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Glossary

bioinformatics	Statistical or computational approaches to biological data or research tools.
gene expression	A measure of the relative expression of each gene from the mRNA extracted from (pooled) cells.
genome	All of the DNA sequence in the genome.
genomic	The use of data from all genes in the genome.
graph or network	A mathematical structure modelling or depicting the relationships between elements.
metagene	A consistent signal of expression for a collection of genes such as a biological pathway, derived from singular value decomposition.
microarray	A high-throughput technique to measure presence or abundance of nucleic acid sequences from binding to probes.
mutation	A change in DNA sequence that disrupts gene function.
pathway	A series of biomolecules that produces a particular product or biological function.
RNA-Seq	The generation of transcriptome data from sequencing RNA.
synthetic lethal	Genetic interactions where inactivation of multiple genes is inviable (or deleterious) which are viable if inactivated separately.
tumour suppressor	A gene potentially causes cancer, typically by disruption of functions which protect the cell from cancer.

Acronyms

ANOVA	Analysis of Variance.
AUROC	Area Under the Receiver Operating Characteristic (curve).
BiSEp	Bimodal Subsetting Expression.
DNA	Deoxyribonucleic Acid.
FDR	False Discovery Rate.
HPC	High Performance Computing.
mtSLIPT	Synthetic Lethal Interaction Prediction Tool (against mutation).
NeSI	New Zealand eScience Infrastructure.
PI3K	Phosphoinositide 3-kinase.
ROC	Reciever Operating Characteristic (curve).
siRNA	Short Interfering RNA.
SLIPT	Synthetic Lethal Interaction Prediction Tool.
Slurm	Simple Linux Utility for Resource Management.
TCGA	The Cancer Genome Atlas (genomics project).

Chapter 6

Simulation and Modelling of Synthetic Lethal Pathways

Simulation and modelling of synthetic lethality in gene expression was revisited in greater detail in this chapter, building upon the results which supported the use of SLIPT (in Section 3.3). In Chapter 3, a procedure for generating simulated data with underlying (known) synthetic lethal partners of a query gene, such as *CDH1*, was developed (as described in Section 3.2.2) by sampling from a multivariate normal distribution based on a statistical model of synthetic lethality in gene expression data (as described in Section 3.2.1). This simulation framework was applied to simulated data (in Section 3.3), including simple correlation structures to assess the statistical performance of the SLIPT methodology and support its use as a computational approach for detecting synthetic lethal candidates from expression data throughout this thesis (Chapters 4 and 5).

While this basic framework provided some support for the use of SLIPT, further investigations with simulations were conducted to assess the strengths and limitations of the SLIPT methodology, compare it to alternative statistical approaches to synthetic lethal detection, and assess its performance under more complex correlation structures. Together these simulation investigations assess the performance of the SLIPT methodology, including on pathway graph structures (e.g., those discussed in Chapter 5). These results can indicate whether the SLIPT methodology robustly detects known synthetic lethal partners (and how it compares to other bioinformatics strategies) or is suitable for wider genomics applications.

These simulation investigations continue to utilise the multivariate normal simulation procedure (as applied in Section 3.3) with further refinements. The SLIPT methodology (and the χ^2 test) were applied across a range of parameters (including altering the quantiles for detecting synthetic lethal direction) and compared to correlation as a predictor of synthetic lethality. These simulations included thousands of non-synthetic lethal genes and correlations with the query gene (as performed in Section 3.3).

A refined simulation procedure was developed specifically to extend the methodology described in Section 3.2 to utilise pathway graph structures for the correlation structures of simulated datasets (as described in Section 3.4.2). This methodology can be applied to simulated correlation structures across simple graph structures to test specific network modules or use pathway structures based on biological pathways. Thus graph structure and simulation approaches were combined to test whether a gene locus in a pathway affects detection by SLIPT and whether SLIPT performance is affected by pathway structure. The simulation procedure based on graph structures was applied in a computational pipeline across many parameter combinations using high-performance computing resources (as discussed in Section 2.5.3) and the core simulation functions have been released as a software package for wider use to test bioinformatics and statistical methods on graph structures (as described in Section 3.5.3).

6.1 Synthetic Lethal Detection Methods

The SLIPT methodology (as it has been applied throughout Chapters 4 and 5) was compared for alternative computational approaches to detecting synthetic lethality in simulated gene expression data. As discussed in Section 3.3, this procedure enabled testing the ability of SLIPT to detect known synthetic lethal partner genes by sampling from a statistical model of synthetic lethality. While comprehensive benchmarking has not been performed, several approaches to synthetic lethal detection are considered (e.g., Pearson correlation, the χ^2 test, and testing for bimodality) to evaluate the strengths of the SLIPT methodology, including modifications to the parameters of SLIPT. The following comparisons of simulations of computational detection of synthetic lethality with different statistical rationales were perfomed to show the strengths of SLIPT, evaluate whether it is appropriate for further application in genomics research, and identify limitations which may be addressed with further developments.

6.1.1 Performance of SLIPT and χ^2 across Quantiles

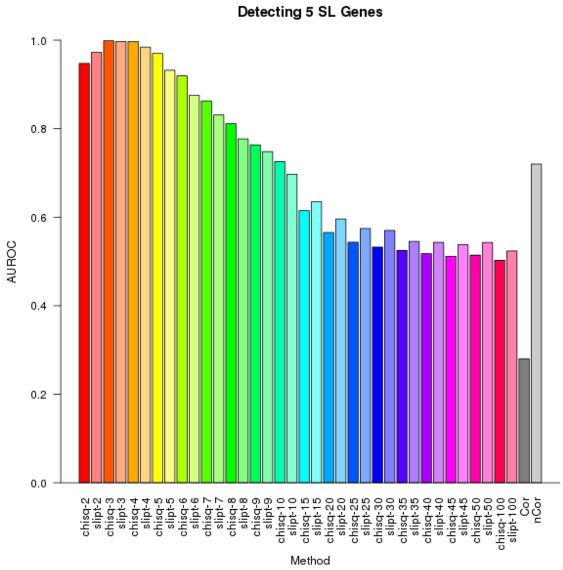
Simulated datasets with synthetic lethal partner genes were generated using the multivariate normal simulation procedure (as described in Section 3.2.2) with performance assessed using area under receiver operating characteristic (AUROC) analysis (as described in Section 2.3.5). Synthetic lethal detection was compared for modifications to the SLIPT methodology (as described in Section 3.1), namely that the quantiles used to define low and high expression were varied. Rather, than $1/3$ (as used throughout this thesis) the samples below the lowest $1/n$ quantile and above the highest $1/n$ quantile were used for SLIPT (and the χ^2-test) to detect samples that exhibited low and high expression levels respectively. The quantiles tested ranged from two, splitting at the $1/2$ quantile (the median), to 100, using the lowest (1%) and highest (99%) percentiles.

This enabled testing of the threshold for low expression of genes which is most able to distinguish synthetic lethal genes, even with higher-order synthetic lethal interactions (as discussed in Section 3.2.1). Both SLIPT with the directional criteria for synthetic lethality and significance of the equivalent χ^2 test were performed for each quantile. Pearson correlation was also tested on simulated continuous expression data for synthetic lethal detection in simulated data, considering both positive and negative correlations separately as predictors of synthetic lethality for comparison with χ^2 based approaches, using discrete categories of gene function deriving from quantiles.

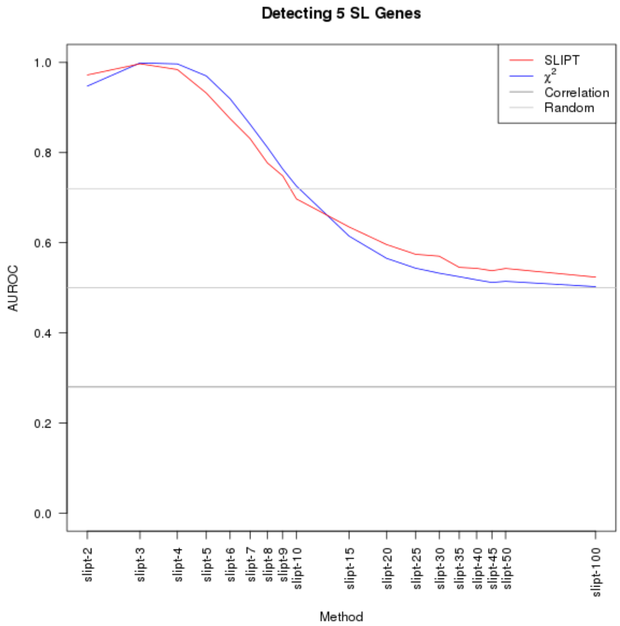
The results presented throughout this Section use the example of five synthetic lethal partners to illustrate the differences in performance between the standard SLIPT procedure (slipt-3) to n quantiles (slipt-n), the χ^2-test on the same quantiles, and positive or negative correlation. However, similar results across different numbers of known synthetic lethal genes are shown in Appendix J. The synthetic lethal detection procedures were compared with 10,000 simulations of a small dataset of 100 genes and 1000 samples without correlation structure between genes (as performed in Section 3.3.2).

As shown in Figure 6.1, the $1/3$-quantiles previously used have optimal performance and SLIPT has a comparable or higher performance than the χ^2-test alone across quantiles. Pearson correlation was also tested as a predictor of synthetic lethality (i.e., whether highly positive or negative correlations with the query gene detected synthetic lethal partners). Positive correlation performed worse than random (with an AUROC lower than 0.5) as thus coexpression of genes was not predictive of synthetic lethality in simulated data. Conversely, negative correlation was predictive of synthetic lethality, consistent with synthetic lethal gene activity being mutually exclusive. However, neither correlation approach performed as well as the optimal quantiles for the SLIPT procedure or the χ^2-test.

These results are shown in both a bargraph and lineplot to show the individual results of each parameter, and to compare SLIPT with the χ^2-test side-by-side across



(a) Barplot of χ^2, SLIPT, and correlation.



(b) Lineplot of χ^2, SLIPT, and correlation.

Figure 6.1: **Performance of χ^2 and SLIPT across quantiles**. Synthetic lethal detection (of 5 genes) with quantiles as on the axes. The barplot uses the same hues for each quantile (grey for correlation) and darker for χ^2 (and positive correlation). The line plot (with log-scale quantiles) is coloured according to the legend. SLIPT and χ^2 perform similarly, peaking at 1/3-quantiles and converging to random (0.5). Negative correlation had higher performance than positive correlation but not optimal quantiles for SLIPT or χ^2.

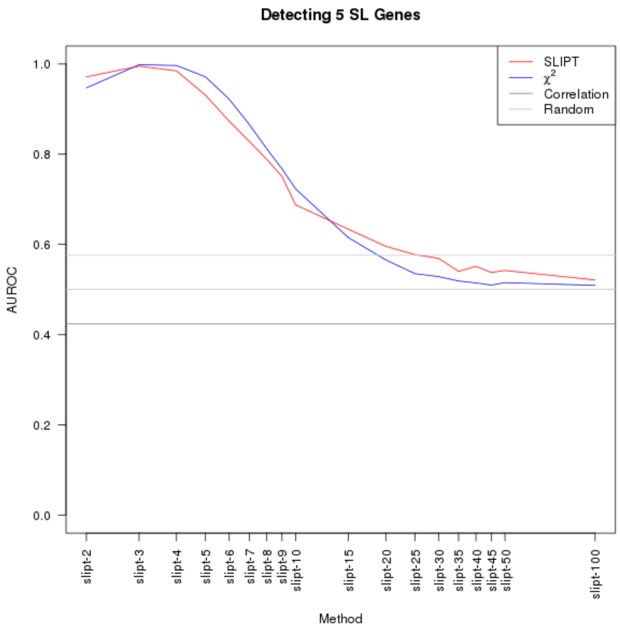


Figure 6.2: **Performance of χ^2 and SLIPT across quantiles with more genes**. Synthetic lethal detection (of 5 genes in 20,000) with quantiles as in axis labels. The line plot (with log-scale quantiles) is coloured according to the legend. As for simulations with fewer genes, SLIPT and χ^2 perform similarly, peaking at $^1/_3$-quantiles and converging to random (0.5). Negative correlation had higher performance than positive correlation but not optimal quantiles for SLIPT or χ^2.

quantiles. Similarly, these plots are given for detecting a range of known synthetic lethal partners in the simulations in Appendix Figures J.1 and J.2. These demonstrate that the findings shown for five synthetic lethal genes are robust across different numbers of underlying synthetic lethal genes.

The synthetic lethal detection procedures were also tested with 1000 simulations of a larger dataset of 20,000 genes and 1000 samples. While fewer simulations gives a less accurate receiver operating characteristic (ROC) result, this is sufficient to replicate the above findings with a feasible number of genes in a human gene expression dataset and assess the impact of a higher proportion of non-synthetic lethal genes (potential false positives). Simulated datasets of this size were also used in Section 3.3.2 to test the specificity in a number of genes similar to that in experimental datasets for cancer genomes. As shown in Figure 6.2, the above findings were replicated in simulations of a larger dataset with 20,000 genes. These were also robustly replicated across varying numbers of underlying synthetic lethal genes (as shown in Appendix Figure J.3).

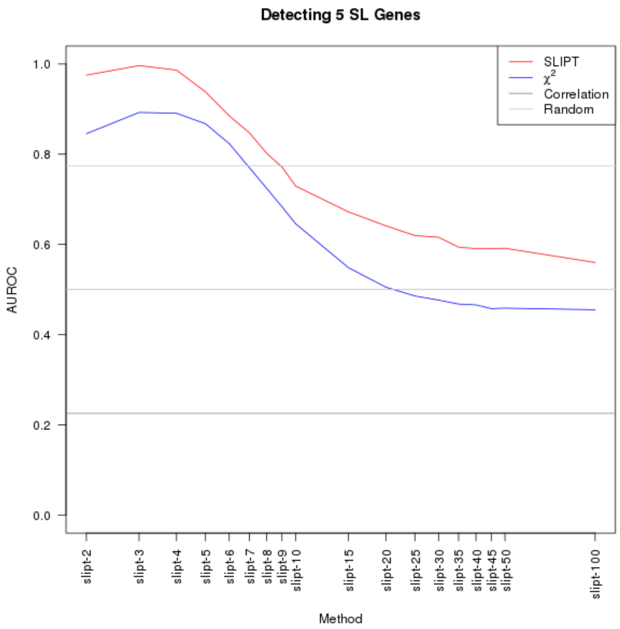


Figure 6.3: **Performance of χ^2 and SLIPT across quantiles with query correlation**. Synthetic lethal detection (of 5 genes in 100 including 5 query correlated) with quantiles as in axis labels. The line plot (with log-scale quantiles) is coloured according to the legend. SLIPT performs consistently higher than χ^2 due to higher specificity. Negative correlation performed modestly.

