

ALEdb 1.0: A Database of Mutations from Adaptive Laboratory Evolution Experimentation

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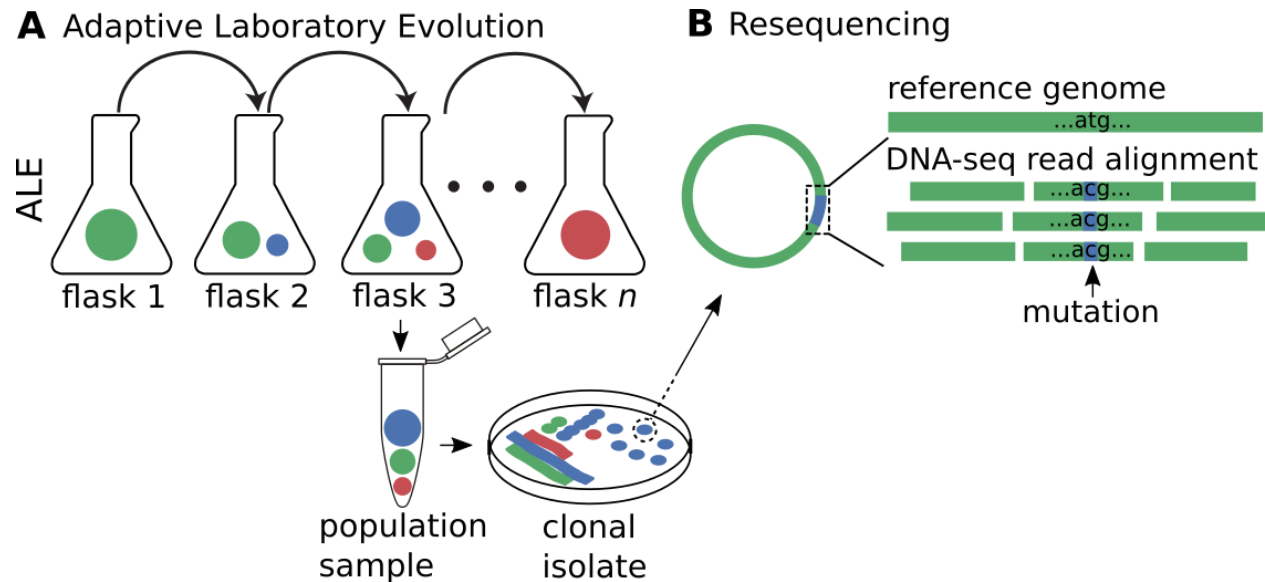
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ABSTRACT

Full genomic sequences are readily available, but their functional interpretation remains a fundamental challenge. Adaptive Laboratory Evolution (ALE) has emerged as an experimental approach to discover causal mutations that confer desired phenotypic functions. Thus, ALE not only represents a controllable experimental approach to systematically discover genotype-phenotype relationships, but it also allows for the revelation of the series of genetic alterations required to acquire the new phenotype. Numerous ALE studies have appeared in the literature providing a strong impetus for developing structured databases to warehouse experimental evolution information and make it retrievable for large-scale analysis. Here, the first step towards establishing this capability is presented: ALEdb (<http://aledb.org>). This initial release contains over 11,000 mutations that have been discovered in ALE experiments. ALEdb is the first of its kind; (1) it is a web-based platform that comprehensively reports on ALE acquired mutations and their conditions, (2) it reports key mutations using previously established trends, (3) it enables a search-driven workflow to enhance user mutation functional analysis, (4) it allows exporting of mutation query results for custom analysis, (5) it has a bibliome that describes the underlying published literature, and (6) contains experimental evolution mutations from multiple model organisms. Thus, ALEdb is an informative platform which will become increasingly revealing as the number of reported ALE experiments and identified mutations continue to expand.

27

INTRODUCTION



28 **Figure 1. A** An illustration of a batch ALE experiment where both a clonal and population sample are
29 isolated from an intermediate (i.e., midpoint) flask. The petri dish represents streaking methodology
30 for isolating a clonal colony from a population. **B** An illustration of how the resequencing process
31 leverages a reference genome sequence and DNA-seq reads to identify mutations in an ALE sample.

32 Adaptive Laboratory Evolution (ALE) is a tool for the study of microbial adaptation. The typical execution
33 of an ALE experiment involves cultivating a population of microorganisms in defined conditions (i.e., in a
34 laboratory) for a period of time that enables the selection of improved phenotypes. Standard model
35 organisms, such as *E. coli*, have proven well suited for ALE studies due to their ease of cultivation and
36 storage, fast reproduction, well known genomes, and clear traceability of mutational events [1]. With the
37 advent of accessible whole genome resequencing, associations can be made between selected
38 phenotypes and genotypic mutations [2].

