Elsevier Editorial System(tm) for Journal of Theoretical Biology

Manuscript Draft

Manuscript Number: JTB-D-16-01015

Title: Genetic algorithm applied to parameter estimation of bacterial growth modeling

Article Type: Regular paper

Keywords: Predictive microbiology; Mathematical modeling of LAB and L. monocytogenes growth; Parameter estimation by inverse engineering; Multi-objective optimization using genetic algorithms

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Abstract: Predictive microbiology is nowadays one of the main tools to understand microbial interactions and to assess the quantitative risk in foods. Several models have been developed in order to predict microorganism growth. The resulting model equations for the growth of interacting microorganisms include a number of parameters which must be determined for the specific conditions to be modeled. The most effective method to determine these parameters is inverse engineering. When it is required to fit more than one experimental growth curve simultaneously, the process is more complex since it is necessary to apply a multi-objective optimization procedure. In the present report a genetic algorithm is presented which is applied to obtain the best parameter values of a mechanistic model that permit the construction of the front of Pareto with 50 individuals or phenotypes. The method was applied to the growth of lactic acid bacteria (LAB) and Listeria monocytogenes, resulting in very low errors of 0.23 and 0.25 for the LAB and L. monocytogenes between model and experimental values, respectively. The method is very adequate for application in determining parameter values adjusted by inverse engineering giving very good results.

1. Cover Letter

Instituto de Materiales de Misiones
CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS
UNIVERSIDAD NACIONAL DE MISIONES

Posadas, November 14 2016

Dear Editor

I am attaching in the web page of The journal of Theoretical Biology a full paper entittled "Genetic algorithm applied to parameter estimation of bacterial growth modeling" by Alejandro Pedrozo and Carlos E. Schvezov of the Instituto de Materiales de Misiones (IMAM-UNaM), Misiones, Argentina to be considered for publication in your Journal.

Sincerely,

Carlos Schvezov

Director

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Genetic algorithm applied to parameter estimation of bacterial growth modeling

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Abstract

Predictive microbiology is nowadays one of the main tools to understand microbial interactions and to assess the quantitative risk in foods. Several models have been developed in order to predict microorganism growth. The resulting model equations for the growth of interacting microorganisms include a number of parameters which must be determined for the specific conditions to be modeled. The most effective method to determine these parameters is inverse engineering. When it is required to fit more than one experimental growth curve simultaneously, the process is more complex since it is necessary to apply a multi-objective optimization procedure. In the present report a genetic algorithm is presented which is applied to obtain the best parameter values of a mechanistic model that permit the construction of the front of Pareto with 50 individuals or phenotypes. The method was applied to the growth of **lactic acid bacteria** (LAB) and *Listeria monocytogenes*, resulting in very low errors of 0.23 and 0.25 for the LAB and *L. monocytogenes* between model and experimental values, respectively. The method is very adequate for application in determining parameter values adjusted by inverse engineering giving very good results.

Keywords: predictive microbiology; bacterial interactions; parameter estimation; genetic algorithm.

1. Introduction

Growth and occupancy of any microorganism in different ecological niches depend on several environmental factors as well as the metabolic functions of cohabiting cells, since the microorganisms in common environment do not typically occur in axenic culture. The presence of one microorganism often can inhibit or delay the growth of their neighboring cells (negative interactions) due to consumption of shared resources (competition) or the release of toxic compounds (inhibitory activity) (Freilich et al., 2011, Stubbendieck et al., 2016). These interactions between microorganisms are usually used in food technology as a tool to extend the shelf life of fermented products to which a starter culture may be added. Lactic acid bacteria (LAB) are starter cultures able to compete with food-borne pathogens and/or food spoilage bacteria (Vignolo et al., 2012). They produce several inhibitory compounds, which can either be unspecific metabolites such as acetic acid, phenyllactic acid, indolelactic acid, etc. (Rodríguez-Pazo et al., 2013, Dallagnol et al., 2015) or more complex and specific compounds such as bacteriocins (Alvarez-Sieiro et al., 2016). In this sense, several works support the effectiveness of the inhibitory metabolites from LAB for controlling the growth of *Listeria* (*L.*) *monocytogenes* (Naz et al., 2013; Wemmenhove et al., 2016; Saraoui et al., 2016), being one of the main pathogen involved in ready-to-eat foods (Williams et al., 2011; Gómez et al., 2015). In order to understand better the responses of the microorganisms to the key controlling factors in the food environment, and develop the means to interpolate calculated microbial responses, emerges the predictive microbiology. This is nowadays one of the main tools to understand microbial interactions and to assess the quantitative risk in foods (Isabelle and André, 2006; Pérez-Rodríguez et al., 2013). In this work particularly, LAB behavior and capacity of inhibiting and/or altering pathogenic bacteria is studied. Several models have been developed in order to predict microorganism growth, which can be separated in two categories; phenomenological and mechanistic models. The firsts one are based on observations and measurements such as the following models: the logistic model (Gibson et al., 1987) which describes the growth by adjusting the experimental curve using four parameters; the trilinear model (Buchanan et al., 1997) in which each phase is described by a linear curve; the logistic model which includes growth delay (Rosso et al., 1996), and also uses four parameters which provides a good fitting capacity. On the other hand, the most current mechanistic model derived to describe the growth of microorganisms was developed by Baranyi and Roberts (1994).
An earlier work to describemicrobial interaction was based on the Lokta-Volterra model for two species in competition (Vereecken, et al 2000). Then, the logistic model was modified by Gimenez and Dalgaard (2004) to model the growth of interacting microorganisms growing in the same culture media and based on models of competing species. Later, Le Marc extended the

model (Le Marc et al., 2009), including a new parameter, the critical population density in which one of the species (lactic acid bacteria) inhibits the growth of other microorganisms. The resulting model equations for the growth of interacting microorganisms include a number of parameters which must be determined for the specific conditions to be modeled. The most effective method to determine these parameters is inverse engineering. However, when it is required to fit more than one experimental growth curve simultaneously, the process is more complex; since in order to obtain the values it is necessary to apply a multi-objective optimization procedure. In this way, the whole growth parameters are estimated simultaneously, which may have better results than sequential parameters estimation (Van Der Linden, et al. 2010).

