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·综 述·

# 酶祖先序列重建与定向进化

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**摘 要：**酶祖先序列重建是指通过计算机算法推导来自灭绝生物的祖先酶的氨基酸序列的技术。通常可分为 6 个步骤，依次为现代酶的核酸/氨基酸序列收集、多序列比对、系统发育树构建、祖先酶序列的计算机推测、基因克隆、酶学性质表征。该方法广泛应用于研究分子在行星时间尺度上对环境条件不断变化的适应性和进化机制。随着酶在生物催化领域中扮演越来越重要的角色，该方法逐渐成为研究酶序列、结构和功能关系的有力手段。同时，祖先酶大多具有温度稳定性、突变稳定性等特性，使其成为进一步定向进化的理想蛋白质支架。文中综述了酶祖先序列重建的计算机算法、应用和常用计算机软件，并结合最新研究进展，展望其在酶定向进化领域中的应用前景。

**关键词：**祖先序列重建，定向进化，祖先酶，酶结构与功能关系，生物催化

# Enzyme ancestral sequence reconstruction and directed evolution

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**Abstract:** The amino acid sequence of ancestral enzymes from extinct organisms can be deduced through *in silico* approach termed ancestral sequence reconstruction (ASR). ASR usually has six steps, which are the collection of nucleic acid/amino acid sequences of modern enzymes, multiple sequence alignment, phylogenetic tree construction, computational deduction of ancestral enzyme sequence, gene cloning, and characterization of enzyme properties. This method is widely used to study the adaptation and evolution mechanism of molecules to the changing environmental conditions on planetary time scale. As enzymes play key roles in biocatalysis, this method has become a powerful method for studying the relationship among the sequence, structure, and function of enzymes. Notably, most of the ancestral enzymes show better temperature stability and mutation stability, making them ideal protein scaffolds for further directed evolution. This article summarizes the computer algorithms, applications, and commonly used computer software of ASR, and discusses the potential application in directed

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evolution of enzymes.

**Keywords:** ancestral sequence reconstruction, directed evolution, ancestral enzyme, enzyme structure-function relationships, biocatalysis

自然界中，酶经过亿万年的进化，形成复杂又精巧的结构以行使其功能[1-2]。了解其如何进化成现在的结构与功能有助于我们更好地了解地球早期地质生物化学演化[3]、物种的起源与进化[4]和结构与功能关系的演化[5-6]等。祖先序列重建技术 (Ancestral sequence reconstruction，ASR)，为解决部分这些基本问题提供了新的思路[7]，特别是酶祖先序列重建技术。该技术通常可分为 6 个步骤，即搜集同源序列集、序列集多序列比对、系统发育树构建、计算机工具推测祖先序列、实验室克隆、酶学性质表征 (图 1)。ASR 使得研究人员能够 (1) 重建和“复活”，即在体内或体外合成灭绝的酶，以研究它们与现代酶的差异；(2) 识别在时间尺度上改变酶功能的关键氨基酸变化；(3) 从垂直的角度研究酶序列，结构与功能的关系[8]等。同时，前寒武纪时期地球的极端环境如高温和原始细胞仅依赖于少数酶系的假设，祖先酶通常具有更好的热稳定性[9-10]、高异源表达量[11-13]、低 pH 活力[14]、高活性[9]、催化混杂性 (Catalytic promiscuity)[15-16]等，更符合现代绿色生物制造对工业酶的需求[17]。除此之外，祖先酶还有突变耐受性，在酶定向进化技术广泛应用于工业酶定制的背景下[18]，祖先酶相较于现代酶展现出更好地作为突变亲本和蛋白质支架的能力[19]。基于此，本综述介绍了酶祖先序列重建技术的发展历程和常用工具，重点讨论了其在酶定向进化领域的应用前景。

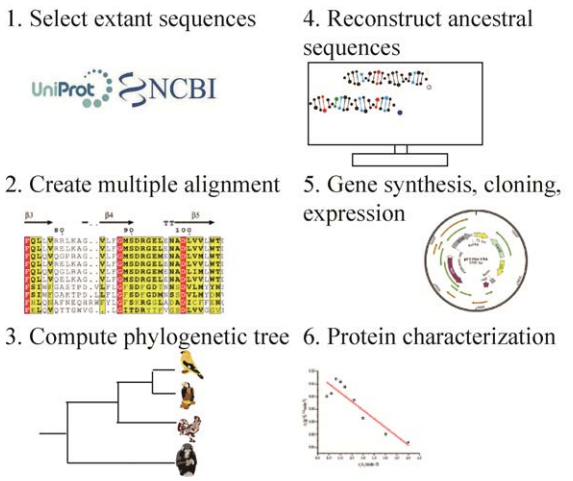


**图 1　酶祖先序列重建技术流程**
Fig. 1　Process of ASR of enzymes.

## 1　酶祖先序列重建的发展历程及其应用

### 1.1　酶祖先序列重建算法的发展历程

从已知的现存蛋白质序列中推导出古代/祖先蛋白质序列相对合理的近似值的设想最初是由 Pauling 和 Zuckerkandl 在 20 世纪 60 年代提出 (图 2)[20]。然而，它一直是一个理论概念，直到 20 世纪 90 年代，随着生物信息学的进步，蛋白质序列的日益增加和分子生物学的进步使得祖先序列编码的蛋白质能够在实验室中分子克隆。1990 年，Stackhouse 等首次报道了酶祖先序列的实验室重建，作者使用最大简约法 (Maximum parsimony，MP) 重建了生活于上新世 (530 万年前至 258.8 万年前) 的沼泽水牛和河牛共同祖先的核糖核酸酶和两个可能是现代核糖核酸酶进化过程中的中间产物序列，并在实验室中表征了它们的性质[21]。MP 算法重建的祖先蛋白序列被定义为能够通过最少的点突变转化为其后代序列的序列，后续使用 MP 算法又复活了祖先溶菌酶[22]和祖先糜酶[23] (表 1)。1995 年，Yang 等在其设计的 PAML 软件包中实现了通过最大似然法 (Maximum likelihood，ML) 重建祖先序列[24]，自此 MP 算法逐渐被淘汰。1997 年，Zhang 等比较了 MP、ML 和他们开发的基于距离的贝叶斯算法 (Distance-based Bayesian) 推断祖先氨基

