

# **Kineome and Phosphoproteome Analysis with Multi-immunoblotting and Antibody Microarrays**

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# Gene Families in Human Genome

|                             |      |        |
|-----------------------------|------|--------|
| ■ Metabolic enzymes         | 3142 | (9.2%) |
| ■ Signalling proteins       | 6166 | (21%)  |
| ■ Protein kinases           | 515  | (1.7%) |
| ■ Protein phosphatases      | 138  | (0.5%) |
| ■ Transcription factors     | 1850 | (6.0%) |
| ■ Receptors                 | 1543 | (5.0%) |
| ■ Extracellular mediators   | 376  | (1.2%) |
| ■ Other signalling proteins | 1722 | (5.6%) |

~30,000 genes (100%)

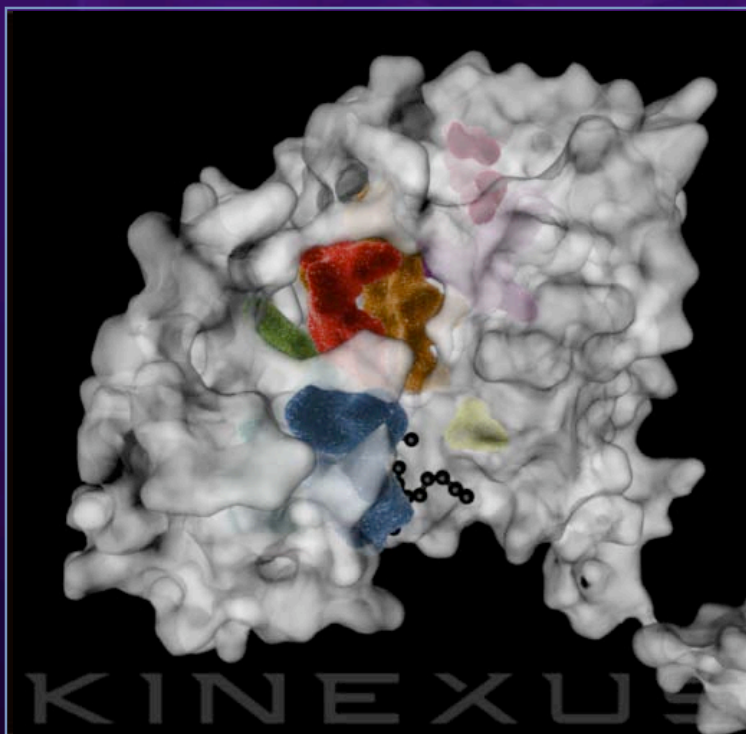
\*based on Venter et al. (2001) Science



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# Protein Kinases are Major Drug Targets



*EGF receptor 3D structure with  
kinase inhibitor drug Tarceva*

## ■ Kinase inhibitors are proven drugs

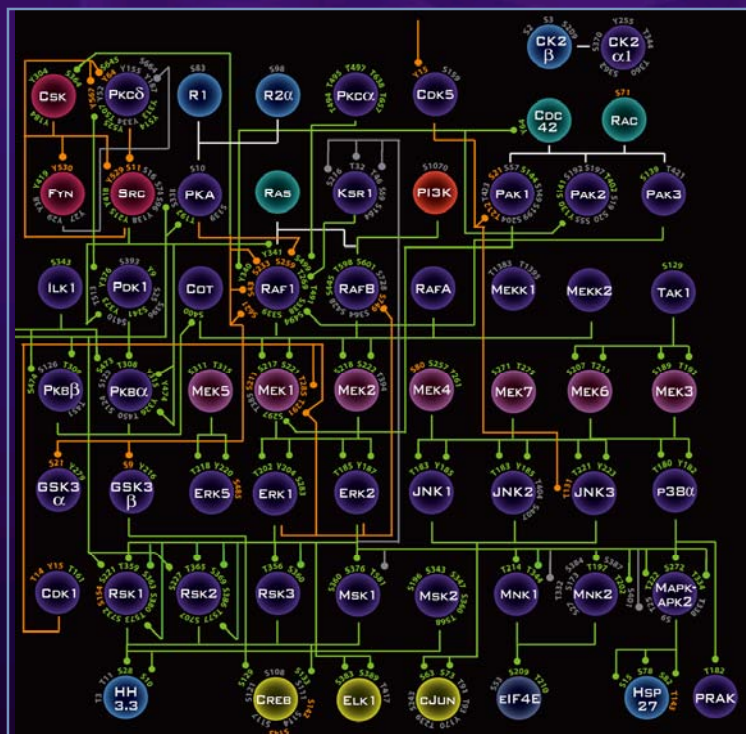
### ■ 11 approved in last 6 years

- Gleevec (Novartis, \$2.2B in 05)
- Avastin (Genetech/Roche, >\$1.5B in 06)
- Herceptin (Genetech, \$0.75B in 05)
- Erbitux (Imclone/Merck, \$0.67B in 05)
- Iressa (AstraZeneca, \$0.39B in 04)
- Rapamune (Wyeth, ~\$0.3B in 05)
- Sprycel (BristolMyersSquibb, >\$0.3B in 07)
- Tarceva (Genetech/Roche, \$0.28B in 05)
- Sutent (Pfizer, >\$0.2B in 06)
- Nexavar (Onxy Pharm/Bayer, >\$0.15B in 06)
- Fasudil (Asahi Kasei, N/A)

### ■ >75 in clinical trials

### ■ >500 in pre-clinical trials

# Protein Kinase Drug Targets



- More than 30% of pharmaceutical drug discovery is focused on protein kinases
- Over 400 human diseases linked to defects in kinase signalling
- Only ~ 24 of 515 protein kinases targeted for drug screening

# Human Protein Kineome

| Group           | Number<br>in group | Number<br>with TM<br>domains | Number<br>with two<br>catalytic<br>domains | Number<br>pseudo<br>genes | Known<br>Pho-Ser<br>sites | Known<br>Pho-Thr<br>sites | Known<br>Pho-Tyr<br>sites |
|-----------------|--------------------|------------------------------|--------------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| <b>AGC</b>      | 63                 | 0                            | 0                                          | 6                         | 44                        | 38                        | 17                        |
| <b>ATYPICAL</b> | 38                 | 2                            | 0                                          | 1                         | 15                        | 2                         | 6                         |
| <b>CAMK</b>     | 74                 | 0                            | 8                                          | 39                        | 24                        | 31                        | 3                         |
| <b>CK1</b>      | 12                 | 1                            | 0                                          | 5                         | 0                         | 0                         | 0                         |
| <b>CMGC</b>     | 61                 | 0                            | 0                                          | 12                        | 41                        | 10                        | 18                        |
| <b>OTHER</b>    | 82                 | 4                            | 1                                          | 21                        | 17                        | 11                        | 6                         |
| <b>RGC</b>      | 5                  | 5                            | 0                                          | 3                         | 0                         | 0                         | 0                         |
| <b>STE</b>      | 47                 | 0                            | 0                                          | 6                         | 29                        | 19                        | 5                         |
| <b>TK</b>       | 90                 | 55                           | 4                                          | 5                         | 38                        | 5                         | 215                       |
| <b>TKL</b>      | 43                 | 12                           | 0                                          | 6                         | 39                        | 9                         | 5                         |
| <b>Total</b>    | <b>515</b>         | <b>79</b>                    | <b>13</b>                                  | <b>104</b>                | <b>247</b>                | <b>125</b>                | <b>275</b>                |



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## Human Phosphoproteome

- Number of human phospho-sites known: ~19,540
- Number of human phosphoproteins identified: ~7,000
- Percent serine phosphorylation sites: 58.8%
- Percent threonine phosphorylation sites: 11.5%
- Percent tyrosine phosphorylation sites: 29.7%
- Percent tyrosine phosphorylation sites according to Gygi and Mann lab data: 0.7-1.8%
- Number of tyrosine phosphosites known: 5804
- Minimum number of human phosphosites:  
 $5804 \times 80 = 464,320$



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# Human Phosphoproteome

- Likely number of human phosphosites: ~1,000,000
- Number of proteins: ~30,000
- Average number of sites per protein: ~33
- Average number of amino acids per protein: 500
- Frequency of phosphorylation sites: 1 every 15 aa
- Average number phospho-sites per kinase: ~2000
- Average number phospho-sites per phosphatase: ~7250
- Minimum number of connections in phosphorylation networks: ~2,000,000
- Likely number of connections in phosphorylation networks: ~10,000,000



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# Human Phosphoproteome

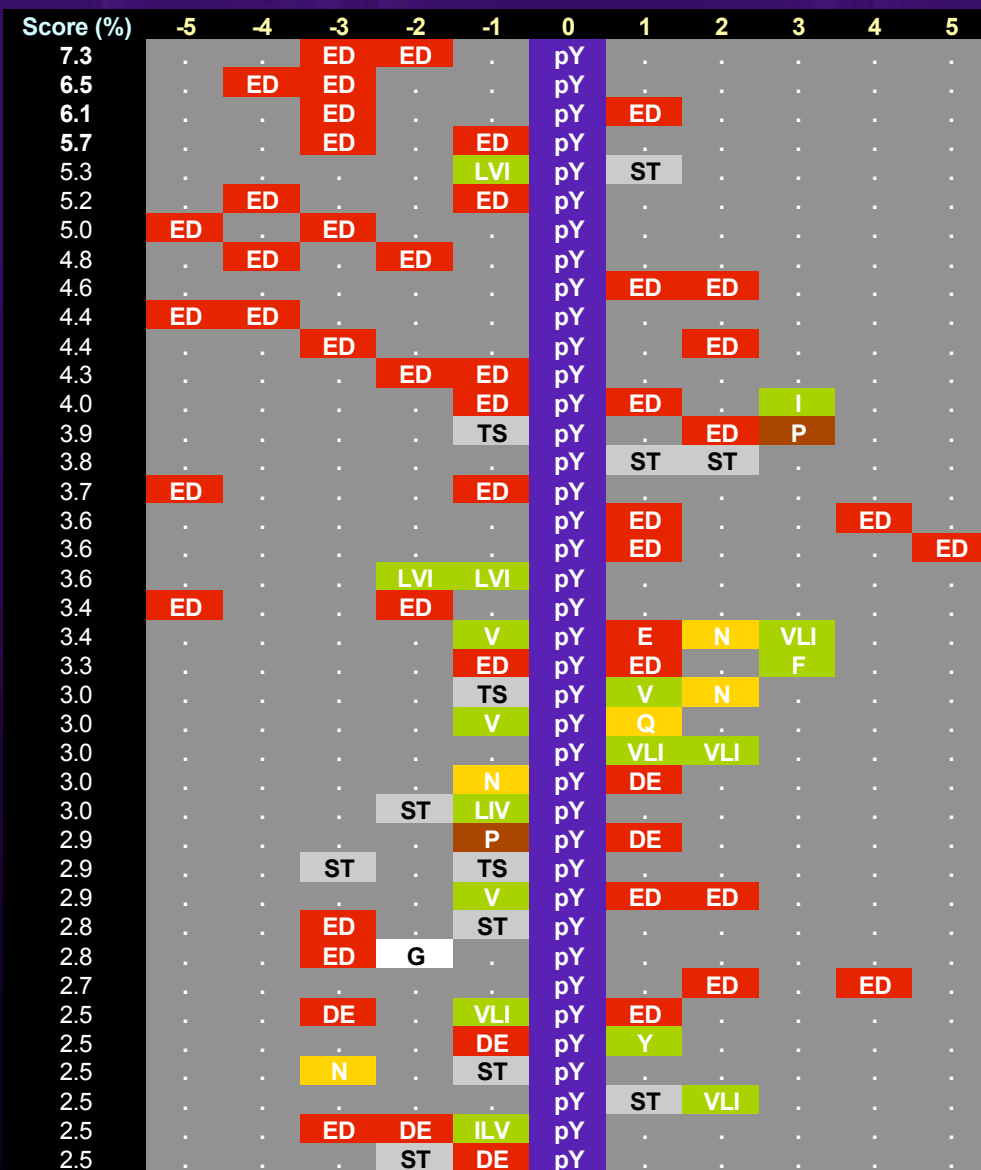
- The human and mouse phosphoproteomes are 46% different.
- Percent human phospho-sites conserved in mouse: 71.4
- Percent human phospho-sites conserved in frog: 14.4
- Percent human phospho-sites conserved in worm: 5.9
- Percent human phospho-sites conserved in yeast: 1.7