39 Beginning with a starting strain, an ALE experiment can be executed by serially passing a selected culture
40 to a fresh flask of media (Figure 1A), enabling the strain passed to continue acquiring mutations under
41 the experimental conditions without dilution of resources. Strains propagated during ALEs are assumed
42 to be those that outcompeted their competition due to adaptive mutations. Additional methods to
43 perform ALEs have been reviewed [2,3]. Whole genome comparative sequencing, or resequencing, is
44 used to identify mutations within evolved strains relative to the evolution's starting strain (Figure 1B).
45 ALE experiments can additionally involve replicate ALEs: identical evolutions that are often executed in
46 parallel. Replicate ALEs can reveal the dynamics of adaptation by enabling research into converging
47 genotypes within an experiment [4].

48 ALE methods have become important scientific tools in the study of evolutionary phenomena and have
49 contributed to research in basic discovery and applied fields. Evolutionary biologists seek to examine the
50 dynamics and repeatability of evolution and to better understand the relationship between genotypic
51 and phenotypic changes [5]. ALE methods, along with the plummeting cost of sequencing, has greatly
52 enabled their efforts, resulting in a variety of insights into adaptive evolution. ALE has often
53 demonstrated that (1) increases in fitness diminish with each new adaptive mutation [6], (2) genotypic
54 convergence through mutations can occur on the level of functional complexes [7], and, (3) interactions
55 between mutations may cause nonlinear fitness effects [8].

56 ALE methods have also been leveraged in the applied research of synthetic biology to engineer microbes
57 for commodity, industrial, and biopharmaceutical chemical synthesis [2]. Comprehensive whole genome
58 rational design is rarely achievable due to the complexity of biological systems [4,9]. The inability to
59 provide for comprehensive solutions in genome engineering can result in strains which cannot maintain
60 homeostasis, such as strains which cannot tolerate the concentrations of products they were designed to
61 produce. ALE has been used to produce adaptive mutations that provide solutions for the gaps left by
62 current rational genome engineering methods [10]. ALE can therefore complement rational genome
63 engineering in the work to provide for a comprehensive whole genome solution to an application [2,9].

64 Accurately interpreting the results of an ALE requires the identification of causal mutations for observed
65 adaptations. Identifying causal mutations requires a clear understanding of the mechanistic effects of
66 mutations on cellular components and systems. Due to the complexity of cellular systems, interpreting
67 the effects of mutations has proven to be a primary challenge in ALE [4,9]. A common approach to
68 mutation functional analysis is a literature search on the mutation target (e.g., a given annotated ORF).
69 Functional studies of genetic targets have traditionally served as primary resources for interpreting
70 mutation effects, providing information on a sequence's biological function. Published ALE results can
71 enhance approaches to identify and understand new adaptive mutations since they describe the fitness
72 of an allele relative to its predecessor. Researchers can therefore work to identify and understand their
73 ALE mutations by considering published adaptive mutations in conditions similar to their own ALEs.

74 A review of ALE methods [2] lists 34 separate ALE studies. Each study reports on novel combinations of
75 selection conditions and the resulting microbial adaptive strategies. Large scale analysis of ALE results
76 data from such consolidation efforts could be a powerful tool for identifying and understanding novel
77 adaptive mutations. No current online platform exists for ALE experimental result consolidation.

78 A web platform named *ALEdb* (aledb.org) has been created to meet the need for accessible consolidated
79 ALE mutations, conditions, and publication reporting. ALEdb additionally includes features to search for
80 specific mutations, report key mutations, and export mutation data for custom analysis. With these
81 features, ALEdb serves to fill the gap in the field of experimental evolution for an accessible resource of
82 consolidated experimental evolution mutations.

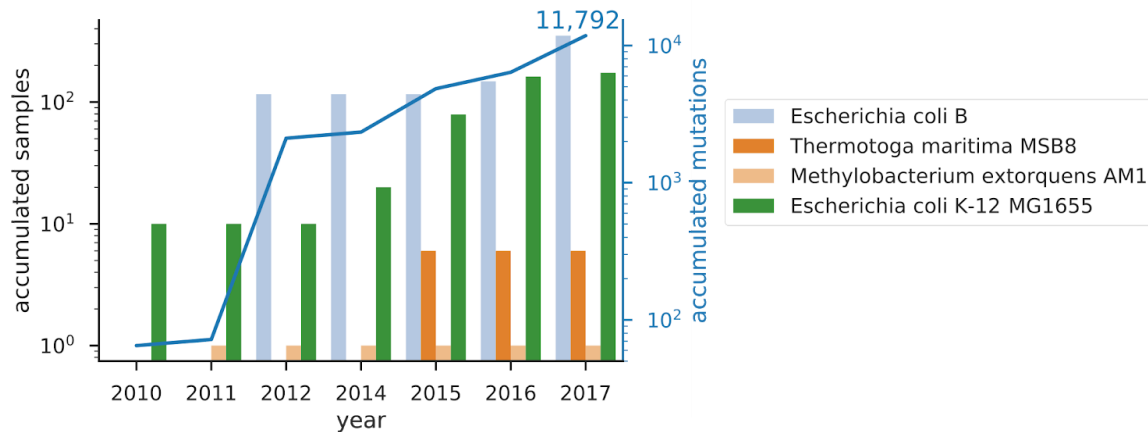
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RESULTS