酸序列的准确性[25]。在 ML 算法中，使用进化的统计模型来计算序列中与树的最深节点相关联的每个位置上的每种氨基酸的可能性。祖先序列被定义为其中每个残基在其相关位置存在的可能性最大的残基集合。贝叶斯算法则将与系统发育树拓扑、分支长度和替换模型相关的不确定性集成到祖先序列计算中[7]。当氨基酸序列的离散度较低时，这 3 种方法都能给出可靠的推断。然而，当序列分歧程度较高时，ML 和基于距离的贝叶斯算法比 MP 算法给出更准确的结果。2002 年，Zhang 等利用基于距离的贝叶斯算法和 MP 算法重建高等灵长类动物核糖核酸酶的祖先序列并在实验室中表征其酶学性质。作者研究发现，MP 算法推测的祖先序列与贝叶斯推论获得祖先序列大体一致[26]。2006 年，Williams 等将真实祖先序列的热力学性质与由 MP、ML 和贝叶斯算法推断的“祖先序列”的性质进行比较。作者发现 MP 和 ML 算法高估了“祖先序列”的热稳定性，而贝叶斯算法则不会高估“祖先序列”的热稳定性[27]。Hall 等比较了 ML 和贝叶斯算法的理论精度，发现贝叶斯算法推断的 DNA 序列比 ML 算法推断的 DNA 序列更准确，但对于推断的蛋白质序列则相反[28]。2012 年，Hobbs 报道了分别使用 ML 和贝叶斯算法从芽孢杆菌的最后一个共同祖先中重建结构复杂的核心代谢酶 3-异丙基苹果酸脱氢酶。这种酶的贝叶斯版本也是嗜热的，但表现出异常的催化动力学[29]，说明贝叶斯版本比其 ML 版本的祖先序列包含更多的序列错误。

综上所述，ML 和贝叶斯算法是目前祖先序列重建的首选算法，尤其是 Yang 等开发的 PAML 软件包中的 Codeml 模块[24]，然而两者的优劣，不同的研究对象具有不同的结论，目前尚无广泛认可的证据说明二者的优劣。

### 1.2 酶祖先序列重建的应用

酶祖先序列重建首先被用作探索进化假说和进化过程的工具[22,30]，继而被用作地球生物学的重要研究手段，研究分子在行星时间尺度上对不断变化的环境的适应性[31]。2010 年，Harms 等探讨了酶祖先序列重建作为重要手段，研究酶的历史突变对其功能多样化的影响的优势及示范性案例[6]。作者提出通过对祖先酶的序列重建和生化性质评估，有望对塑造蛋白质进化的物理化学决定因素和蛋白质结构的历史决定因素提供新的见解[6]。2013 年，Risso 等对 A 类 β-内酰胺酶进化中的几个前寒武纪节点进行了序列重建、异源表达和生化特征分析。这些结果支持前寒武纪生命是嗜热的推论，蛋白质在自然进化过程中从催化底物杂乱无章的“多面手”进化成“专家”的观点(图 3)。作者还强调了前寒武纪蛋白质在实验室中

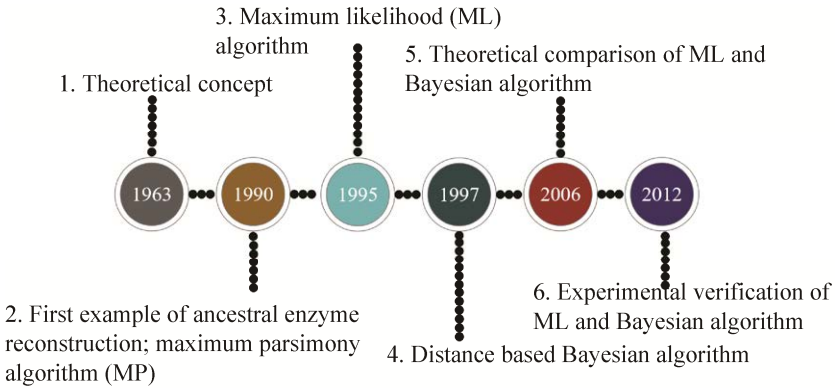


**图 2 祖先序列重建算法的发展历程**
Fig. 2 The history of ASR algorithm.

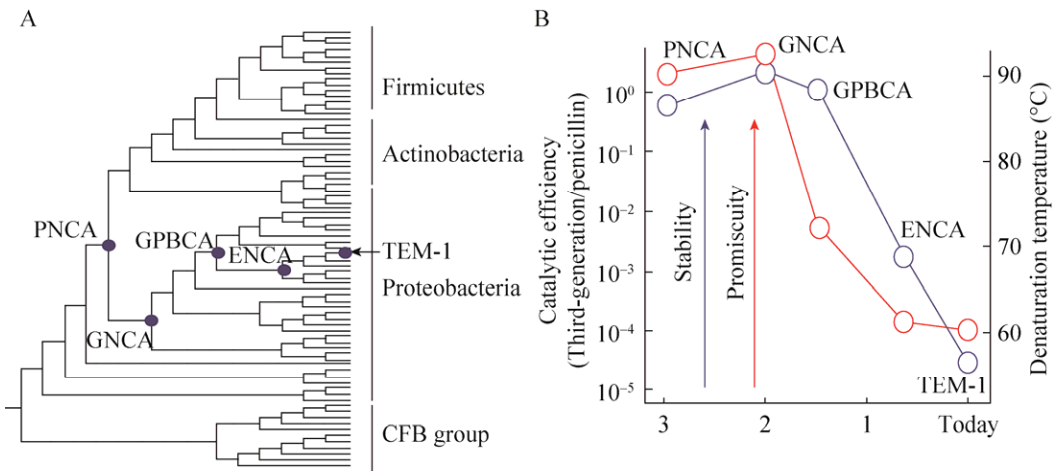


**图 3　实验室序列重建的前寒武纪 β-内酰胺酶的稳定性和混杂性[32]**

Fig. 3　The stability and promiscuousness of Precambrian β-lactamase reconstructed in the laboratory[32]. (A) A tree derived from phylogenetic analysis of 75 modern lactamase sequences. (B) The biophysical and chemical properties of Precambrian lactamase.

序列重建在生物技术领域的潜力。因为高稳定性和催化混杂性是蛋白质支架在分子设计和定向进化方面的有利特征[32]。

后续一系列研究表明，序列重建的祖先酶通常表现出高稳定性[10,33]、高活性[34]、底物混杂性[11,35]和催化混杂性等[36]，这些特征有助于酶的进一步进化[37]，并引起了广泛的关注[8,17,19,37-39]。基因组和后基因组时代的序列数据库的指数增长，加上系统发育学和生物信息学的进步，已经将 ASR 转变为几乎例行的计算程序。因此，酶祖先序列重建技术有成为常规的挖掘和设计性能优异的工业酶的工具的发展趋势[9,12,40-42]。如 2018 年，Gumulya 等通过 ASR 技术获得了能够承受高温和长时间反应的 P450 酶和酮醇酸还原异构酶。CYP3 家族的脊椎动物祖先体中的 P450 酶(CYP3_N1) 比现存人体中的 CYP3A4 对溶剂的耐受性更强，同时以相当的效率催化相似的底物范围。此外，与现存的大肠杆菌来源的酮醇酸还原异构酶相比，祖先酶的比活力提高了 8 倍，热稳定性也更好[9]。为了确定祖先 CYP3 是热稳定性的推断的稳健性，作者进一步构建了祖先序列预测中不确定性最大的 10 个位点的祖先酶库，在 1 023 个突变样本中，77%的突变体能够正常表达，大多数突变体表现出类似的热稳定性，其中 222 个突变体的热稳定性明显高于 CYP3_N1。作者提出即使只使用最近祖先的序列数据也可以设计出耐热蛋白质。2019 年，Chaloupkova 等描述了酶祖先序列重建具有获得多功能催化剂的潜力[43]。