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# Phospho-Tyrosine Sites Frequency



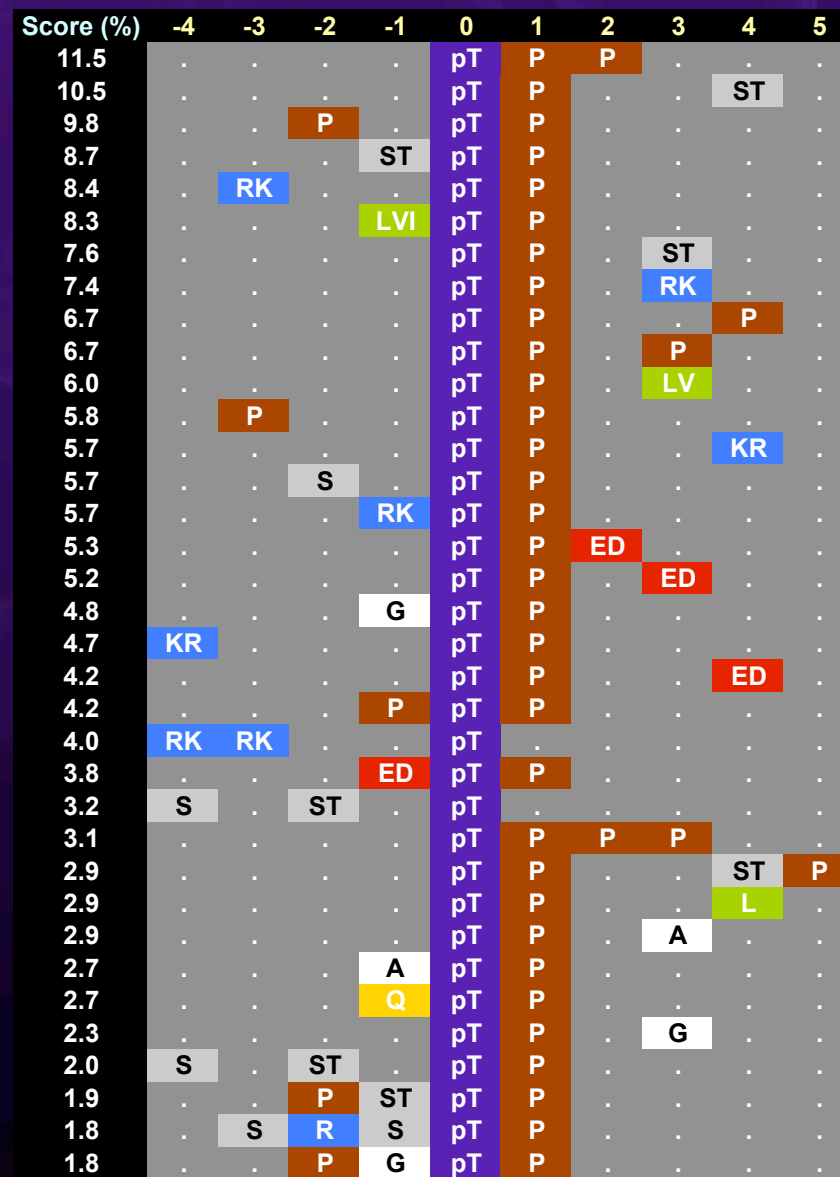
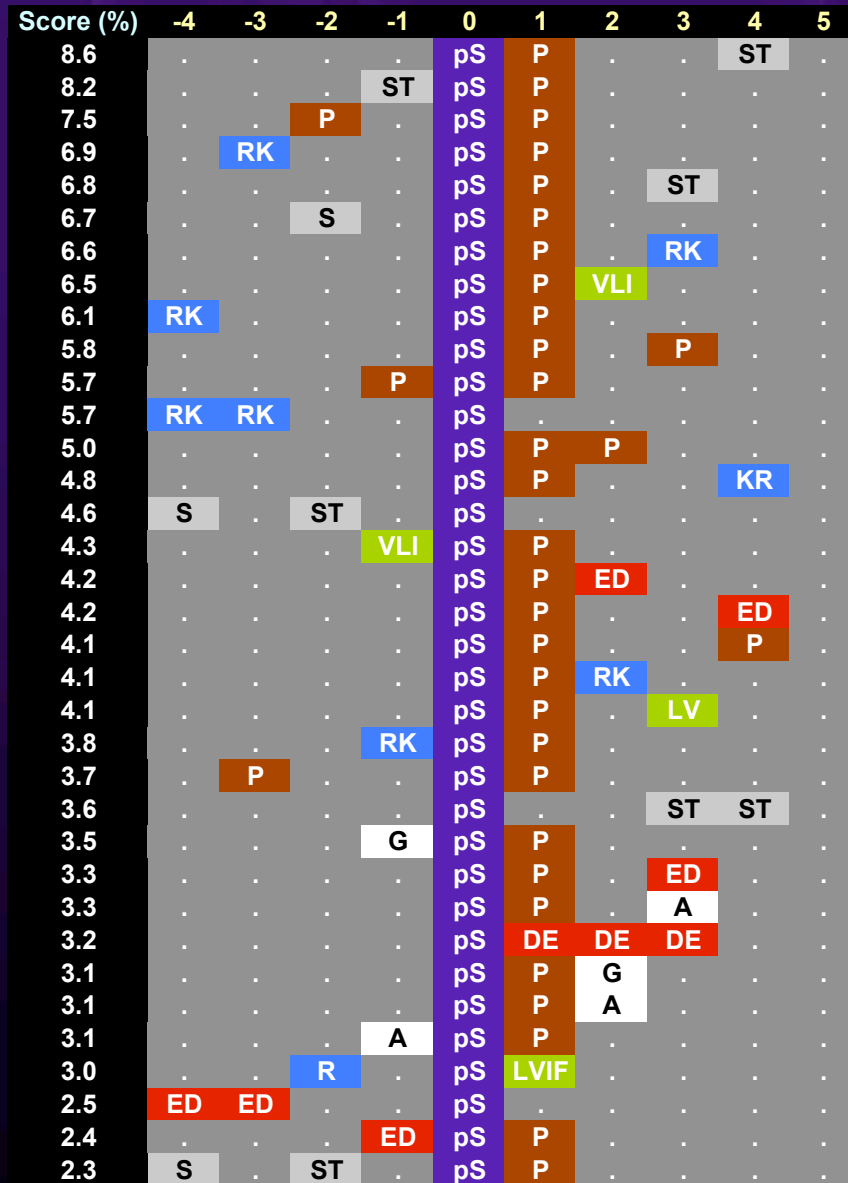
|             |       |                     |
|-------------|-------|---------------------|
| Acidic      | DE    | Asp/Glu             |
| Basic       | KR    | Lys/Arg             |
| Weak Basic  | NQ    | Asn/Gln             |
| Weak polar  | M     | Met                 |
| Hydrophobic | FILVY | Phe/Ile/Leu/Val/Tyr |
| Kink        | P     | Pro                 |
| Polar       | ST    | Ser/Thr             |
| Phospho-AA  | pSpT  | pSer/pThr           |
| Phospho-AA  | pY    | pTyr                |
| Neutral     | AG    | Ala/Gly             |
| Any AA      | .     | Variable            |



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# Phospho-Serine/Threonine Sites Frequency



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# Protein Kinase Specificity

- Percent phospho-sites within 10 aa of N-terminus: **1.4%**
- Percent phospho-sites within 10 aa of C-terminus: **4.1%**

|             |       |                     |
|-------------|-------|---------------------|
| Acidic      | DE    | Asp/Glu             |
| Basic       | KR    | Lys/Arg             |
| Weak Basic  | NQ    | Asn/Gln             |
| Weak polar  | M     | Met                 |
| Hydrophobic | FILVY | Phe/Ile/Leu/Val/Tyr |
| Kink        | P     | Pro                 |
| Polar       | ST    | Ser/Thr             |
| Phospho-AA  | pSpT  | pSer/pThr           |
| Phospho-AA  | pY    | pTyr                |
| Neutral     | AG    | Ala/Gly             |
| Any AA      | .     | Variable            |