A typical method to simplify this kind of problems consists on assuming that the effect of one microorganism is negligible for the growth of the other. Thereby it is possible to fit one growth curve, and then, with the information obtained from the last step, it is possible to fit the growth curve of the other microorganism, whose growth is affected by the other microorganism. However, with this procedure suboptimal results may be obtained due to the fact that it is assumed that the growth of one microorganism is not perturbed by the presence of the other; and if the growth curve, that is fitted first, presents high experimental errors, the parameters obtained for the second curve will present high errors too, despite the fact that the second curve may have less experimental errors.

In the present report a genetic algorithm is presented which is applied to obtain the best parameter values of the mechanistic model developed by Baranyi and Roberts (1994) and modified by Le Marc et al. (2009). The algorithm is applied to the growth of interacting LAB with *L. monocytogenes*, and the results are presented and analyzed.

2. Material and methods

2.1. The Model Equations

The following set of equations were used to model the growth of LAB and *L. monocytogenes* (Le Marc et al., 2009):

$$\frac{1}{LAB[t]}\frac{dLAB[t]}{dt} = \frac{Q^{LAB}[t]}{1+Q^{LAB}[t]}\mu_{max}^{LAB}\left(1-\frac{LAB[t]}{LAB_{max}}\right)\left(1-\frac{LM[t]}{LM_{CPD}}\right) \quad (1.a)$$

$$\frac{dQ^{LAB}[t]}{dt} = \mu_{max}^{LAB}Q^{LAB}[t] \quad (1.b)$$

$$\frac{1}{LM[t]}\frac{dLM[t]}{dt} = \frac{Q^{LM}[t]}{1+Q^{LM}[t]}\mu_{max}^{LM}\left(1-\frac{LAB[t]}{LAB_{CPD}}\right)\left(1-\frac{LM[t]}{LM_{max}}\right) \quad (1.c)$$

$$\frac{dQ^{LM}[t]}{dt} = \mu_{max}^{LM}Q^{LM}[t] \quad (1.d)$$

Subject to the following initial condition:

$$LAB[0] = LAB_0;\ LM[0] = LM_0;\ Q^i(0) = \left(e^{\mu_{max}^i * lag^i} - 1\right)^{-1}; \quad (1.e)$$

Where the variables are:

LAB[t]: concentration of *LAB* at time *t*;
LM[t]: concentration of *LM (L. monocytogenes)* at time *t*;
$Q^{LAB}[t]$: physiological state of *LAB* at time *t*;
$Q^{LM}[t]$: physiological state of *L. monocytogenes* at time *t*;
And the parameters are:
μ_{max}^{LAB} : maximum growth rate of *LAB*;
μ_{max}^{LM} : maximum growth rate of *L. monocytogenes*;
LAB_{max}: maximum concentration of LAB compatible with the given substrate;
LM_{max}: maximum concentration of *L. monocytogenes* compatible with the given substrate;
LAB_{CPD}: threshold concentration of *LAB* inhibiting growth of *LM*;
lag^{LAB}: delay time for *LAB*;
lag^{LM}: delay time for *L. monocytogenes;*
LAB_0*:* initial microbial load of Lactic acid bacteria;
LM_0*:* initial microbial load of *L. monocytogenes;*

superscript *i* represents the set *LAB, LM.*

Each term in the equations represents the following:

$\frac{Q^i[t]}{1+Q^i[t]}$:

This is a factor introduced by Baranyi and Roberts (1994) to correct the growth curve due to the lag phase in a mechanistic way. At low times, the value of the physiological state is near one, so the growth is slower; for higher times the physiological state is larger than one. As time

increases, this factor tends to one and represents the stage in which most of bacteria have changed to an exponential phase.

$\left(1-\frac{i[t]}{i_{max}}\right)$:

This is a factor to model the transition from the exponential to the stationary phase. At low times, its value is near one; for higher times this factor tends to zero and the growth is negligible.

$\left(1-\frac{i[t]}{i_{CPD}}\right)$:

This is a factor to model the competition intra-species in co-culture. When the concentration of species *i* is near i_{CPD}, the effect of inhibition becomes important. For *L. monocytogenes*, it is assumed that $LM_{CPD} = LM_{max}$.

In order to solve equation (1), Wolfram Mathematica 9.0 was used. In particular, the differential ordinary equations systems were solved with a commad "NDSolve" inside the software, and a Runge-Kutta 4-5 was set as solver.

2.2. The Genetic Algorithm for Selecting Parameter Values

The basic process to determine the values of the parameters is based on selecting those which best fit the model solutions with the experimental results. In such case there are always differences between the model and the experimental results which are called residues. The regression method widely used to find the parameter values that best fit the experimental with the model results consist on minimizing the squared error, which in the present case is the sum of the square of all residues. Considering the complexity of the differential equations (1), it is necessary to use non-conventional methods in order to find the values of the parameters that minimize the squared error (McKellar and Lu, 2003).

On the other hand, the squared error for both curves must be minimized simultaneously, so it is a multi-objective optimization problem. The most common method applied to solve this problem consists on transforming, by a suitable combination, the set of objectives in only one objective through a weighed addition method. In this method, the new objective function is the sum of each objective with a given weight determined by the user and related to the specific problem. In particular, in the present report the criterion proposed by Sun and Li (2014) is adopted in which the new objective function ϕ can be written as:

$$\phi = \sum_{i=1}^{2} w_i \sum_{j=1}^{n_{exp}} (X_{ji} - \hat{X}_{ji})^2 \qquad (2.a)$$

$$w_i = {\sigma_{ii}}^{-1} = \frac{\left(\sum_{j=1}^{n_{exp}} X_{ji}\right)^{-1}}{\sum_{i=1}^{2}\left(\sum_{j=1}^{n_{exp}} X_{ji}\right)^{-1}} \qquad (2.b)$$

Where, w_i is the weight of each objective; X_{ji} y $\hat{X}_{ji}$ are the experimental and calculated data, respectively, of the concentration of bacteria i at the experimental point j; n_{exp} is the number of experimental points. The values assigned to the weights w_i, are the inverse of the elements of the main diagonal of the covariance matrix of the errors.