除此之外，酶祖先序列重建还被用于探究宇宙生命共同祖先的环境温度[44]；生物分子机器复杂性的进化[45]；酶催化功能的起源与进化，如尿酸酶[46]、解毒酶[47]；揭示抗癌药物格列卫选择性的详细原子机制[48]；促进蛋白质结晶[49]；提高苯丙氨酸/酪氨酸解胺酶疗法的潜力[50]；CMGC 组激酶特异性的进化机制[15]和调控进化[51]；代谢途径进化的大规模分析[52]；丝氨酸-苏氨酸激酶变构激活的起源[53]等 (表 1)。

## 2　酶祖先序列重建常用工具

### 2.1　多序列比对

序列的搜集和选择是 ASR 的第一步，通常

表 1 酶祖先序列重建的应用部分实例

**Table 1 Some case studies of using ASR**

| Enzyme | ASR protocol | Biological significance | Published year | References |
|---|---|---|---|---|
| RNase | MP | Reconstruct the evolutionary history of RNases | 1990 | [21] |
| Lysozyme | MP | Explore the theory of molecular evolution | 1990 | [22] |
| Chymase | MP | Explore the origin and evolution of chymase | 1996 | [23] |
| RNase | Bayesian and MP | RNase role in the related antiviral activity | 2002 | [26] |
| Alcohol dehydrogenase | PAML | Global ecosystem change | 2005 | [31] |
| Thioredoxin | PAML | Understand the evolution of thioredoxin | 2011 | [14] |
| 3-isopropylmalate dehydrogenase | PAML and MrBayes | Origin and evolution of thermophily | 2012 | [29] |
| Glucosidase | PAML | Molecular mechanisms and evolutionary forces of glucosidase | 2012 | [30] |
| Nucleoside diphosphate kinase | PAML | Environmental temperature of the universal common ancestor of life | 2013 | [44] |
| β-lactamase | Bayesian | Ancient properties of biomolecules as well as the intracellular and extracellular environments hosting these proteins | 2013 | [32] |
| Malate and lactate dehydrogenase | PAML | How are new genes created? | 2014 | [54] |
| Bacterial ribonuclease H1 | PAML | How the diverse thermostabilities of bacterial ribonuclease H1 | 2014 | [33] |
| Uricase | PAML | Evolutionary history and metabolic insights of ancient mammalian uricases | 2014 | [46] |
| CMGC protein kinase | Lazarus | Evolutionary mechanisms of kinase plasticity | 2014 | [15] |
| Imidazole glycerol phosphate synthase | PhyloBayes 3.0 | Origin and evolution of complex enzyme | 2014 | [55] |
| Tyrosine kinase | PAML | Molecular mechanism of 3000-fold difference in Gleevec affinity between Src and Abl | 2015 | [48] |
| Lactate and malate dehydrogenase | PAML | Biochemical and evolutionary mechanisms of altered substrate specificity | 2016 | [56] |
| Hydroxynitrile lyase | PAML | To test the hypothesis that ancestral enzymes were more promiscuous than their modern descendants | 2016 | [16] |
| Tryptophan synthase | PRANK | Whether primordial enzyme complexes from extinct species displayed a similar degree of functional sophistication | 2016 | [57] |
| Adenylate kinase | PAML | Characterize the molecular mechanisms underlying thermoadaptation of enzyme catalysis | 2017 | [58] |
| Ligninolytic peroxidase | PAML | Analyzing the evolutionary pathway leading to the most efficient lignin-degrading peroxidases characterizing Polyporales species | 2017 | [59] |
| Transaminase | PAML | Date mining of new enzymes | 2017 | [42] |
| Chalcone isomerase | FastML | Vertical explore enzyme structure-function relationship | 2018 | [60] |
| Pyruvate decarboxylase | PAML | To further the understanding of bacterial enzymes | 2018 | [61] |
| Diterpene cyclase | MEGA7 | The potential of ancestor sequence reconstruction to produce remodeling cyclase with enhanced stability, activity | 2018 | [11] |
| Ketol-acid reductoisomerase | Bayesian-based tool | Engineering highly functional thermostable proteins | 2018 | [9] |

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续表 1

| Enzyme | ASR protocol | Biological significance | Published year | References |
|---|---|---|---|---|
| Vertebrate CYP3 P450 | FastML | Engineering highly functional thermostable proteins | 2018 | [9] |
| Cyclohexadienyl dehydratase | PAML | Reveals the molecular evolution process behind the emerging enzymes. | 2018 | [62] |
| Carboxylic acid reductase | FastML, PAML, and Ancescon | Date mining of new enzymes | 2019 | [40] |
| Methyl-parathion hydrolase | PAML | Characterize the functional evolution of methyl-parathion hydrolase | 2019 | [63] |
| Nucleoside diphosphate kinase | PAML | Discuss the biological adaptation to the climatic changes | 2019 | [4] |
| Endoglucanases | PAML | Date mining of new enzymes | 2019 | [64] |
| Kinase | Phylobot | To study the evolution of kinase regulation | 2019 | [51] |
| Dehalogenase | PAML | Exploring the Evolutionary Mechanism of enzyme promiscuous | 2019 | [43] |
| L-arginine Oxidase | FastML | Elucidate how enzymes attain their characteristic functions | 2019 | [65] |
| L-arginine Oxidase | FastML | Excellent catalytic performance and soluble expression properties of ancestral enzymes | 2019 | [41] |
| Triosephosphate isomerase | PAML | The evolution of enzyme obligated oligomer | 2019 | [66] |
| Flavin-containing monooxygenase | PAMLX | Demonstrates the power of ancestral-sequence reconstruction as a strategy for the crystallization of proteins. | 2020 | [49] |
| Fungal laccase | PAML | Date mining of new enzymes and directed evolution | 2020 | [12] |
| Ser-Thr kinase | PAML | Ancient origins of allosteric activation in a Ser-Thr kinase | 2020 | [53] |
| Phenylalanine/tyrosine ammonia lyase | Mega7 and PAML | Exploring the therapeutic potential of modern and ancestral phenylalanine/tyrosine ammonia lyases | 2020 | [50] |
| 3-isopropylmalate dehydrogenase | PAML | Engineering highly functional thermostable proteins | 2020 | [67] |
| Tryptophan synthase | FastML | Analysis of allosteric communication in a multienzyme complex | 2020 | [45] |