| Protein Kinase | -7  | -6 | -5    | -4  | -3   | -2  | -1   | 0    | 1     | 2    | 3    | 4     | 5   |
|----------------|-----|----|-------|-----|------|-----|------|------|-------|------|------|-------|-----|
| CLK1           | .   | KR | .     | KR  | .    | KR  | .    | pSpT | .     | .    | R    | .     | .   |
| PKC-eta        | A   | R  | .     | .   | R    | KR  | R    | pSpT | F     | R    | R    | .     | .   |
| PKC-alpha      | .   | R  | RF    | R   | R    | KR  | G    | pSpT | FL    | KR   | KRQ  | .     | .   |
| PKA            | .   | .  | .     | KR  | KR   | KR  | RN   | pSpT | FILVY | I    | F    | D     | .   |
| Akt/PKB        | .   | .  | R     | .   | R    | STA | STA  | pSpT | FL    | .    | .    | .     | .   |
| PKC-delta      | .   | .  | R     | .   | R    | K   | G    | pSpT | F     | .    | .    | .     | .   |
| PKC-gamma      | .   | .  | R     | R   | R    | K   | KG   | pSpT | F     | KR   | KR   | K     | A   |
| DMPK-E         | .   | .  | KR    | K   | R    | R   | R    | pSpT | LV    | .    | .    | .     | .   |
| Pim1           | .   | .  | KR    | KR  | R    | KR  | L    | pSpT | .     | .    | .    | .     | .   |
| RSK1           | .   | .  | KR    | .   | R    | R   | .    | pSpT | .     | .    | .    | .     | .   |
| PKC-epsilon    | .   | .  | .     | K   | R    | KRQ | G    | pSpT | V     | R    | R    | .     | .   |
| SLK1           | .   | .  | .     | R   | R    | F   | G    | pS   | FILVY | R    | R    | FILVY | .   |
| ZIPK           | .   | .  | .     | KR  | R    | R   | R    | pS   | .     | .    | .    | .     | .   |
| NIMA           | .   | .  | .     | NR  | FLM  | KR  | KR   | pS   | IVMR  | IVMR | FIMV | FIM   | .   |
| PKC-beta       | .   | .  | FL    | KR  | R    | KQ  | G    | pSpT | FM    | K    | K    | .     | A   |
| AMPK           | .   | .  | ILMV  | HKR | HKR  | HKR | .    | pSpT | .     | .    | .    | ILMV  | .   |
| DCK1-b2        | .   | .  | FILMV | R   | R    | .   | .    | pSpT | FILMV | .    | .    | .     | .   |
| MAPKAPK2       | FIL | .  | ILV   | .   | R    | .   | L    | pT   | ILMV  | .    | .    | .     | .   |
| CHK1           | .   | .  | ILMV  | .   | KR   | .   | .    | pSpT | .     | .    | .    | .     | .   |
| CaMK1          | .   | .  | FILMV | .   | R    | .   | .    | pSpT | FILMV | .    | .    | .     | .   |
| CaMK2          | .   | .  | FILVY | .   | KR   | .   | .    | pSpT | ILMV  | DE   | .    | .     | .   |
| CaMK4          | .   | .  | FILVY | .   | R    | .   | .    | pSpT | .     | .    | .    | .     | .   |
| PKC-zeta       | .   | .  | F     | .   | R    | .   | .    | pSpT | FM    | FM   | .    | .     | .   |
| PKC-mu         | .   | .  | VL    | ALV | R    | .   | M    | pSpT | .     | .    | .    | .     | .   |
| MSK1/2         | .   | .  | .     | .   | R    | .   | .    | pSpT | .     | .    | .    | .     | .   |
| PAK            | .   | .  | .     | .   | KR   | R   | .    | pSpT | .     | .    | .    | .     | .   |
| Phos.Kinase    | .   | .  | .     | .   | KR   | .   | FILM | pS   | FILMV | FKR  | IL   | FIL   | .   |
| PDK1           | .   | .  | F     | .   | .    | .   | F    | pSpT | FY    | .    | .    | .     | .   |
| LKB1           | .   | .  | .     | .   | .    | L   | .    | pT   | .     | .    | .    | .     | .   |
| mTOR           | .   | .  | .     | .   | .    | .   | F    | pT   | Y     | .    | .    | .     | .   |
| MEK3           | .   | .  | .     | .   | .    | .   | .    | pT   | G     | Y    | .    | .     | .   |
| CK1d           | .   | .  | E     | EF  | DpS  | AGT | G    | pS   | I     | FGIY | GFI  | FG    | FLP |
| CK1g           | .   | .  | Y     | EY  | DYpS | AD  | AG   | pS   | I     | FGIY | GFI  | FG    | FLP |
| BARK           | .   | .  | .     | .   | .    | E   | .    | pS   | .     | .    | .    | .     | .   |
| ATM            | .   | .  | .     | .   | ILMP | .   | DE   | pS   | Q     | E    | .    | .     | .   |
| DNAPK          | .   | .  | .     | .   | .    | E   | .    | pS   | Q     | E    | .    | .     | .   |
| SIM            | .   | .  | .     | .   | .    | .   | .    | pT   | E     | Y    | .    | .     | .   |
| CK2            | .   | .  | .     | .   | .    | .   | .    | pSpT | .     | DE   | DEpS | DE    | .   |
| ERK1           | .   | .  | .     | .   | .    | P   | .    | pSpT | P     | .    | .    | FIY   | .   |
| p38 MAPK       | .   | .  | .     | .   | G    | P   | QM   | pS   | P     | I    | .    | .     | .   |
| CDK2           | .   | .  | R     | .   | .    | P   | M    | pSpT | P     | .    | KR   | K     | .   |
| CDK4           | .   | .  | .     | .   | .    | P   | L    | pSpT | P     | I    | P    | HKR   | .   |
| CDK5           | .   | .  | .     | .   | .    | P   | .    | pSpT | P     | .    | K    | .     | .   |
| CDK1           | .   | .  | .     | .   | .    | .   | KR   | pS   | P     | KR   | KR   | .     | .   |
| GSK3           | .   | .  | .     | .   | .    | .   | .    | pS   | .     | .    | .    | pS    | P   |
| TGF-betaR1     | .   | .  | .     | .   | .    | .   | .    | pS   | S     | .    | pS   | .     | .   |

# Kinases Genes in Diverse Genomes

|                 | Kinase Genes / Total Genes |          |        |
|-----------------|----------------------------|----------|--------|
| ■ Human         | 515                        | / 30,000 | (1.7%) |
| ■ Fly           | 319                        | / 13,338 | (2.4%) |
| ■ Worm          | 437                        | / 18,266 | (2.4%) |
| ■ Yeast         | 122                        | / 6,144  | (2.0%) |
| ■ Mustard plant | 1049                       | / 25,706 | (4.1%) |

\*based on Intern. HGS Consortium (2001) Nature



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# Protein Kinase Catalytic Domain Conservation - High

| Kinase Name            | Mouse | Rat | Dog | Chicken | Fly | Worm | Mustard Plant | Rice | Budding Yeast | Neurospora | SUM |
|------------------------|-------|-----|-----|---------|-----|------|---------------|------|---------------|------------|-----|
| Average Conserv.       | 89    | 89  | 91  | 77      | 50  | 47   | 43            | 44   | 41            | 44         | 615 |
| CK2 $\alpha$ 1/CSNK2A1 | 98    | 98  | 100 | 97      | 88  | 78   | 75            | 76   | 59            | 70         | 839 |
| AMPK $\alpha$ 2/PRKAA2 | 97    | 98  | 98  | 90      | 60  | 58   | 46            | 46   | 42            | 80         | 715 |
| GSK3 $\beta$           | 96    | 96  | 99  | 95      | 70  | 74   | 63            |      | 54            | 62         | 709 |
| p38 $\alpha$ /MAPK14   | 95    | 99  | 95  | 96      | 68  | 62   | 45            | 46   | 52            | 49         | 707 |
| CDK7                   | 93    | 95  | 92  | 94      | 66  | 60   | 57            | 58   | 48            | 42         | 705 |
| PKAC $\beta$ /PRKACB   | 97    | 97  | 88  | 90      | 83  | 80   | 35            |      | 54            | 55         | 679 |
| CDK5                   | 99    | 99  | 99  | 98      | 79  | 74   |               |      | 55            | 60         | 663 |
| CK1 $\delta$ /CSNK1D   | 99    | 99  | 99  | 97      | 61  |      | 74            | 61   |               | 71         | 661 |
| ERK2/MAPK1             | 99    | 99  | 96  | 98      | 81  | 81   | 50            |      | 50            |            | 654 |
| CK1 $\alpha$ /CSNK1A1  | 92    | 91  | 100 | 99      | 70  | 79   | 59            | 60   |               |            | 650 |
| MAP2K1/MEK1            | 98    | 99  | 91  | 95      | 63  | 52   | 36            | 33   | 42            | 39         | 648 |
| AurA/STK6              | 82    | 83  | 89  | 71      | 50  | 57   | 57            | 53   | 43            | 55         | 640 |
| PAK1                   | 97    | 98  | 96  | 94      | 51  | 53   |               | 49   | 45            | 40         | 623 |
| FRAP/FRAP1             | 98    | 98  | 99  | 93      | 53  | 51   | 40            | 46   | 41            |            | 619 |
| NDR2/STK38L            | 97    |     | 99  | 97      | 71  | 67   | 50            | 49   | 47            | 42         | 619 |
| CK1 $\epsilon$ /CSNK1E | 98    | 99  | 99  | 95      | 84  |      | 63            | 74   |               |            | 612 |
| PDHK3/PDK3             | 97    | 97  | 99  | 92      | 58  | 53   | 38            | 39   |               | 37         | 610 |
| PKAC $\alpha$ /PRKACA  | 98    | 98  | 98  | 95      | 82  | 83   |               |      | 48            |            | 602 |
| CLK2                   | 96    | 96  | 98  | 88      | 58  | 62   | 49            |      |               | 46         | 593 |
| CDK11/CDC2L6           | 87    | 96  | 96  | 79      | 85  | 60   | 44            | 41   |               |            | 588 |
| CDK9                   | 98    | 98  | 98  | 90      | 73  | 51   |               |      | 41            | 34         | 583 |
| NDR1/STK38             | 98    | 97  | 95  | 93      | 80  | 66   | 49            |      |               |            | 578 |
| RIOK2                  | 83    | 81  | 84  | 67      | 45  | 41   | 45            | 45   | 40            | 47         | 578 |
| MST2/STK3              | 94    | 96  | 98  | 98      | 46  | 54   | 43            | 43   |               |            | 572 |
| p70S6K/RPS6KB1         | 99    | 99  | 99  | 98      | 72  | 60   | 43            |      |               |            | 570 |
| PKC $\epsilon$ /PRKCE  | 97    | 98  | 98  | 82      | 57  | 57   |               |      | 46            | 33         | 568 |
| PIK3R4                 | 95    | 95  | 97  | 91      | 41  | 29   | 30            | 26   | 26            | 30         | 560 |
| PRP4/PRPF4B            | 95    | 95  | 98  | 90      | 43  | 41   | 33            | 48   |               |            | 543 |
| RIOK1                  | 80    | 82  | 89  | 75      | 48  | 42   | 46            |      | 38            | 41         | 541 |
| TRRAP                  | 98    | 98  | 98  | 93      | 49  | 24   | 27            |      | 27            | 27         | 541 |
| CaMKK2                 | 89    | 92  | 91  | 74      | 54  | 55   | 40            |      |               | 45         | 540 |
| CDK2                   | 96    | 72  | 96  |         | 70  |      |               | 71   | 64            | 64         | 533 |
| OSR1/OXSR1             | 95    | 95  | 97  | 86      | 56  | 52   | 49            |      |               |            | 530 |
| PDK1/PDPK1             | 94    | 95  | 94  | 90      | 43  | 37   | 38            | 39   |               |            | 530 |
| BRAF                   | 99    | 87  | 93  | 89      | 45  | 40   | 33            | 42   |               |            | 528 |
| AMPK $\alpha$ 1/PRKAA1 | 96    | 96  | 98  | 83      |     | 58   | 50            | 46   |               |            | 527 |
| JNK3/MAPK10            | 98    | 99  | 98  | 84      | 78  | 70   |               |      |               |            | 527 |
| AKT2                   | 98    | 97  | 97  |         | 61  |      | 40            | 39   | 49            | 45         | 526 |
| NLK                    | 99    | 99  | 99  | 98      | 76  | 54   |               |      |               |            | 525 |
| CaMK2 $\delta$         | 92    | 92  | 91  | 68      | 74  | 67   | 39            |      |               |            | 523 |