6.1.1.1 Correlated Query Genes affects Specificity

As discussed in Section 3.3.2.2, positively correlated genes (with the query gene) have an impact on the performance of synthetic lethal detection. SLIPT was able to distinguish these correlated genes from synthetic lethal partners and hence is likely to have a higher specificity in datasets which include positively correlated genes with the query gene (as expected in gene expression data). The synthetic lethal detection procedures were compared with 10,000 simulations of a small dataset of 100 genes (with 5 correlated with the query gene) and 1000 samples otherwise without correlation structure between genes. As shown in Figure 6.3, this specificity is reflected in the increased AUROC performance values for SLIPT (in contrast to Figure 6.1). This specificity can be attributed to the directional criteria (as described in Section 3.1) since the χ^2-test alone performs comparatively poorly with positively correlated genes.

The synthetic lethal detection procedures were also compared with 1000 simulations of a larger dataset of 20,000 genes (with 1000 correlated with the query gene) and 1000 samples otherwise without correlation structure between genes. This simulation

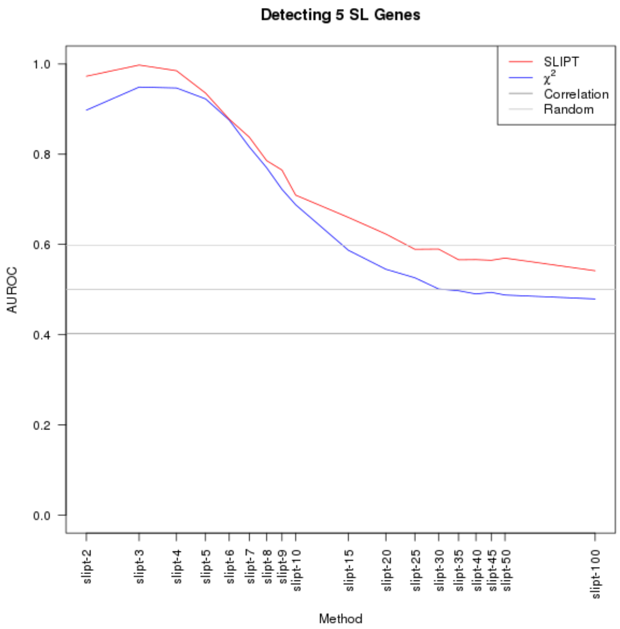


Figure 6.4: **Performance of χ^2 and SLIPT across quantiles with query correlation and more genes**. Synthetic lethal detection (of 5 genes in 20,000 including 1000 query correlated) with quantiles as in axis labels. The line plot (with log-scale quantiles) is coloured according to the legend. SLIPT performs consistently higher than χ^2 due to higher specificity. Negative correlation performed modestly.

increases the number of genes (and proportion of negative genes) to those comparable with a human gene expression dataset while maintaining a comparable 5% of positively correlated genes. As shown in Figure 6.4, SLIPT still outperforms χ^2 or negative correlation and is optimal at the $^1/_3$-quantile. The difference between SLIPT and χ^2 was less pronounced in a larger dataset with many weakly correlated genes. The greater specificity of SLIPT than the χ^2-test to distinguish positively correlated non-synthetic lethal genes is not as evident with a large number of negative genes (as potential false positives). However, specificity is an important consideration in large-scale genomics analysis where there are potentially many false positives.

Nevertheless, SLIPT with $^1/_3$-quantiles (as performed throughout Chapters 4 and 5), had higher performance than when other quantile thresholds were used, particularly when positive correlations were present (replicating the Section 3.3.2.2). These findings hold across different numbers of underlying synthetic lethal genes (as shown in Figures J.5 and J.6).

Together these results support the use of SLIPT, particularly the use of quantiles as thresholds for gene function and specific use of $1/3$-quantiles which perform well compared to other quantiles. A particular concern in the design of SLIPT for expression data whether the samples sizes are sufficient when the data are divided into quantiles. The SLIPT methodology further performed better for $1/3$-quantiles (and other moderate values) than χ^2 or correlation as a predictor of synthetic lethality. These results were irrespective of sample size or p-value threshold since the results replicated across sample sizes and the AUROC values were independent significance thresholds. Using a moderate number of quantiles for SLIPT ensures that there are a sufficient number of samples expected below and above them so that deviations from these are statistically detectable. These quantiles were also optimal for the χ^2 test which uses the same expected values asthe SLIPT directional conditions.

6.1.2 Alternative Synthetic Lethal Detection Strategies

The SLIPT approach (and χ^2) to detect synthetic lethality from binning expression to estimate gene function also outperformed correlations, which use continuous data directly. Correlation performing poorly as a synthetic lethal detection strategy is consistent with there not necessarily being a relationship between synthetic lethal partners, which can be in distinct biological pathways, expressed at different times or in different cell types. Nevertheless, correlation is among the alternative detection methods considered in further detail.

The BImodal Subsetting ExPression (BiSEp) R package (Wappett, 2014) for using bimodality to detect synthetic lethality (Wappett *et al.*, 2016) was also considered, along with a linear regression approach. These statistical methods span a range of computational approaches to detecting synthetic lethality and serve to compare alternatives to SLIPT, supporting its design and application. However, these comparisons are able provide supporting data from statistical modelling and simulations for the viability of the SLIPT methodology for synthetic lethal discovery in cancer (as demonstrated in Chapter 4) and further applications.

6.1.2.1 Correlation for Synthetic Lethal Detection

As expected, negative (Pearson) correlation performed better than positive correlation at detecting synthetic lethality (shown in Section 6.1.1). However, neither correlation approach performed as well as SLIPT or the χ^2 test as a predictor of synthetic lethal gene partners. It is notable that negative correlation still often performed considerably better than random chance.

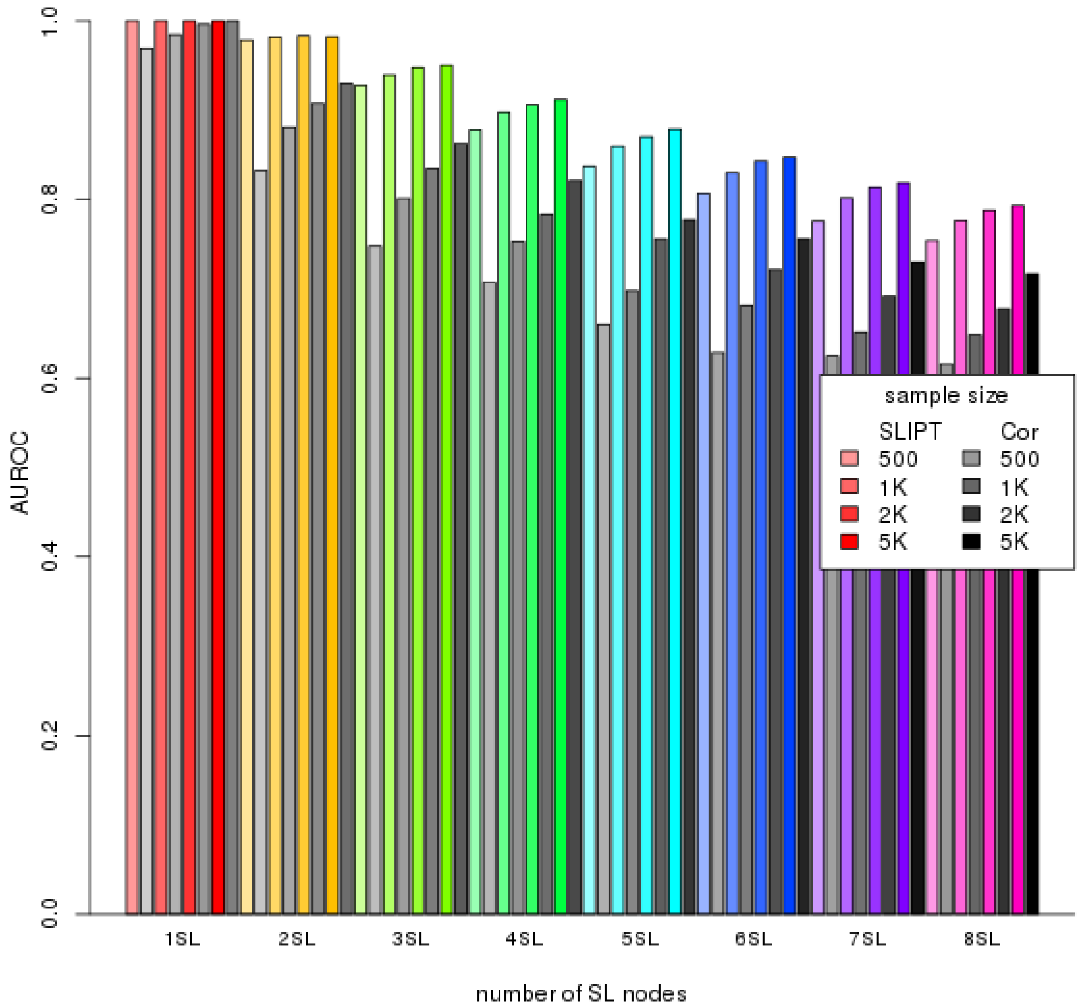


Figure 6.5: **Performance of negative correlation and SLIPT**. Synthetic lethal detection with SLIPT was compared to negative (Pearson) correlation across parameters. SLIPT consistently outperformed correlation. Both approaches had lower performance for more synthetic lethal partners and for lower sample sizes. 10,000 simulations were performed with correlation structure.

Negative correlation was compared directly to the SLIPT methodology (as described in Section 3.1) across numbers of known synthetic lethal partners and sample size (ranging from 500 to 5000). This comparison used 1000 simulations of a dataset with 20,000 genes and synthetic lethal genes from within a network (sampled as in Section 3.4.2) with a 0.8 correlation between adjacent genes. In a direct comparison of SLIPT and negative correlation (shown in Figure 6.5), SLIPT consistently has higher performance in simulated data across parameter values and (inverse) correlation-based approaches perform modestly in comparison. Thus using thresholds to categorise expression data (as performed by SLIPT and χ^2) does not compromise the performance of these methods by losing continuous data that would be used for calculating correlations.

Both SLIPT and correlation had poorer performance with increasing numbers of the synthetic lethal genes to detect, while they had higher performance in higher sample sizes, as expected (as previously observed for SLIPT in Section 3.3). Thus the issue with detection of greater numbers of synthetic lethal genes is not specific to SLIPT but occurs across computational methods of synthetic lethal discovery in (simulated) expression data and likely stems from cryptic higher-order synthetic lethal interactions (as conservatively assumed in Section 3.2.1).

6.1.2.2 Testing for Bimodality with BiSEp

Extensive attempts were also made to compare SLIPT to the BiSEp methodology (Wappett *et al.*, 2016), a statistical approach to identify synthetic lethal gene pairs from mutually exclusive relationships using bimodal distributions. This synthetic lethal detection methodology is also designed for expression analysis in cancer and is readily available as an (open-source) R package (Wappett, 2014), a practice which facilitates adoption and testing of the methodology on the same datasets and simulations procedures as previously used for SLIPT.

The BiSEp package is designed for global testing of all potential gene pairs in the genomes for synthetic lethality rather than focusing on the search space of potential partners of the query gene. This approach was unable to detect synthetic lethal gene pairs in the The Cancer Genome Atlas (TCGA) breast cancer expression dataset (Koboldt *et al.*, 2012). However, this may be due to stringent thresholds under the multiple testing of millions of potential gene pairs.

For a direct comparison with the query-based SLIPT approach, the source code of the BiSEp R functions was modified to test solely for the partners of a specific gene. This approach was still unable to detect synthetic lethal partners of *CDH1* in TCGA breast cancer expression data (Koboldt *et al.*, 2012), even with the detection thresholds for bimodality and significance greatly relaxed from those which the package defaults to.

To circumvent multiple testing issues, BiSEp only tests gene pairs for synthetic lethality between genes with a detectable bimodal distribution. However, even with relaxed thresholds, bimodal distributions were not detectable in the normalised TCGA data

(Koboldt *et al.*, 2012). Such normalisation Ritchie *et al.* (2015) is standard practice for expression datasets generated from microarrays or RNA-Seq and therefore BiSEp may not be appropriate to apply to this data. However, it is noted that BiSEp may also use other data types such as DNA copy number or cell line data for which it may be more applicable (Wappett *et al.*, 2016).

Nevertheless, attempts were made to test BiSEp on simulated datasets with underlying synthetic lethal genes (using the procedures described in Sections 3.2.2 and 3.4.2). However, BiSEp was also unable to detect genes with bimodal distributions of genes (and thus unable to detect synthetic lethality) in a limited number of computationally intensive simulations. Therefore investigations on a wider range of parameters were not performed.

6.2 Simulations with Graph Structures

The simulations of synthetic lethality performed in Section 3.3 included correlated blocks of genes as a rudimentary representation of pathway structure and co-regulated genes. The simulation procedure was enhanced here to account for more complex pathway structures by sampling from multivariate normal distributions with correlation structure derived from graph structures (as described in Section 3.4.2). This approach enabled the simulation of synthetic lethal pathways with known correlation structure and synthetic lethal partners (of a gene not in the pathway). Using this procedure, the performance of SLIPT was evaluated under simple controlled correlation structures and complex correlations, such as those derived from biological networks (e.g., those described in Chapter 5). The SLIPT methodology was tested in artificially constructed networks to evaluate the effect of pathway structure on synthetic lethal detection These included large biologically feasible pathways to ensure that the SLIPT methodology is robust under complex correlation structures and applicable to such complex genomics data.