以目标序列作为“Query”在 NCBI、Uniprot 等数据库中搜索同源序列，导出同源性序列以待进一步多序列比对 (Multiple sequence alignment，MSA) 分析。MSA 是生物信息学许多领域的核心，已经发展出连串的软件及比对结果修饰工具 (表 2)，其中使用较多的软件为 ClustalW[68]和 MUSCLE (Multiple sequence comparison by log-expectation)[69]。虽然 ClustalW 更加常用，但 MUSCLE 更为精确，且对于常规大小的数据集，其运行速度是 ClustalW 的 2–5 倍。软件直接输出的比对结果一般不能直接用于构建系统发育树，需要借助修饰软件或者手动去除长尾序列等进一步的修饰，如 trimAl[70]，一个自动比对修剪的工具，可以从比对中去除排列不佳的区域以提高后续分析的质量。

用于 MSA 的序列丰度和准确的序列比对结果对系统发育树的精确构建具有重要的影响。平均氨基酸一致性百分率可用于评估多序列比对结果的可靠性。Ogden 和 Rosenberg 的研究表明，当该值大于 50%时，那么序列比对准确性对系统发育树构建的影响就微乎其微[71]。

### 2.2 系统发育树的构建

系统发育树 (Phylogenetic tree，PT) 是由分支和连接的节点组成的，代表不同物种与它们祖先的关系或基因蛋白质序列与其祖先序列的关系。树内部节点代表“假定的祖先”，分支的长度代表祖先和它后代之间的变异程度。构建系统发育树的软件和算法有很多，其中 Mega 是使用最为广泛的软件，涵盖了邻接法、最大简约法、最

大似然法等算法[72]。MrBayers 是广泛认可的使用贝叶斯推论 (Bayesian inference) 构建系统发育树的软件[73]。Ogden 等和 Hall 等使用更接近生物学进化过程的模拟数据,比较了多种不同建树方法构建的树与正确的树的接近程度,同时考察了系统发育树拓扑结构和分支长度的准确性。两项研究结果表明,贝叶斯推论法比最大似然法稍准确,然后是最大简约法,而邻接法是准确性最低的方法[71,74]。

系统发育树构建是祖先序列重建技术的关键步骤,系统发育树算法的基本原理、可靠性检验方法和常用软件使用可以参考由陈士超等翻译的美国 Barry G. Hall 著作的《轻松构建系统发育树使用操作方法和理论》[75]和相关文献综述[76]。

### 2.3 祖先序列重建

ML 和贝叶斯算法是目前 ASR 最常使用的计算机算法,其中由著名华裔科学家、伦敦大学统计遗传学教授杨子恒开发的 PAML 软件包[24]是目前应用最广泛的祖先序列重建软件 (表 2),并免费提供给学术研究使用。PAML 并不是最好的系统发育树构建软件,但在系统发育树的基础上可以非常有效地进行:进化参数估计、进化假说检验、分歧时间估计和正选择估计等。CodeML 是 PAML 软件包下的一个程序,在估算蛋白编码序列同义替换的非同义替换速率、检测序列是否已经受正选择和 ASR 中受到广泛的认可。使用 CodeML 进行序列重建需要已经比对好的序列文件和树文件,还需要一个配置好的控制文件。

MrBayers是基于贝叶斯算法重建祖先序列的常用软件,它需要输入比对好的 Nexus 格式文件和一些指令来执行它的工作。祖先序列重建计算机算法的更多理论基础可以参考 Merkl 等的综述[7-8]。

除此之外,还有系列 ASR 综合工具被开发出来,如 PhyloBot[77]、FastML[78]和基于 Mega 的软件包 (表 2),它们将 ASR 所需的软件 (多序列比对、系统发育树构建、祖先序列重建) 集成到一个用户界面中,还包括可视化工具,极大地简化了重建过程。如 2016 年,Hanson-Smith 等开发的基于网络的工具 PhyloBot[77]。它专门为不熟悉生物信息学的科学家设计,可以在网络浏览器上运行,不需要在用户的电脑上安装。用户只需上传 FASTA 格式的蛋白质序列集合,为作业创建一个唯一的名称,并指定外类群 (Outgroup),然后使用网站默认的设置即可启动作业。外类群的定义是与内类群序列关系远于内类群序列内部相互关系的一条或多条序列。Mega 软件中也整合了 ASR 工具 (Ancestors) 和时间进化树分析工具 (Computer timetree)。2020 年,Carletti 等建立了一个来自灭绝有机体的序列重建蛋白质的网络数据库[79]。它包含

**表 2 祖先序列重建常用工具**

**Table 2 Common tools for ASR**

| Name | Web site | Function | References |
|---|---|---|---|
| ClustalW | https://www.genome.jp/tools-bin/clustalw | MSA | [68] |
| MUSCLE | http://www.drive5. com/muscle | MSA | [69] |
| trimAl | http://trimal.cgenomics.org | Automated alignment trimming | [70] |
| CD-HIT | http://cd-hit.org | Clustering and comparing large sets of protein or nucleotide sequences | [80] |
| Mega | https://www.megasoftware.net | MSA-PT-ASR | [71] |
| MrBayers | http://nbisweden.github.io/MrBayes/index.html | PT-ASR | [72] |
| PAML | http://abacus.gene.ucl.ac.uk/software/paml.html | ASR | [24] |
| PhyloBayes 3 | http://www.phylobayes.org | PT-ASR | [81] |
| FastML | https://fastml.tau.ac.il | ASR | [78] |
| PhyloBot | http://www.phylobot.com. | MSA-PT-ASR | [77] |
| Revenant | http://revenant.inf.pucp.edu.pe | A database of resurrected proteins | [79] |

来自文献的 84 个序列重建蛋白质的精选集合，每种蛋白质都有广泛的注释，包括结构、生化和生物物理信息。上述综合工具的开发与运用，一定程度上降低了 ASR 应用所需的计算机技术门槛。