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# Protein Kinase Gene Expression - High

| Kinase Name   | Liver | Lung | Spleen | Testes | Kidney | Prostate | Uterus | Pancreas | Adrenal | Ovary | Thyroid | Thymus | Stomach | Cervix | Salivary Gland | Lymph Node | Tonsil | Colon | Heart | Esophagus | Fallopian Tube | Parathyroid Gland | Bladder | Breast | Epididymus | Pericardium | Vagina | Trachea | Spinal Cord | Whole Brain | Pituitary | SUM |
|---------------|-------|------|--------|--------|--------|----------|--------|----------|---------|-------|---------|--------|---------|--------|----------------|------------|--------|-------|-------|-----------|----------------|-------------------|---------|--------|------------|-------------|--------|---------|-------------|-------------|-----------|-----|
| AVERAGE       | 3.4   | 3.3  | 3.3    | 3.3    | 3.3    | 3.3      | 3.3    | 3.3      | 3.2     | 3.2   | 3.1     | 3.2    | 3.3     | 3.4    | 3.1            | 3.4        | 3.3    | 3.2   | 2.9   | 3.3       | 3.1            | 3.0               | 2.8     | 2.7    | 2.7        | 2.2         | 2.0    | 2.1     | 2.1         | 2.0         | 1.9       | 92  |
| HIPK1         | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 3      | 3       | 4           | 4           | 122       |     |
| PINK1         | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 3       | 4      | 4          | 3           | 4      | 4       | 4           | 4           | 4         | 122 |
| MAPKAPK2      | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 3                 | 4       | 4      | 4          | 4           | 3      | 4       | 4           | 3           | 4         | 121 |
| ARAF          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 3              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 3      | 4          | 3           | 4      | 4       | 4           | 4           | 4         | 120 |
| G11           | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 3      | 3     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 3           | 4      | 4       | 4           | 3           | 4         | 120 |
| NEK9          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 3      | 3       | 2           | 4           | 4         | 120 |
| PDGFRA        | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 3                 | 4       | 4      | 4          | 4           | 4      | 4       | 4           | 3           | 2         | 120 |
| ABL/ABL1      | 3     | 4    | 4      | 4      | 4      | 4        | 4      | 3        | 4       | 4     | 4       | 4      | 4       | 4      | 3              | 4          | 3      | 4     | 4     | 4         | 4              | 3                 | 4       | 4      | 4          | 4           | 4      | 4       | 4           | 4           | 4         | 119 |
| ARG/ABL2      | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 3      | 4       | 4      | 4              | 3          | 4      | 4     | 4     | 3         | 4              | 3                 | 4       | 4      | 3          | 4           | 4      | 4       | 4           | 4           | 4         | 119 |
| BCR           | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 2      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 3                 | 4       | 3      | 3          | 4           | 4      | 4       | 4           | 4           | 4         | 119 |
| DLK/MAP3K12   | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 3      | 2       | 3           | 4           | 3         | 119 |
| PDGFRB        | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 3              | 4          | 3      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 4       | 3           | 3           | 3         | 119 |
| SNRK          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 2       | 3           | 3           | 3         | 119 |
| TAF1          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 3      | 3       | 3           | 3           | 3         | 119 |
| CK1e/CSNK1E   | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 3      | 4       | 4      | 3              | 4          | 3      | 4     | 4     | 4         | 4              | 4                 | 3       | 3      | 4          | 4           | 3      | 4       | 4           | 4           | 4         | 118 |
| MAST4         | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 2     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 4       | 4           | 4           | 0         | 118 |
| YSK1/STK25    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 3          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 1       | 3           | 4           | 3         | 118 |
| CDK9          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 3      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 3       | 3      | 4          | 4           | 3      | 3       | 3           | 3           | 4         | 117 |
| DDR1          | 4     | 4    | 3      | 4      | 4      | 4        | 3      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 3          | 3      | 4     | 3     | 4         | 4              | 4                 | 3       | 4      | 4          | 3           | 4      | 4       | 4           | 4           | 4         | 117 |
| FAK/PTK2      | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 3       | 4      | 4       | 4      | 4              | 4          | 3      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 2          | 4           | 2      | 4       | 4           | 4           | 3         | 117 |
| MNK1/MKNK1    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 3              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 3          | 4           | 4      | 3       | 3           | 2           | 3         | 117 |
| MST3/STK24    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 3          | 4      | 4     | 4     | 3         | 3              | 4                 | 4       | 2      | 3          | 4           | 3      | 4       | 4           | 4           | 4         | 117 |
| PAK3          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 3      | 4     | 4     | 3         | 3              | 4                 | 3       | 3      | 4          | 3           | 3      | 4       | 4           | 4           | 4         | 117 |
| PIM1          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 3     | 4     | 4         | 3              | 3                 | 4       | 3      | 2          | 3           | 4      | 4       | 4           | 4           | 4         | 117 |
| JNK3/MAPK10   | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 3          | 4      | 4     | 1     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 2       | 3           | 4           | 3         | 116 |
| MST2/STK3     | 3     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 3      | 4              | 4          | 3      | 4     | 4     | 3         | 4              | 4                 | 3       | 4      | 3          | 3           | 3      | 4       | 4           | 4           | 4         | 116 |
| NEK7          | 3     | 4    | 4      | 3      | 4      | 4        | 4      | 4        | 4       | 4     | 3       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 3       | 3           | 2           | 3         | 116 |
| PDHK4/PDK4    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 4           | 4      | 2       | 2           | 2           | 2         | 116 |
| CaMKK2        | 4     | 4    | 4      | 3      | 4      | 4        | 4      | 3        | 4       | 4     | 4       | 4      | 4       | 4      | 3              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 3          | 2           | 3      | 4       | 3           | 4           | 3         | 115 |
| Erk5/MAPK7    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 3              | 4          | 4      | 4     | 4     | 4         | 4              | 3                 | 4       | 3      | 4          | 4           | 4      | 3       | 3           | 2           | 2         | 115 |
| GPRK5/GRK5    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 3       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 3     | 4         | 4              | 3                 | 4       | 4      | 2          | 4           | 4      | 3       | 3           | 3           | 3         | 115 |
| MAPKAPK3      | 4     | 4    | 4      | 3      | 4      | 4        | 3      | 3        | 4       | 4     | 4       | 4      | 4       | 3      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 3          | 3           | 3      | 3       | 4           | 3           | 4         | 115 |
| NEK4          | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 3       | 4      | 4              | 4          | 4      | 4     | 4     | 4         | 4              | 4                 | 4       | 4      | 4          | 3           | 4      | 2       | 2           | 2           | 3         | 115 |
| PKD2/PRKD2    | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 3       | 3      | 4              | 4          | 4      | 3     | 4     | 4         | 4              | 4                 | 2       | 3      | 3          | 3           | 3      | 4       | 4           | 4           | 4         | 115 |
| ILK           | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 3       | 4      | 4              | 2          | 4      | 3     | 4     | 4         | 4              | 3                 | 3       | 3      | 3          | 3           | 3      | 4       | 4           | 4           | 4         | 114 |
| MAP2K2/MEK2   | 4     | 4    | 4      | 4      | 4      | 4        | 4      | 4        | 4       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 3      | 3     | 4     | 4         | 4              | 3                 | 4       | 1      | 3          | 2           | 3      | 4       | 4           | 4           | 4         | 114 |
| PKACβ/PRKACB  | 3     | 4    | 4      | 3      | 4      | 4        | 4      | 4        | 4       | 4     | 3       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 3     | 4         | 4              | 4                 | 4       | 4      | 4          | 2           | 2      | 3       | 3           | 4           | 4         | 114 |
| CaMK2γ        | 3     | 4    | 4      | 3      | 4      | 4        | 4      | 2        | 3       | 4     | 4       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 3     | 4         | 4              | 3                 | 4       | 4      | 4          | 3           | 3      | 3       | 4           | 4           | 3         | 113 |
| CK1γ2/CSNK1G2 | 4     | 3    | 4      | 4      | 4      | 4        | 4      | 3        | 4       | 3     | 3       | 4      | 4       | 4      | 4              | 4          | 4      | 4     | 3     | 3         | 4              | 4                 | 4       | 3      | 3          | 4           | 3      | 4       | 3           | 4           | 3         | 113 |
| ERK1/MAPK3    | 4     | 4    | 4      | 4      | 4      | 3        | 4      | 3        | 4       | 4     | 4       | 4      | 4       | 3      | 4              | 3          | 3      | 4     | 4     | 4         | 3              | 3                 | 3       | 3      | 2          | 4           | 4      | 4       | 4           | 4           | 4         | 113 |



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# Protein Kinase Gene Expression

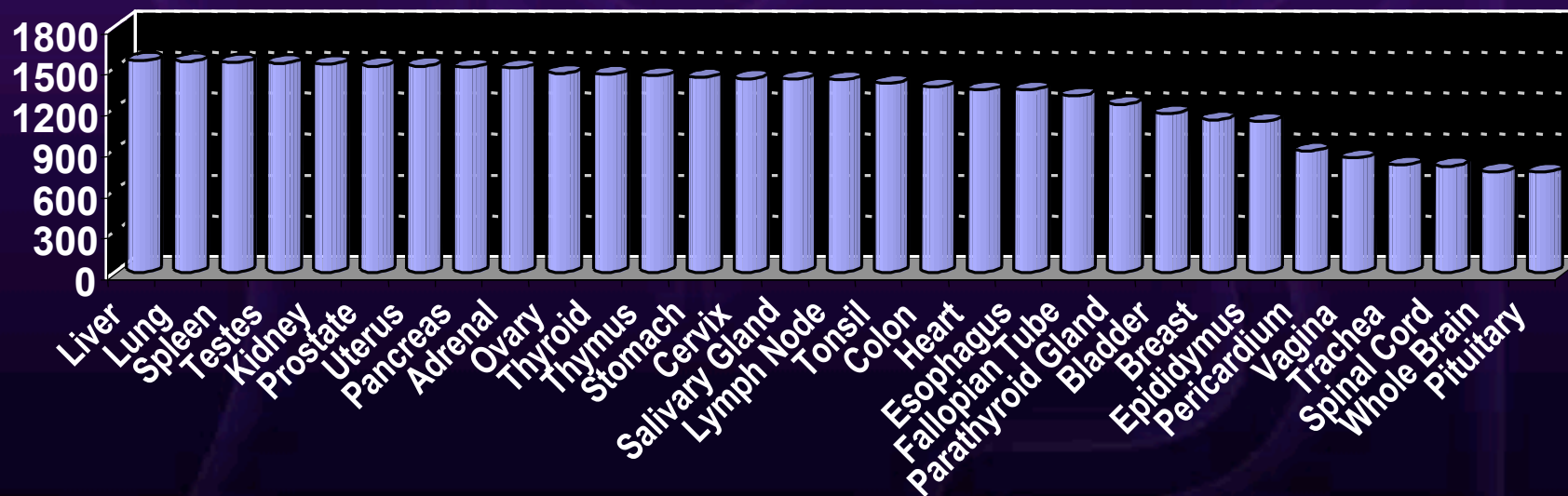
| Expression Score | Kinase Number | Kinase Percent |
|------------------|---------------|----------------|
| 100 to 122       | 161           | 35             |
| 80 to 99         | 161           | 35             |
| 60 to 79         | 73            | 16             |
| 40 to 59         | 38            | 8              |
| 20 to 39         | 13            | 2.8            |
| 0 to 19          | 16            | 3.5            |
| <b>Total</b>     | <b>462</b>    |                |

