <i>Applied Bioinformatics</i> , 3 , 1–5.	Could not run (does not accept custom arrays), no info in documentation
DAVID	Entrez Gene ID	Found NOT annotations for genes <i>RSF1</i> and <i>EDF1</i>	Dennis <i>et al.</i> 2003, 4 , <i>Genome Biology</i> , R60.	Only enrichment