## 3 酶祖先序列重建与定向进化

### 3.1 现代酶融合祖先酶氨基酸残基

2001 年，Miyazaki 等开发了一种基于 ASR 方法的耐热酶理性设计方法 (图 4)，作者将推断的 3-异丙基苹果酸脱氢酶的祖先氨基酸残基引入现代嗜热酶中，在测试的 7 个突变体中，有 5 个突变体表现出比野生酶更高的热稳定性，且突变对酶的催化活性没有明显影响[82]，这一实验结果与原核生物共同祖先是极端嗜热的假说是一致的。该团队进一步将祖先氨基酸残基引入中温酶异柠檬酸脱氢酶，以验证该方法的普适性[83]。5 个突变酶中有 4 个比野生型异柠檬酸脱氢酶具有更高的热稳定性，实验结果表明，在现代蛋白质序列中加入祖先氨基酸残基提高蛋白质的热稳定性具有广泛适用性。2006 年，Watanabe 等进一步探究了嗜热酶 3-异丙基-苹果酸脱氢酶的非保守氨基酸位点引入祖先氨基酸残基的效果。在测试的 12 个突变体中，至少有 6 个表现出比原始酶更高的热稳定性。结果表明，祖先残基对热稳定性的影响并不取决于位点残基的保守程度，这表明这些突变蛋白的稳定性与序列保守无关，而与引入的残基的古老程度有关[84]。后续使用该方法成功提升了多种酶的稳定性和活性，如漆酶的 pH 稳定性和热稳定性[85]、甘油激酶的热稳定性[86]、木质素过氧化物酶的热稳定性和比酶活[87]、8-淀粉酶的热稳定性和活力[88]、漆酶和多功能过氧化物酶的热稳定性[89]、甘氨酰-tRNA 合成酶的热稳定性和活力[90]，人工 L-苏氨酸 3-脱氢酶的热稳定性和活性[91]。

### 3.2 祖先酶作为定向进化的支架

传统酶基因挖掘一般采用菌种筛选、功能宏基因组等技术。菌种筛选以特定催化活性为导向，筛选的成功率受通量筛选方法效率的限制，功能宏基因组方法依赖实体样品，研发周期长，成本高[92]。ASR 与其他工程方法不同，它基于对非保守功能空间的概率搜索来生成新序列，在给定准确的序列比对输入的情况下，使每个输出都具有很高的功能性。如果输入数据集有足够的变化，得到的祖先通常会与现有序列有很大差异，甚至

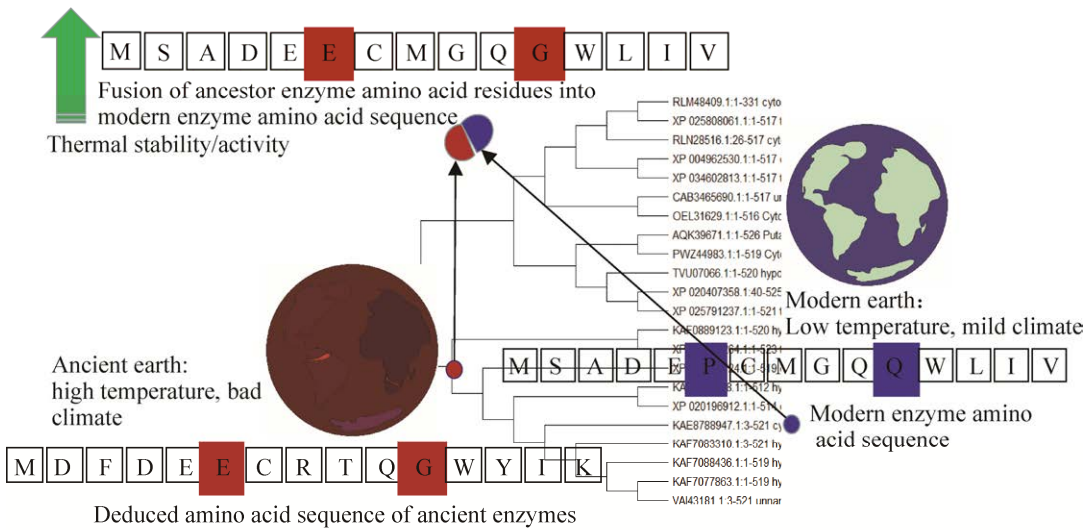


**图 4 现代酶融合祖先酶氨基酸残基提高其稳定性或活性**

Fig. 4 Fusion of modern enzyme with ancestral enzyme amino acid residues to improve its stability or activity.

同源性<30%。这使得研究人员可以发现其他方法无法获得的有益突变，包括协调的突变集。这些可以改变由蛋白质全序列状态决定的特征，包括在热或其他压力下的稳定性[40]。因此，祖先酶作为支架为新的酶活性的进化提供了强有力的起点[93]。表 3 总结了文献报道中祖先酶较现代酶的优势特性的部分实例 (表 3)。

2020 年，Gomez-Fernandez 等报道了祖先酶序列重构与定向进化结合的应用实例。作者重建了几个可以追溯到 5 亿–2.5 亿年之间的真菌漆酶的祖先序列。与现代漆酶不同的是，序列重建的中生代漆酶很容易被酵母分泌，具有相似的动力学参数，更广泛的稳定性和明显的 pH 活性。作者进一步对祖先酶进行了定向进化，以提高其催化 1,3-环戊二酮的氧化速率[12]，获得的 P163R-V165R 双突变体的氧化速率是祖先亲本的 1.6 倍。

## 4 结论与展望

祖先序列重建在分子生物学和蛋白质工程中的广泛应用一直很缓慢，部分原因是这种方法需要大量的计算专业知识。同时，ASR 在一定程度上不可避免地存在不确定性，因为不可能确定哪个序列在历史上是真实存在的，而只能推断最可能的祖先序列。目前的研究表明，ASR 在表型水平上得到了很大程度的验证，且重建的祖先蛋白往往表现出更好的热稳定性，低 pH 活力，较高的表达水平。随着序列数据量的指数增长，加上系统发育学和生物信息学的进步，尤其是具有交互界面的综合性网站的开发，已经大大减少了该方法使用所需的计算机专业知识。此外，全基因合成成本的不断降低和标准的分子生物学方法的进步也大大促进了酶祖先序列重建的应用。祖先酶的序列重建技术，在研究分子进化与酶结构与功能关系的同时，向挖掘耐高温、高活性的新酶发展是未来趋势。随着定向进化技术与半理性设计技术的发展，祖先酶的稳定性，尤其是突变稳定性成为其独特优势，可以有效避免酶定向进化中的酶活力与稳定性不能兼得的问题。此外，详细的计算构象分析支持，通过改变其构象状态的集合，祖先蛋白可能进化为新的或更优异的现代功能酶。因此笔者相信，在不远的将来，不仅现

**表 3 祖先酶与现代酶酶学性质比较**

**Table 3 Comparison of the properties of ancestral enzymes and modern enzymes**

| Enzymes | Ancestral enzymes compared with modern enzymes | References |
|---|---|---|
| Carboxylic acid reductases | AncCARs had a Tmup to 35 °C higher, with half-lives up to nine times longer than the greatest previously observed | [40] |
| L-arginine oxidase | Improved thermal stability | [65] |
| Ketol-acid reductoisomerases | Ancestral enzyme showed an 8 fold higher specific activity than the cognate *Escherichia coli* form at 25 °C, which increased 3.5 fold at 50 °C. | [9] |
| Cytochromes P450 | $^{60}T_{50}$ of 66 °C and enhanced solvent tolerance | [9] |
| Diterpene cyclases | Increased thermostability and substrate acceptance | [11] |
| 3-isopropylmalate dehydrogenase | Thermally stable and catalytic properties adapted to low-temperature reactions | [67] |
| Phenylalanine/tyrosine ammonia lyases | All ancestral enzymes displayed increased thermostability | [50] |
| β-lactamase | Ancestral β-lactamases display denaturation temperature enhancements (~35 °C) and enhanced substrate promiscuity | [32] |
| ω-transaminase | Ancestral transaminases demonstrated novel and superior activities up to 20 fold. In most cases, the ancestral proteins were also easier to overproduce, and demonstrated comparable or improved thermostability | [42] |
| Laccase | The ancestral laccases were readily secreted by yeast, with similar kinetic parameters, a broader stability, and distinct pH activity profiles | [12] |