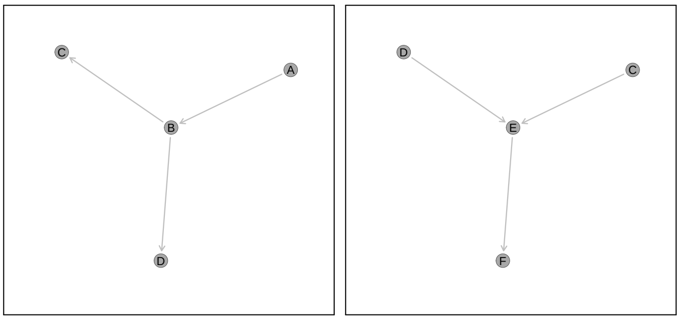
These simulations combine the approach of prior simulation analyses (in Sections 3.3 and 6.1) with the graph structures for biological pathways (as used in Chapter 5). This enabled testing whether subtle or large differences in pathway structure affect synthetic lethal detection, whether inhibiting relationships (or inverse correlations) between genes affect synthetic lethal detection, and whether synthetic lethal detection varies by which gene is synthetic lethal and which genes are closely linked within the pathway structure. In addition, large numbers of synthetic lethal genes and biologically feasible numbers of genes (with many non-synthetic lethal genes) were tested to replicate the findings of Sections 3.3 and 6.1 in correlated structures derived from pathway graphs, including examples of biological pathways from Reactome (Croft *et al.*, 2014).

Simple and more complex constructed graph structures were used to demonstrate the impact of pathway structure of the performance of SLIPT for synthetic lethal detection in simulations. In addition, more complex constructed graph structures were compared to the phosphoinositide 3-kinase (PI3K) and $G_{\alpha i}$ signalling pathways derived from Reactome which were used for simulation of pathway structures of biological complexity (as shown in Figure 5.1 and Appendix Figure 5.4).

6.2.1 Performance over Graph Structures

6.2.1.1 Simple Graph Structures

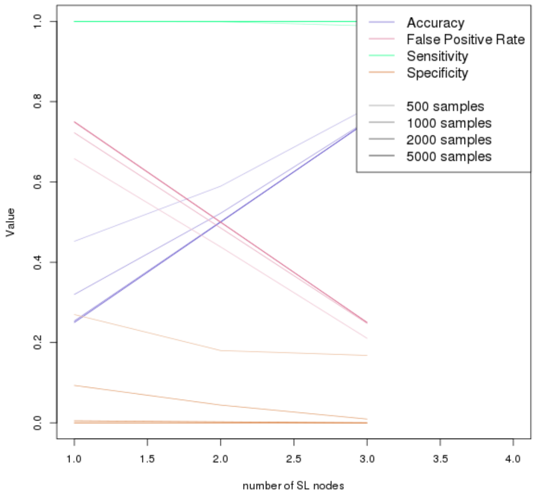
Simple pathway modules were used to test the effect of pathway structure on the performance of detecting synthetic lethal partners within graph structures. For an intial comparison, the graph structures (shown by Figure 6.6) were used where a gene has one upstream regulator and two downstream (Figure 6.6b) or a gene has two upstream regulators and one downstream gene (Figure 6.6b). SLIPT had a high performance in these simulations, detecting randomly selected synthetic lethal partners in both of these small simple networks (shown in Figure 6.7 and Appendix Figure K.1).



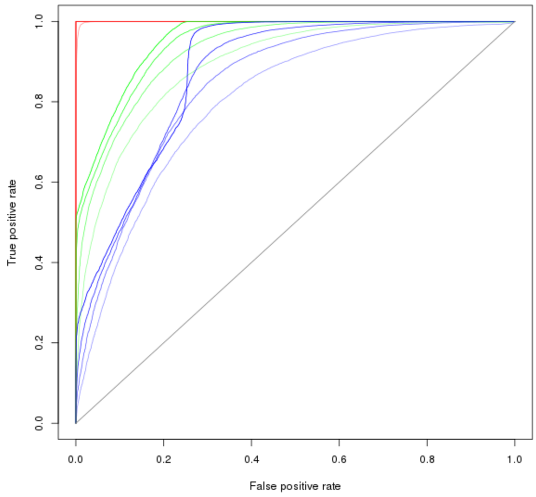
(a) Diverging Module (b) Converging module

Figure 6.6: **Simple graph structures.** These simple graph structures used to demonstrate the simulation procedure. These are examples of a pathway diverging or converging respectively which enabled testing the importance of direction in pathway structures. These are used with both activating and inhibiting relationships as shown.

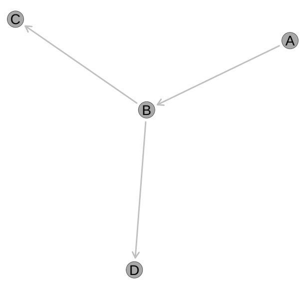
As previously observed (in Section 3.3), performance declined with higher numbers of synthetic lethal genes and lower sample sizes. However, the sensitivity of SLIPT as a binary classifier was high. Synthetic lethal partners are often distinguishable for non-



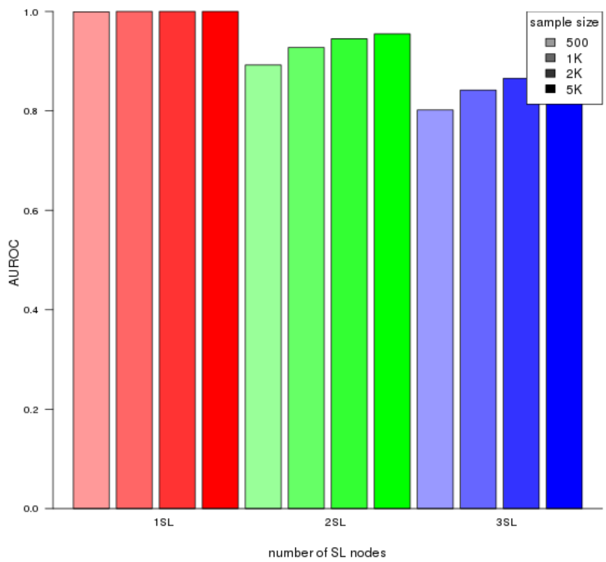
(a) Statistical evaluation



(b) Receiver operating characteristic



(c) Graph Structure



(d) Statistical performance

Figure 6.7: **Performance of simulations on a simple graph.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution generated from a diverging graph structure. Performance of SLIPT declines for more synthetic partners but this is mitigated by increased sample sizes (in darker colours). This manifests as a decline in specificity and the false positive rate. For each parameter value, 10,000 simulations were used. Colours of the ROC curves in Figure 6.7b correspond to the parameters in Figure 6.7d.

synthetic lethal genes, even in simple highly correlated networks. The small number of genes and their high correlation has an impact on the ROC curves for higher numbers of synthetic lethal partners which are skewed compared to those observed previously. Specificity cannot be tested if all potential partner genes are synthetic lethal, which limits the number of synthetic lethal genes that can be tested.

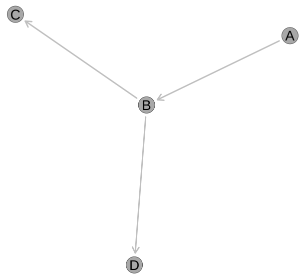
These results were consistent between the pathway modules of diverging (as shown in Figure 6.8a) and converging signals (as shown in Figure 6.8b). The AUROC performance and underlying curves were strikingly similar between these graph structures (as shown in Figure 6.7 and Appendix Figure K.1). Thus the performance of SLIPT was not perturbed by pathway structure, specifically the direction of pathway relationships since these graph structures also demonstrate pathways in opposite direction. In a direct comparison (shown in Figure 6.8c), the performance of simulations did not differ across parameter values in these simple graphs and therefore SLIPT is robust to pathway direction.

6.2.1.2 Constructed Graph Structures

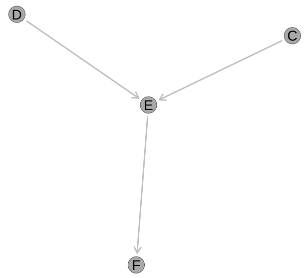
A more complex graph structure was used to examine the performance of detecting synthetic lethal partners with SLIPT in simulated expression data with pathway correlation structures. For a simple chain of genes representing a very linear pathway (shown in Figure 6.9), the above findings were generally replicated. SLIPT had high performance across parameter values in small networks but was still lower for higher numbers of synthetic lethal genes and lower sample sizes.

When detecting synthetic lethal genes with SLIPT as a binary classifier, the performance differences were primarily due to changes in specificity, as the small numbers of synthetic lethal genes still had highly significant p-values. Despite lower specificity and performance in ROC curves, the accuracy increased and false positive rate decreased desirably with higher numbers of synthetic lethal genes due to the high sensitivity and the high proportion of synthetic lethal genes detected. Therefore the use of adjusted p-values for SLIPT as a binary classifier appear to be appropriate for detecting synthetic lethal partners, even in strongly correlated pathways, at least in these small-scale test cases.

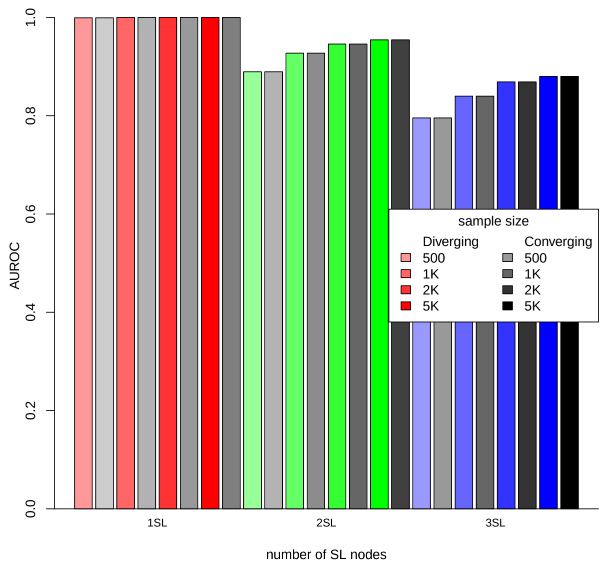
An artifact of these small test cases led to the skewed ROC curves (as discussed in Section 6.2.1.1), which may be the result of the low number of non-synthetic lethal genes to identify as true negatives, affecting the accuracy of specificity. This issue does not occur in larger, more complex graph structures, even with modest total numbers of genes and high correlations (as shown in Section 6.3). This issue is unlikely to occur



(a) Diverging Module



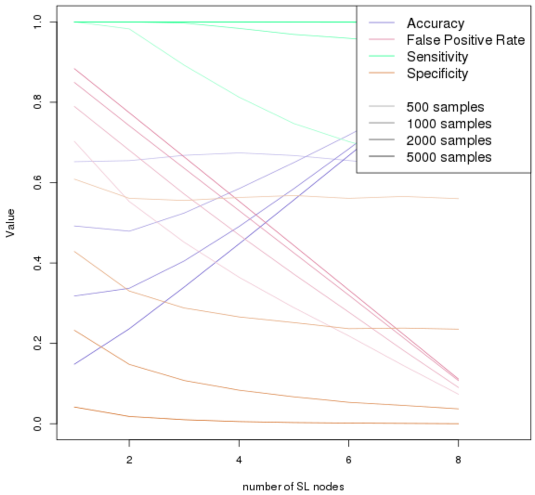
(b) Converging module



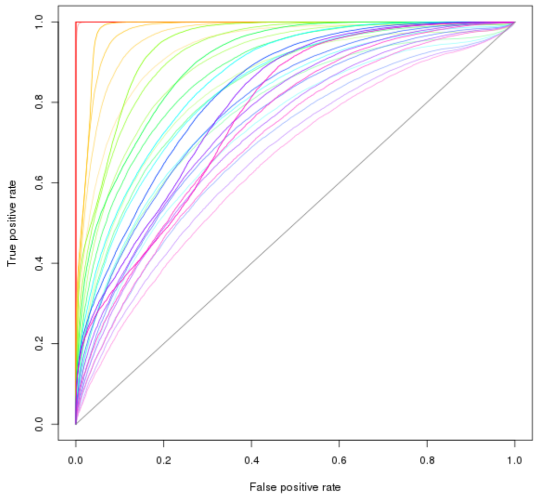
(c) Performance between Graph Structures

Figure 6.8: **Performance of simulations is similar in simple graphs.** The AUROC values for simulations of multivariate normal distributions based on each graph structure yielded indistinguishable performance across parameter values in 10,000 simulations.

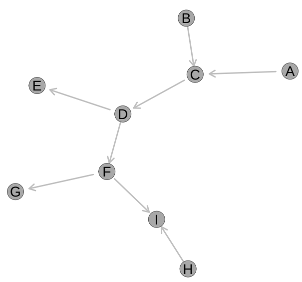
in large expression datasets with many non-synthetic lethal genes, as shown previously (in Section 3.3 and 6.2.1.1) with graph structures in larger datasets (in Section 6.2.4).



