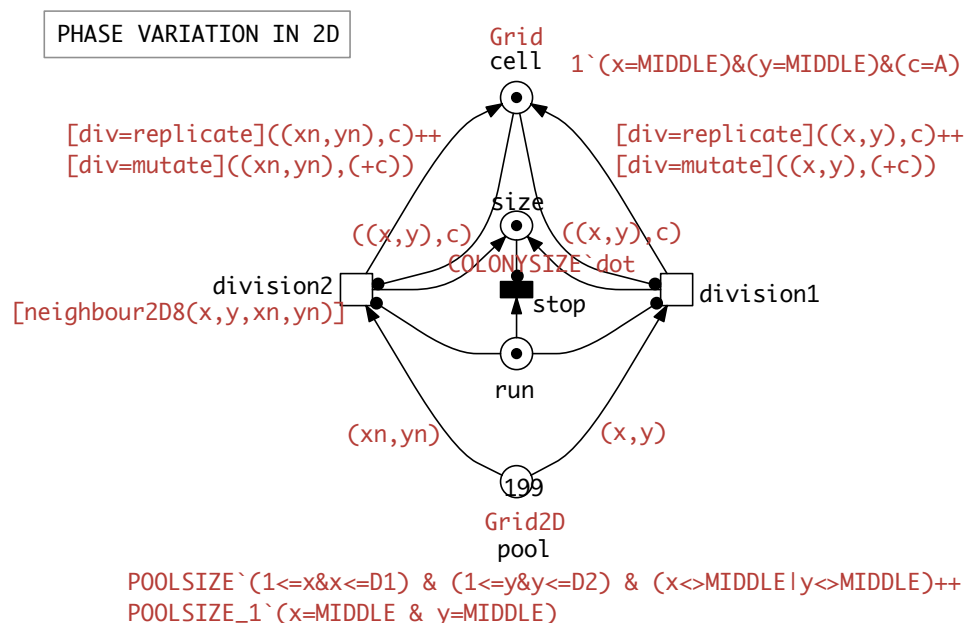


*This form is a summary description of the model entitled “Phase variation in cell colony growth” proposed for the Model Checking Contest @ Petri Nets. Models can be given in several instances parameterized by scaling parameters. Colored nets can be accompanied by one or many equivalent, unfolded P/T nets. Models are given together with property files (possibly, one per model instance) giving a set of properties to be checked on the model.*

## Description

This coloured (stochastic) Petri net has been designed to study phase variation in bacterial cell colonies growing in a 2-dimensional space. Phase variation is a common microbial stochastic mechanism, in which gene expression is controlled by a reversible genetic mutation, re-arrangement, or modification. Here, we consider a colony of bacteria with two phenotypes A and B, which develop over time by cell division. Cell division may involve cell mutation, and back-mutation alternates phenotypes. The observation starts with one bacterium of phenotype A in the spatial centre. In the stochastic setting, we are interested in the proportion of phenotypes in the cell generations, and how their spatial distribution evolves over time. The Markov chain (marking graph) is finite, but its size explodes already for small parameter values. Thus, phase variation has been explored by stochastic simulation; for more details see [GHLS13] and [PGHLS13].



*Coloured Petri net constructed with Snoopy [HHL+13]. The 2-dimensional space is colour-encoded as  $D \times D$  rectangular grid as demonstrated in the model 'Diffusion in 2D'. Division1: the offspring goes to the parent's position, division2: the offspring goes to one of the (up to) eight neighbouring positions. If there is mutation, then it is always the mutant who goes to the neighbouring position. The number of bacteria on a given grid position is limited by the number of available tickets, which are provided on the coloured place 'pool'; shown is the total number of tokens of any colour. The model enters a dead state (by the firing of the transition 'stop') when the specified colony size (parameter CS) has been reached. This requires always exactly CS-many firings (CS - 1 - because we start with one bacterium, plus one firing of stop).*

## References

- GHLS13** D Gilbert, M Heiner, F Liu and N Saunders: Colouring Space - A Coloured Framework for Spatial Modelling in Systems Biology; In Proc. PETRI NETS 2013, Milano, Springer, LNCS, volume 7927, pages 230–249, June 2013.
- PGHLS13** O Parvu, D Gilbert, M Heiner, F Liu and N Saunders: Modelling and Analysis of Phase Variation in Bacterial Colony Growth; In Proc. CMSB 2013, Vienna, Springer, LNCS/LNBI, volume 8130, pages 78-91, September 2013.
- HHL+13** M Heiner, M Herajy, F Liu, C Rohr and M Schwarick: Snoopy a unifying Petri net tool; In Proc. PETRI NETS 2012, Hamburg, Springer, LNCS, volume 7347, 398407, June 2012.