存的酶，它们序列重建的祖先酶也可以在实验室的工作台上进行定向进化，并且很快就能收获酶祖先序列重建与定向进化结合带来的研究成果。

## REFERENCES


[1] Ouzounis CA, Coulson RMR, Enright AJ, et al. Classification schemes for protein structure and function. Nat Rev Genet, 2003, 4(7): 508-519.

[2] 张锟, 曲戈, 刘卫东, 等. 工业酶结构与功能的构效关系. 生物工程学报, 2019, 35(10): 1806-1818.
Zhang K, Qu G, Liu WD, et al. Structure-function relationships of industrial enzymes. Chin J Biotech, 2019, 35(10): 1806-1818 (in Chinese).

[3] Garcia AK, Kacar B. How to resurrect ancestral proteins as proxies for ancient biogeochemistry. Free Radical Biol Med, 2019, 140: 260-269.

[4] Harada M, Nagano A, Yagi S, et al. Planktonic adaptive evolution to the sea surface temperature in the Neoproterozoic inferred from ancestral NDK of marine cyanobacteria. Earth Planet Sci Lett, 2019, 522: 98-106.

[5] Napolitano S, Reber RJ, Rubini M, et al. Functional analyses of ancestral thioredoxins provide insights into their evolutionary history. J Biol Chem, 2019, 294(38): 14105-14118.

[6] Harms MJ, Thornton JW. Analyzing protein structure and function using ancestral gene reconstruction. Curr Opin Struct Biol, 2010, 20(3): 360-366.

[7] Merkl R, Sterner R. Ancestral protein reconstruction: techniques and applications. Biol Chem, 2016, 397(1): 1-21.

[8] Merkl R, Sterner R. Reconstruction of ancestral enzymes. Perspect Sci, 2016, 9: 17-23.

[9] Gumulya Y, Baek JM, Wun SJ, et al. Engineering highly functional thermostable proteins using ancestral sequence reconstruction. Nat Catal, 2018, 1(11): 878-888.

[10] Trudeau DL, Kaltenbach M, Tawfik DS. On the potential origins of the high stability of reconstructed ancestral proteins. Mol Biol Evolut, 2016, 33(10): 2633-2641.

[11] Hendrikse NM, Charpentier G, Nordling E, et al. Ancestral diterpene cyclases show increased thermostability and substrate acceptance. FEBS J, 2018, 285(24): 4660-4673.

[12] Gomez-Fernandez BJ, Risso VA, Rueda A, et al. Ancestral resurrection and directed evolution of fungal mesozoic laccases. Appl Environ Microbiol, 2020, 86(14): e00778-20

[13] Musil M, Konegger H, Hon J, et al. Computational design of stable and soluble biocatalysts. ACS Catal, 2018, 9(2): 1033-1054.

[14] Perez-Jimenez R, Inglés-Prieto A, Zhao ZM, et al. Single-molecule paleoenzymology probes the chemistry of resurrected enzymes. Nat Struct Mol Biol, 2011, 18(5): 592-596.

[15] Howard CJ, Hanson-Smith V, Kennedy KJ, et al. Ancestral resurrection reveals evolutionary mechanisms of kinase plasticity. eLife, 2014, 3: e04126.

[16] Devamani T, Rauwerdink AM, Lunzer M, et al. Catalytic promiscuity of ancestral esterases and hydroxynitrile lyases. J Am Chem Soc, 2016, 138(3): 1046-1056.

[17] Alcalde M. When directed evolution met ancestral enzyme resurrection. Microb Biotechnol, 2017, 10(1): 22-24.

[18] 曲戈, 张锟, 蒋迎迎, 等. 2018 诺贝尔化学奖: 酶定向进化与噬菌体展示技术. 生物学杂志, 2019, 36(1): 1-6, 19.
Qu G, Zhang K, Jiang YY, et al. The Nobel Prize in chemistry 2018: the directed evolution of enzymes and the phage display technologies. J Biol, 2019, 36(1): 1-6, 19 (in Chinese).

[19] Risso VA, Sanchez-Ruiz JM. Resurrected ancestral proteins as scaffolds for protein engineering//Alcalde M. Directed Enzyme Evolution: Advances and Applications. Cham: Springer, 2017: 229-255.

[20] Pauling L, Zuckerkandl E. Chemical paleogenetics: molecular ‘restoration studies’ of extinct forms of life. Acta Chem Scand, 1963, 17: S9–S16.

[21] Stackhouse J, Presnell SR, McGeehan GM, et al. The

ribonuclease from an extinct bovid ruminant. FEBS Lett, 1990, 262(1): 104-106.

[22] Malcolm BA, Wilson KP, Matthews BW, et al. Ancestral lysozymes reconstructed, neutrality tested, and thermostability linked to hydrocarbon packing. Nature, 1990, 345(6270): 86-89.

[23] Chandrasekharan UM, Sanker S, Glynias MJ, et al. Angiotensin Ⅱ-forming activity in a reconstructed ancestral chymase. Science, 1996, 271(5248): 502-505.

[24] Yang ZH, Kumar S, Nei M. A new method of inference of ancestral nucleotide and amino acid sequences. Genetics, 1995, 141(4): 1641-1650.

[25] Zhang JZ, Nei MS. Accuracies of ancestral amino acid sequences inferred by the parsimony, likelihood, and distance methods. J Mol Evol, 1997, 44(1): S139-S146.

[26] Zhang JZ, Rosenberg HF. Complementary advantageous substitutions in the evolution of an antiviral RNase of higher primates. Proc Natl Acad Sci USA, 2002, 99(8): 5486-5491.

[27] Williams PD, Pollock DD, Blackburne BP, et al. Assessing the accuracy of ancestral protein reconstruction methods. PLoS Comput Biol, 2006, 2(6): e69.

[28] Hall BG. Simple and accurate estimation of ancestral protein sequences. Proc Natl Acad Sci USA, 2006, 103(14): 5431-5436.

[29] Hobbs JK, Shepherd C, Saul DJ, et al. On the origin and evolution of thermophily: reconstruction of functional precambrian enzymes from ancestors of *Bacillus*. Mol Biol Evol, 2012, 29(2): 825-835.