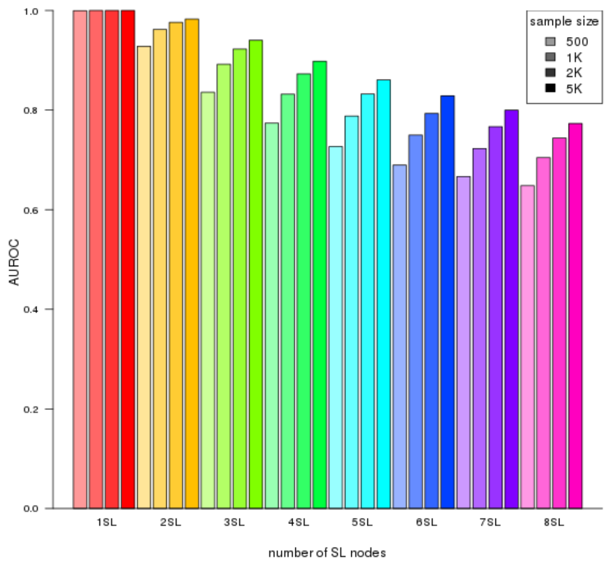
(a) Statistical evaluation



(b) Receiver operating characteristic



(c) Graph Structure



(d) Statistical performance

Figure 6.9: **Performance of simulations on a pathway.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution generated from a pathway structure. Performance of SLIPT declines for more synthetic partners and lower sample sizes (in darker colours). For each parameter value, 10,000 simulations were used. Colours of the ROC curves in Figure 6.9b correspond to the parameters in Figure 6.9d.

6.2.2 Performance with Inhibitions

Simulations of synthetic lethality in expression data were also performed with correlation structures derived from graphs containing inhibiting relationships (as are commonplace in biological pathways) which produce negative correlations. As shown in Figure 6.10, these are not an issue for detection by SLIPT. Rather, the SLIPT procedure performs well on simple graph modules with highly negative correlations. With synthetic lethal detection based on p-value (adjusted by False discovery rate (FDR)), there was higher specificity, higher accuracy, and lower false positive rate in an inhibitory graph than the same graph with activating relationships (as shown by Figure 6.7).

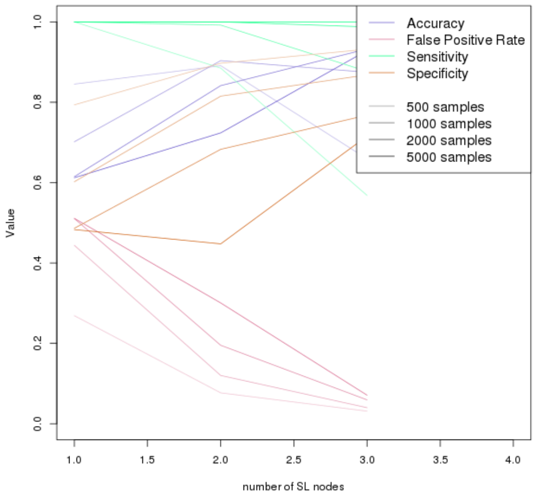
The ROC curves for an inhibiting graph also showed consistently high specificity, irrespective of detection threshold, with only the upper extreme of the curve exhibiting a skew below random performance (as shown in Figure 6.10). Nevertheless, the AUROC values show a high performance across parameter values, particularly avoiding issues with higher numbers of synthetic lethal partners (as observed in Section 6.2.1.1). However, performance was marginally lower for higher numbers of synthetic lethal genes to detect and lower sample sizes, consistent with previously observations.

Negatively correlated simulated datasets are also unperturbed by minor differences in graph structure, such as changing in the direction of the graph module. As observed for activating relationships in these graph modules, the performance was highly concordant between the graph modules (shown by similar results in Figures 6.10 and K.2).

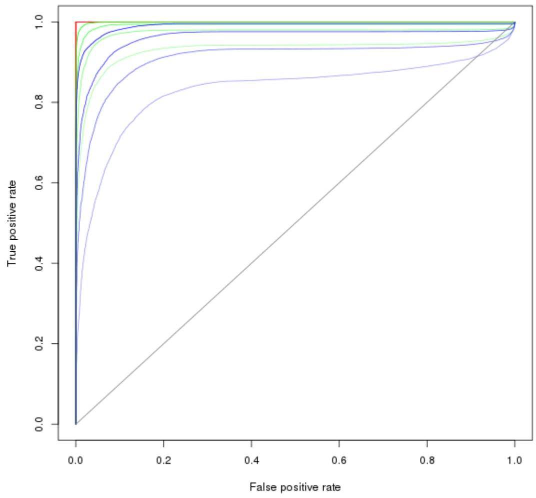
Detection of synthetic lethality by SLIPT in simulated data with inhibiting relationships outperforms simulations with activating relationships in the same graph structure (as shown in Figure 6.11). Thus SLIPT was robust in gene expression datasets with inverse correlations and performed well in them, at least in simple test cases. This is important because such relationships occur frequently in biological pathways and therefore the findings inferred from graph structures without inhibiting relationships are a conservative estimate.

The SLIPT methodology likely performs better in biological pathways (which contain negative correlations) than the graph structures discussed previously (in Section 6.2.1). This is likely since negative correlations lead to synthetic lethal partners and inversely correlated genes which are positively correlated with the query gene. As previously shown, the SLIPT methodology performs well with specificity against positively correlated query genes (in Sections 3.3.2.2 and 6.1.2.1).

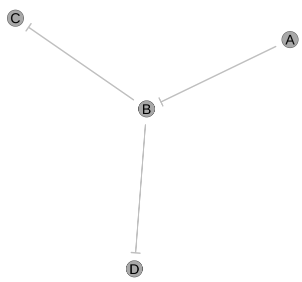
Similarly, more complex graph structures with entirely inhibiting relationships (negative correlations) also perform desirably with SLIPT as a binary classifier and have



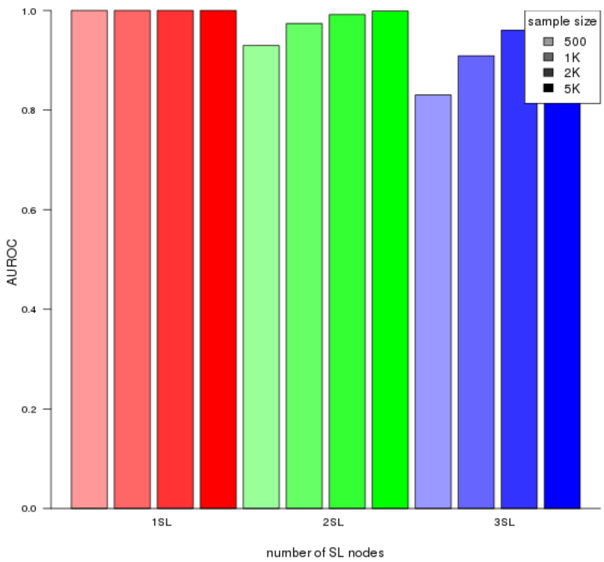
(a) Statistical evaluation



(b) Receiver operating characteristic

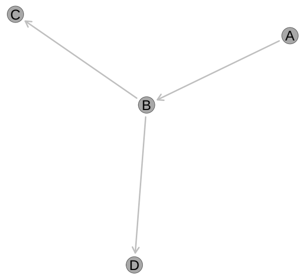


(c) Graph Structure

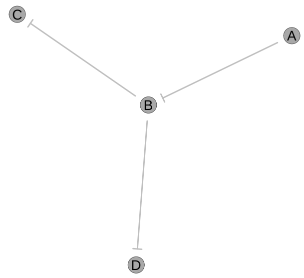


(d) Statistical performance

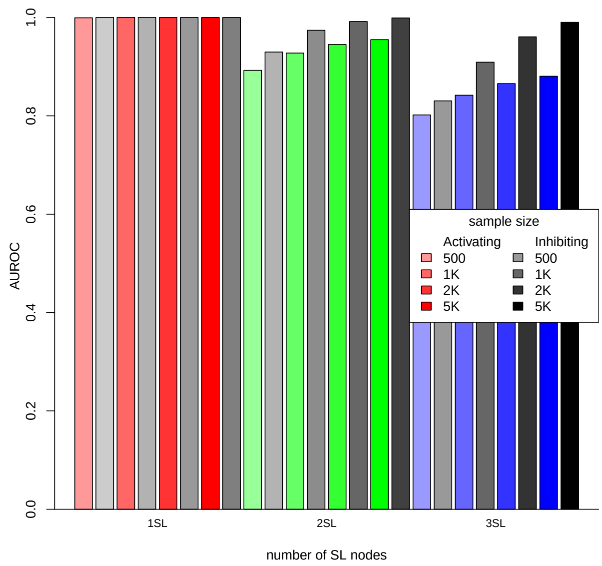
Figure 6.10: **Performance of simulations on a simple graph with inhibition.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution generated from an inhibiting graph. Performance of SLIPT declined for more synthetic partners and lower sample sizes. For each parameter value, 10,000 simulations were used. Colours of the ROC curves in Figure 6.10b correspond to the parameters in Figure 6.10d.



(a) Activating Graph



(b) Inhibiting Graph

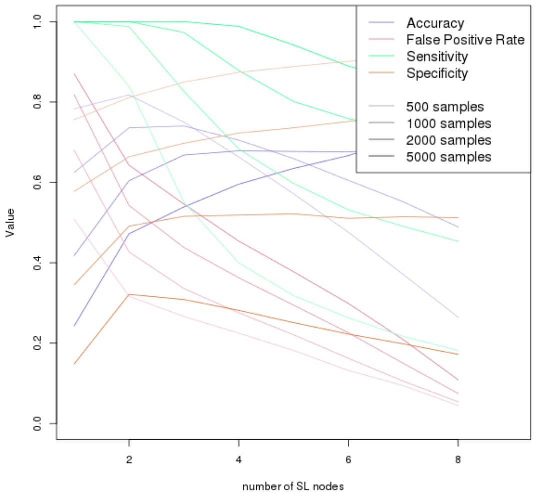


(c) Performance between Graph Structures

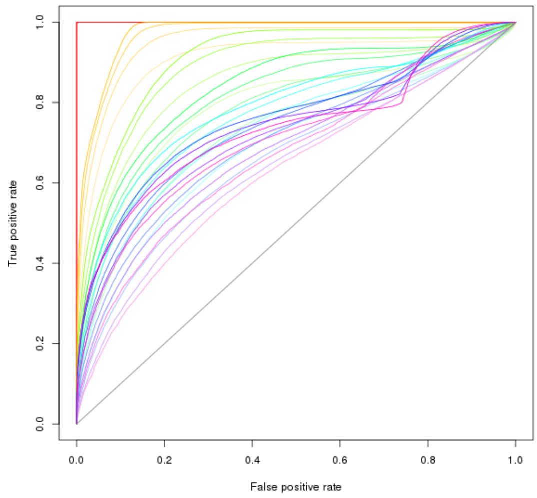
Figure 6.11: **Performance is higher on a simple inhibiting graph.** The AUROC values for simulations of multivariate normal distributions based on inhibitions in the graph structure yielded consistently higher performance across parameter values in 10,000 simulations.

high performance across increasing numbers of synthetic lethal genes, particularly for sufficiently high sample sizes (as shown by Appendix Figure K.3). However, this is not necessarily the case for graph structures with a combination of activating and inhibiting relationships (i.e., containing positive and negative correlations). As shown by Appendix Figure K.4, such a mixed network structure does not necessarily have high performance across parameters as observed for purely inhibiting networks.

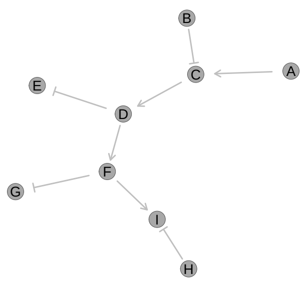
These still appear to have desirably high sensitivity, high accuracy, and low false positive rate for detecting more synthetic lethal genes, despite poor specificity. The



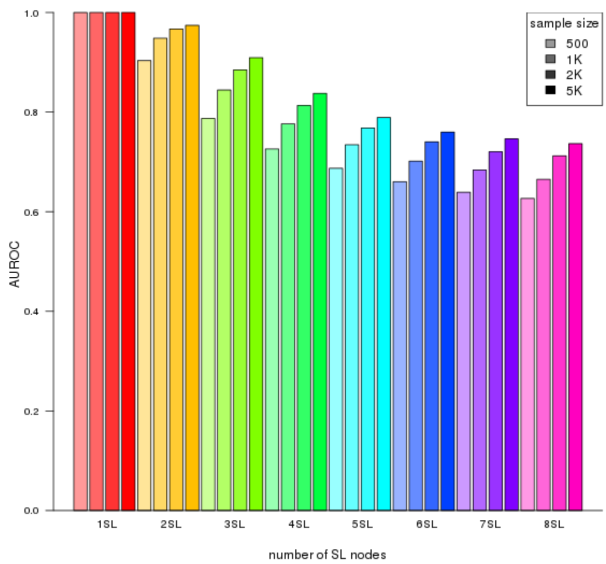
(a) Statistical evaluation



(b) Receiver operating characteristic



(c) Graph Structure



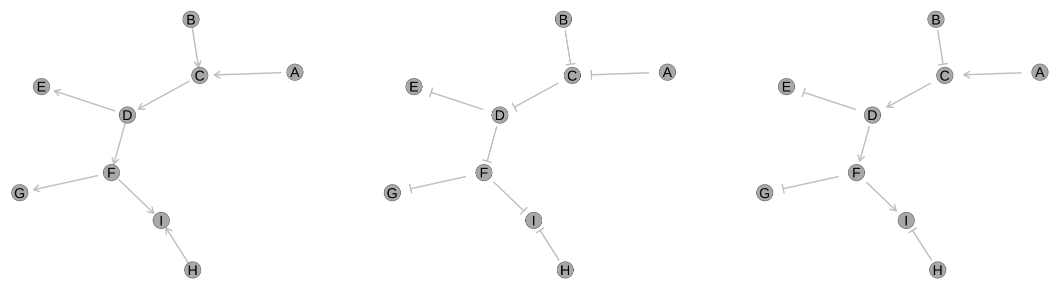
(d) Statistical performance

Figure 6.12: **Performance of simulations on a constructed graph with inhibition.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution generated from pathway structure with a combination of inhibitions. Performance of SLIPT declines for more synthetic partners and lower sample sizes. For each parameter value, 10,000 simulations were used.