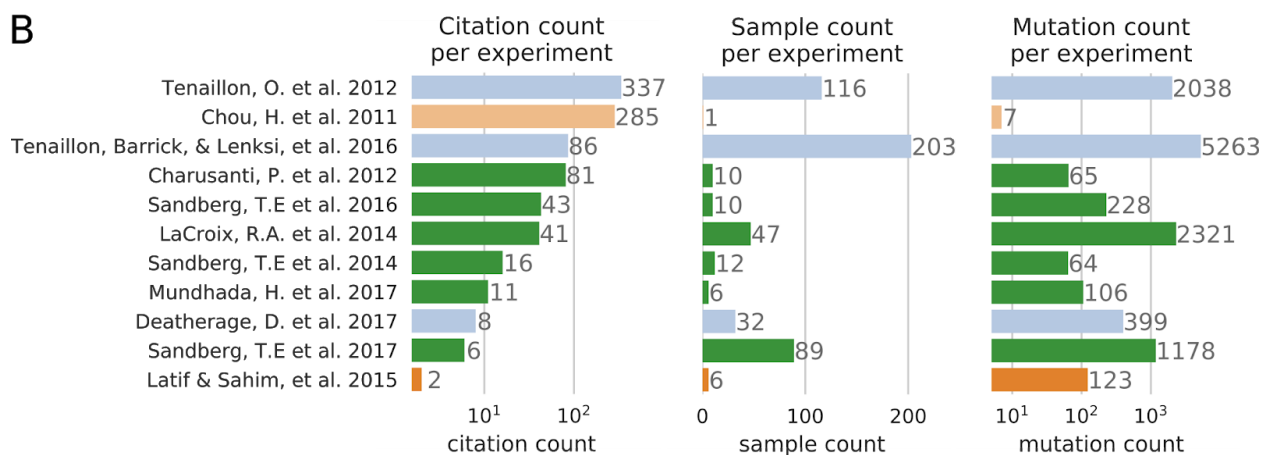
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A web platform to accelerate ALE data to knowledge

A Sample and mutation accumulation in ALEdb



B

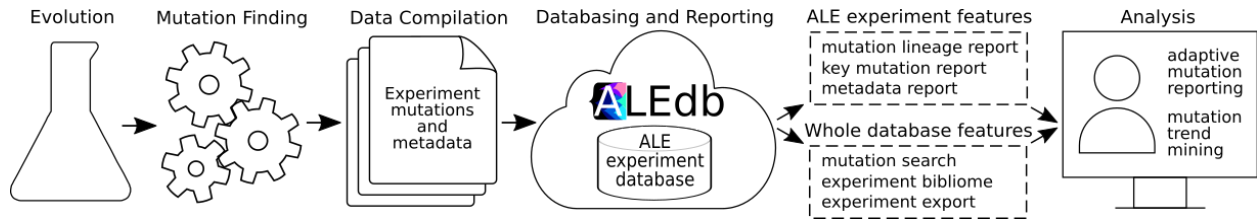


85 **Figure 2 A** A graph of the accumulation of sequenced samples and mutations in ALEdb. **B** Each
 86 publication's sample and mutation contribution to ALEdb along with their citations at the time of
 87 ALEdb's initial release. Citation counts were acquired from Google Scholar (scholar.google.com).

88 The need for consolidated and accessible ALE experiment reporting has resulted in the generation of the
 89 web platform *ALEdb* (aledb.org). Eleven published ALE experiments, with a total of four distinct strains,
 90 532 samples and 21522 observed mutations, serve as an initial seeding data set (Figure 2).

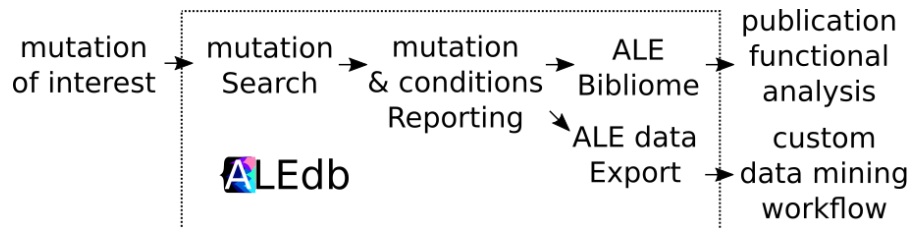
91 Experimental evolution studies explore the solution space of a genome optimization problem through
 92 mutational events. This element of exploration has lead to a rich diversity of published ALE experimental
 93 conditions [2]. Those experimental conditions currently represented in ALEdb are genetic perturbations
 94 [11], stress inducing environments [12], different carbon sources [13–15], and evolution duration [5].