[30] Voordeckers K, Brown CA, Vanneste K, et al. Reconstruction of ancestral metabolic enzymes reveals molecular mechanisms underlying evolutionary innovation through gene duplication. PLoS Biol, 2012, 10(12): e1001446.

[31] Thomson JM, Gaucher EA, Burgan MF, et al. Resurrecting ancestral alcohol dehydrogenases from yeast. Nat Genet, 2005, 37(6): 630-635.

[32] Risso VA, Gavira JA, Mejia-Carmona DF, et al. Hyperstability and substrate promiscuity in laboratory resurrections of Precambrian β-lactamases. J Am Chem Soc, 2013, 135(8): 2899-2902.

[33] Hart KM, Harms MJ, Schmidt BH, et al. Thermodynamic system drift in protein evolution. PLoS Biol, 2014, 12(11): e1001994.

[34] Takenaka Y, Noda-Ogura A, Imanishi T, et al. Computational analysis and functional expression of ancestral copepod luciferase. Gene, 2013, 528(2): 201-205.

[35] Babkova P, Sebestova E, Brezovsky J, et al. Ancestral haloalkane dehalogenases show robustness and unique substrate specificity. ChemBioChem, 2017, 18(14): 1448-1456.

[36] Kürten C, Carlberg B, Syrén PO. Mechanism-guided discovery of an esterase scaffold with promiscuous amidase activity. Catalysts, 2016, 6(6): 90.

[37] Gumulya Y, Gillam EMJ. Exploring the past and the future of protein evolution with ancestral sequence reconstruction: the 'retro' approach to protein engineering. Biochem J, 2017, 474(1): 1-19.

[38] Risso VA, Martinez-Rodriguez S, Candel AM, et al. *De novo* active sites for resurrected Precambrian enzymes. Nat Commun, 2017, 8: 16113

[39] Risso VA, Sanchez-Ruiz JM, Ozkan SB. Biotechnological and protein-engineering implications of ancestral protein resurrection. Curr Opin Struct Biol, 2018, 51: 106-115.

[40] Thomas A, Cutlan R, Finnigan W, et al. Highly thermostable carboxylic acid reductases generated by ancestral sequence reconstruction. Commun Biol, 2019, 2: 429.

[41] Nakano S, Minamino Y, Hasebe F, et al. Deracemization and stereoinversion to aromatic D-amino acid derivatives with ancestral L-amino acid oxidase. ACS Catal, 2019, 9(11): 10152-10158.

[42] Wilding M, Peat TS, Kalyaanamoorthy S, et al. Reverse engineering: transaminase biocatalyst development using ancestral sequence reconstruction. Green Chem, 2017, 19(22): 5375-5380.

[43] Chaloupkova R, Liskova V, Toul M, et al. Light-emitting dehalogenases: reconstruction of multifunctional biocatalysts. ACS Catal, 2019, 9(6):

4810-4823.

[44] Akanuma S, Nakajima Y, Yokobori SI, et al. Experimental evidence for the thermophilicity of ancestral life. Proc Natl Acad Sci USA, 2013, 110(27): 11067-11072.

[45] Schupfner M, Straub K, Busch F, et al. Analysis of allosteric communication in a multienzyme complex by ancestral sequence reconstruction. Proc Natl Acad Sci USA, 2020, 117(1): 346-354.

[46] Kratzer JT, Lanaspa MA, Murphy MN, et al. Evolutionary history and metabolic insights of ancient mammalian uricases. Proc Natl Acad Sci USA, 2014, 111(10): 3763-3768.

[47] Bar-Rogovsky H, Hugenmatter A, Tawfik DS. The evolutionary origins of detoxifying enzymes: the mammalian serum paraoxonases (pons) relate to bacterial homoserine lactonases. J Biol Chem, 2013, 288(33): 23914-23927.

[48] Wilson C, Agafonov RV, Hoemberger M, et al. Using ancient protein kinases to unravel a modern cancer drug's mechanism. Science, 2015, 347(6224): 882-886.

[49] Nicoll CR, Bailleul G, Fiorentini F, et al. Ancestral-sequence reconstruction unveils the structural basis of function in mammalian FMOs. Nat Struct Mol Biol, 2020, 27(1): 14-24.

[50] Hendrikse NM, Larsson HA, Gelius SS, et al. Exploring the therapeutic potential of modern and ancestral phenylalanine/tyrosine ammonia-lyases as supplementary treatment of hereditary tyrosinemia. Sci Rep, 2020, 10: 1315.

[51] Sang DJ, Pinglay S, Wiewiora RP, et al. Ancestral reconstruction reveals mechanisms of ERK regulatory evolution. eLife, 2019, 8: e38805

[52] Psomopoulos FE, Van Helden J, Médigue C, et al. Ancestral state reconstruction of metabolic pathways across pangenome ensembles. Microbial Genomics, 2020, 6(11): e000429.

[53] Hadzipasic A, Wilson C, Nguyen V, et al. Ancient origins of allosteric activation in a Ser-Thr kinase. Science, 2020, 367(6480): 912-917.

[54] Boucher JI, Jacobowitz JR, Beckett BC, et al. An atomic-resolution view of neofunctionalization in the evolution of apicomplexan lactate dehydrogenases. eLife, 2014, 3: e02304.

[55] Reisinger B, Sperl J, Holinski A, et al. Evidence for the existence of elaborate enzyme complexes in the Paleoarchean era. J Am Chem Soc, 2014, 136(1): 122-129.

[56] Steindel PA, Chen EH, Wirth JD, et al. Gradual neofunctionalization in the convergent evolution of trichomonad lactate and malate dehydrogenases. Prot Sci, 2016, 25(7): 1319-1331.

[57] Busch F, Rajendran C, Heyn K, et al. Ancestral tryptophan synthase reveals functional sophistication of primordial enzyme complexes. Cell Chem Biol, 2016, 23(6): 709-715.

[58] Nguyen V, Wilson C, Hoemberger M, et al. Evolutionary drivers of thermoadaptation in enzyme catalysis. Science, 2017, 355(6322): 289-294.

[59] Ayuso-Fernández I, Martínez AT, Ruiz-Dueñas FJ. Experimental recreation of the evolution of lignin-degrading enzymes from the Jurassic to date. Biotechnol Biofuels, 2017, 10: 67.

[60] Kaltenbach M, Burke JR, Dindo M, et al. Evolution of chalcone isomerase from a noncatalytic ancestor. Nat Chem Biol, 2018, 14(6): 548-555.

[61] Buddrus L, Andrews ESV, Leak DJ, et al. Crystal structure of an inferred ancestral bacterial pyruvate decarboxylase. Acta Crystallogr F, 2018, 74(Pt 3): 179-186.

