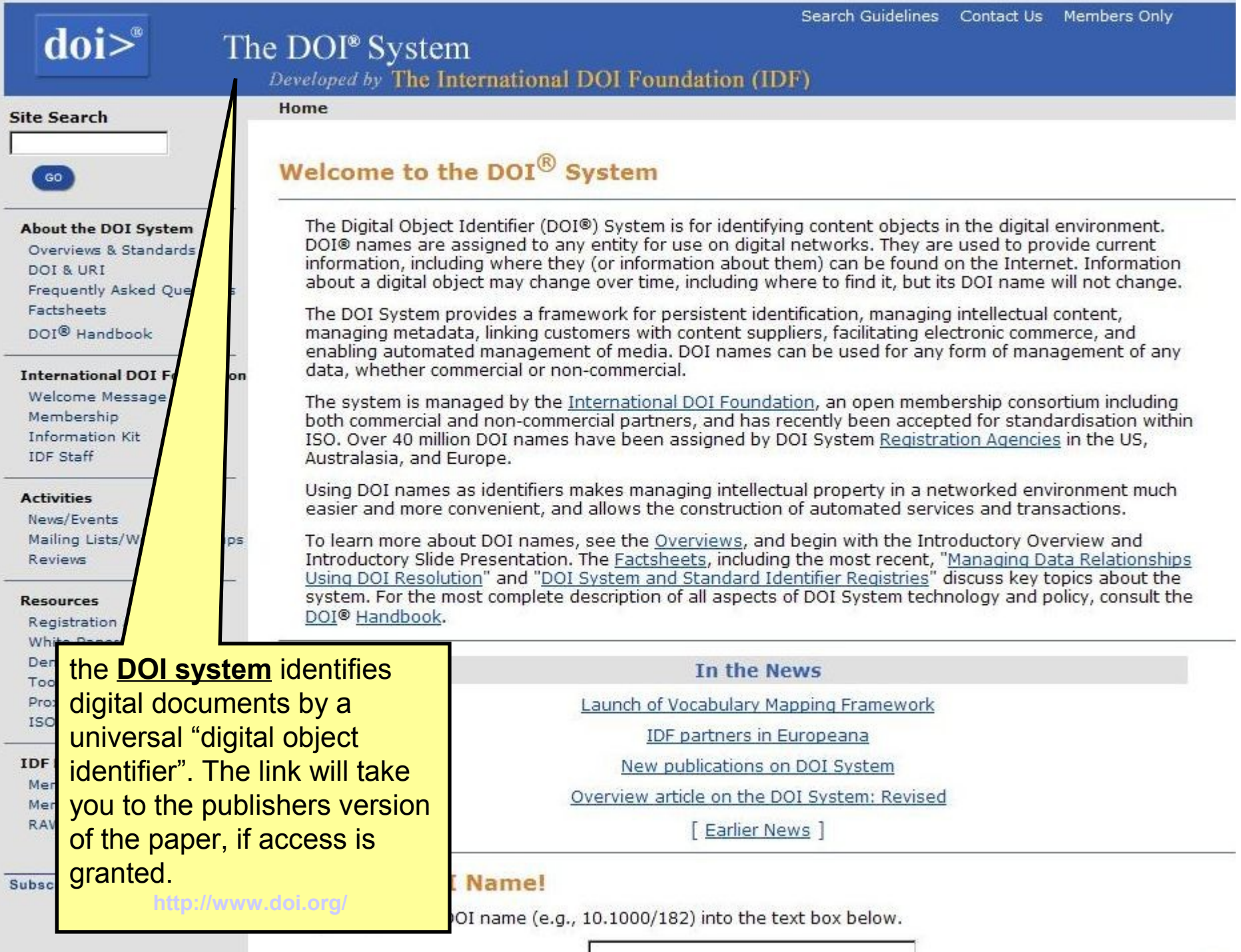
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Genome sequence of the recombinant protein production host *Pichia pastoris*

Kristof De Schutter[1,2,7], Yao-Cheng Lin[3,4,7], Petra Tiels[1,5,7], Annelies Van Hecke[1,5], Sascha Glinka[6], Jacqueline Weber-Lehmann[6], Pierre Rouzé[3,4], Yves Van de Peer[3,4] & Nico Callewaert[1,5]

The methylotrophic yeast *Pichia pastoris* is widely used for the production of proteins and as a model organism for studying peroxisomal biogenesis and methanol assimilation. *P. pastoris* strains capable of human-type N-glycosylation are now available, which increases the utility of this organism for biopharmaceutical production. Despite its biotechnological importance, relatively few genetic tools or engineered strains have been generated for *P. pastoris*. To facilitate progress in these areas, we present the 9.43 Mbp genomic sequence of the GS115 strain of *P. pastoris*. We also provide manually curated annotation for its 5,313 protein-coding genes.

