Challenging times for bioinformatics

SIR—On July 28, 1995, the timely public release of the complete DNA sequence of *Haemophilus influenzae* Rd made the world of biology richer, in one day, by 1,830,137 characters of genetic information encoding a complete bacterial construction kit of 1,743 predicted protein genes[1]. While marveling at the advances in sequencing technology one wonders how biologists will cope with the onslaught of data from this and other genome projects. To illustrate recent advances in informatics technology, we have analyzed the open reading frames of *H.influenzae* with the aim of adding as much biological knowledge as possible to each predicted protein sequence. The computer runs were completed in three days using the GeneQuiz software system[2] which identifies likely protein functions from amino acid sequence information in a completely automated fashion. This resulted in a new set of 148 putative protein functions in *H.influenzae* (see Table) and confirmed most of the 1,007 already identified by TIGR[1].

A thorough and reliable analysis of several thousand protein sequences in one week requires more efficient tools than are currently used for routine database searches. Our approach to high efficiency biosequence informatics mirrors that of high-throughput sequencing: to start with existing technology, add robotics, and focus on solutions for rate-limiting steps. In computerized sequence analysis, the analogue of a robot is a program which embodies key rules learned from a human expert and applies these at high speed. The GeneQuiz system was developed during the analysis of yeast chromasomes and mycoplasma sequences[3]. It combines such an expert module with existing methods, such as programs for similarity searches in sequence databas-

New protein functions in *H.Influenzae* identified by automated sequence analysis.

0017		Formate acetyltransferase 1
0052		C4-dicarboxylate-binding periplasmic
0053	3D	RspB. Starvation sensing
0121		UDP-*N*-acetylmuramate alanine ligase
0126		ABC transporter
0131		Major ferric iron binding
0135		AraJ. Processing of arabinose polymers
0136		ThdF. Possible thiophene and furan oxidation
0146		C4-dicarboxylate-binding periplasmic
0164	#f	NADH:ubiquinone oxidoreductase subunit A
0167		NADH:uniquinone oxidoreductase
0168	#f	NADH:uniquinone oxidoreductase
0182		Glucokinase
0183		Na(+)-linked D-alanine glycine permease
0184		Esterase D
0258	#f	Glycosyl transferase
0270		Nifr3. Nitrogen fixation
0281		Proline/betaine transporter PPII
0291		Mercuric transport. Periplasmic component
0299		Prepilin peptidase dependent protein D
0323		Lactoylglutathione lyase
0333		tRNA (uracil-5-)-methyltransferase
0336		Mog. Molybdopterin biosynthesis
0345	3D	YojB. 4Fe-4S iron sulphur centre, electron transfer
0346		MauN. 4Fe-4S iron sulphur centre, electron transfer
0347		Cytochrome *c*
0350		AmpG. Permease in beta-lactamase induction
0364		Penicillin-binding protein 5
0376		HesB. Nitrogen fixation cluster
0388		*O*-syaloglycoprotein endopeptidase
0389		Slp. Outer membrane
0393		GTP-binding
0409		TagE. Cell wall degradation
0412		Drap deaminase
0415		Thiamine biosynthesis
0417		Thiamine biosynthesis
0418		Proline/betaine transporter PPII
0456		ATP-binding pyrimidine kinase
0464		Ribose 5-phosphate isomerase A
0469		Histidinol dehydrogenase
0493		IS150 transposase
0494		NapA. Acid phosphatase
0508		MenG. Menaquinone biosynthesis
0509		MenA. Menaquinone biosynthesis
0519		NupC. Nucleooside permease.
0520		Activator of pyruvate formate-lyase 1
0565		Phosphoglycolate phosphatase, chromosomal
0572	3D	PmpA peroxisomal membrane
	#m	protein + Glutharedoxin
0574	3D	Peptidyl-prolyl *cis-trans* isomerase
0597		Hydrolase
0624	#m	Fmv+Fmu ATP-binding protein
0626		Large conductance mechanosensitive channel
0634		Nifr3. Nitrogen fixation
0647		Mg(2+) transport ATPase C
0658		ABC transporter
0718		VacJ. Intercellular spreading
0723		TrkH. Potassium uptake
0731	#f	Phenoxazinone synthase
0740	3D	Phosphomannomutase
0756		NlpD. Cell wall degradation
0773		3-oxoadipate CoA-transferase subunit B
0831		Cell wall formation
0837		CpxR. Transcriptional regulator
0852		Multidrug resistance protein B
0858		Methenyl-THF synthetase
0860		SpoU. rRNA methylase
0864	3D	Elongation factor G
0881		Octaprenyl-diphosphate synthase
0906		Nitrogen fixation
0929		Glutathionylspermidine synthetase
0934		NrfF. Cytochrome *c* biogenesis
0935		HelX. Cytochrome *c* biogenesis
0936		Ccl1. Cytochrome *c* biogenesis
0948		Virulence-associated protein B
0955		Ttk. Transcriptional regulator
0959		Chitobiase
0961		Histidine triad zinc binder
0963		FAD synthetase
0964		MviN. Virulence factor
0965		30s ribosomal protein S20
0977		Cell filamentation
0979		Nifr3. Nitrogen regulation
0988	3D	3-Isopropylmalate dehydratase
1001		Sporulation
1004		Peptidyl-prolyl *cis-trans* isomerase
1007		LytB. Penicillin tolerance
1010		3-hydroxyisobutyrate dehydrogenase
1019		Thiamine-binding. Periplasmic
1020		Transport system permease
1021		ABC transporter
1027	3D	L-Xylulose Kinase
1028		C4-dicarboxylate-binding. Periplasmic
1031		Malate dehydrogenase
1032		IclR family of transcriptional regulators
1043	3D	Ferredoxin
1070	#f	HrpA. ATP-dependent helicase
1089		ABC transporter
1090		HelB. Cytochrome *c* biogenesis
1091		HelC. Cytochrome c biogenesis
1092		Cytochrome c biogenesis
1093		Pyoverdine transport. Cytochrome *c* biogenesis
1094		Ccl1/Ycf5. Cytochrome *c* biogenesis
1095		HelX. Cytochrome *c* biogenesis
1104		Sialic acid transporter
1125		Transaldolase B
1147		IIA-ntr. Nitrogen fixation.
1148		ABC transporter
1153	3D	Hypoxanthine phosphoribosyltransferase
1155		Activator of anaerobic ribonucleoside-triphosphate reductase
1165		Thioredoxin
1203		Phosphate acetyltransferase
1252		ABC transporter
1280		IS150 transposase
1289		Nap57/cbf5/yhba family
1300		ABC transporter
1301		Carbonic anhydrase
1309	3D	Ferredoxin
1315		Sodium/myo-inositol cotransporter
1337	3D	Phosphomannomutase
1342		ABC transporter
1349		Bacterioferritin
1364		Transcriptional regulator
1366		Acyl carrier phosphodiesterase
1368		Zinc proteinase
1437		Exodeoxyribonuclease small subunit
1439		Transketolase
1454		CycZ. Cytochrome biogenesis
1463	3D	Phosphomannomutase
1476		Transcription repressor
1546		DNA repair
1555		FtsX. Cell division
1590		DNA polymerase III subunits γ and τ
1607		HemM. Glutamyl-tRNA dehydrogenase
1648		Amidotransferase
1652		Extracellular endopeptidase
1665		Pepsinogen A-4
1684	3D	Polyferredoxin
1688		NADH:Ubiquinone oxidoreductase
1693		ModA. Molybdate-binding periplasmic
1694		Molybdenum transport
1695		*N*-acetylmannosamine transferase
1696		Glycosyltransferase in succinoglycan biosynthesis
1698		Glucosyltransferase in lipopolysacharide synthesis
1721		IS904 transposase
1723		Nitrogen fixation cluster, ATP-binding
1730		Bifunctional urea amidolyase