95 Strains can often adapt to these conditions with a variety of different evolutionary strategies, leading to
 96 different beneficial mutations. This leads to a diversity in the mutations across ALE experiments. This rich
 97 variety of databased conditions and mutations have made ALEdb an attractive research resource, and
 98 further implementation has now made this information accessible through the web.



99 **Figure 3** An illustration of the flow of experimental evolution data to the generation of result reports
 100 for end users and their analysis.

101 ALEdb's feature set was developed in response to the challenge of accessible ALE mutation reporting for
 102 an ALE experiment pipeline [16]. ALEdb's features enable intuitive navigation through consolidated ALE
 103 experiment data by providing two categories of features: those that describe individual ALE experiments,
 104 and those that describe all consolidated experiment data. To describe individual ALE experiments, ALEdb
 105 generates reports that detail ALE the mutation lineages, key mutations, and experimental conditions per
 106 ALE sample. To describe all consolidated ALE experiment data, ALEdb provides a mutation search feature,
 107 the ability to export the mutation data from one or more ALE experiments as spreadsheets, and an
 108 itemization of all publications that describe the databased mutations (Figure 3). ALEdb thus provides for
 109 an unmet need in the experimental evolution community: a platform to search and explore consolidated
 110 experimental evolution mutation data.



111 **Figure 4** An illustration of the flow of mutation functional analysis using ALEdb. Each step within
 112 the ALEdb group is the name of a user feature on the ALEdb platform.

113 Mutation functional analysis is a major challenge in experimental evolution. Besides systems biology
 114 modeling methods, this task often involves searching the literature for similar results. The ALE mutations,
 115 conditions, and publications being consolidated into ALEdb can be leveraged in this work. ALEdb enables
 116 a search-driven workflow which can enhance a user's mutation functional analysis by reporting if
 117 mutations similar to theirs have occurred in published ALE experiments. Through ALEdb's *Search* feature,
 118 users can query for mutations of interest using multiple descriptive parameters and become aware of
 119 any databased ALE experiments that manifest similar mutations. Knowing these experiments, users can
 120 review the conditions and key mutation reports which characterize their results and refer to their
 121 associated publications through ALEdb's *Bibliome* page. These publications ultimately describe adaptive

122 mutations and their functional analysis, which could be leveraged by users to better understand similar
 123 mutations in their own study. ALEdb additionally includes the ability to *Export* mutation data for users
 124 interested in leveraging ALE data in applications beyond this platform (Figure 4).

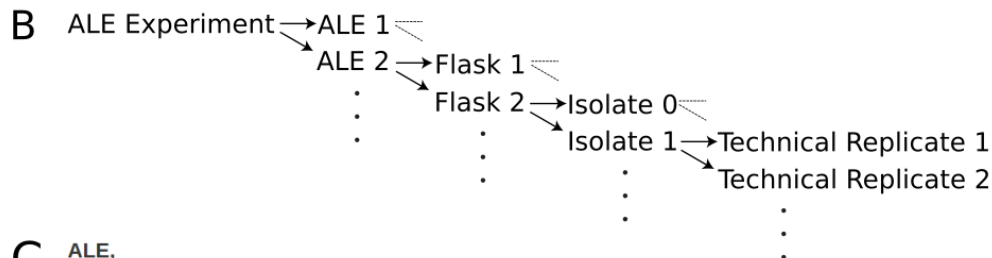
125 ALEdb's features are described in the following sections. With ALEdb already consolidating a significant
 126 amount of ALE experiments, the final section of this study demonstrates how ALEdb can currently be
 127 used as a data resource for experimental evolution.

128 Mutation search and reporting

129 ALEdb implements mutation *Search* to enable users to quickly find mutations of interest. Search returns
 130 a report of mutations for all databased samples according to the following mutation descriptors: gene,
 131 genome position range, mutation type, sequence change, protein change, and experiment.

A

Position	Mutation Type	Sequence Change	Gene	Details	GLU A4 F66 I1 R1	GLU A4 F149 I1 R1	GLU A4 F237 I1 R1	GLU A4 F403 I0 R1	GLU A4 F403 I1 R1
1,551,659	SNP	G → A	adhP	P69S (CCA → ICA)		1.00			
3,998,893	DEL	Δ5 bp	corA	coding (220-224/951 nt)		1.00	1.00	1.00	1.00
3,999,402	DEL	(GGC) ₂ → 1	corA	coding (729-731/951 nt)	1.00				
1,292,256	MOB	IS1 (-) +8 bp	hns, tdk	intergenic (-110/-488)		1.00	1.00	1.00	1.00
3,203,742	SNP	G → A	ttdA	V11V (GTG → GTA)				0.21	



