**Supplementary file S4**

**A.** Inferred expression and flux alteration in various type of cancers compared to the normal stage. There is significant biases among the two methods when their constituent genes were classified based on their expression or flux alteration. In total, we have identified 19 distinct genes with significant changes, either in normal or cancerous states. The figure is pseudo-colored; only significant categories are shown in yellow and black



**B.** Summary information for all gene expression data used for flux variability analysis of 8 normal/cancer cells

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Name | GEO ID | Sample | Normal | Cancer | PMID | References |
| Breast | GSE10780 | 185 | 143 | 42 | 19266279 | Chen et al. [1](#_ENREF_1) |
| Ovary | GSE38666 | 45 | 20 | 25 | 23762861 | Lili et al. [2](#_ENREF_2) |
| Gastric | GSE64951 | 94 | 31 | 63 | - | - |
| Leukemia-CLL | GSE50006 | 279 | 32 | 247 | - | - |
| Cervical | GSE63514 | 52 | 24 | 28 | 26056290 | den Boon et al. [3](#_ENREF_3) |
| Renal | GSE53757 | 144 | 72 | 72 | 24962026 | von Roemeling et al. [4](#_ENREF_4) |
| Liver | GSE45436 | 134 | 39 | 95 | - | - |
| Colorectal | GSE37364 | 65 | 38 | 27 | 25405986 | Valcz et al. [5](#_ENREF_5) |

**C.** Mathematica script for applying the E-Flux method:

*(\*---------------------------------------------------------------------------------------*

*Import a file containing gene-product relationships, then flatten data*

*----------------------------------------------------------------------------------------\*)*

A=Import[FileNameJoin[{rootdir,"gpr\_data","gpr\_new.xlsx"}]]

Length[A];

gpr=Flatten[A];

*(\*---------------------------------------------------------------------------------------*

*Replace Or/And through the input file with mathematical operators in order to change each gpr into a mathematical relation, then delete duplicate data*

*----------------------------------------------------------------------------------------\*)*

geneRules=ToExpression[StringReplace[#,{" || "->"~Or~"," && "->"~ And~",x: NumberString:>"\""~~ StringSplit[x,"."][[1]]~~"\""}]]&/@gpr;

p1=geneRules//.Or->(Or@@DeleteDuplicates[{##}]&);

p1=p1//.And->(And@@DeleteDuplicates[{##}]&);

expressionFunctions=p1//.{Or->Plus,And->Min};

TableForm@Transpose[{Range[Length[expressionFunctions]],expressionFunctions}][[30;;50]]

*(\*---------------------------------------------------------------------------------------*

*Import gene expression data, then in metabolic model we find reactions in which genes are available in the gene expression input data*

*----------------------------------------------------------------------------------------\*)*

{expressionDataPlus}=Import[FileNameJoin[{rootdir,"expression\_data","normal\_vs\_cancer","with\_entrez\_id","renal-normal-average.xls"}]];

expressionDataPlus[[2;;-1,2]]=ToString/@Round/@expressionDataPlus[[2;;-1,2]];

expressionRulesPlusICM=Rule@@@expressionDataPlus[[2;;-1,{2,3}]];

newUpperBounds=expressionFunctions//.expressionRulesPlusICM;

posListGood=Flatten@Position[NumberQ/@newUpperBounds,True];

posListBad=Complement[Range[Length[newUpperBounds]],posListGood];

Length/@{newUpperBounds,posListGood,posListBad}

*(\*---------------------------------------------------------------------------------------*

*Apply E-Flux algorithm to the reactions mentioned above and calculate new lower and upper bounds for such reactions. LB and UB of the rest of the reactions would not be changed. B is a matrix containing lower and upper bounds for the recon1 metabolic model.*

*----------------------------------------------------------------------------------------\*)*

newUpperBounds[[posListGood]]=(newUpperBounds[[posListGood]]/Max[newUpperBounds[[posListGood]]])\*Max[B[[1,All,2]]];

newUpperBounds[[posListBad]]=B[[1,posListBad,2]];

newLowerBounds=B[[1,All,1]];

p1=Transpose[{posListGood,B[[1,posListGood,1]]}];

p1=Select[p1,Abs[#[[2]]]>0.&];