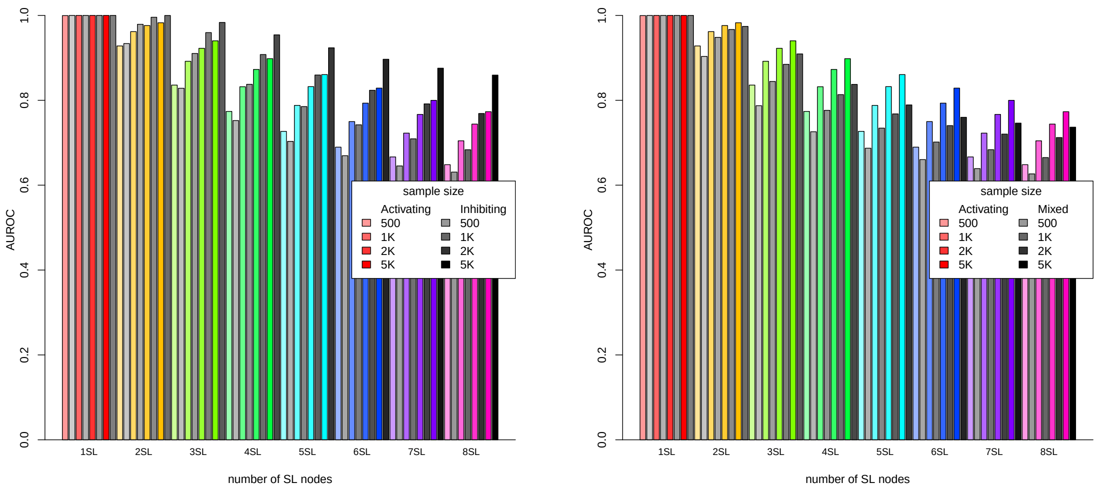
ROC curves were particularly skewed for high proportions of the network being synthetic lethal and may stem from low numbers of true negative genes to detect (as discussed in Section 6.2.1.1). In a direct comparison of performance (shown in Figure 6.13), the purely inhibiting graph had consistently higher performance than the activating one, as observed for simpler graphs (as shown in Figure 6.11).

In contrast, the combination of activating and inhibiting relationships had slightly lower performance across parameters compared to the same graph structure with activating relationships. Therefore correlation structure can impact on the performance of SLIPT in a graph network, in either direction, specifically the addition of negative correlations. However, this may be an artifact of the simulation procedure as synthetic lethal genes from the correlation structure were randomly selected (without regard to their relationships), with the query gene added to ensure that conditions for synthetic lethal relationships were met.

This system for simulating inhibitory pathways is not ideal since it can lead to synthetic lethal gene combinations, by randomly selecting them, which are unlikely to occur in biological pathways. These randomly selected synthetic lethal genes may account for the detection results being suboptimal (i.e., difficult to detect synthetic lethal partners) compared to previous investigations. It is expected that inversely correlated synthetic partner genes will be highly expressed in a mutually exclusive manner such that at least one of them will be compensating for loss of the query gene in most samples, leading to a weak synthetic lethal signature in expression data in this case. Furthermore, this case may not be representative of empirical biological data with synthetic lethal partners of tumour suppressor genes which are commonly inversely correlated to the query gene (to some extent) and therefore it is unlikely that they are strongly negative correlated with each other, unless they are synthetic lethal partners of each other as well. It is plausible that many synthetic lethal partner genes will serve to separately compensate for the loss of query gene function and be positively correlated with each other. Nonetheless, these simulations demonstrate that correlation structure (particularly negative correlations) have an impact on the detection of synthetic lethality. However, SLIPT was still able to perform well across graphs with different activating and inhibiting relationships, and the perturbations in performance were marginal, particularly those reducing performance compared to an activating network.



(a) Activating Graph (b) Inhibiting Graph (c) Mixed Graph



(d) Performance between Graphs (a) and (b) (e) Performance between Graphs (a) and (c)

Figure 6.13: **Performance is affected by inhibition in graphs.** The AUROC values for simulations of multivariate normal distributions based on graph structure containing only inhibitions in the graph structure yielded consistently higher performance across parameter values in 10,000 simulations. A combination of activating and inhibiting relationships had lower performance but was more similar to the activating graph.

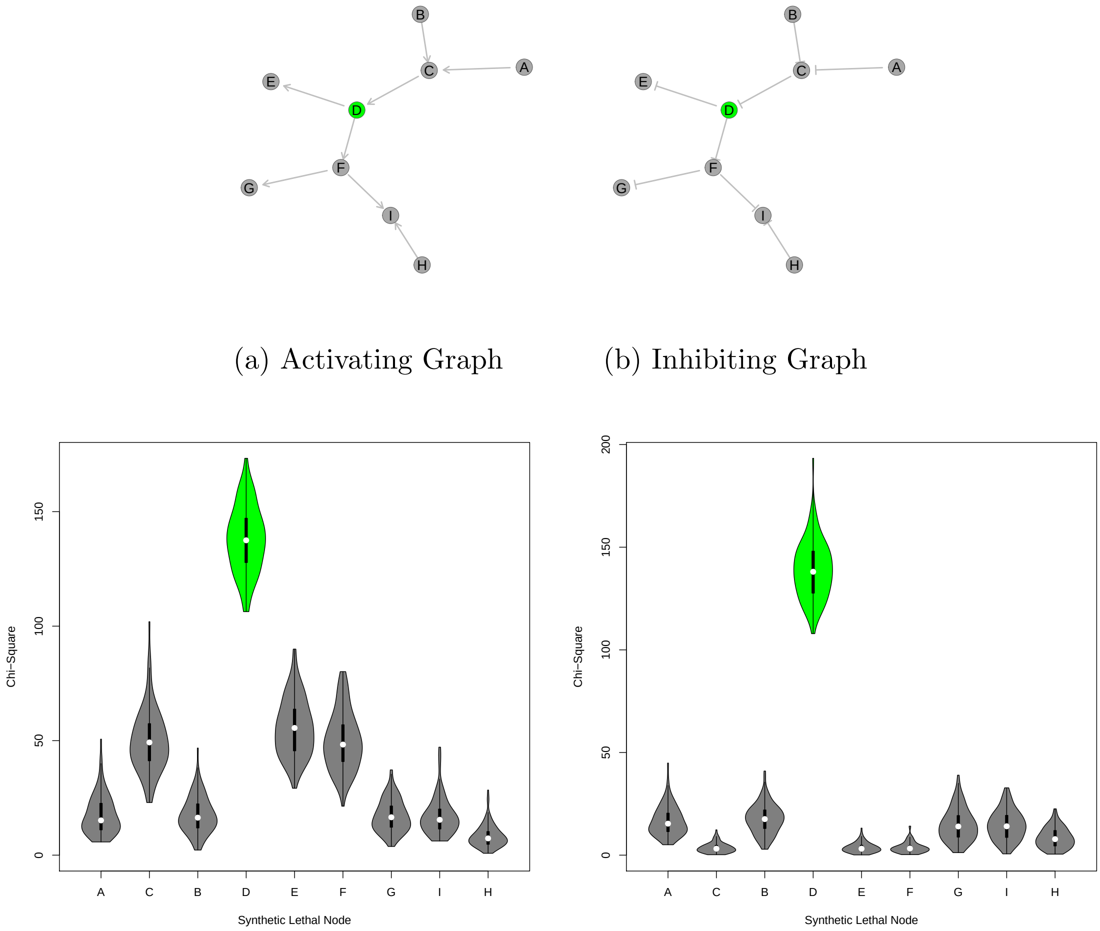
6.2.3 Synthetic Lethality across Graph Structures

Synthetic lethal genes were distinguishable from highly correlated genes in simple cases (as shown by ROC analysis). However, correlated genes may lead to low specificity and high false positive rates. Negative correlations do not affect specificity this way but they may perturb the correlation structure between synthetic lethal partner genes, making it difficult to detect many of them with high sensitivity. Synthetic lethal genes have been selected randomly in simulations so far, which is a limited approach. To examine the impact of pathway relationships in more detail, specific genes were selected to be synthetic lethal within a network over replicate simulations. These simulations with a fixed synthetic lethal gene were performed to demonstrate their impact on the detection of other genes in the network.

For detection of a synthetic lethal gene in an activating graph structure (as shown in Figure 6.14a), the χ^2 values were clearly distinguishable from other genes (shown in Figure 6.14c). Simulations were performed for each gene being the synthetic lethal partner. For each synthetic lethal gene, it had the highest χ^2 value amongst 20,000 genes, including the highly correlated graph network (as shown in Appendix Figure K.5). Despite optimal performance for SLIPT detecting one synthetic lethal gene in a ROC curve (as shown in Figure 6.9), irrespective of detection threshold, the highly correlated genes would be detected as false positives by SLIPT as a binary classifier (as described in Section 3.1). In particular, the genes that were adjacent in the pathway to the synthetic lethal gene "D" had high test statistics which could be false positives (as shown in Figure 6.14c). This was not specific to example of gene "D", with the neighbouring genes of each synthetic lethal having higher χ^2 values (as shown in Appendix Figure K.5).

The synthetic lethal signal propagates from the true synthetic lethal gene throughout the network. As such, the genes nearer to (i.e., more highly correlated with) the true synthetic lethal gene had higher test statistics and were more likely to be detected by SLIPT as false positives. The adjacent genes of synthetic lethal partners being false positives may account for the higher concordance of synthetic lethal pathways than genes between SLIPT in TCGA data (Koboldt *et al.*, 2012) and the siRNA screen (Telford *et al.*, 2015) than individual gene results (in Chapter 4). False positive genes are more likely to be involved in a synthetic lethal pathway, being correlated with a true synthetic lethal gene. Synthetic lethal pathways are likely to contain many genes detected by SLIPT, giving a consensus in the pathway over-representation analysis. SLIPT is also able to detect true synthetic lethal partners or prioritise those most likely to be experimentally validated. Genes with the strongest support (i.e, higher χ^2 values and more significant p-values) are more likely to be the underlying synthetic lethal gene.

The immediately adjacent genes in an inhibiting graph (Figure 6.14b) did not have elevated χ^2 test statistics or a significant inverse effect (as shown in Figure 6.14d). Therefore true synthetic lethal partners were highly distinguishable from other genes with inhibiting relationships. This was shown for each gene in the graph structure as



(a) Activating Graph (b) Inhibiting Graph

(c) χ^2 distribution for Activating Graph (d) χ^2 distribution for Inhibiting Graph

Figure 6.14: **Detection of synthetic lethality within a graph structure.** The gene "D" was designated to be synthetic lethal and the χ^2 value from SLIPT was computed for each gene across each graph structure. The χ^2 values were computed in 100 simulations of datasets of 20,000 genes including the graph structure and 1000 samples. Adjacent genes exhibited lower χ^2 values with inhibiting relationships.

the synthetic lethal partner (shown in Appendix Figure K.6). These results support SLIPT as an appropriate approach to distinguish synthetic lethal partners in biological pathways (which frequently have inhibitions), including those relevant to cancer growth and inhibition.

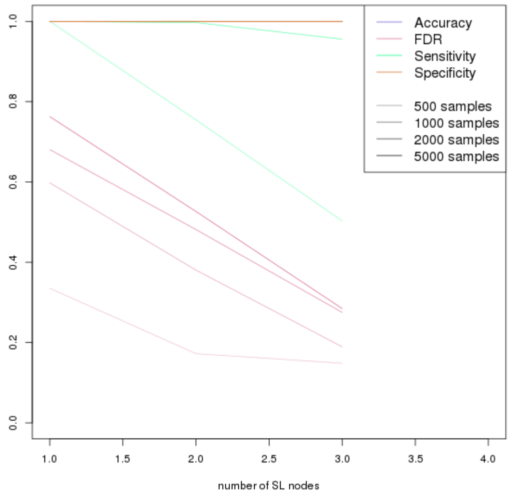
The 2[nd] degree neighbours of the synthetic lethal gene still exhibited moderate χ^2 values (and are moderately correlated with the synthetic lethal gene). These genes could be false positives, as shown for an activating graph structure, although inhibitory relationships (i.e., negative correlations) lead to more differences in test statistics between correlated genes and underlying synthetic lethal partners (as shown in Appendix Figure K.6). Simulations in a graph containing a combination of activating and inhibiting relationships exhibits either of these χ^2 profiles, depending on which gene is synthetic lethal and the relationships between genes (as shown in Appendix Figure K.7). In this case, the synthetic lethal gene is distinguishable and inhibitory relationships make it easier to detect with SLIPT.

These results contrast with randomly selecting multiple synthetic lethal genes (as shown in Figure 6.13), where the performance of SLIPT was impeded by the inhibitory relationships between synthetic lethal partners. The randomly selected synthetic lethal genes, with negative correlations between them, which had poor performance due to an artifact in the simulation process resulting in biologically implausible synthetic lethal genes. The results with one synthetic lethal partner were sufficient to show the impact of synthetic lethal partners on neighbouring (correlated) genes. It is plausible that the synthetic lethal signatures in expression data would propagate similarly through a network from multiple synthetic lethal partners.

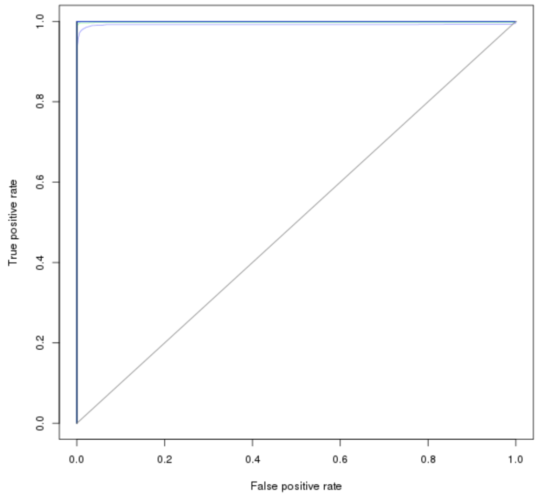
6.2.4 Performance within a Large Simulated Datasets

The performance of SLIPT with higher numbers of true partners to detect may have been affected by the high proportion of synthetic lethal partners (i.e., fewer true negatives) in small networks (as noted in Section 6.2.1.1). The performance of SLIPT increased with the addition of more non-synthetic lethal genes, particularly the specificity (as shown in Sections 3.3 and 6.1). The correlated genes from graph structures (as used in Section 6.2.1) were included in a larger simulated dataset to assess the performance of SLIPT for a synthetic lethal pathway in the context of thousands of genes, as occurs in expression datasets.