C

ALE, Flask, Isolate, Technical Replicate	Clonal or Population	Species	Strain	Additional Strain Details	Media	Substrate	Temperature
A4 F403 I0 R1	population	E. Coli	511145	BOP27	M9	Glucose(2g/L), NH4Cl(1g/L), KH2PO4(3g/L), O2	37.0
A4 F403 I1 R1	clonal	E. Coli	511145	BOP27	M9	Glucose(2g/L), NH4Cl(1g/L), KH2PO4(3g/L), O2	37.0

132 **Figure 5 A** An example mutation lineage report where samples are represented as columns, ordered
133 from left to right as earliest to latest in an ALE. Rows describe the specific mutations manifested
134 within the sample set, and values contained within cells represent the allele frequency. This format
135 enables researchers to intuitively identify important mutational patterns, such as the fixed mutations
136 within the *corA* gene and *hns/tdk* intergenic region. Columns are described with the experiment
137 name, then an ALE (A#), flask (F#), isolate (I#), and technical replicate (R#) value to serialize samples.
138 Population samples will always be described by an isolate number (I#) of 0 and will be the only
139 sample types to carry allele frequencies less than 1.0. The information describing each mutation is
140 generated by the mutation finding stage described in Figure 2 and details a mutations type, genomic
141 region, and potential product effects. **B** The illustrated ordering of sample columns from left to right
142 in the mutation lineage report. **C** An ALE experiment metadata report.

143 Mutation search, along with most other mutation reporting mechanisms on ALEdb, present their results
144 in the form of mutation tables (Figure 5A). Each ALE experiment can be described as a series of mutation
145 sets relative to an ALE's starting strain. Ordering sample mutation sets as columns from earliest to latest
146 (Figure 45, 5B) in an ALE serves to render intuitive visualizations of temporal mutational trends. The
147 occurrence of a mutation in a sample is annotated with an allele frequency within the intersection of the
148 mutation row and sample column. Mutation tables therefore describe the lineage of an ALE's final
149 sample, or endpoint, according to the mutations that manifest during an evolution.

150 Researchers investigating ALE experiments require reporting that enables them to quickly understand
151 which mutations are likely causal for adaptations; the mutation tables built by ALEdb are designed to
152 meet this need. Among the many mutations that manifest within an ALE experiment, mutation rows that
153 describe multiple alleles of a gene will cluster together according to their positions on the genome. This
154 is illustrated with the mutated *corA* within Figure 5A. Due to the chronological sorting of the sample
155 columns per ALE, a mutation that fixes across samples will manifest as an unbroken sequence of cells in a
156 mutation row annotated with an allele frequency. This is illustrated with both the *hns/tdk* and *corA*
157 mutations in Figure 5A. These two patterns are obvious to an observer and serve well to describe the
158 adaptive mutational trends in ALE experiments.

159 ALE experiment mutations cannot be completely understood without considering the experiment's
160 conditions. ALEdb includes reports that describe an ALE experiment's strain, substrate, and environment
161 (Figure 5C). This experiment metadata can additionally be exported as spreadsheets for analysis
162 workflows external to ALEdb.

163 Consolidated ALE knowledge

164 A key component in the utility of ALEdb is the per experiment knowledge built from the databased
165 mutations. The *Bibliome* feature itemizes the publications that studied the ALE mutations databased
166 within ALEdb. Users can leverage the mutation functional analysis within these publications toward
167 understanding any similar mutations in their experimental evolutions.

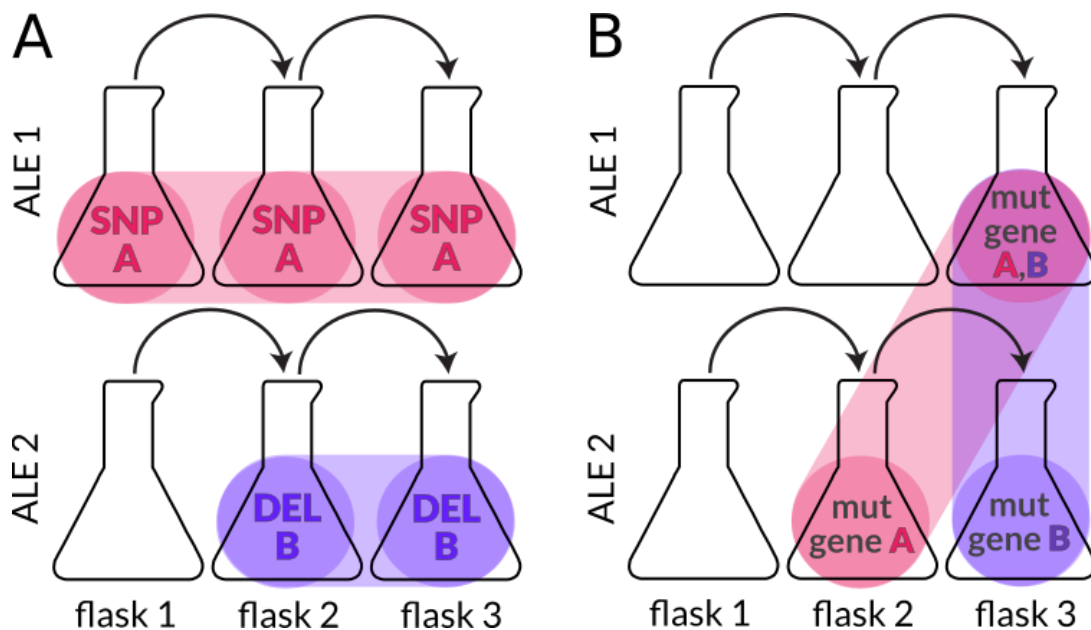
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ALE experiment mutation export

