4.3.2 - DNA Nebulization

Parameters

Parameter Name	Variable	Default Value	Parameter Range	Description
FRAG_NB_M	Unknown macro: 'mathinline'	1	>0	fragmentation intensity ("rotor speed", controls the slope of the fragment size distribution)
FRAG_NB_LAMBDA	Unknown macro: 'mathinline'	900	>0	limiting molecule size below which it is very difficult to break a molecule with the shear field
FRAG_NB_THOLD	Unknown macro: 'mathinline'	0.1	Unknown macro: 'mathinline'	threshold on the fraction of molecules below which thermodynamics equilibrium is assumed

Algorithm

Already early reports on results from RNA-Seq experiments based on nebulization observed reads accumulating at the 5'-end of transcripts and around the center, especially of shorter transcript forms. These observations coincide well with breakpoint distributions obtained by a theoretical model of mechanical breakage that considers molecules as rigid stiffs, in which breakpoints recursively accumulate around the midpoint of iteratively broken fragments. According to this model, the average expected fragment size depends on the length of the nebulized DNA molecule: comparatively short molecules accumulate higher breaking probabilities during the time it takes to fragment the longer molecules in the transcript population.

In the light of these preliminary studies, we simulate nebulization by an iterative two-step process: first, a random orientation of the molecule in the shear field—i.e., the point (Unknown macro: 'mathinline') where the shearing stress is applied—is determined by random sampling under a Gaussian function centered at a molecule's midpoint. Subsequently, the breaking probability Unknown macro: 'mathinline' is deduced from the exponential:

Unknown macro: 'mathblock'

where Unknown macro: 'mathinline' is the molecule length, Unknown macro: 'mathinline' is a parameter that describes the limiting size below which molecules are very unlikely broken by the shearing field; Unknown macro: 'mathinline' is a parameter describing the force of the shear field and determines the steepness of the slope in the resulting fragment size distribution; Unknown macro: 'mathinline' finally is a constant that adjusts Unknown macro: 'mathinline' to be 0.5 for a size exactly between Unknown macro: 'mathinline' (Unknown macro: 'mathinline') and (Unknown macro: 'mathinline'). In our model, a Bernoulli trial on Unknown macro: 'mathinline' determines whether a simulated break incurs at a given position. Recursive breaking continues until thermodynamics equilibrium as assumed by convergence of the fraction of breaks per time unit in the library falling below a given threshold (*t*=1%).