[62] Clifton BE, Kaczmarski JA, Carr PD, et al. Evolution of cyclohexadienyl dehydratase from an ancestral solute-binding protein. Nat Chem Biol, 2018, 14(6): 542-547.

[63] Yang G, Anderson DW, Baier F, et al. Higher-order epistasis shapes the fitness landscape of a xenobiotic-degrading enzyme. Nat Chem Biol, 2019, 15(11): 1120-1128.

[64] Barruetabeña N, Alonso-Lerma B, Galera-Prat A, et al. Resurrection of efficient Precambrian endoglucanases for lignocellulosic biomass hydrolysis. Commun Chem, 2019, 2: 76.

[65] Nakano S, Niwa M, Asano Y, et al. Following the

evolutionary track of a highly specific L-arginine oxidase by reconstruction and biochemical analysis of ancestral and native enzymes. Appl Environ Microbiol, 2019, 85(12): e00459-19.

[66] Schulte-Sasse M, Pardo-Ávila F, Pulido-Mayoral NO, et al. Structural, thermodynamic and catalytic characterization of an ancestral triosephosphate isomerase reveal early evolutionary coupling between monomer association and function. FEBS J, 2019, 286(5): 882-900.

[67] Furukawa R, Toma W, Yamazaki K, et al. Ancestral sequence reconstruction produces thermally stable enzymes with mesophilic enzyme-like catalytic properties. Sci Rep, 2020, 10: 15493.

[68] Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinformatics, 2002, (1): 2.3.1-2.3.22.

[69] Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res, 2004, 32(5): 1792-1797.

[70] Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T, et al. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics, 2009, 25(15): 1972-1973.

[71] Ogden TH, Rosenberg MS. Multiple sequence alignment accuracy and phylogenetic inference. Syst Biol, 2006, 55(2): 314-328.

[72] Kumar S, Stecher G, Li M, et al. Mega x: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol, 2018, 35(6): 1547-1549.

[73] Ronquist F, Teslenko M, Van Der Mark P, et al. Mrbayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst Biol, 2012, 61(3): 539-542.

[74] Hall BG. Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. Mol Biol Evol, 2005, 22(3): 792-802.

[75] 陈士超, 吴晓运. 轻松构建系统发育树: 实用操作方法和理论. 4 版. 北京: 高等教育出版社, 2016.
Chen SC, Wu XY. Phylogenetic Trees Made Easy: A How-to Manual. 4th ed. Beijing: Higher Education Press, 2016 (in Chinese).

[76] 张丽娜, 荣昌鹤, 何远, 等. 常用系统发育树构建算法和软件鸟瞰. 动物学研究, 2013, 34(6): 640-650.
Zhang LN, Rong CH, He Y, et al. A bird's eye view of the algorithms and software packages for reconstructing phylogenetic trees. Zool Res, 2013, 34(6): 640-650 (in Chinese).

[77] Hanson-Smith V, Johnson A. Phylobot: a web portal for automated phylogenetics, ancestral sequence reconstruction, and exploration of mutational trajectories. PLoS Comput Biol, 2016, 12(7): e1004976.

[78] Ashkenazy H, Penn O, Doron-Faigenboim A, et al. FastML: a web server for probabilistic reconstruction of ancestral sequences. Nucleic Acids Res, 2012, 40(W1): W580-W584.

[79] Carletti MS, Monzon AM, Garcia-Rios E, et al. Revenant: a database of resurrected proteins. Database (Oxford), 2020, 2020: baaa031.

[80] Li WZ, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics, 2006, 22(13): 1658-1659.

[81] Lartillot N, Lepage T, Blanquart S. PhyloBayes 3: a bayesian software package for phylogenetic reconstruction and molecular dating. Bioinformatics, 2009, 25(17): 2286-2288.

[82] Miyazaki J, Nakaya S, Suzuki T, et al. Ancestral residues stabilizing 3-isopropylmalate dehydrogenase of an extreme thermophile: experimental evidence supporting the thermophilic common ancestor hypothesis. J Biochem, 2001, 129(5): 777-782.

[83] Iwabata H, Watanabe K, Ohkuri T, et al. Thermostability of ancestral mutants of *Caldococcus noboribetus* isocitrate dehydrogenase. Fems Microbiol Lett, 2005, 243(2): 393-398.

[84] Watanabe K, Ohkuri T, Yokobori S, et al. Designing thermostable proteins: ancestral mutants of 3-isopropylmalate dehydrogenase designed by using a phylogenetic tree. J Mol Biol, 2006, 355(4): 664-674.

[85] Hamuro Y, Tajima K, Matsumoto-Akanuma A, et al.

Characterization of a thermostable mutant of *Agaricus brasiliensis* laccase created by phylogeny-based design. J Biosci Bioeng, 2017, 124(6): 623-629.

[86] Fukuda Y, Abe A, Tamura T, et al. Epistasis effects of multiple ancestral-consensus amino acid substitutions on the thermal stability of glycerol kinase from *Cellulomonas* sp. NT3060. J Biosci Bioeng, 2016, 121(5): 497-502.

[87] Semba Y, Ishida M, Yokobori SI, et al. Ancestral amino acid substitution improves the thermal stability of recombinant lignin-peroxidase from white-rot fungi, *Phanerochaete chrysosporium* strain UAMH 3641. Protein Eng Des Select, 2015, 28(7): 221-230.

[88] Yamashiro K, Yokobori SI, Koikeda S, et al. Improvement of *Bacillus circulans* β-amylase activity attained using the ancestral mutation method. Protein Eng Des Select, 2010, 23(7): 519-528.

[89] Garcia-Ruiz E, Maté D, Ballesteros A, et al. Evolving thermostability in mutant libraries of ligninolytic oxidoreductases expressed in yeast. Microb Cell Fact, 2010, 9: 17

[90] Shimizu H, Yokobori SI, Ohkuri T, et al. Extremely thermophilic translation system in the common ancestor commonote: ancestral mutants of glycyl-tRNA synthetase from the extreme thermophile *Thermus thermophilus*. J Mol Biol, 2007, 369(4): 1060-1069.

[91] Nakano S, Motoyama T, Miyashita Y, et al. Benchmark analysis of native and artificial NAD+-dependent enzymes generated by a sequence-based design method with or without phylogenetic data. Biochemistry, 2018, 57(26): 3722-3732.

[92] 张建志, 付立豪, 唐婷, 等. 基于合成生物学策略的酶蛋白元件规模化挖掘. 合成生物学, 2020, 1(3): 319-336.
Zhang JZ, Fu LH, Tang T, et al. Scalable mining of proteins for biocatalysis via synthetic biology. Synth Biol J, 2020, 1(3): 319-336 (in Chinese).

[93] Gardner JM, Biler M, Risso VA, et al. Manipulating conformational dynamics to repurpose ancient proteins for modern catalytic functions. ACS Catal, 2020, 10(9): 4863-4870.

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