Ubiquitous



Tissue Specific

Relative Total Kinase Expression

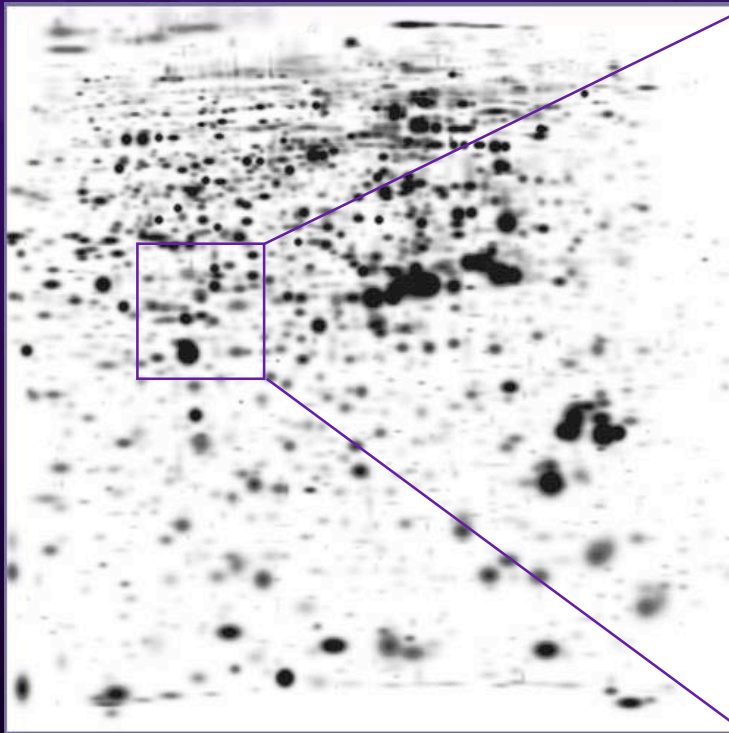


KINEXUS

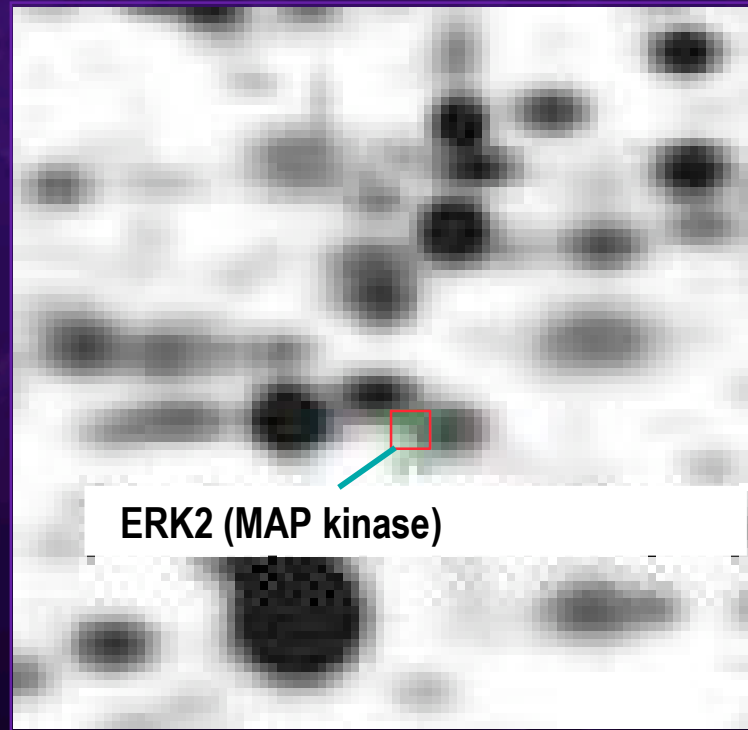
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# 2D Gel Electrophoresis

## Human keratinocyte proteome analysis



*Silver-stained*



ERK2 (MAP kinase)

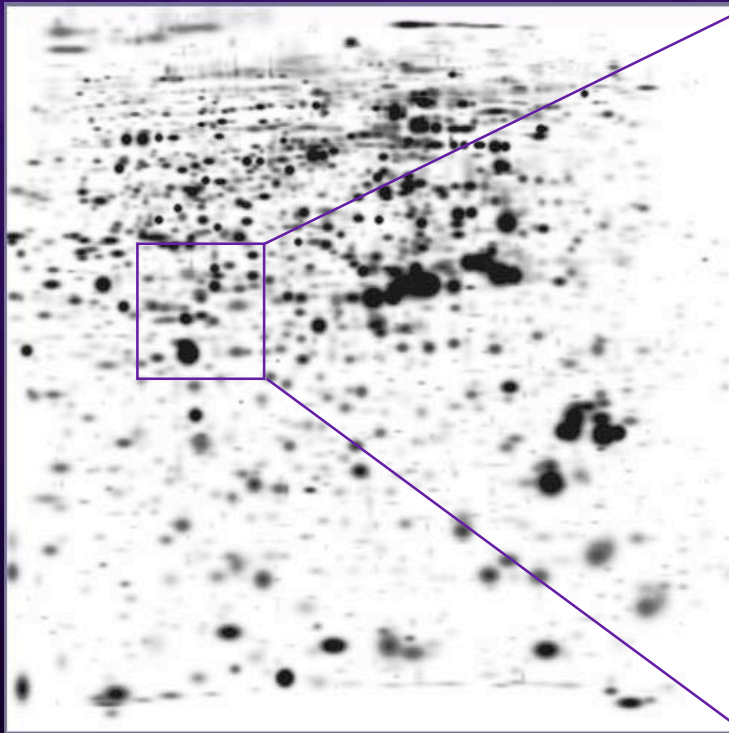


KINEXUS

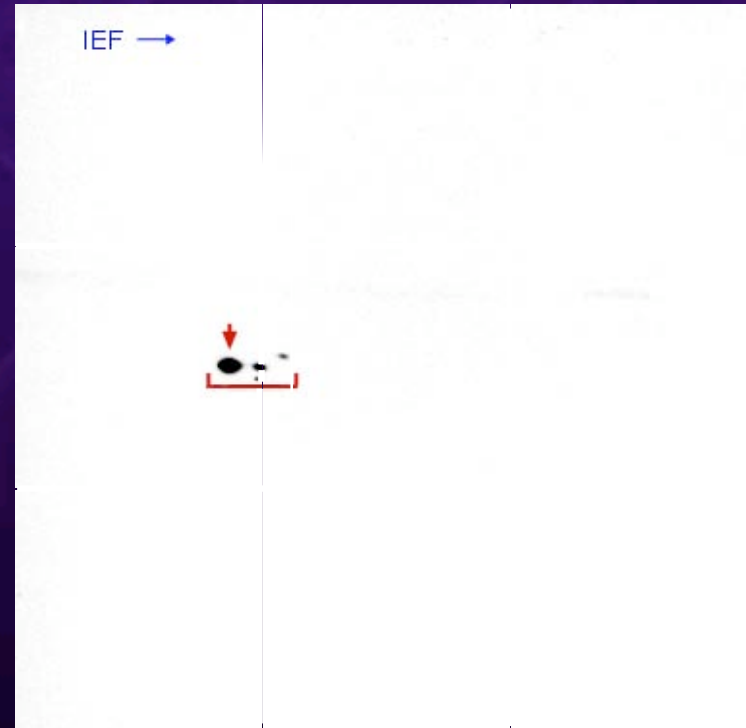
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## 2D Gel Electrophoresis

- Protein kinases are low abundance and usually not detectable by silver staining



*Silver-stained*



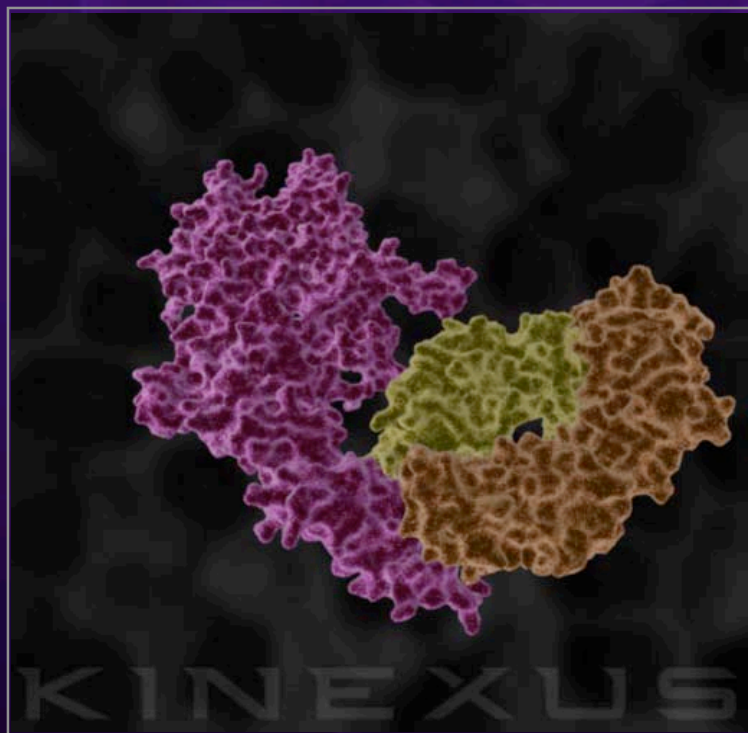
*Immunoblot with ERK1/2 Ab*



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# Kinexus Immunoblotting Analyses



