Using *UIDcollision\_GCpref.r*

Jenna VanLiere Canzoniero, M.D., M.S.

Johns Hopkins University Department of Medicine

Last updated August 17, 2016

1. Description

The file UIDcollision\_GCpref.r contains the primary function UIDerrorGCpref, as well as supporting function estP2same and ecol. UIDerrorGCpref simulates the results of a next-generation sequencing run performed with barcoding and processed into consensus sequences by family. UIDs are created from nucleotides according to probability (0.3, 0.3, 0.2, 0.2). estP2same estimates the probability that at least 2 sample molecules have the same UID. ecol calculates the expected number of samples that will have non-unique UIDs.

1. Usage

UIDerrorGCpref(nsamp, mutFreq, nUID, seed=1, conFrac=0.95, PCRcyc=25, PCRsuc=0.2, minMembers=2)

1. Arguments

|  |  |
| --- | --- |
| nsamp | Number of sample molecules, must be a positive integer |
| mutFreq | Actual underlying frequency of mutation in data, note that if the mutFreq x nsamp does not results in an integer number of mutants, then the number of mutant molecules will be rounded according to rules of round() |
| nUID | Number of possible UIDs, must be a multiple of 4 |
| seed | Random generator seed, default 1 |
| conFrac | Fraction of molecules within each UID family that must agree in order for a consensus sequence to be called. Default 0.95 (95%) |
| PCRcyc | Number of PCR cycles, default 25 |
| PCRsuc | Probability of success for each PCR cycle, default 0.2 |
| minMembers | Minimum number of members of UID family in order for UID consensus to be called |

1. Details

UIDerrorGCpref simulates binding of UID molecules to sample molecules as sampling with replacement. Only 1 locus of mutation is simulated. The UIDs are created according to probability of each nucleotide set in the function. The probability weights for each nucleotide can be changed by adjusting the vector on line 34 of the function. Please note that the r file contains an example at the bottom of how to set up the simulation. You should comment this out before sourcing the file. Please see in-code notes for further details.

1. Value

UIDerrorGCpref returns a numerical vector with the following components in order:

|  |  |
| --- | --- |
| emutFreq1 | Mutation frequency estimated from the data assuming that families without a consensus call are removed from the denominator. |
| err1 | Error of the emutFreq1 compared to mutFreq |
| emutFreq2 | Mutation frequency estimated from the data assuming that all families, including those without a consensus call, are included in the denominator. |
| err2 | Error of emutFreq2 compared to mutFreq |
| ncfreq | Fraction of UID families that did not produce a consensus sequence |
| uUIDs | Number of unique UIDs used |
| nreads | Number of reads expected for sequencing based on success of PCR amplification |