The methylotrophic yeast *Pichia pas*
used yeast species in the production
employed in laboratories around th
basic research and medical applications. It is also an important model organism for the investigation of peroxisomal proliferation and methanol assimilation. The *P. pastoris* expression technology has been commercially available for many years. *P. pastoris* grows to high cell density, provides tightly controlled methanol-inducible transgene expression and efficiently secretes heterologous proteins in defined media. Several *P. pastoris*–produced biopharmaceuticals that are either not glycosylated (such as human serum albumin[2]) or for which glycosylation is needed only for proper folding (such as several vaccines[3]) are already on the market. An important recent breakthrough has been the development of *P. pastoris* strains with human-type N-glycosylation[4–6]. Humanized glycosylation will further increase the importance of *P. pastoris* for biopharmaceutical production; indeed, proteins produced with this system are moving into clinical

of 9.7 Mbp by pulsed-field
igned 13 *P. pastoris* genes to
e of a genetic map makes
chromosome assembly a challenging task, which we completed according to the strategy outlined in **Figure 1a**. We made use of 454/Roche sequencing[12] (GS-FLX version) to highly oversample the genome (20× coverage) and generated 70,500 paired-end sequence tags, to enable the assembly of all but seven contigs into nine 'supercontigs' (plus the mitochondrial genome) using automated shotgun assembly and BLASTN-based contig end-joining (Online Methods and **Supplementary Fig. 1** online). Upon assigning these (super)contigs to the four chromosomes (Online Methods and **Supplementary Fig. 2** online), the order of the supercontigs was determined through PCR and Sanger sequencing of the amplification products. These finishing experiments allowed the reconstruction of the four chromosomal sequences (**Fig. 1b** and **Table 1**), with only two
gaps remaining (one each on chromosomes 1 and 4). A ribosomal

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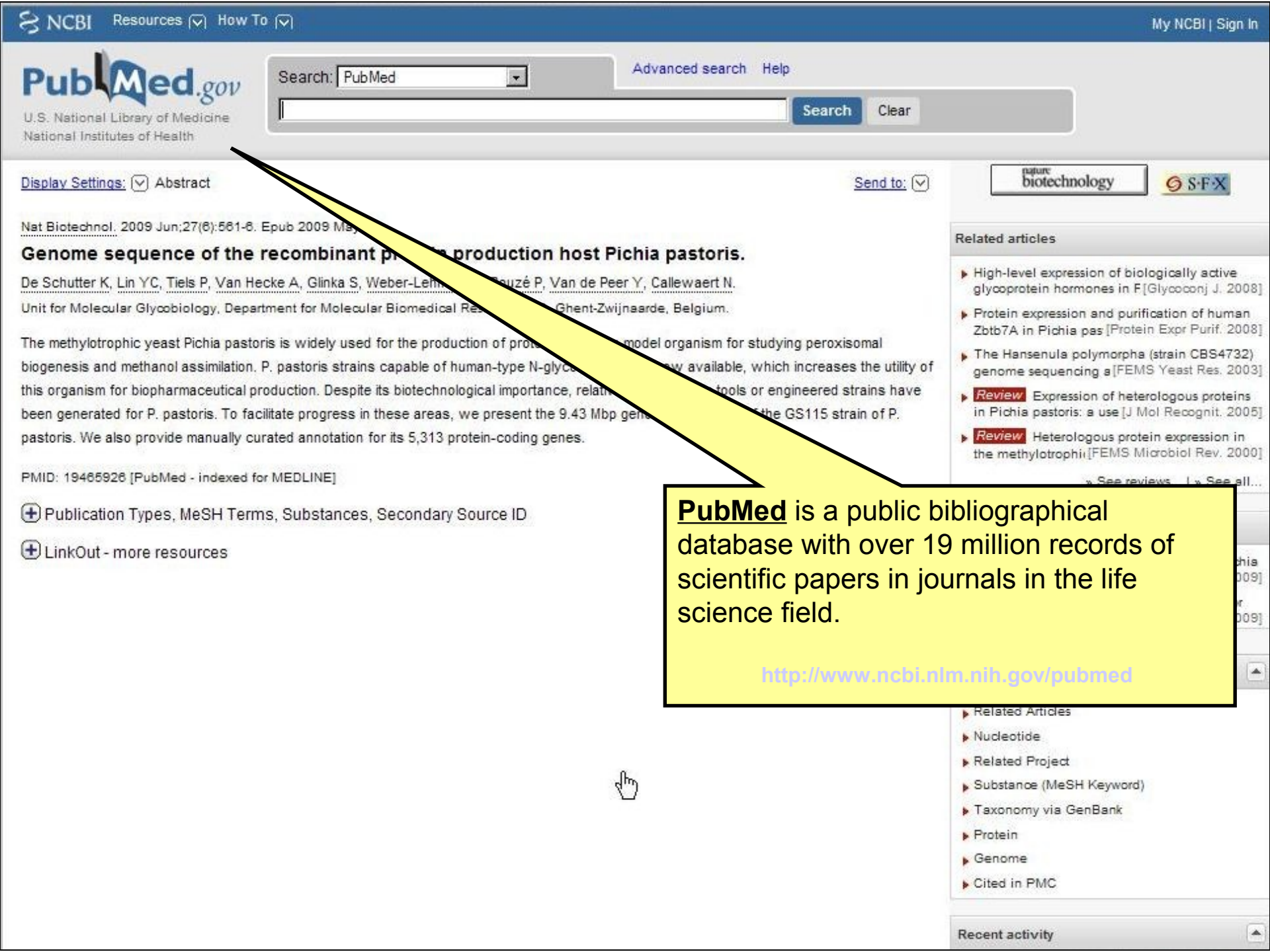
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