The 1680 protein sequences were provided as of July 31, 1995, by the TIGR Internet server[1].Numbers are the gene identifiers in the HIDB database, where 234 means HI0234.#f: Indicate where two or more sequence fragments have been merged due to the identification of putative frameshifts. #m: Potentially incorrectly merged sequences (missing stop codon) are indicated by giving two functions separated by a '+'. 3D: Proteins for which significant sequence similarity was detected to a protein of known 3D structure allowing fairly accurate three-dimensional models to be built[6]. 18 (out of 148) of the new functions identified are the results of database matches with proteins that entered the databases between 16 May 1995 (the submission date of the TIGR analysis) and Aug 3, 1995. In addition we have classified an additional 55 new functions from database hits as 'tentative' and another 254 as 'marginal'. These results are accessible over the World Wide Web (http://www.sander.embl- heidelberg.de/genequiz/haemophilus.html) in the form of 'living' features which are automatically updated as new data arrives.

es, enhanced by frameshift detection, pattern searches, family analysis, structure prediction, knowledge navigation tools and information parsers.[4]

Proposed protein functions (see table and the TIGR table[1]) are, for the most part, implied from the known functions of related proteins. Depending on the species, context and evolutionary distance, however, the similarity of function between related proteins varies substantially. The assigned functions should therefore be treated as plausible hypotheses (or "putative identification"[1]), ranging from certain to approximate.

The 148 new functions include, among others, 65 enzymes, 16 transporters and 7 proteins involved in electron transfer. These have increased the overall assignment levels for *H. influenzae* to 9% - implied known 3D structure, 65% - clear functional assignment, 82% - clear database similarity hit. The distribution of functional roles, classified in nine classes similar to those of Riley[5], is very similar to that of the currently available *Escherichia coli* sequences.

This analysis of *H.influenzae* protein sequences illustrates how automated computational sequence analysis can be rapid, exhaustive and accurate up to a level normally reached through extensive work of sequence analysis experts. Keeping pace with future advances in sequencing technology by imaginative informatics methods will be a continuing challenge.