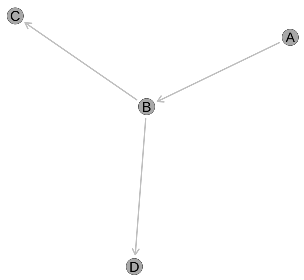
The simulations performed in Section 6.2.1.1 were replicated within a dataset of 20,000 genes with the remainder being composed of non-synthetic lethal genes without correlation structure. The specificity in a higher number of synthetic lethal genes did not affect performance in a simple graph structure (as shown in Figure 6.15). For a graph of highly correlated genes within a gene expression dataset, SLIPT had high the performance detecting of synthetic lethal genes in the network within a larger dataset. In this case, a reduction in sensitivity resulted in poorer performance. A high number of non-synthetic lethal genes were correctly identified, with a low false positive rate and high accuracy. Thus the use of stringent χ^2 p-value thresholds (adjusted by FDR) are



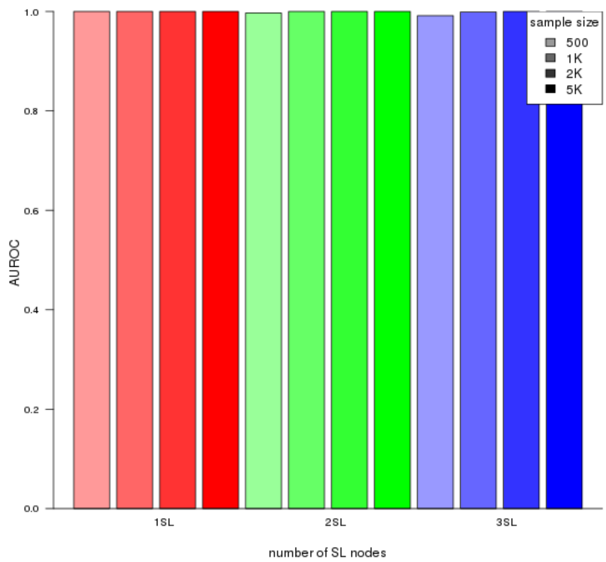
(a) Statistical evaluation



(b) Receiver operating characteristic



(c) Graph Structure



(d) Statistical performance

Figure 6.15: **Performance of simulations including a simple graph.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution (without correlation structure apart from the graph shown). Performance of SLIPT was high across parameters for detecting synthetic lethality in the graph structure within a larger dataset. The sensitivity decreased for a greater number of true positives to detect but the specificity remained high with a low false positive rate.

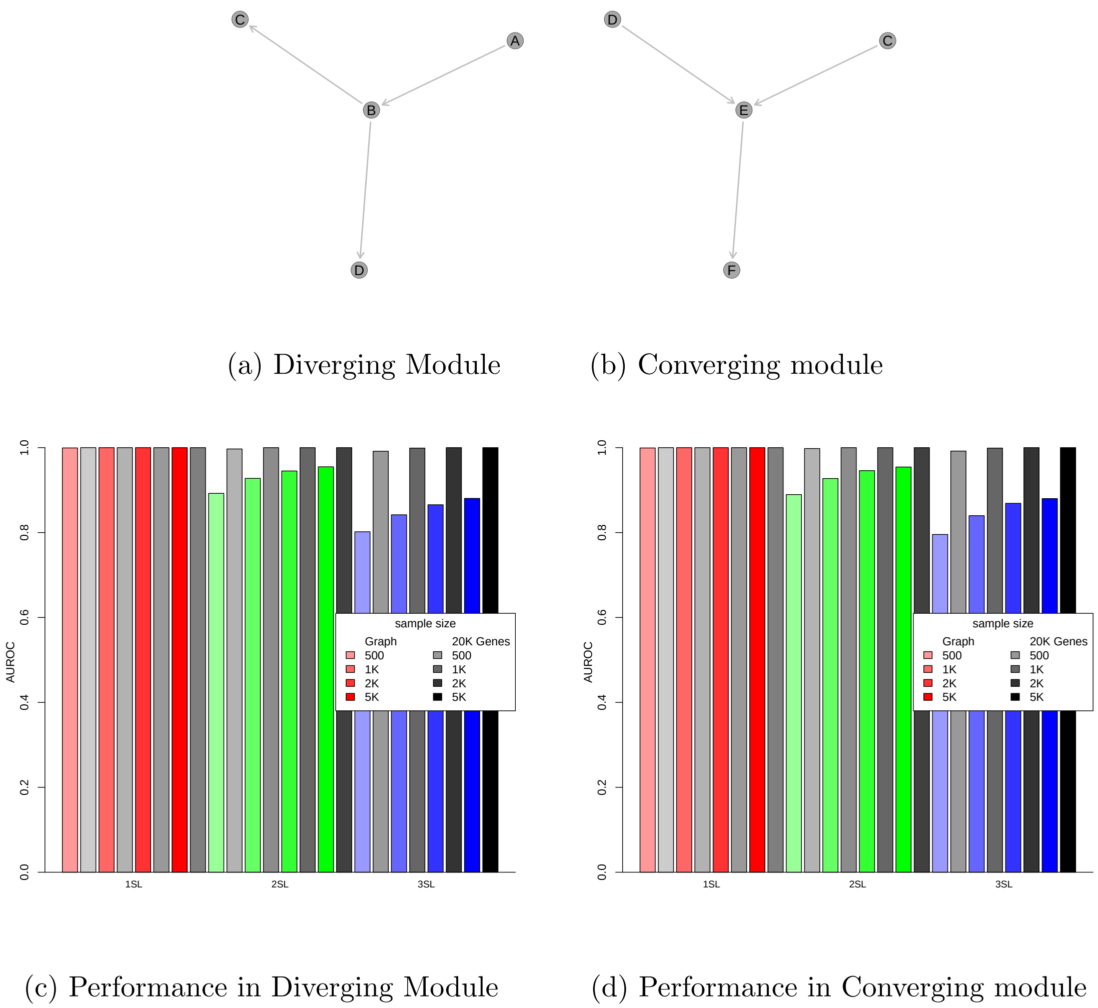
suitable for testing for synthetic lethality in gene expression data across the number of genes in human and cancer data.

In a direct comparison with simulations in the graph structure alone (as performed in Section 6.2.1.1), detection of synthetic lethality with SLIPT performed consistently better in a larger dataset with many true negative genes to detect (as shown in Figure 6.16). The SLIPT methodology had a high specificity and low false positive rate, which is desirable. SLIPT is therefore applicable to large gene expression datasets, where these are important considerations since the number of negative genes often vastly outnumbers the number of positive genes to detect.

Performance was not necessarily higher with more non-synthetic lethal genes in an inhibiting graph structure. The performance of simulations of an entirely inhibiting graph structure did not improve within a larger dataset. Rather, the performance in the inhibiting graph structure was similar to simulations of the graph structure in isolation. Biological pathways commonly contain inhibiting relationships (and inverse correlations), although they are unlikely to occur across an entire pathway. In graph structures with inhibitions included in a larger dataset, the performance of synthetic lethal detection by SLIPT was sometimes higher than in graph structure simulated alone (as shown in Figure 6.17). However, these did not perform as well as the equivalent graph structures without inhibitory relationships within a similar dataset. It is expected that the findings based on these simulations of genes with pathway structures in smaller datasets (as described in Section 6.2.1) will be relevant to larger datasets. The simulation results in these inhibiting graph structures perform comparably or higher with more non-synthetic lethal genes to distinguish from them even with inhibitory relationships within the graph structure

This poorer performance of inhibitory graph structures may be due to highly negatively correlated genes being false positives. These genes will be positively correlated with the query gene if they are negatively correlated with a synthetic lethal partner (i.e., within a synthetic lethal pathway). The SLIPT procedure performs well at distinguishing these positively correlated genes, as previously shown (in Sections 3.3.2.2 and 6.1.1.1). These false positives will also be a minority amongst a larger dataset of non-synthetic lethal genes without correlation to the query or synthetic lethal genes.

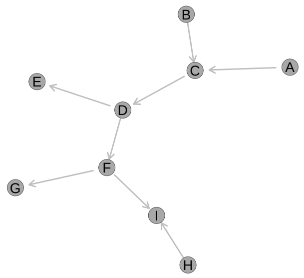
It more likely that the poorer performance stems from negative correlations between synthetic lethal genes which makes them more difficult to individually detect (as observed in Section 6.2.2). As discussed in Section 6.2.3, this is likely an artifact of the simulation procedure selecting random synthetic lethal genes with strong inhibitory



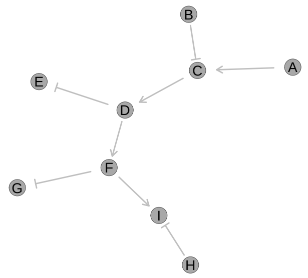
(c) Performance in Diverging Module (d) Performance in Converging module

Figure 6.16: **Performance on a simple graph improves with more genes.** Simulations were performed with each of the graph structures to detect synthetic lethal partners within them. In either structure, performance of detection in a dataset containing on the graph structure (in colour) was lower than testing the graph structure within a larger dataset of non-synthetic lethal genes (without correlations).

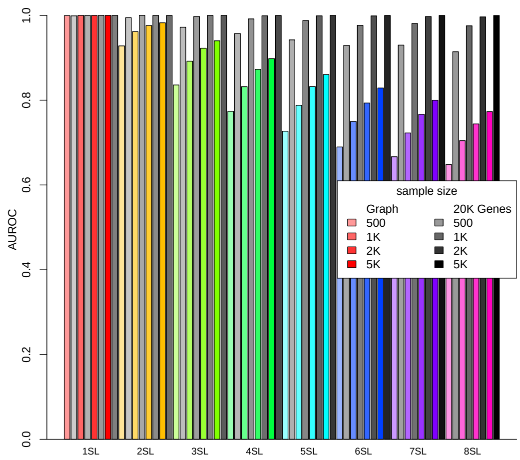
relationships between them. Therefore the poorer performance for inhibiting graphs within larger datasets is not cause for concern because the cases where SLIPT performs poorly are likely to be combinations of simulated synthetic lethal genes that are not likely to occur within biological pathways. This simulation procedure has included higher-order synthetic lethal to produce the weakest signal of synthetic lethality for individual partner genes which are still detectable by SLIPT.



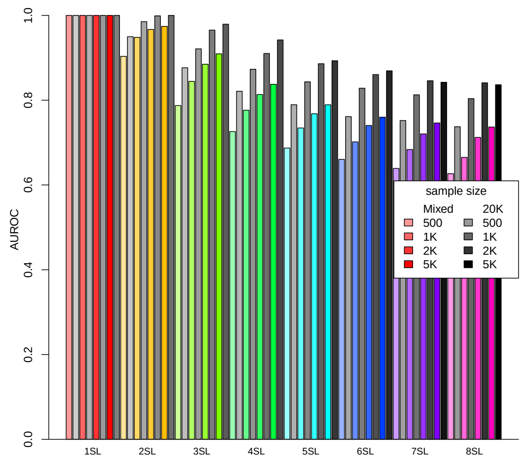
(a) Graph Structure



(b) Inhibiting Structure



(c) Performance in Graph



(d) Performance with Inhibitions

Figure 6.17: **Performance on an inhibiting graph improves with more genes.** Simulations were performed in a graph structure with activating and inhibiting relationships to detect synthetic lethal partners within them. In contrast to an activating graph, performance of detection in a dataset containing only the graph structure (in colour) was as much lower than testing the graph structure within a larger dataset of non-synthetic lethal genes (without correlations) in an inhibiting graph structure with negative correlations.

6.3 Simulations in More Complex Graph Structures

Investigations with simulations based on graph structures were extended to larger graphs, enabling more synthetic lethal genes within a pathway and modelling the complexity of a biological pathway. Sensitivity declines over a greater range for the number of synthetic lethal partners in a larger network with a trade-off with specificity (as shown in Appendix Figures K.8–K.10). However, the accuracy declined for greater numbers of synthetic lethal partners and the false positive rate peaks at intermediate values. In this range, differencse between simulations varied with greater sample size. The AUROC results were similar between these more complex graph structures, although the larger graph (Appendix Figure K.10) differed in sensitivity and specificity for SLIPT as a binary classifier. This difference be due to different proportions of synthetic lethal and non-synthetic lethal genes to detect, since these graphs (as shown in Appendix Figures K.8 and K.9) had fewer genes.

While the graph structures (of similar size) were highly distinct, they had similar performance profiles across parameters. SLIPT is therefore robust across pathway structures and is more affected by the number or proportion of genes to detect. Findings from previous simulations in similar correlation structures (in Section 3.3) should be applicable to expression data with more complex correlation structures, such as those occurring in biological pathways. Specifically, synthetic lethal partners are distinguishable from closely correlated geness in the context of a biological pathway networks, irrespective of thresholds (shown by ROC) and with the sensitivity and specificity of SLIPT as a binary classifier (as used in Chapters 4 and 5).

The findings for inhibitory graph structures were replicated with larger more complex graph structures with inhibiting relationships and more synthetic lethal genes to detect (shown in Appendix Figures K.11–K.14). In each graph structure, simulations entirely with inhibiting relationships (Appendix Figures K.11, K.13, and K.15) had higher performance than the equivalent graph with entirely activating relationships (Appendix Figures K.8, K.9, and K.10) or a combination of activating and inhibiting relationships (Appendix Figures K.12, K.14, and K.16). While the presence of negative correlations subtly affects the performance of SLIPT, the methodology is robust across the exact structures of genes and is therefore applicable to detecting synthetic lethal genes in a range of (synthetic lethal) biological pathways with different structural relationships.