Due to the non-linearity of the differential equations 1 (a-d) and the objective function 2 (a-b), for convergence of the solutions using conventional optimization methods as the Newton-like methods, good initial values of the state variables and the parameters are required. Otherwise, the solution method may fail in finding global solutions and converge to local minimum (Rangaiah and Bonilla-Petriciolet, 2013).

In addition, minimizing the objective function 2a indirectly may increase the probability of missing some optimal solutions in the case of integrated objective functions, which show a duality gap due to non-convexity (Silva and Biscaia, 2003). In such case, rather than obtaining a unique solution, multi-objective optimization provides a family of solutions, which is called Pareto-optimal set. This set is built with all vector solutions, which improve at least in one of the objectives without degrading the values of the other objectives. Each vector solution included in the Pareto-optimal set is called non-dominated solution. The image of Pareto-optimal set is called Pareto front (Abraham and Jain, 2005).

In the present work, the Pareto front is obtained employing a genetic algorithm. This kind of algorithm have been applied to solve optimization problems (Silva and Biscaia, 2003; Meneses and Echeverri, 2007 ; Din et al, 2016) giving very good results. Genetic algorithms are based on biological evolution as the conceptual framework for their search process and they consists on representing the set of adjusting parameters (the phenotype) by a binary chain (genotype). The squared errors for the phenotypes are evaluated (Figure 1) and the best or more suitable genes are determined and selected, as well as the direction of evolution towards better individuals.

The starting population of individuals is random, and their genotypes are subjected to operations of selection, crossover (with crossover probability) and mutation (with mutation probability) in order to duplicate the number of individuals. The best individuals are selected to belong to the next generation by means of an elitist algorithm of the type NSGA II (Deb et al., 2002) as illustrated in Figure 2.

The population is classified through an elitist algorithm (Deb et al., 2002). The classification by ranks is as follows: Rank 1 is assigned to non-dominated individuals of whole population, then

these individuals are removed from the population, and rank 2 is assigned to non-dominated individuals of the residual population. This procedure continues until every individual gets a rank.

Apart from the rank of each individual, there may be a second criteria for the selection of the best individuals. In the elitist algorithm NSGA II (Deb et al., 2002), this criteria is based on the crowd distance, which is an index to keep the diversity of results and to avoid the convergence of all individuals to a cluster solution. In the present problem, a cluster solution with near zero squared error for both curves is desired, in such case the crowd distance was substituted for the Euclidean distance of the objectives to the origin. At first the crowd distance was established as second criteria, but preliminary results of this research proved that good solution near the origin may disappear, due to its low crowd distance, when all individuals have rank 1.

The mating selection is done through a tournament where two individuals are selected randomly, their rank and their origin distance are compared (lower rank is selected; in case of the same rank, lower origin distance is selected), and the best individuals are chosen. With the selected individuals from the previous step, couples of them are generated randomly. Then, for each couple a random number between 0 and 1 is generated, if this number is less than the crossover probability, a piece of their genotype is exchanged randomly (Figure 3). Then for each individual of the last stage and for each of their genes a random number between 0 and 1 is generated, if this number is less than the mutation probability, its gene changes (Figure 3).

In order to pass to the next generation, the whole population must be reduced to the initial number, therefore a selection operation is applied through the comparison among the ranks and their origin distances. Lower rank individuals are selected, and in case they have the same rank, lower origin distances are selected.

It is necessary to select a convergence criteria to decide when the search is stopped. Different option of this may be chose, like the maximum number of iteration (Din, et al. 2016) or minimum convergence speed (Silva and Biscaia, 2003). In this work such criteria is as follow: If no better solutions appear after 10 iterations of the algorithm, the search is ended.

2.3. Monte-Carlo analysis

In order to determinate the uncertainty of the fitted parameters obtained by means, the proposed algorithm Monte-Carlo analysis was applied (Poschet et al., 2003). In addition, the Monte-Carlo analysis was used to compare multi-objective optimization and the conventional fit.

The conventional fit was applied as follow: the effect of inhibition of *L. monocytogenes* is neglected towards the lactic acid bacteria, then it is possible to get the parameters of lactic bacteria growth by means of a regression method. Then with this set of parameters, the growth curve of *L. monocytogenes* is fitted using equation 1.c and 1.d. In this case, the algorithm used

for minimizing the squared errors in the regression method was the proposed genetic algorithm, but with mono-objective function.

For each algorithm, Monte Carlo simulation was performed using 3000 runs. Then, the mean and standard deviation (SD) of each parameter is calculated and the results of each model are compared.

3. Results and discussion

"The regression method described above was applied to specific population evolution of the interacting LAB and *Listeria monocytogenes* species (Tas5611, Tas5612) obtained from Combase (www.combase.cc) and produced in the Tasmanian Institute of Agriculture (Australia)."

The iteration process applied in the present report, starts with a population of individuals with large initial squared errors that with the successive iterations decreases to acceptable values. An example of this kind of simulation is showed in Figure 4. At generation zero the best solution presents high SE of the order of 6 and 8 for LAB and *L. monocytogenes* respectively, but for the thirtieth generation, the errors diminished below 1 for both bacteria.

The evolution of parameters to give better solutions is shown in Table 1 for another simulation. In this table, the best individual is chosen by the criteria described above, and its parameters are shown for different simulations. The evolution direction of parameters is not trivial since the targets (squared errors) are complex functions of them. At the ultimate iteration, each individual belongs to Pareto-optimal set and many of them are locally optimal solutions of the problem. The best solution is chosen using the objective function (2.a), in this case, it presents SE of 0.23 and 0.25 for LAB and *L. monocytogenes* respectively.