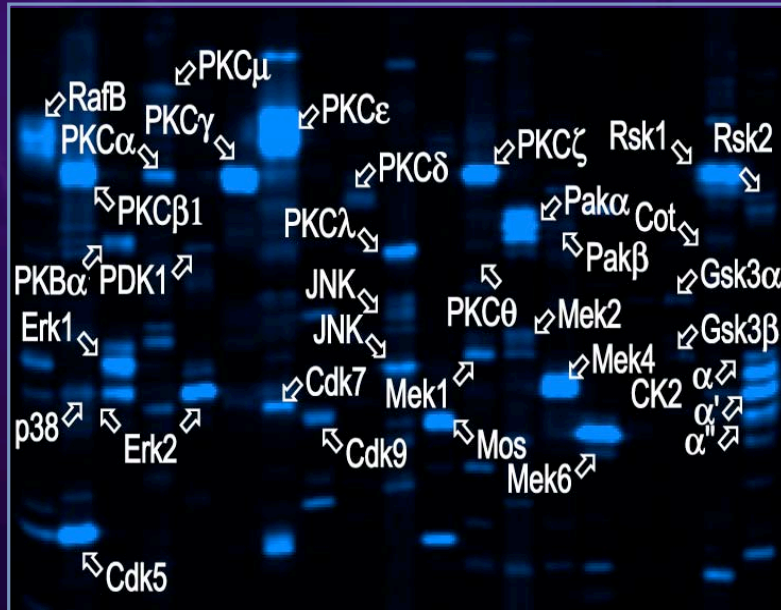
- Kinetworks™ proprietary analysis uses over 650 of the world's best antibodies
  - Targets low abundance cell signalling proteins
  - Quantifies protein expression and phosphorylation
  - More accurate and quantitative than competing methods



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# Kinetworks™ Multi-Immunoblotting



*Mouse Brain KPKS-1.2  
Protein Kinase Screen Gel A*

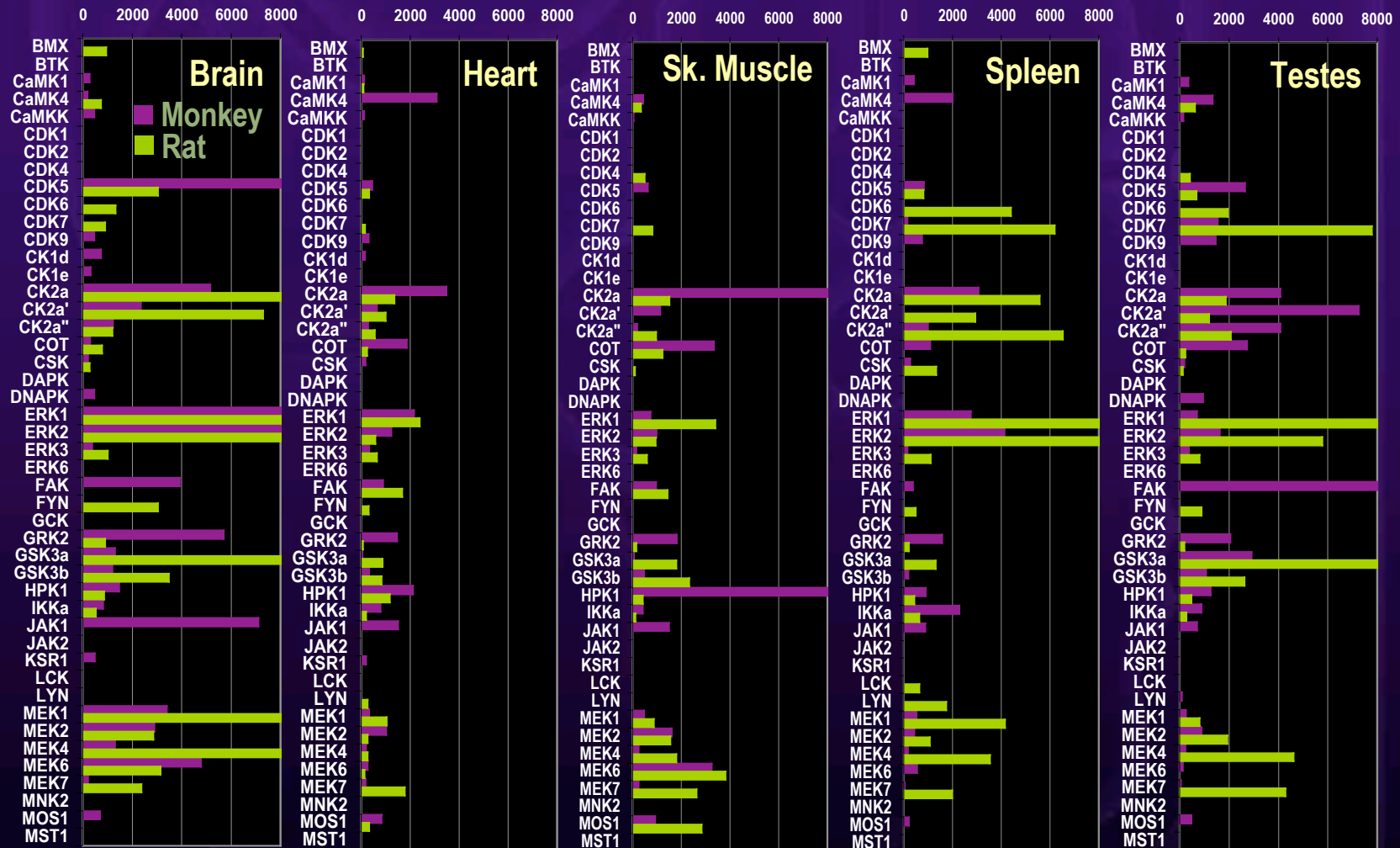
- **Accurate** - based on immunoreactivity and M.W.
- **Quantitative** - ECL signals detected over 2000-fold range
- **Sensitive** - ~350 µg cell lysate protein needed
- **Reproducible** - ±10-20% S.D.
- **Broad utility** - works in human, rat, mouse, etc.



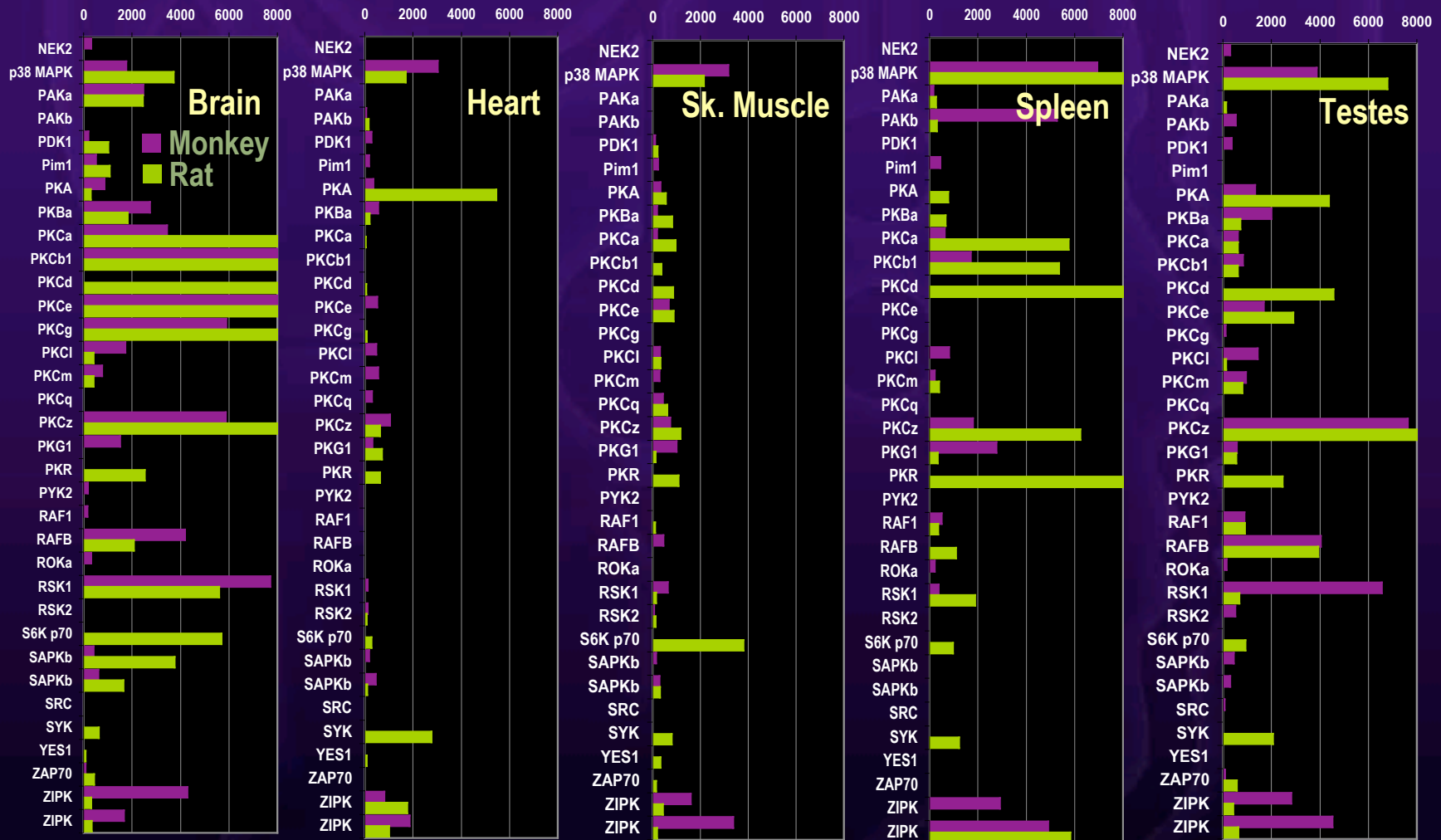
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# Tissue Differences in Protein Kinase Expression