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1. Fleischmann, R.D. et al., *Science* **269** 496-512 (1995).
2. Scharf, M. *et al. in Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology* (eds Altman, R., Brutlag, D., Karp, P., Lathrop, R. & Searls, D.) 348-353 (AAAI Press, Menlo Park, CA, 1994).
3. Bork, P. *et al., Molec. Microbiol.* **16** 955–967 (1995).
4. Etzold, T. & Argos P. *Comput. appl. Biosci.* **9** 49–57 (1993).
5.Riley, M., *Microbiol.Rev.* **57** 862 (1993)
6. Vriend, G., *J.molec.Graph.* **8** 52-56 (1990).

Late experience alters vision

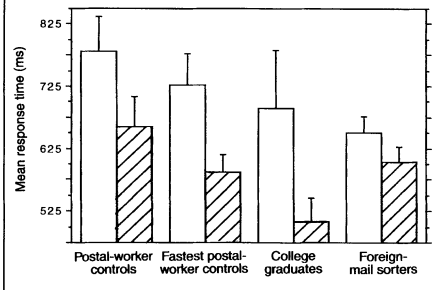
SIR — A visual target is said to 'pop out' if it is detected quickly and without serial search. Targets that differ from distractors by the presence of primitive visual features (for example colour, orientation) pop out whereas targets that differ based on other features do not[1]. Treisman and Gelade[1] proposed that primitive features are processed in specialized modules, called feature maps. This hypothesis is consistent with neuroscientific evidence for spatially segregated cortical areas specialized for processing such features[2].

A similar pop-out effect occurs for letters and digits: a letter target is detected more efficiently among digits than among letters (and vice versa)[3], suggesting that category information about alphanumeric stimuli is computed automatically and in parallel in much the same way as information about primitive visual features is processed. Consistent with this hypothesis, brain-damaged patients can be selectively impaired at letter recognition over number recognition[4] and vice versa[5], suggesting that letter and digit recognition are carried out by distinct maps. Recordings from human extrastriate visual cortex using chronically implanted electrodes also suggest segregation of letter and digit processing[6].

Letter and digit recognition are not innate, so if the functional architecture of vision makes a distinction between letters and numbers, the environment must be helping to shape that architecture. How might this happen? The fact that neural learning is correlation-based suggests that correlations in the environment may provide an answer. Stimuli within a category (letters) tend to occur together (in text) and are thus encoded in close temporal proximity, but stimuli between categories (letters and digits) co-occur less often. This statistical feature could interact with correlation-based learning in the brain to lead to maps for letter and digit recognition. We have implemented this hypothesis in a Hebbian neural network[7] which, when presented with inputs that satisfy co-occurrence statistics, spontaneously self-organized to produce distinct letter and digit maps.

The co-occurrence hypothesis predicts a smaller category effect in subjects who regularly see letters and digits together. Such subjects should have less segregated maps and, as a result, letters should not pop out as much from digits. To test this prediction, we compared the effect in foreign-mail sorters at the Philadelphia Air Mail Facility with that in control subjects. Foreign-mail sorters spend roughly 4 hours per day processing Canadian zip codes in which letters and digits occur together (for example, M5S 1A4).

As predicted, foreign-mail sorters showed a reduced category effect compared with postal-worker controls, as measured both by the absolute difference in and ratio of response times in the letter-among-letters (LL) and letter-among-digits (LD) conditions (see figure). The sorters were, however, faster than controls, presumably because of their extensive experience with rapid tasks. To ensure that these results were not the result of a floor effect, we excluded the three slowest postal-worker controls (out of 16) for one analysis and used



Mean reaction times (with standard error bars) for correct responses on target-present trials in both letter-among-letter (LL; open bars) and letter-among-digit (LD; shaded bars) conditions for 16 postal-worker controls, the 13 fastest postal-worker controls, 8 college graduates and 10 foreign-mail sorters. All the foreign-mail sorters had been sorting foreign mail for at least 6 months before their participation. A computer presented subjects with a target letter followed by a circular array of letters or digits, and subjects were asked to press a key indicating whether or not the target was present. Details are based on experiment 1 of ref. 13. *a*, Foreign-mail sorters compared with postal-worker controls. LD trials were reliably faster than LL trials ($t(25) = 4.367$, $P < 0.001$, one-tailed). Foreign-mail sorters showed a smaller alphanumeric category effect than did the postal-worker controls as measured both by the difference between the LL and LD conditions ($t(24) = 1.816$, $P < 0.05$, one-tailed) and by the LL/LD ratio ($t(24) = 1.864$, $P < 0.05$, one-tailed). *b*, Foreign-mail sorters against fastest postal-worker controls. LD trials were significantly faster than LL trials ($t(22) = 4.785$, $P < 0.001$, one-tailed) and the foreign-mail sorters showed a reduced category effect as measured by the LL–LD difference ($t(21) = 1.881$, $P < 0.05$, one-tailed) and by the LL/LD ratio ($t(21) = 2.060$, $P < 0.05$, one-tailed). *c*, Foreign-mail sorters against college graduates. Again, the LD condition was significantly faster than the LL condition ($t(17) = 3.208$, $P < 0.01$, one-tailed and the foreign-mail sorters showed a reduced category effect as measured by the LL–LD difference ($t(16) = 2.271$, $P < 0.05$, one-tailed) and by the LL/LD ratio ($t(16) = 2.792$, $P < 0.01$, one-tailed).