Table 1. Parameters evolution

		Iteration			
	Units	0	10	100	Ultimate
μ_{max}^{LAB}	(h^{-1})	0.1856	0.1522	0.1499	0.1497
LAB_{max}	$Log_{10}(CFU/ml)$	9.3664	8.9458	9.1783	9.3752
lag^{LAB}	(h)	15.1270	8.8544	7.4621	6.5843
LAB_0	$Log_{10}(CFU/ml)$	2.6988	3.1111	3.1380	3.089
μ_{max}^{LM}	(h^{-1})	0.0630	0.0953	0.0971	0.1027
LM_{max}	$Log_{10}(CFU/ml)$	5.9964	6,0707	5,8069	5,8284
lag^{LM}	(h)	25.298	10.9648	17.4279	21.0400
LM_0	$Log_{10}(CFU/ml)$	2.9638	2.6473	2.7220	2.7858
LAB_{CPD}	$Log_{10}(CFU/ml)$	9.1969	8.7812	8,8242	8.7905
SE BAL		3.3357	0.3275	0.2451	0.2307

SE LM		10.7725	0.5115	0.2539	0.2500

With this population the front of Pareto is built. At the end of the search, in order to choose good individuals the objective function is calculated using equation 2. The best individuals are those with the smallest objective functions. The set of parameter values of the governing equation, represented by this individual or phenotype, is used to solve the governing equations 1(a-d). Then, the evolution of the interacting bacteria populations are plotted and compared with the experimental results.

A front of Pareto built from an initial population of 50 individuals and employing the genetic algorithm described here is shown in Figure 5. The crossover probability and mutation probability were fixed in 90 % and 4 %, respectively. This front is obtained in the 251-th generation of individuals, which after each generation gives a lower error and converges to a given point in the front. The coordinates in Figure 5 are the squared errors for each bacteria. In this front, each point represents the squared error of each phenotype when used as parameters in the governing Equation 1. Each of the 50 phenotypes in the front of Pareto are valid solutions; however the best must be chosen. The criterion employed in the present report, as described before, is based in the calculation of the target function defined in equation 2.a. The results are shown in Figure 6.

On other hand, many other individuals have objective function values close to 0.24, being the first individual in Figure 6 the one with the smallest weighted error. If this individual or phenotype is chosen as the set of parameter values to be input in the governing equation, the results of the evolution model for both interacting bacteria are represented in Figure 7. The full lines are the model results and the points are the experimental results obtained from the literature (Tasmanian Institute of Agriculture). It can be concluded that there is a very good fitting between model and experimental results with a squared error of 0.23 for the L and of 0.25 for the *L. monocytogenes*. Moreover, it shows that the genetic algorithm developed and presented in this report is adequate and gives very good results.

3.1 Monte-Carlo simulation

The results of Monte-Carlo analysis are shown in Table 2 Numerical values of the parameters obtained by using both fit method are similar. Both means values, the standard deviation (SD) and the average of squared errors (both SE BAL and SE LM) are similar too. However, conventional fit results may not capture the effect of interacting phenomena.

The critical density population of lactic acid bacteria (LAB_{CPD}) is a parameter to quantify the interaction between bacteria. A numerical value of LAB_{CPD} higher than LAB_{CPD} means the growth of the pathogen is not inhibited by LAB when this bacteria achieves its maximum

density population. This case is obtained by conventional fit, and the calculated average of LAB_{CPD} is higher than the average of LAB_{max}.

Instead, multi-objective optimization calculated values of LAB_{CPD} lower than the average of LAB_{max}, which means BAL inhibits the growth of the pathogen before it achieves its maximum density population. Due to the kind of co-culture analyzed, the results obtained by multi-objective optimization captures better the biological behavior.

Table 2. Monte-Carlo analysis for both fit methods.

		Multi-objective optimization		Conventional fit	
	Units	Mean	SD	Mean	SD
μ_{max}^{LAB}	(h^{-1})	0.1513	0.0152	0.1352	0.0109
LAB_{max}	$Log_{10}(CFU/ml)$	9.3141	0.3917	8.9529	0.1935
lag^{LAB}	(h)	7.7645	5.2278	4.7590	4.5665
LAB_0	$Log_{10}(CFU/ml)$	3.1508	0.2308	3.0842	0.4145
μ_{max}^{LM}	(h^{-1})	0.0965	0.0152	0.1023	0.0211
LM_{max}	$Log_{10}(CFU/ml)$	5.9515	0.2681	5.8589	0.2384
lag^{LM}	(h)	16.555	9.1005	18.8027	9.7552
LM_0	$Log_{10}(CFU/ml)$	2.7263	0.2116	2.6403	0.3941
LAB_{CPD}	$Log_{10}(CFU/ml)$	8.9601	0.3238	9.1596	0.3424
SE BAL		0.5367	0.2322	0.5312	0.2230
SE LM		0.5127	0.2246	0.4931	0.2205

4. Conclusions

A coupled ordinary differential equation to describe the growth of interacting microorganisms method was solved, LAB and *L. monocytogenes*, using a mechanistic model. The parameters of the differential equations were determined by inverse engineering using a genetic algorithm that permits the construction of the front of Pareto: 50 individuals or phenotypes were used to build the front. The best individuals are chosen minimizing the objective function. The predictions given by the model results are very close to the experimental values with very low errors of 0.23 and 0.25 for the LAB and *L. monocytogenes* between model and experimental values.

The Monte-Carlo analysis show that the mean values and standard deviation of parameters obtained by using conventional and multi-objective fit are similar, but the results of multi-objective optimization describes better the biological behavior in co-culture.

The method is very adequate for application in determining parameter values adjusted by inverse engineering giving very good results.

5. Acknowledgements

This study was supported by Comite Ejecutivo de Desarrollo e Inovación Tecnologica (CEDIT), Misiones, Argentina and, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina.