## Scaling parameter

Parameter name	Parameter description	Chosen parameter values
$D, CS$	$D$ – grid size, i.e. there are $D \times D$ grid positions; $CS$ – colony size, i.e. the colony will grow up to $CS$ tokens (bacteria); $CS$ does not influence the size of the model.	2, 5, 10, 20, 30 (for $D$ ); 10, 100 (for $CS$ )

## Size of the model

Parameter	Number of places	Number of transitions	Number of arcs
$D = 2$	14 $[3 \cdot (D \cdot D) + 2]$	65	$193 + 2 \cdot 129$
$D = 5$	77	677	$2029 + 2 \cdot 1353$
$D = 10$	302	3137	$9409 + 2 \cdot 6273$
$D = 20$	1202	13457	$40369 + 2 \cdot 26913$
$D = 30$	2702	30977	$92929 + 2 \cdot 61953$

## Structural properties

<b>ordinary</b> — all arcs have multiplicity one .....	X
<b>simple free choice</b> — all transitions sharing a common input place have no other input place .....	X (a)
<b>extended free choice</b> — all transitions sharing a common input place have the same input places .....	X (b)
<b>state machine</b> — every transition has exactly one input place and exactly one output place .....	X (c)
<b>marked graph</b> — every place has exactly one input transition and exactly one output transition .....	X (d)
<b>connected</b> — there is an undirected path between every two nodes (places or transitions) .....	✓
<b>strongly connected</b> — there is a directed path between every two nodes (places or transitions) .....	X
<b>source place(s)</b> — one or more places have no input transitions .....	✓
<b>sink place(s)</b> — one or more places have no output transitions .....	X
<b>source transition(s)</b> — one or more transitions have no input places .....	X
<b>sink transitions(s)</b> — one or more transitions have no output places .....	X
<b>loop-free</b> — no transition has an input place that is also an output place .....	X
<b>conservative</b> — for each transition, the number of input arcs equals the number of output arcs .....	X
<b>subconservative</b> — for each transition, the number of input arcs equals or exceeds the number of output arcs .....	X
<b>nested units</b> — places are structured into hierarchically nested sequential units <sup>(e)</sup> .....	X

## Behavioural properties

<b>safe</b> — in every reachable marking, there is no more than one token on a place .....	X
<b>deadlock</b> — there exists a reachable marking from which no transition can be fired .....	✓ (f)
<b>reversible</b> — from every reachable marking, there is a transition path going back to the initial marking .....	X (g)
<b>quasi-live</b> — for every transition $t$ , there exists a reachable marking in which $t$ can fire .....	✓
<b>live</b> — for every transition $t$ , from every reachable marking, one can reach a marking in which $t$ can fire .....	X

(a) the net is not ordinary in all its 10 instances (see all aforementioned scaling parameter values).

(b) the net is not ordinary in all its 10 instances (see all aforementioned scaling parameter values).

(c) the net is not ordinary in all its 10 instances (see all aforementioned scaling parameter values).

(d) the net is not ordinary in all its 10 instances (see all aforementioned scaling parameter values).

(e) the definition of Nested-Unit Petri Nets (NUPN) is available from <http://mcc.lip6.fr/nupn.php>

(f) Reached by firing of the transition stop; there is a very large number of dead states, corresponding to the token distributions over the grid.

(g) There is no counteraction to the token increase.

## Size of the marking graphs

Parameter	Number of reachable markings	Number of transition firings	Max. number of tokens per place	Max. number of tokens per marking
$D = 2, CS = 10$	7 716	?	10	?
$D = 2, CS = 100$	18 989 219 241 (10)	?	100	?
$D = 5, CS = 10$	476 385 615 597 (11)	?	10	?
$D = 5, CS = 100$	$> 5.5 \cdot 10^{25}$	?	100	?
$D = 10, CS = 10$	?	?	10	?
$D = 10, CS = 100$	?	?	100	?
$D = 20, CS = 10$	?	?	10	?
$D = 20, CS = 100$	?	?	100	?
$D = 30, CS = 10$	?	?	10	?
$D = 30, CS = 100$	?	?	100	?

## Other properties

A realistic scenario considers about 26 synchronous generations (which may develop in the wet lab in 24 hours), yielding a colony size of approximately  $67 \cdot 10^6$ . In our simulation experiments, we used a  $101 \times 101$  grid. Obviously, this configuration is far beyond of what can be currently explored by constructing the complete state space with state-of-the-art hardware & software techniques.