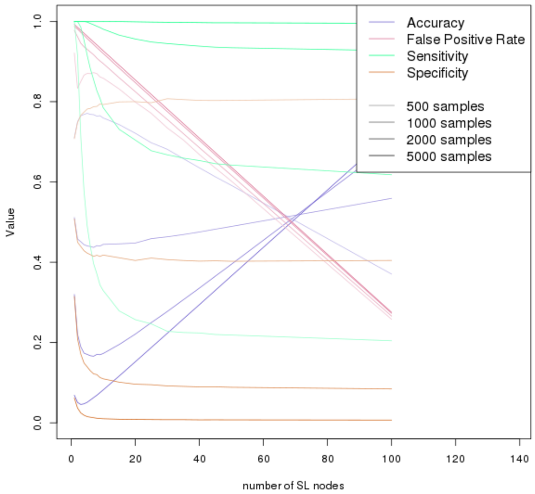
6.3.1 Simulations over Pathway-based Graphs

Simulations of synthetic lethality in gene expression with correlation structures thus far have used simple blocks of correlated genes (as used in Section 3.3) or have ben derived from constructed graph structures (as used in Section 6.2). These have been used to make inferences on the impact of correlation structure but it remains to be shown whether these findings are reproducible in the complexity of the biological network structure. Specifically, SLIPT was tested on simulated data with known underlying simulated synthetic lethal partners (as described in Section 3.2.2) with multivariate normal correlation structure derived from biological pathways (as described in Section 3.4.2).

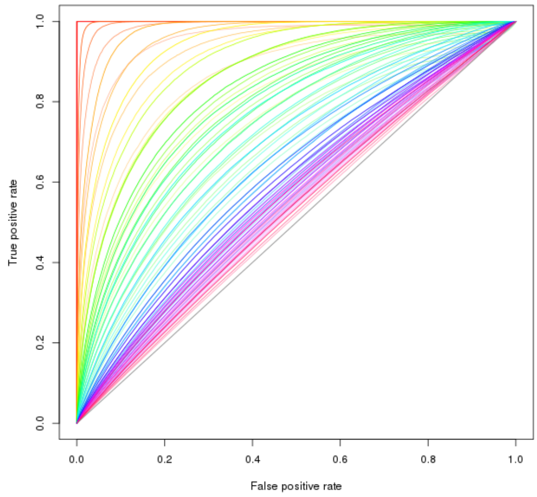
The Reactome pathway structure for the PI3K cascade (as used in Chapter 5) was used to demonstrate the simulation procedure for detecting synthetic lethality in the graph structure of a biological pathway. This pathway has clear directionality, with related signalling pathways among those identified to be synthetic lethal candidates (in Chapter 4). The PI3K pathway has a relatively moderate size (138 genes) and complexity. It is therefore suitable for comparison to previous graph structures of a similar scale (50–100 genes) with the complexity of a biological pathway.

The performance of synthetic lethal detection with SLIPT, in simulated expression data based on the Reactome PI3K pathway (as shown in Figure 6.18), was concordant with previous findings. SLIPT had high performance when detecting a low number of synthetic lethal genes which decreased for high numbers of synthetic lethal genes or lower sample sizes. In particular, the performance of simulations in the PI3K pathway closely resembled the simulation results for constructed graphs of similar scale and complexity (as shown in Appendix Figures K.8 and K.9). Using thresholds based on the χ^2 p-value (adjusted by FDR), simulations in the biological PI3K pathway had a higher sensitivity and lower specificity. While the performance decreased for more synthetic lethal genes to detect within the simulated PI3K pathway, this primarily involved a reduction in sensitivity to detect synthetic lethal genes rather than false positives, as the false positive rate decreased, the accuracy increased, and the specificity was relatively unperturbed (being more dependent on sample size). Thus SLIPT was stringent in an example of a biological graph structure and is appropriate for detection of synthetic lethal genes in complex correlation structures in gene expression data involving biological pathways.

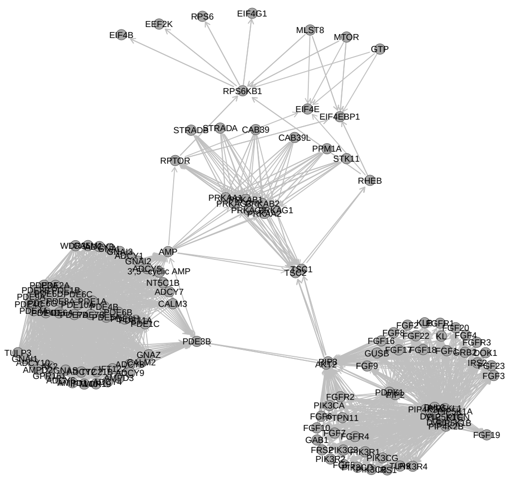
These simulations were replicated in the more complex $G_{\alpha i}$ signalling pathway (of 292 genes), which was one of the most well supported synthetic lethal pathways with loss of *CDH1* in cancer (in Chapters 4 and 5). This pathway showed similar relationships between sensitivity, specificity, and false positive rate with number of synthetic lethal partners and sample size (as shown in Appendix Figure K.17). While the overall performance was lower than for smaller networks structures, many of the findings from previous networks were replicated in a larger more complex biological network. In the $G_{\alpha i}$ signalling pathway, SLIPT had high performance for detecting



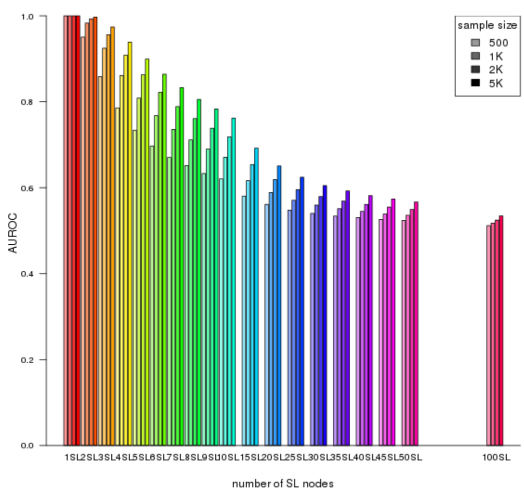
(a) Statistical evaluation



(b) Receiver operating characteristic



(c) Graph Structure



(d) Statistical performance

Figure 6.18: **Performance of simulations on the PI3K cascade.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution based on the Reactome PI3K cascade. Performance of SLIPT was high across parameters for detecting synthetic lethality in the graph structure within a larger dataset. The performance decreased for a greater number of true positives to detect but the accuracy increased with a low false positive rate.

low numbers of synthetic lethal genes and was highly stringent against false positives for higher numbers of synthetic lethal genes.

6.3.2 Pathway Structures in a Large Simulated Datasets

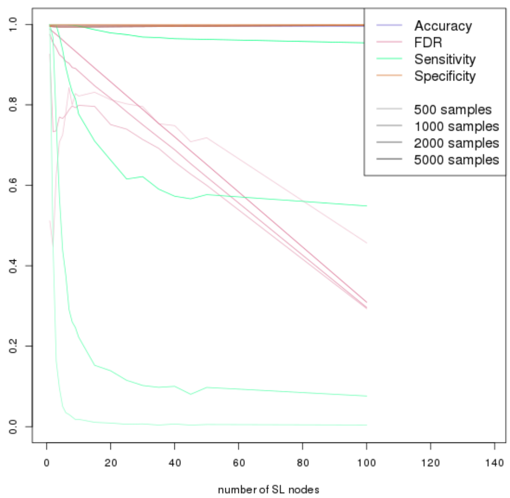
Simulations were also performed with graph structures from biological pathways included in a larger dataset to simulate gene expression data of the scale typical for human and cancer studies. These simulations (as discussed in Section 6.2.4) showed a higher specificity and therefore SLIPT had higher performance. The simulated PI3K pathway (as shown in Figure 6.19) was no exception, with high performance across parameter values, which remained high up to many genes. While the sensitivity decreased for high numbers of synthetic lethal genes to detect within the PI3K pathway, the SLIPT methodology remained accurate, with high specificity in a large simulated gene expression dataset.

Therefore the SLIPT methodology is a highly stringent approach suitable to be applied for detecting synthetic lethal genes and pathways within highly complex expression data with biological pathway structure. Even the poorly performing simulations were highly stringent, with low false positive rates, which are an important consideration in a gene expression data with many non-synthetic lethal genes. The enrichment of true synthetic lethal partners among detected genes makes SLIPT valuable for triage of candidate synthetic lethal partners for further validation and for pathway analysis.

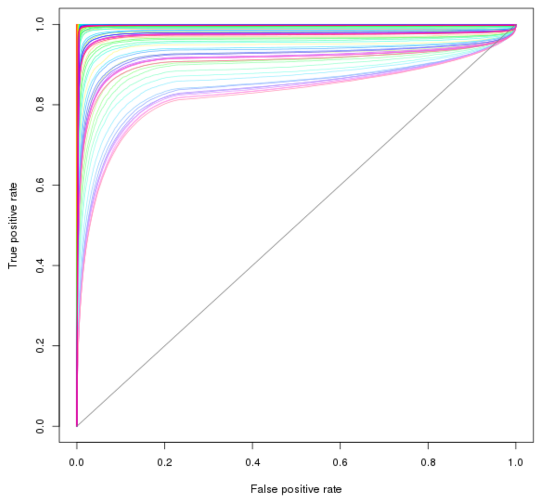
The performance of SLIPT in simulations of synthetic lethality within biological pathways was markedly higher in the context of a larger dataset of thousands of genes. As shown in a direct comparison with the graph structures alone (as shown in Figure 6.20c), performance was consistently higher across parameters in pathways of biological complexity from the Reactome database (Croft *et al.*, 2014) such as PI3K cascade and the $G_{\alpha i}$ signalling pathway (shown in Figure 6.20d and Appendix Figure K.18).

These biologically complex graph structures, based on the Reactome pathways, assumed activating relationships to test synthetic lethal detection with SLIPT in the context of complex correlation structures. Inhibiting relationships were not distinguished in the Reactome database (Croft *et al.*, 2014). However, these investigations with pathway-based graph structures indicate that the findings in constructed graphs (as used in Section 6.2) are relevant to gene expression data containing real correlated pathways. Furthermore, previous comparisons between simulations with inhibiting relationships indicated that the performance of synthetic lethal detection in an equivalent graph structure with inhibitory relationships will likely be higher.

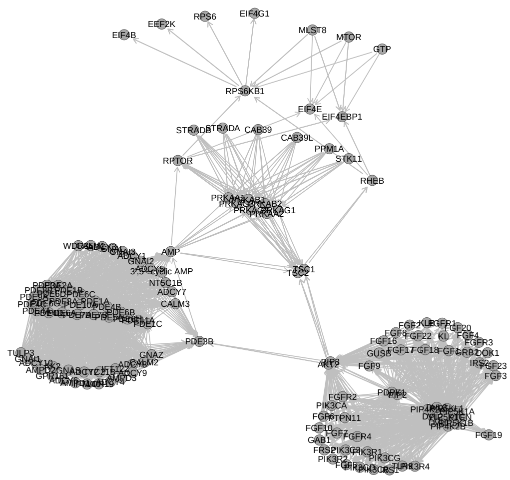
Non synthetic lethal genes, inversely correlated with the underlying synthetic lethal partners, were distinguishable by SLIPT with high specificity. Synthetic lethal genes were detectable with reasonable performance in large scale simulated gene expression



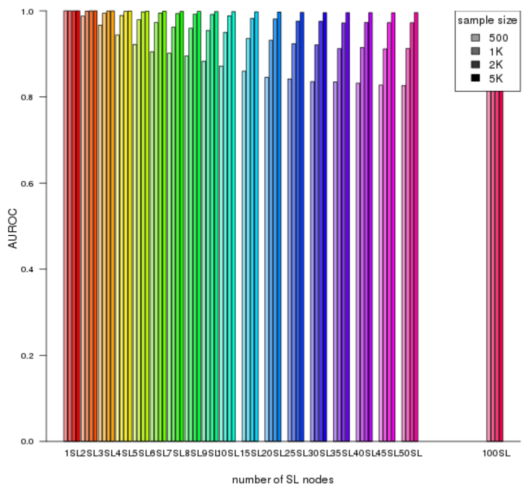
(a) Statistical evaluation



(b) Receiver operating characteristic



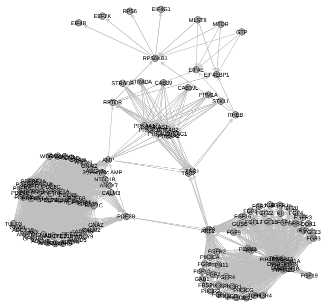
(c) Graph Structure



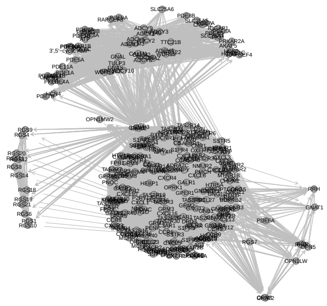
(d) Statistical performance

Figure 6.19: **Performance of simulations including the PI3K cascade.** Simulation of synthetic lethality was performed by sampling from a multivariate normal distribution (without correlation structure apart from the Reactome PI3K cascade). Performance of SLIPT was high across parameters for detecting synthetic lethality in the graph structure within a larger dataset. The sensitivity decreases for a greater number of true positives to detect but the specificity remains high with a low false positive rate.