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424
425 **Figure captions**
426
427 **Figure 1.** Schematic of the process to obtain the attributes of individuals.
428 **Figure 2.** Schematic of the generation of a new Population (adapted from Meneses and
429 Echeverri, 2007).
430 **Figure 3.** Crossover and mutation operations (De Castro, 2006).
431 **Figure 4.** Pareto Front evolution.
432 **Figure 5.** Front of Pareto obtained with an initial population of 50 individuals.
433 **Figure 6.** Objective function calculated for 50 hundred individuals using Equation 2.
434 **Figure 7.** Experimental and model results produced with the selection of parameters with a
435 genetic algorithm.

***Highlights (for review)**

Highlights

- Predictive microbiology to asses microbial interactions
- Mathematical modeling of LAB and *L. monocytogenes* growth.
- Parameter estimation by inverse engineering.
- Multi-objective optimization using genetic algorithms

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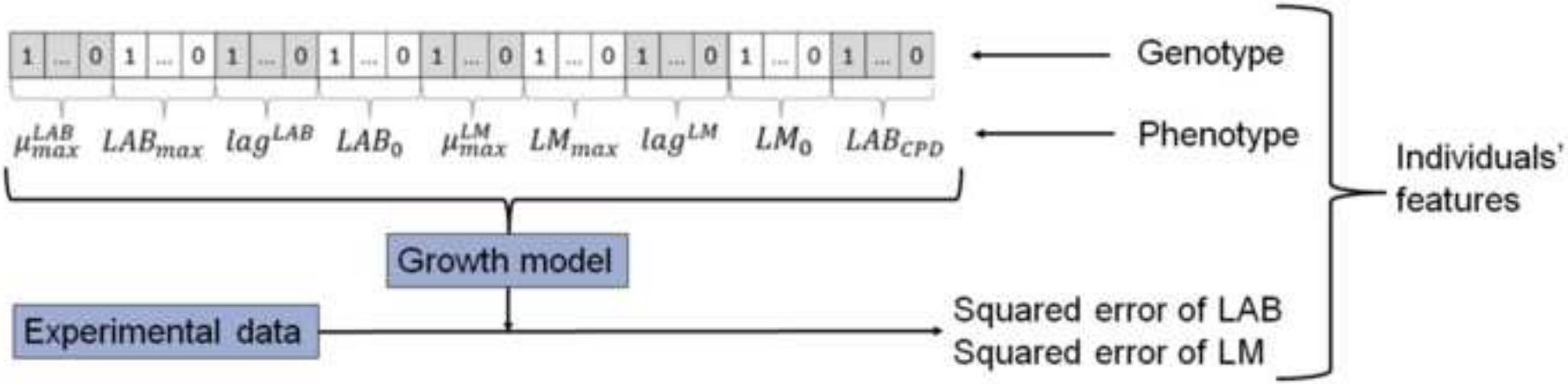