# Tissue Differences in Protein Kinase Expression

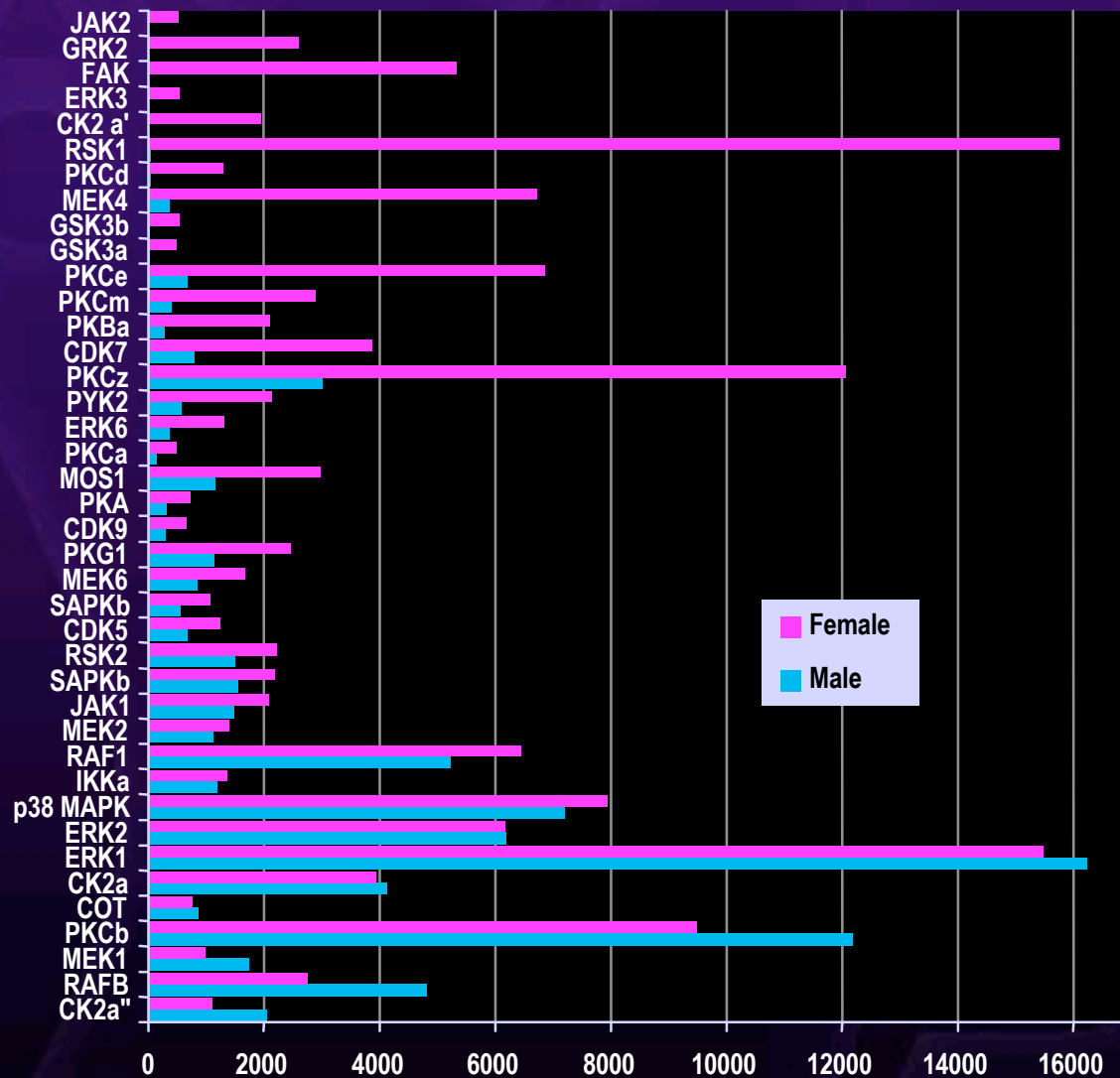


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# Gender Differences in Protein Kinase Expression

Mouse  
Submandibular  
Gland

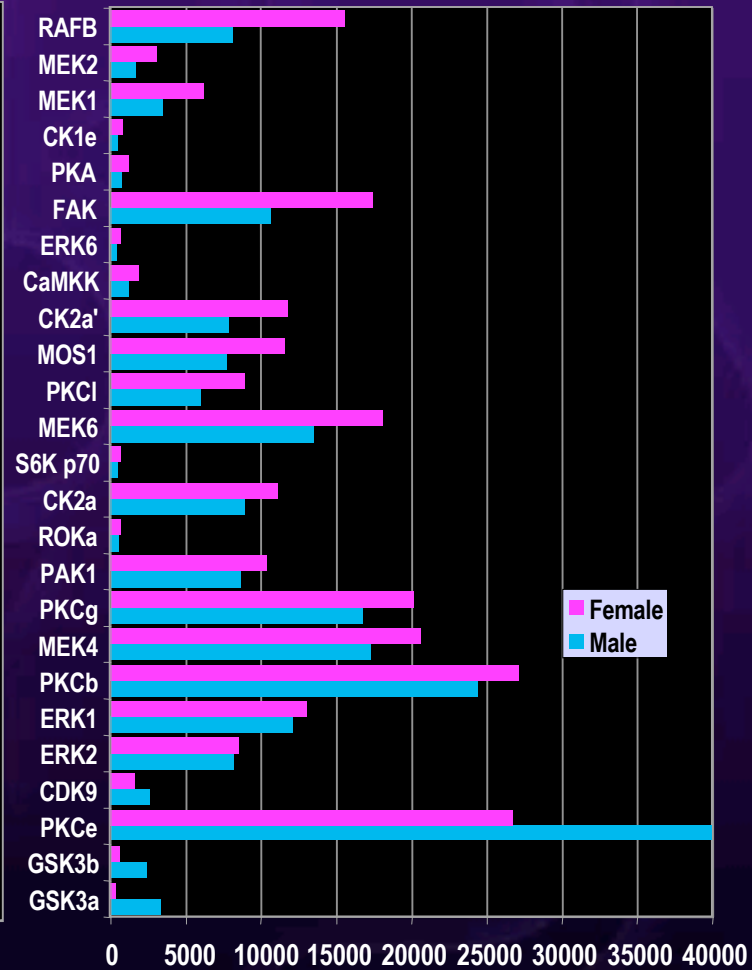
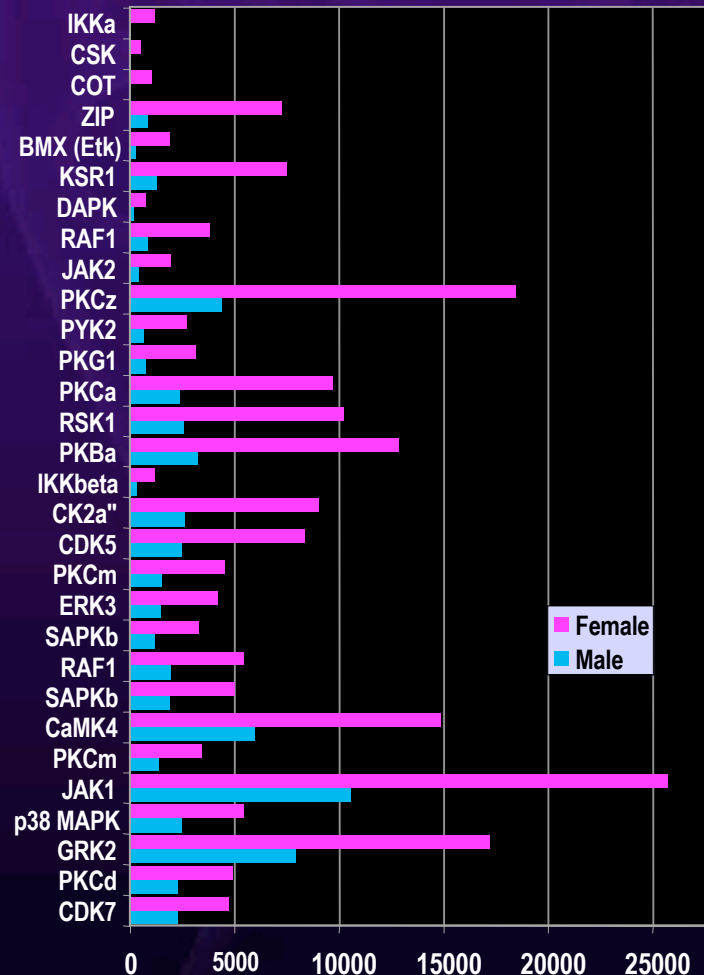


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# Gender Differences in Protein Kinase Expression

Mouse  
Brain



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# True Functional Proteomics

~30,000

Genes



mRNAs



INACTIVE

Proteins



ACTIVE

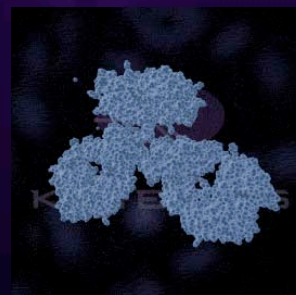
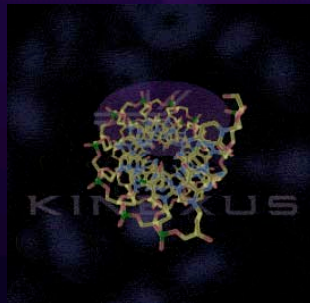
Phospho-  
Proteins

1,000,000  
Phospho-sites



PHENOTYPE

- Disease status correlates best with phosphoproteins
- Phosphoproteins will provide the richest diversity of biomarkers



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# Phospho-Site Multi-Immunoblot

## Jurkat T Cells -Staurosporin Treated

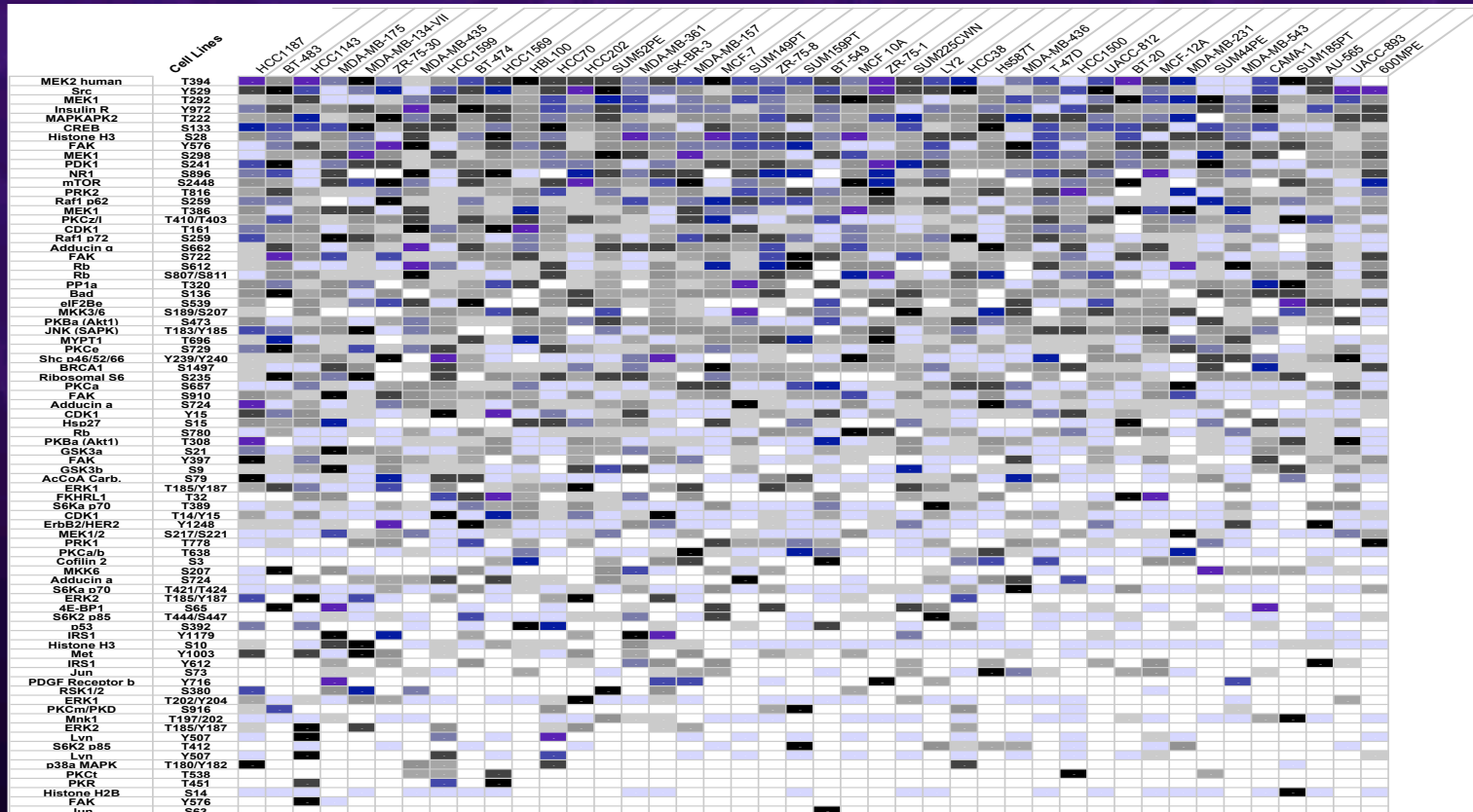


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# Phospho-Site Multi-Immunoblot