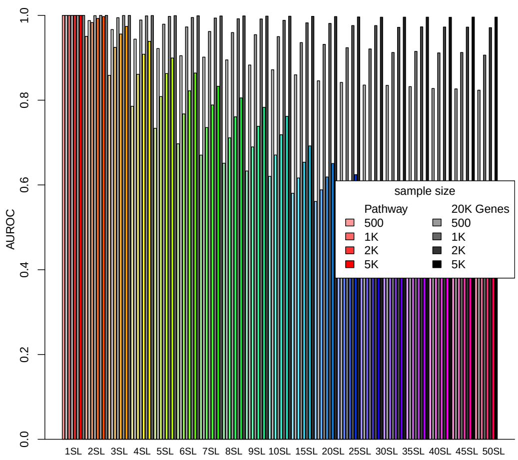
data and highly (positively) correlated genes in pathway structures. These findings serve as a conservative estimate for the performance of SLIPT to detect synthetic lethal



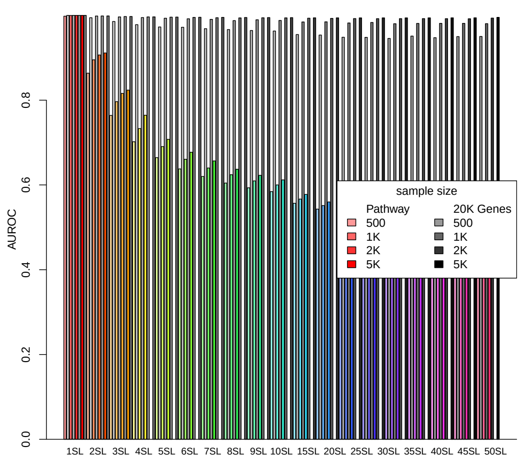
(a) The PI3K cascade



(b) $G_{\alpha i}$ signalling



(c) Performance in the PI3K cascade



(d) Performance in $G_{\alpha i}$ signalling

Figure 6.20: **Performance on pathways improves with more genes.** Simulations were performed in a graph structures for the PI3K cascade and $G_{\alpha i}$ signalling pathways structures to detect synthetic lethal partners within them. As for constructed graphs, performance of detection in a dataset containing only the graph structure (in colour) was as much lower than testing the graph structure within a larger dataset of non-synthetic lethal genes (without correlations) for both graphs of biological complexity.

genes within a synthetic lethal biological pathway in empirical data. Synthetic lethal genes are distinguishable from correlated genes, to varying extents, in simulations. False positives are also more likely to occur within the same (synthetic lethal) pathways. Therefore SLIPT is both effective at triage of synthetic lethal candidates within a biological pathway and at identifying synthetic lethal pathways in high dimensional gene expression data.

6.4 Discussion

6.4.1 Simulation Procedure

Simulations were performed to assess the performance of the SLIPT methodology (as described in Section 3.1 and with modifications) for detecting known underlying synthetic lethal partners of a query gene. The simulation results supported the findings in empirical data (in Chapters 4 and 5) by addressing whether the methodology used to generate them was accurate or had desirable statistical performance in controlled simulated conditions. These investigations included adjusting parameters such as the numbers of synthetic lethal genes which were known in empirical data to assess the performance of the SLIPT methodology across simulation parameters and characterise the datasets for which SLIPT performs well. Simulation and statistically modelling also enabled comparison of the SLIPT methodology to other statistical approaches to synthetic lethal detection in expression data.

These simulations were based on a statistical model of synthetic lethality (as described in Section 3.2.1) which was designed stringently to ensure that if synthetic lethality was detectable in the simulated datasets it would also be detectable by the same methodology in empirical expression data. The model of synthetic lethality made conservative assumptions such as the low threshold of expression for gene function or the inclusion of cryptic higher-order synthetic lethality (when testing pairwise). These assumptions decrease the likelihood that synthetic lethal signatures would be detectable in expression data. Thus it is reassuring that synthetic lethality wes still detectable in under many simulation parameters as the performance of SLIPT would be expected to be higher were these assumptions to be violated in empirical data.

The simulation procedure (as described in Section 3.2.2) wes designed as a computational pipeline with arguments passes to scripts. The SLIPT methodology and simulation of expression from graph structures were both used as R (R Core Team, 2016) software packages developed and released for this project (as described in Section 3.5). This design ensurd that the simulations can be robustly applied across parameters with consistency between simulations apart from the differences discussed. The simulation procedure is also flexible to simulating other datasets, including synthetic lethal relationships and pathway correlation structures, should these be relevant to future investigations or bioinformatics tool development. The computational pipeline is also compatible with parallel computing and made use of High Performance Computing (HPC) infrastructure provided by the New Zealand eScience Infrastructure (NeSI) using the Simple Linux Utility for Resource Management (Slurm) submission system (as described in Section 2.5.3). This parallel computing pipeline enabled extensive investigations into synthetic lethality in simulated data, including approximately 2 million cpu-hours on NeSI.

6.4.2 Comparing Methods with Simulated Data

Attempts were made to implement alternative synthetic lethal detection approaches such as linear regression and the BiSEp R package (discussed in Section 6.1). However, those tested were ineffective at detecting synthetic lethality in multivariate normal simulated data in comparison to SLIPT. While some of the published synthetic lethal detection methods (Jerby-Arnon *et al.*, 2014; Lu *et al.*, 2015) did not provide reproducible software releases for direct comparison, some of the central assumptions used in their design were tested by the statistical methods considered for synthetic lethal detection in expression data.

BiSEp took considerably more time to compute predictions than SLIPT or χ^2, which limited the number of simulations that were feasible and made it difficult to apply across parameters in the simulation pipeline (even when using supercomputing infrastructure as discussed in Section 2.5.3). The computationally intensive nature of the BiSEp procedure does not appear to be the issue for detecting synthetic lethal genes in TCGA data or simulations, although it has made more extensive simulations challenging. Rather, BiSEp was not suitable in either case since the TCGA data was normalised with `voom` (Ritchie *et al.*, 2015) and simulated data was generated by sampling from a multivariate normal distribution. In either case, even subtle bimodal signatures in expression data were not consistently detectable or sufficient to detect synthetic lethality.

The BiSEp methodology may perform better on other data types but it cannot be directly compared with the results for SLIPT throughout this thesis which have used normalised or (multivariate) normally distributed data. Since it requires bimodal distributions, BiSEp was not suitable for stringently normalised expression data nor would it be expected to perform on (ranked) pathway metagenes. Thus SLIPT represents a distinct approach more suitable for these data types whereas BiSEp may be applicable to other applications in which bimodal distributions are more frequent.

This investigation also demonstrate that implementing scientific software from other research groups is not a trivial exercise, even when released as an open-source R package. Therefore, the above results were used to evaluate SLIPT and compare it to other statistical rationales. A comprehensive comparison to contemporary synthetic lethal detection approaches (and those released in the future) or further benchmarking is left to an impartial researcher to evaluate. The above findings showed that the SLIPT approach was able to detect synthetic lethal genes in simulated data with comparable or better performance than a range of distinct statistical techniques and was appropriate for use throughout this thesis.

6.4.3 Design and Performance of SLIPT

The simulation procedure using sampling from a multivariate normal distribution was used throughout the majority of the simulation investigations in this thesis. This approach has the advantages of emulating the continuous normalised expression data used for gene expression analysis and enabled the simulation of correlation structures (as discussed in Section 3.3). These simulations scaled to datasets of comparable scale to those used in gene expression anlaysis with thousands of genes. The SLIPT methodology was shown to perform robustly across large numbers of genes and simple correlation structures. This included high specificity against genes positively correlated with the query gene for which the directional SLIPT methodology was more suited to distinguishing synthetic lethal genes from than the χ^2 test without directional criteria on the number of samples observed.

These findings were expanded upon in this chapter. Specifically, different quantiles were compared for SLIPT and the χ^2 test. These approaches using threshold based discrete gene function were compared to the Pearson correlation without loss of the continuous expression data. The $1/3$-quantiles for SLIPT (as described in Section 3.1) were optimal for both SLIPT and the χ^2 alone. In addition to being optimal for estimating the significance of synthetic lethal interactions, these quantiles were also optimal for the directional criteria of SLIPT since this method outperformed the χ^2 test and was the most different at the $1/3$-quantile. As previously noted, this difference was more pronounced with positively correlated genes (with the query gene) for which the specificity of SLIPT improved, and was replicated in large datasets with thousands of genes, as occur in human expression data. These results were not simply due to sufficient samples for significant p-values since the performance as determined by AUROC analysis which was independent from significance thresholds. This indicated that the SLIPT methodology (as it has been used in Chapters 4 and 5) was optimal, with the $1/3$-quantile having the highest performance, and as such the parameters used to design it were appropriate.

Both discrete functional approaches (SLIPT and χ^2) were able to outperform negative correlation which supports their use. In particular, this result addressed the concern that arbitary thresholds of low and high gene function (as used by SLIPT) may lose useful data by compressing the spectrum of gene expression into categorical data. However, this does not impede the performance of SLIPT if the quantiles used were optimal. The poorer performance of correlation-based detection of synthetic lethality was consistent with gene function for synthetic lethality being qualitative, that is expression must be sufficient for cell viability and higher expression was not necessary for function (as this is not the case for all genes). Furthermore, the finding that negative correlation outperforms positive correlation is also consistent with co-expression being a poor predictor of synthetic lethality compared to other approaches (Jerby-Arnon *et al.*, 2014), supporting the claims of Lu *et al.* (2015).

Compared with SLIPT, neither correlation approaches nor bimodality signatures were suitable for detecting synthetic lethality in expression data. The correlation-based approaches made assumptions about the relationship between gene expression and function which do not necessarily hold for all genes. Similarly, the bimodal approach was not appropriate for normalised data since deviations from a normal distribution had already been used for ensuring data quality, as is common practice for RNA-Seq data. A linear model or regression approach may also be used to detect synthetic lethality from relationships between expression of genes, which may be improved with conditioning on known synthetic lethal partners with multivariate regression or Bayesian priors. Similarly, synthetic lethal detection could be performed by iteratively conditioning upon the strong candidate from previous analysis. These approaches may be able to better circumvent the issues of high-order synthetic lethality and multiple testing.

Nevertheless, the above findings were sufficient to assess the performance of SLIPT and present an effective straightforward approach to synthetic lethal detection in gene expression data. Further development with linear models, Bayesian inference approaches, or comparison to existing synthetic lethal approaches (e.g., machine learning) remain as future directions. Developing and testing more sophisticated statistical approaches to synthetic lethal detection may benefit from the concepts discussed with regard to the relatively simple SLIPT methodology. Similarly, further comparisons and benchmarking of SLIPT against other computational approaches to synthetic lethal detection in gene expression data is more suitable for an independent researcher and the `slipt` R package has been released (as described in Section 3.5) for this purpose, in addition to further application in research.

6.4.4 Simulations from Graph Structures

The simple correlation structures (as used in Section 3.3) were expanded upon using the multivariate normal simulation procedure to produce correlation structures based on graph structures (as described in Section 6.2). These simulations enabled further investigations into the performance of SLIPT in the context of more complex correlation structures. The simulation of expression from network structures is widely applicable to simulating pathway expression data and as such the `graphsim` R package has been released (as described in Section 3.5).

These investigations show that SLIPT performs robustly across datasets with different correlation structures, including those derived from graphs with the complexity of biological pathways. The SLIPT methodology was able to detect synthetic lethal genes within synthetic lethal pathways across many graph structures. This methodology performed particularly well with synthetic lethal pathways in the context of a larger dataset with a high specificity which supports SLIPT as a stringent approach to synthetic lethal detection in highly dimensional gene expression data. Together these results support the use of SLIPT in biological gene expresssion data since it was able to detect synthetic lethal genes in highly complex correlation structures.

Similarly, the inclusion of inhibitory relationships in graph structures was shown to increase the performance in simple networks, supporting SLIPT being applicable to biological data in which these relationships are common. While these results were not replicated in more complex inhibitory graph structures, this is likely an artifact of the simulation procedure (which randomly selects synthetic lethal genes) generating biologically implausible combinations of synthetic lethal genes which were difficult to detect. When the test statistics in simulations with a synthetic lethal gene were examined in more detail, the test statistics of the synthetic lethal gene were consistently higher and distinguishable from nearby genes in the graph structure. In contrast to previous concerns with inhibiting relationships, these differences were more pronounced with genes which had inhibitory relationships with synthetic lethal genes. While distinguishable from nearby genes in a pathway structure, the genes correlated with synthetic lethal partners still had higher test statistics than more distant genes (similar to observations with correlated genes in Section 3.3).

In addition to being able to detect synthetic lethal genes in a pathway, the proximal genes in a pathway were most likely to be false positives and therefore SLIPT is also able to detect synthetic lethal pathways. SLIPT identifies genes which are likely to be constituent of a synthetic lethal pathway and is more likely to rank underlying synthetic lethal genes with greater significance. Together these findings support the use of SLIPT throughout this thesis, further application of SLIPT, and further development of such strategies for synthetic lethal detection. Similarly, the simulation procedures developed and demonstrated for examining synthetic lethal detection in expression data using graph structures is amenable to further development and investigations into pathway structure in expression data such as predicting biological pathways from expression data or the impact of pathways on differential expression analyses.

6.5 Summary

A statistical model and simulation procedure has been developed to test the performance of the SLIPT methodology in controlled conditions, using multivariate normal distributions. This simulation procedure has been developed into a computational pipeline which was able to test the statistical performance (using stringent assumptions) of SLIPT across many parameters and compare it to alternative synthetic lethal detection strategies. The SLIPT methodology performed well at detecting small numbers of synthetic lethal genes in simple systems. It did not perform as well in more complex systems but neither did alternative strategies. The SLIPT methodology performed well compared to Pearson correlation and similar methods based on the χ^2 test. Thus SLIPT is an effective detection method for synthetic lethal relationships in expression data despite its relatively simple design.

Simulations of more complex datasets were performed, including large numbers of genes, complex correlation structure derived from graph structures, and correlations with the query gene. SLIPT performed robustly across these, including correlation structures based on complex biological pathways. The performance of SLIPT improved in larger datasets, datasets with positive correlations with the query genes, and some graph structures which included inhibiting relationships, namely those datasets that were more representative of gene expression in biological data. SLIPT was both capable of recurrently detecting genes within a synthetic lethal pathway, and distinguishing synthetic lethal genes from correlated with them, even in highly complex correlation structures. Therefore SLIPT is a stringent synthetic lethal detection strategy and is applicable to gene expression as previously demonstrated for the partners of *CDH1* in breast and stomach cancer in this thesis.

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