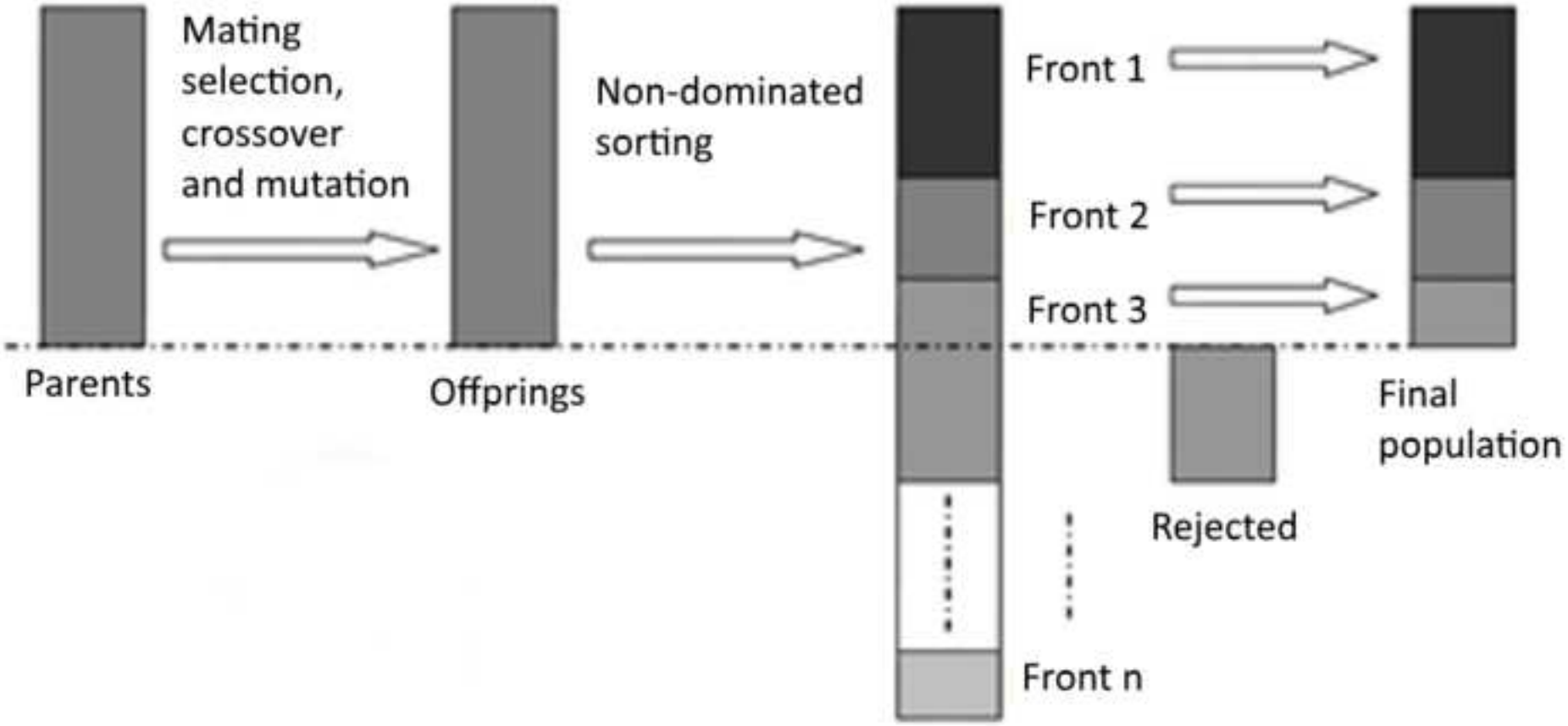

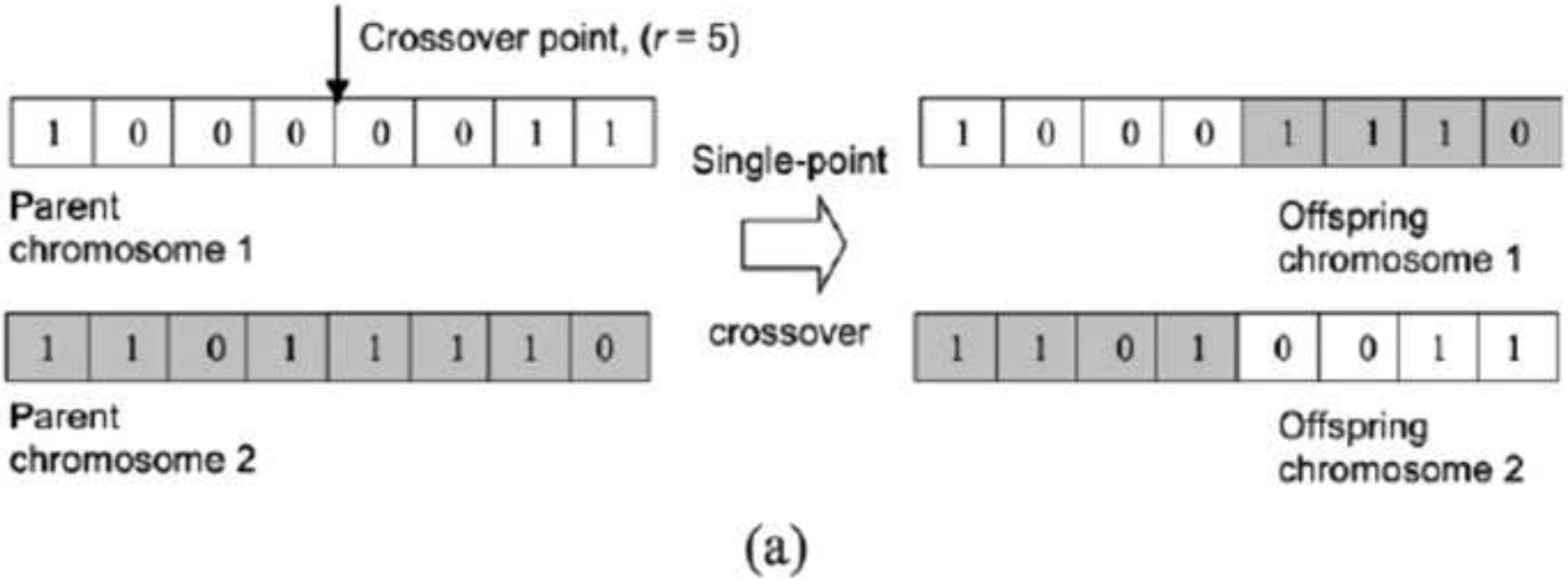

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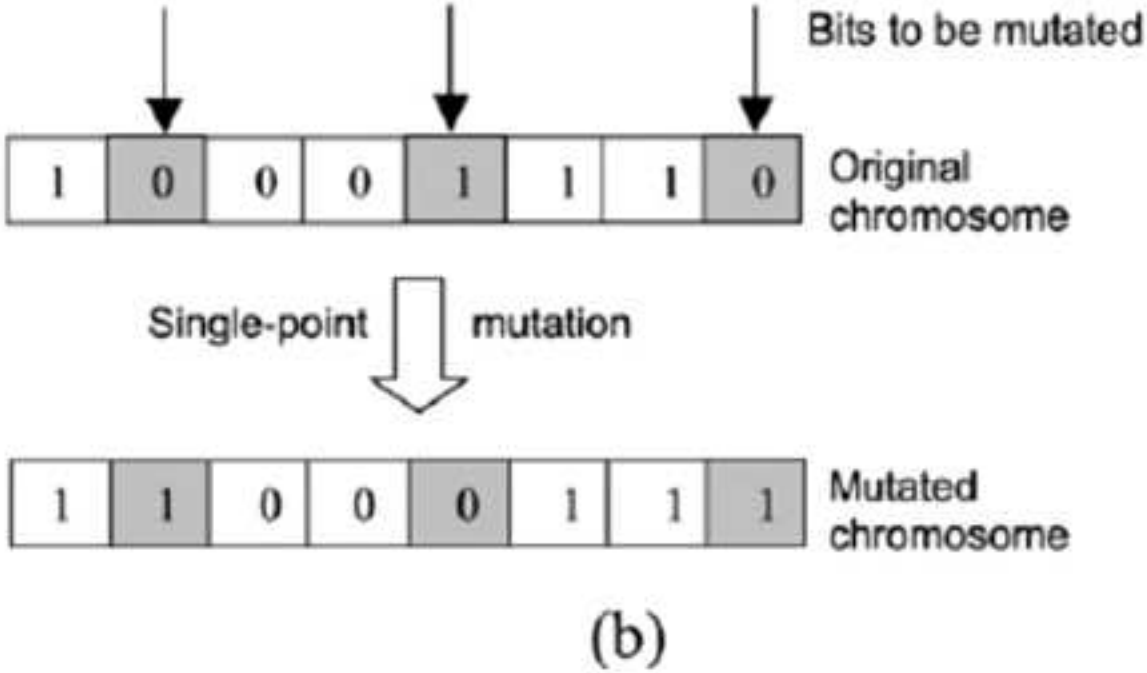