169 ALEdb implements an *Export* feature to give users the freedom to perform any analysis of interest on the
170 hosted data. This feature enables users to extract one or more experiment mutation sets into comma
171 separated value files. Users can then leverage custom analysis pipelines on the ALEdb's data towards
172 generating novel results.

173

Automated ALE experiment key mutation reporting



174 **Figure 6** Intuition for converging and fixed mutation reports. **A** SNP A and DEL B occur in separate
175 ALE replicates and persist through all subsequent flasks. **B** genetic targets A and B are seen to mutate
176 across ALEs.

177 ALEdb includes features that automate the reporting of established ALE adaptive mutation trends. These
178 trends are termed *fixed* and *converged* key mutations, where each trend describes a unique pattern of
179 mutations occurring within or across multiple ALEs in an experiment. These patterns have been used in
180 published ALE studies to identify adaptive mutations [11–14]. The manual consolidation of adaptive
181 mutation evidence can be prone to human error, inconsistent between researchers, and time consuming.
182 The automation of these common analyses contributes to more consistent analysis and more accurate
183 results.

184 A *fixed* mutation is one in which manifests in an ALE's midpoint, or intermediate sample, and is
185 propagated to all following samples in the ALE. The propagation of a mutation from their emergence to
186 an ALE's endpoint may describe the selection of a mutation due to its fitness benefits [13]. This analysis
187 is only possible if an ALE experiment includes midpoint samples, providing the possibility of more than
188 one data point per ALE mutation. The identification of *fixed* mutations is accomplished by organizing

189 mutations according to the ALE's sample chronology and identifying mutations that emerge in a
190 midpoint and manifest in all following samples of the same ALE (Figure 6A). ALEdb's *fixed* mutation
191 reporting automates this analysis and reports results in the format described in Figure 4A.

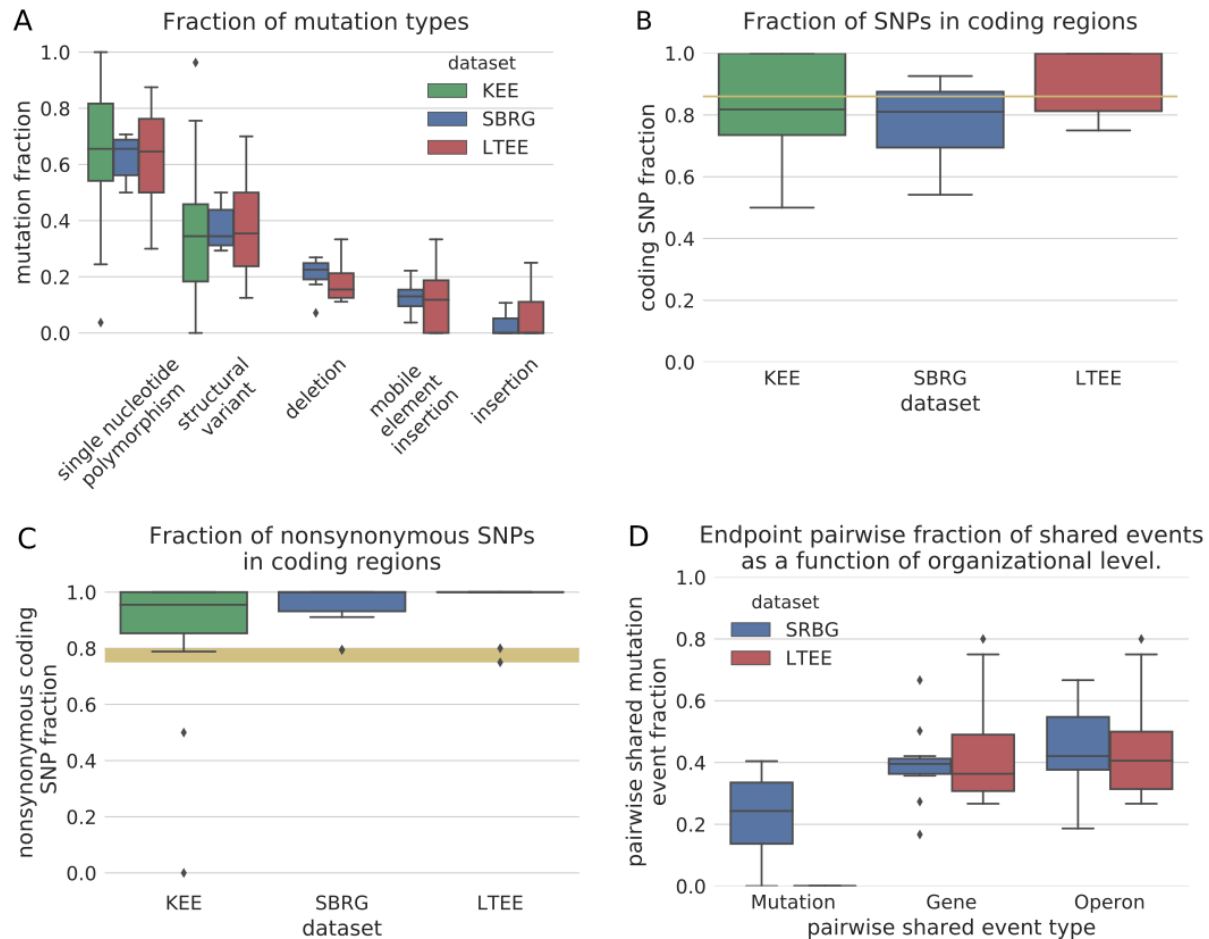
192 A *converged* mutation is one in which manifests in a genetic region seen to be mutated in multiple
193 replicate ALEs (Figure 6B). This phenomenon describes evidence of a potential common adaptive
194 trajectory between microbes exposed to the same conditions and has been leveraged in ALE analysis
195 methods to more quickly identify mutations causal for adaptive phenotypes [13]. ALEdb's *converged*
196 mutation reporting automates this analysis and reports results in the format described in Figure 5A.