Tally[p1[[All,2]]]

newLowerBounds[[p1[[All,1]]]]=-newUpperBounds[[p1[[All,1]]]];

*(\*---------------------------------------------------------------------------------------*

*Export new LB and UB data*

*----------------------------------------------------------------------------------------\*)*

Export[FileNameJoin[{rootdir,"newBounds.xlsx"}],{newLowerBounds,newUpperBounds}]

**D.** The details of biomass composition (which has been used as an objective function) in our study according to the *Recon1\_corrected* model

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomass** | | | |
| **Metabolite name** | **Coefficient** | **Metabolite name** | **Coefficient** |
| L-Alanine | 0.119 | dTMP | 0.00227 |
| L-Arginine | 0.000927 | CMP | 0.00924 |
| L-Asparagine | 0.0121 | GMP | 0.0105 |
| L-Aspartate | 0.693 | UMP | 0.00555 |
| L-Cysteine | 0.00121 | AMP | 0.00555 |
| L-Glutamate | 0.168 | Glycogen | 0.266 |
| L-Glutamine | 0.191 | Sphingomyelin | 0.00241 |
| L-Histidine | 0.0287 | Cholesterol | 0.00827 |
| L-Isoleucine | 0.00371 | Phosphatidylcholine | 0.0172 |
| L-Leucine | 0.0111 | Phosphatidylethanolamine | 0.0201 |
| L-Lysine | 0.00927 | Phosphatidylinositol | 0.0031 |
| L-Methionine | 0.00185 | Phosphatidylserine | 0.00589 |
| L-Phenylalanine | 0.00371 | Monoacylglycerol | 0.00386 |
| L-Proline | 0.018 | Diacylglycerol | 0.00315 |
| L-Serine | 0.0371 | Lysophosphatidylcholine | 0.00126 |
| L-Threonine | 0.0204 | Triacylglycerol | 0.0275 |
| L-Tryptophan | 0.000927 | cholesterol ester | 0.00657 |
| L-Tyrosine | 0.00556 | ATP | 43.153 |
| L-Valine | 0.0121 | H2O | 100 |
| Glycine | 0.139 | ADP | 100 |
| dAMP | 0.00227 | Hydrogene | 100 |
| dCMP | 0.00152 | Phosphate | 100 |
| dGMP | 0.00152 |  |  |

**E.** The details of the compositions of the RPMI-1640 applied to all metabolic models as a cell culture in this studyaccording to the *Recon1\_corrected* model**.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Media: RPMI-1640** | | | |
| **Metabolite name** | **Value** | **Metabolite name** | **Value** |
| Glycine | 0.05 | Biotin | 0.005 |
| L-Arginine | 0.05 | Choline | 0.005 |
| L-Asparagine | 0.05 | (R)-Pantothenate | 0.005 |
| L-Aspartate | 0.05 | Folate | 0.005 |
| L-Cysteine | 0.05 | Nicotinamide | 0.005 |
| L-Glutamate | 0.05 | Pyridoxal | 0.005 |
| L-Histidine | 0.05 | Riboflavin | 0.005 |
| L-Isoleucine | 0.05 | Thiamin | 0.005 |
| L-Leucine | 0.05 | myo-Inositol | 0.005 |
| L-Lysine | 0.05 | Calcium | 1000 |
| L-Methionine | 0.05 | potassium | 1000 |
| L-Phenylalanine | 0.05 | Chloride | 1000 |
| L-Proline | 0.05 | Sodium | 1000 |
| L-Serine | 0.05 | Phosphate | 1000 |
| L-Threonine | 0.05 | D-Glucose | 5 |
| L-Tryptophan | 0.05 | Reduced glutathione | 0.05 |
| L-Tyrosine | 0.05 | O2 | 1000 |
| L-Valine | 0.05 | Fe2+ | 1000 |
| L-Glutamine | 0.5 | L-Alanine | 0.05 |

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