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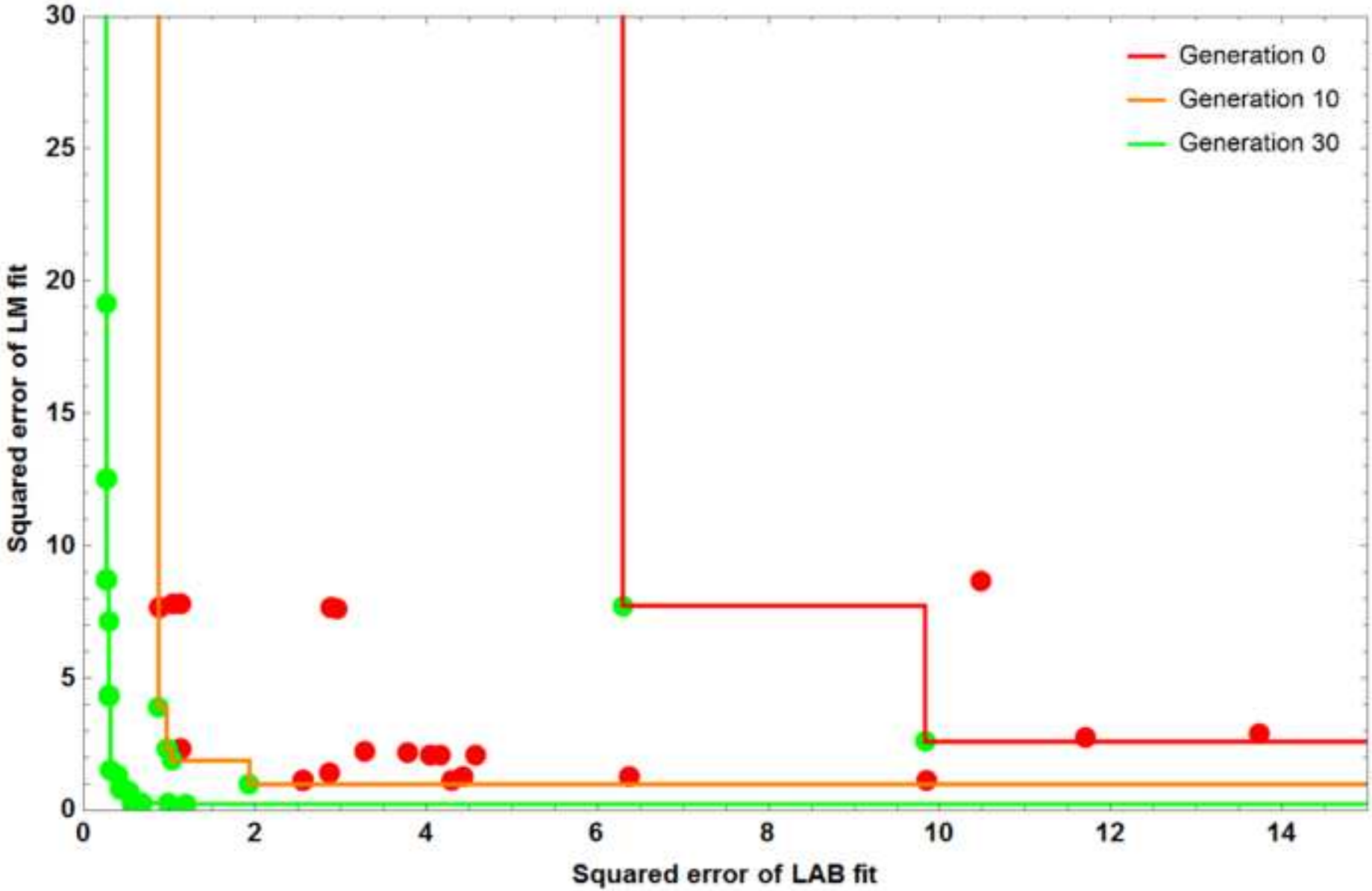