197 Design and implementation

198 ALEdb is implemented and deployed using a standard web application technology stack and a
199 combination of user interface technologies. ALEdb's server-side hosts a MySQL database
200 (<https://www.mysql.com/>), uses the Python based Django web framework for pre-built web application
201 features (<https://www.djangoproject.com/>), and serves the content using Gunicorn
202 (<http://gunicorn.org/>) and Nginx (<https://www.nginx.com/>). ALEdb implements its user-interface with
203 HTML, CSS, and JavaScript along with a combination of essential libraries, including Bootstrap
204 (<https://getbootstrap.com/>), jQuery (<https://jquery.com/>), DataTables (<https://datatables.net/>),
205 mutation-needle-plot (<http://dx.doi.org/10.5281/zenodo.14561>), WebGL Protein Viewer
206 (<http://dx.doi.org/10.5281/zenodo.20980>), and D3 (<https://d3js.org/>).

207

Characterization and comparison of experimental evolution mutation sets



208 **Figure 7** **A** Endpoint mutation type proportion distributions across experiment sets. **B** Endpoint
 209 coding SNP proportion distributions across experiment sets. **C** Endpoint synonymous SNP
 210 distributions across experiment sets. **D** Coding endpoint mutation pairwise parallelism distributions
 211 for the SBRG and LTEE experiment sets. Operons were obtained from DOOR [17].

212 To demonstrate the potential for ALEdb as a consolidated mutation data resource for the field of
 213 experimental evolution, the experiment set databased in ALEdb and generated by the Systems Biology
 214 Research Group (SBRG) [5,10–15,18] was compared to experimental evolutions consolidated as two
 215 different sets. The experimental evolution mutation set from the Dettman and Kassen's studies (KEE)
 216 [19,20] was chosen for this comparison due to its parallel nature with the that of SBRG's. The KEE work
 217 consolidated the mutation data from 12 bacteria based experimental evolutions from the late 2000s and
 218 early 2010s, and established some of the mutation trends used in our comparisons. The Long Term
 219 Experimental Evolution (LTEE) is currently one of the most thoroughly studied experimental evolution
 220 mutation sets and its decades of mutation data has been released to the public [5]. The LTEE experiment
 221 set was chosen to represent a key data set for the field of experimental evolution. Mutation trends
 222 observed in previous publications were investigated across these three experiment sets [7,19,20],

223 resulting in an overall high level of agreement across experiment sets in their recapitulation of these
224 previously observed mutation trends.

225 Mutation type distributions were investigated across experiment sets to compare their endpoint
226 proportions. The mutation finding software used by the SBRG and LTEE [21] describes mutations as
227 single nucleotide polymorphisms (SNP), deletions (DEL), mobile element (MOB), and insertions (INS).
228 The KEE set describes its mutations as SNPs or structural variants (SV), where SVs describe the
229 combination of DEL, MOB, and INS mutations. SNPs are the most common mutation across all datasets,
230 with deletions, mobile elements, and insertions being the most common structural variants across SBRG
231 and LTEE sets respectively (Figure 7A). All experiment sets produced the same mutation type abundance
232 order and demonstrate similar distributions (Figure 7A, Table S1).

233 The frequent manifestation of SNPs in experimental evolution endpoints suggests that they are highly
234 correlated with adaptations. Previous publications investigated the selectivity of SNPs according to the
235 density of open reading frames within bacterial genomes. They proposed that if coding SNPs were more
236 causal for adaptations among all SNPs, their evolution endpoint proportions would be significantly larger
237 than the proportion of coding nucleotides within the *E. coli* bacterial genome (86%) [19]. The coding SNP
238 proportion distributions of all three experiment sets overlap the average bacterial open reading frame
239 genome proportion and don't provide evidence of being statistically different (Figure 7B, Table S2). The
240 SBRG and LTEE distributions are found to be significantly different (Table S3), which may result from the
241 different coding SNP selection characteristics in their executions.

242 Previous studies hypothesized that nonsynonymous SNPs are often selected as adaptive mutations in
243 experimental evolutions [19,20]. Given a SNP, the published range for the fraction of the bacterial
244 genome that can result in a nonsynonymous mutation is given as between 75% and 80%, depending on
245 the species [19,20]. The LTEE and SBRG sets demonstrate significant differences from this range (Table
246 S4). Though the KEE distribution doesn't demonstrate this same significance, it is shown to be
247 significantly similar to both the LTEE and SBRG sets, therefore lending evidence towards it likely being
248 borderline significant (Table S5). Overall, the experiment sets agree in presenting evidence of selection
249 for nonsynonymous SNPs.

250 A common method for finding adaptive mutations in an experimental evolution is through identifying
251 common mutational events between replicate evolutions [7]. Similar mutations that manifest across
252 independent replicate evolutions provide strong evidence towards a beneficial fitness effect. When the
253 starting strain of the replicate evolutions are identical, the repeat manifestation of mutational events is
254 known as parallel evolution [20]. The frequency of parallel evolution among replicates depends on the
255 resolution of genomic details considered. Parallel evolution may be more likely when considering broad
256 levels of organization, such as genes and operons, and less likely when considering higher resolutions,
257 such as sequence positions [19]. A pairwise comparison of coding mutations between replicate evolution
258 endpoints within experiments demonstrates that the broader the functional category of mutated
259 genomic regions considered, the larger the fraction of shared mutation events between endpoints
260 (Figure 7D). The KEE dataset did not include the necessary mutation details to test for parallelism
261 between replicates and therefore could not be compared alongside the SBRG and LTEE experiments. This
262 result recapitulate previous observations of parallelism increasing when considering higher levels of

263 genomic organization [7,19,20] and demonstrate similar proportions of parallelism between the SBRG
264 and LTEE datasets (Table S6).

265 This case study of mutation trend mining demonstrates the use of ALEdb as a source for rich
266 experimental evolution mutation data. The results demonstrate the agreement of the consolidated
267 experimental evolution mutation data with previously established trends and the agreement of trends
268 across data generated from different groups.

269

CONCLUSION

270 ALEdb works to serve the current need for a mutation database in the field of experimental evolution. It
271 is a platform designed for the integration and reporting of ALE mutation datasets and currently
272 integrates the mutation data and published materials of eleven published ALE experiments. Additionally,
273 multiple features are implemented within ALEdb to enable intuitive navigation and analysis. Finally, the
274 case study included in this work demonstrates the potential for ALEdb as a mutation data resource for
275 investigating experimental evolution trends.

276 ALEdb will continue to be developed to meet the needs of consolidating, reporting, and navigating ALE
277 experiment data. This initial release of ALEdb considers previously generated mutation datasets. ALEdb
278 will continue to grow with future inclusion of published ALE experiment results from currently
279 contributing and new research organizations.

280

AVAILABILITY

281 ALEdb is freely available online at <http://aledb.org> and can be accessed with a JavaScript-enabled
282 browser.

283

METHODS

284

Mutation finding pipeline

285 Mutation data currently hosted on ALEdb are generated by the *breseq* mutation finding pipeline [21].
286 Being that these samples come from different projects, various version of *breseq* were used in their
287 mutation data generation. The sequencing reads used to generate the mutation data were subjected to
288 quality control through either *FastQC* and *FastX-toolkit* or *AfterQC* [22–24].

289

Experimental evolution sample selection for case study

290 In comparing the endpoints of the SBRG, KEE, and LTEE experiment sets, strategies for normalizing
291 between experiments of different replicate evolution counts and lengths were necessary. To normalize

292 for approximate evolution length, LTEE samples at 2000 generations were considered endpoints. To
293 normalize for different replicate evolution counts per experiment within the SRBG experiment sets, the
294 average of each result across replicates is taken to represent an experiment. Additionally, no samples
295 containing hypermutator strains were included. Though hypermutator strains are informative, they
296 represent a cellular state that isn't easily comparable to strains with their DNA repair mechanisms intact.

297

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299

CONTRIBUTIONS

300 PVP, BOP, and AMF designed the study. PVP and DG consolidated the data and implemented the
301 software. PVP, BOP, and AMF wrote the paper.

302

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