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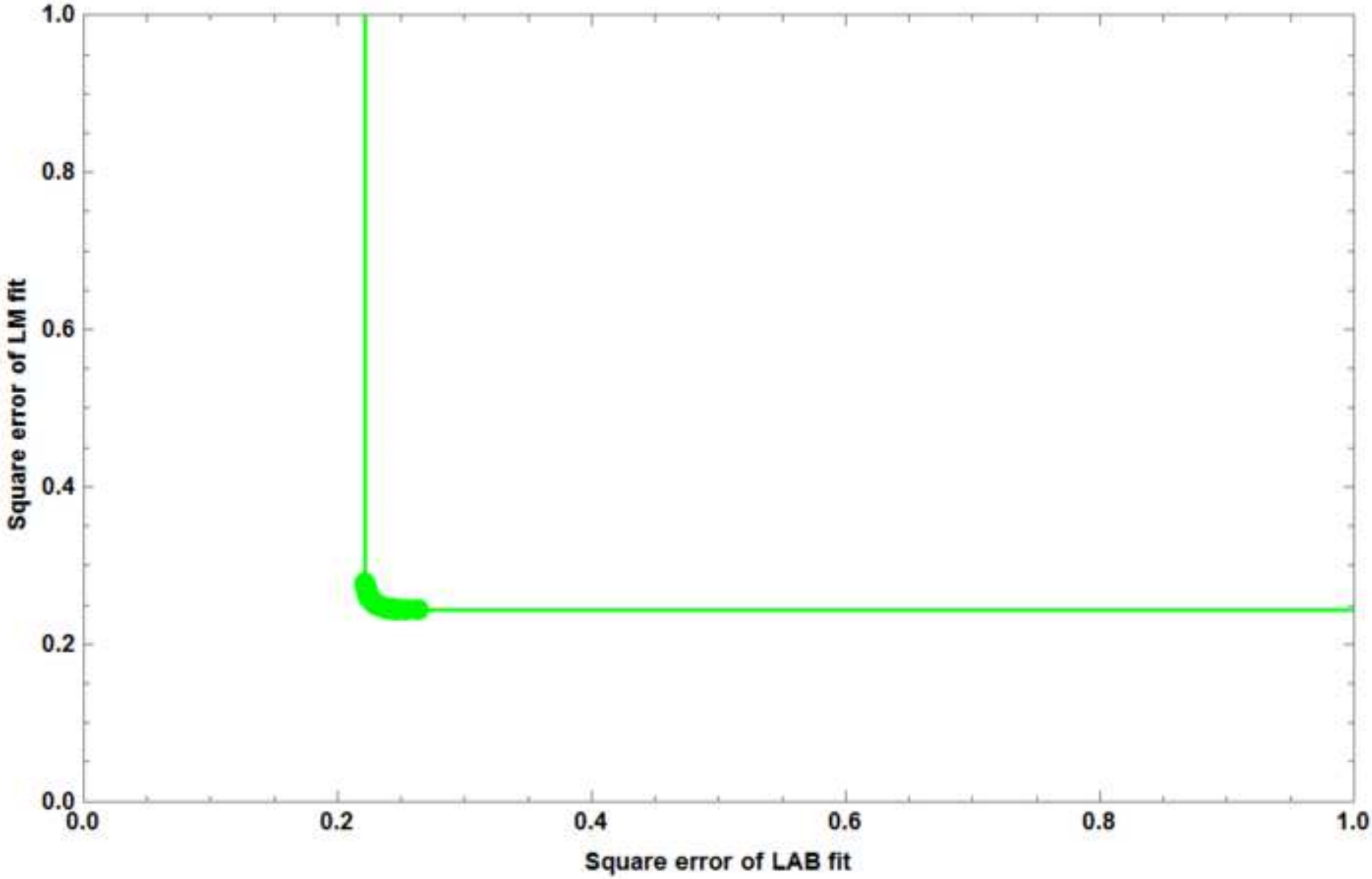

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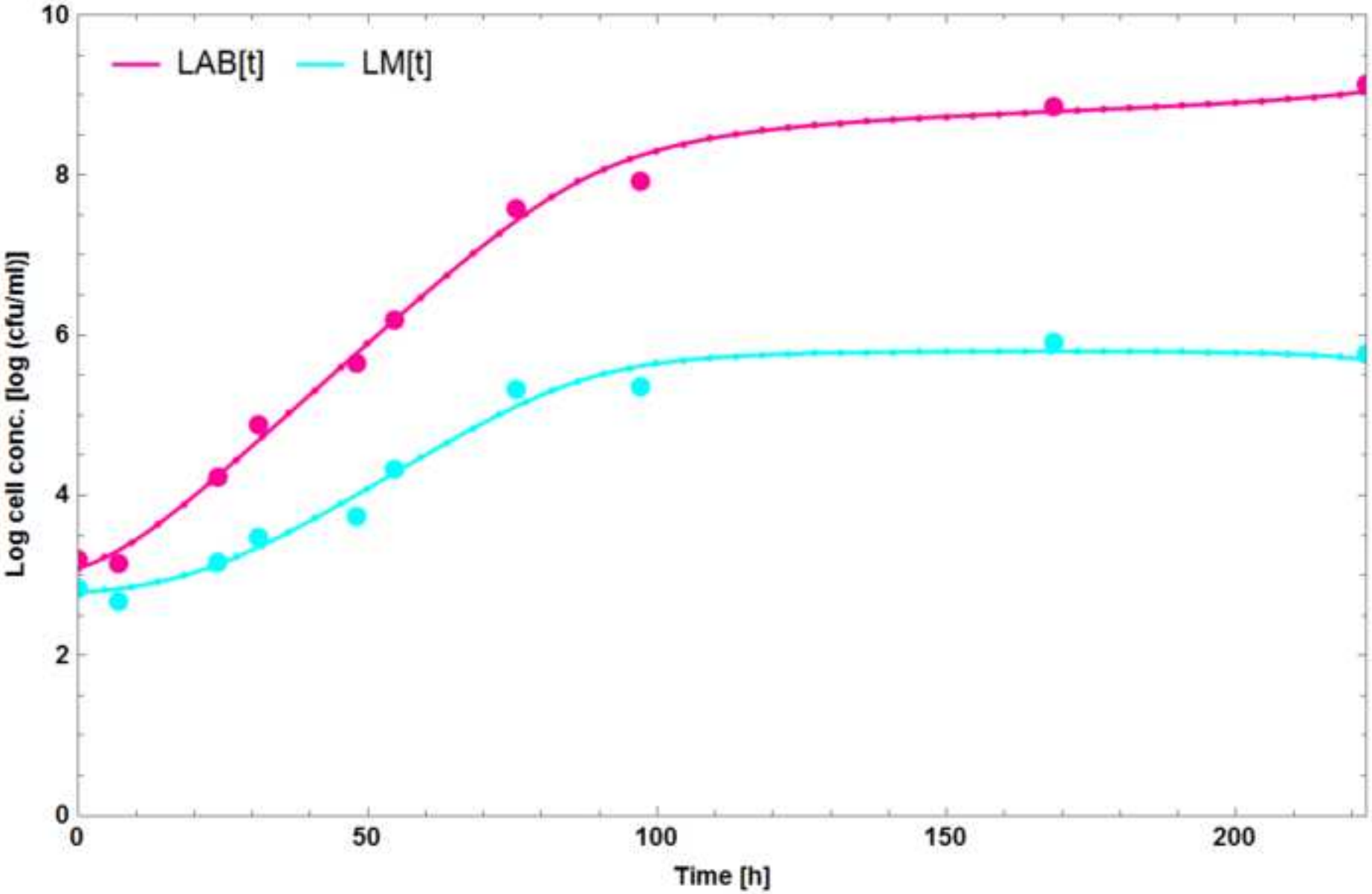

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