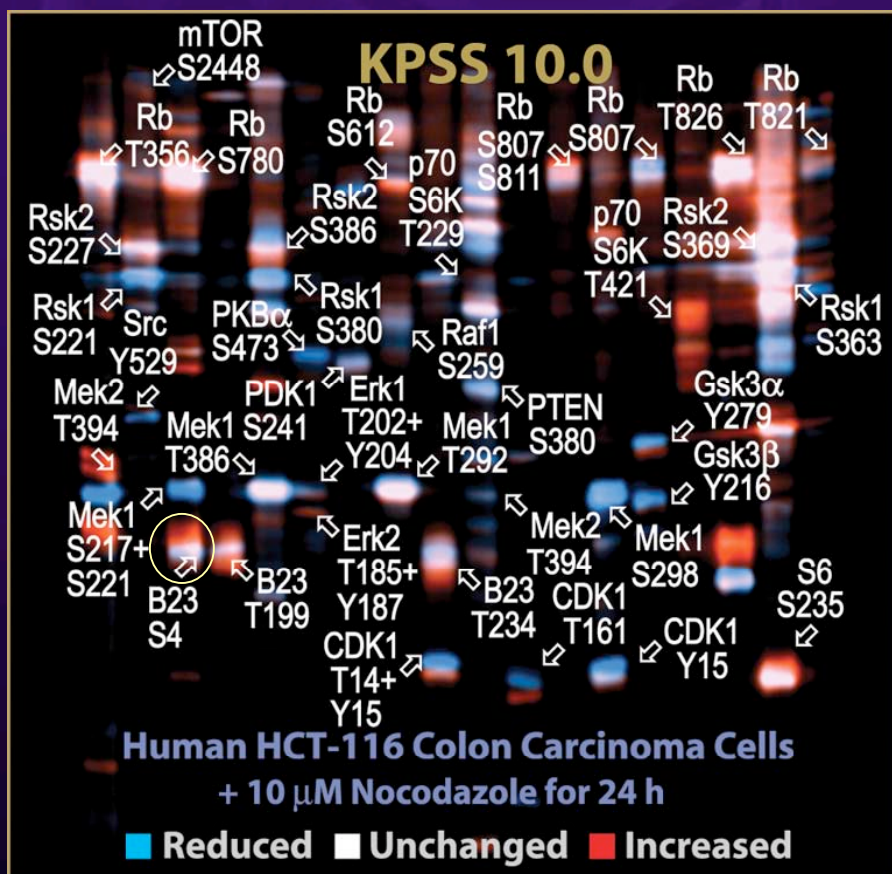
## Human breast tumour cell lines



No two human breast tumour cell lines have similar patterns of phosphorylation.

# Novel Phosphorylation Site Detection

## Drug target discovery



- Detected increased phosphorylation of unknown protein in human colon carcinoma HCT-116 tumour cells in response to a cancer drug with a phospho-site antibody
- Purified and identified protein by mass spectrometry as B23 (also known as nucleophosmin)

Zhang et al. (2004) *J. Biol. Chem.* 279, 35726

# Novel Phosphorylation Site Detection

## Drug target discovery

↙ Phospho-site

Mek1/2: <sup>213</sup>QLID**SM**ANSFVGTRSYMSPERLQ<sup>235</sup>

B23 WT: <sup>1</sup>MED**SM**DMDMSPLRPQNYLFGCE<sup>22</sup>

B23 S4A: <sup>1</sup>---**A**-----<sup>22</sup>

B23 S4E: <sup>1</sup>---**E**-----<sup>22</sup>

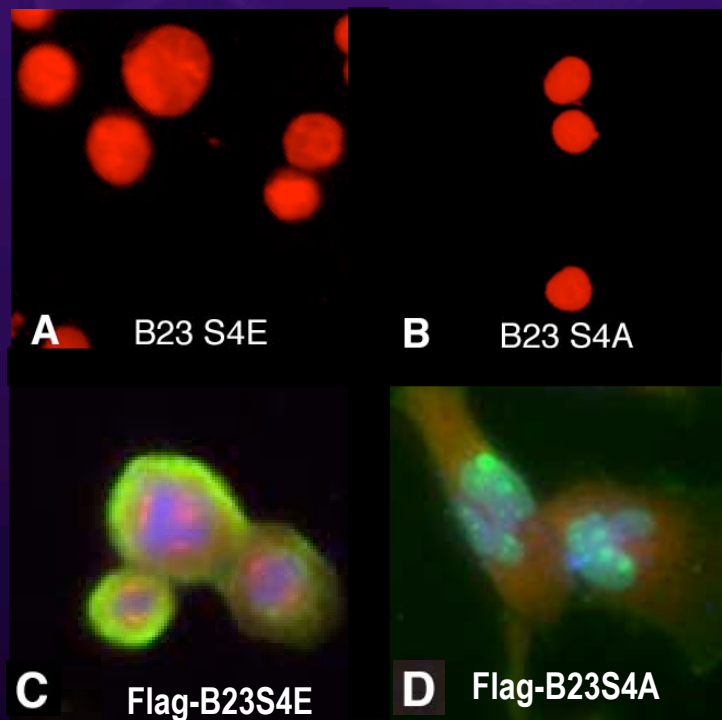
- Phosphorylation of B23 at S4 is directly catalyzed by polo-like kinase 1 in nocodazole-treated HeLa cells via cyclin-dependent kinase 1 activation

*Zhang et al. (2004) J. Biol. Chem. 279, 35726*

# Novel Phosphorylation Site Detection

## Drug target discovery

DAPI=Red



Flag Tag=Green; Tubulin=Red; DAPI=Blue

- Transfected HeLa cervical cancer cells with mutated B23 at phosphorylation site
- Observed increased nuclei size and increased centrosome number

Zhang et al. (2004) *J. Biol. Chem.* 279, 35726

# Gene & Protein Microarrays

## MARKET - 2006

### GENE MICROARRAYS

~US\$ 725 million global;  
6.7% annual growth

### ANTIBODY MICROARRAYS

<US\$ 5 million global;  
>100% annual growth

## PROBES

High fidelity oligo-nucleotides probes are fast & cheap to produce

High fidelity antibody probes are slow & expensive to produce

## PRINTED CHIPS

~81% are commercially produced; Affymetrix has 75% of this market

<50% are commercially produced; Clontech & Sigma are main suppliers

## FACILITIES

Expensive scanners needed to read chips

Expensive scanners needed to read chips

## DATA ANALYSIS

Primarily used for pattern recognition;  
Poor correlation to phenotype; Quantitation software required

Can track protein levels & activity; Better correlation to phenotype and prediction; Quantitation software required

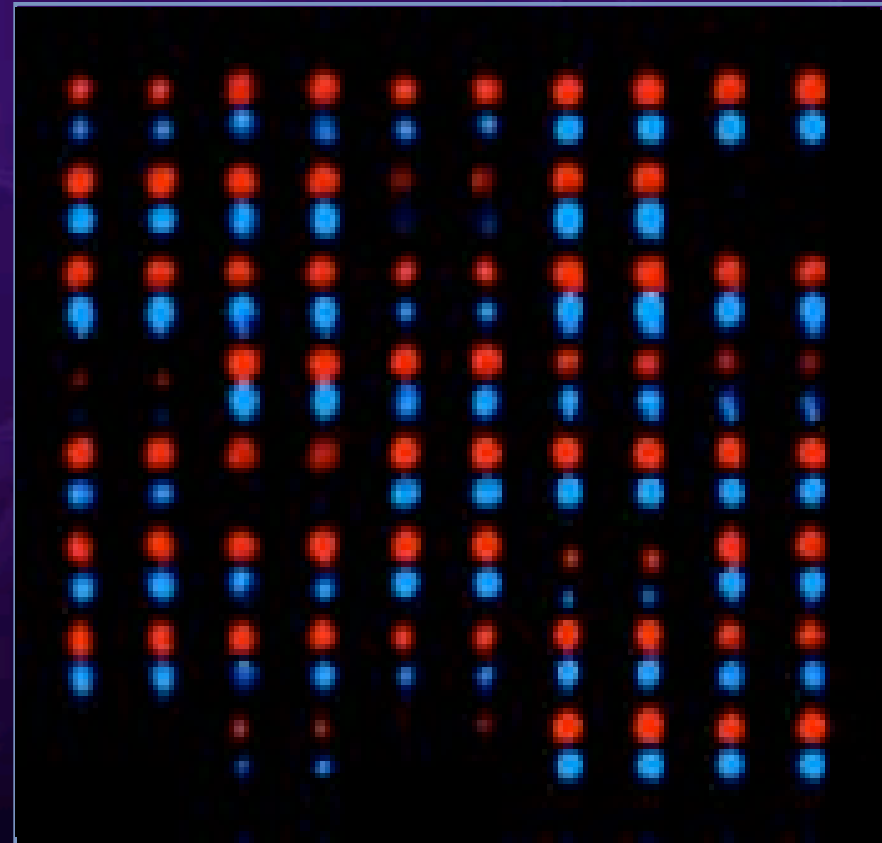


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# Kinex™ Antibody Microarrays

- 650 different antibodies from Kineteworks™ immunoblot service arrayed on a plastic slide
- Two samples analyzed per slide in duplicate
- Kinex™ cost of \$1.15 per protein compared to \$10 - \$36 per protein with Kineteworks™



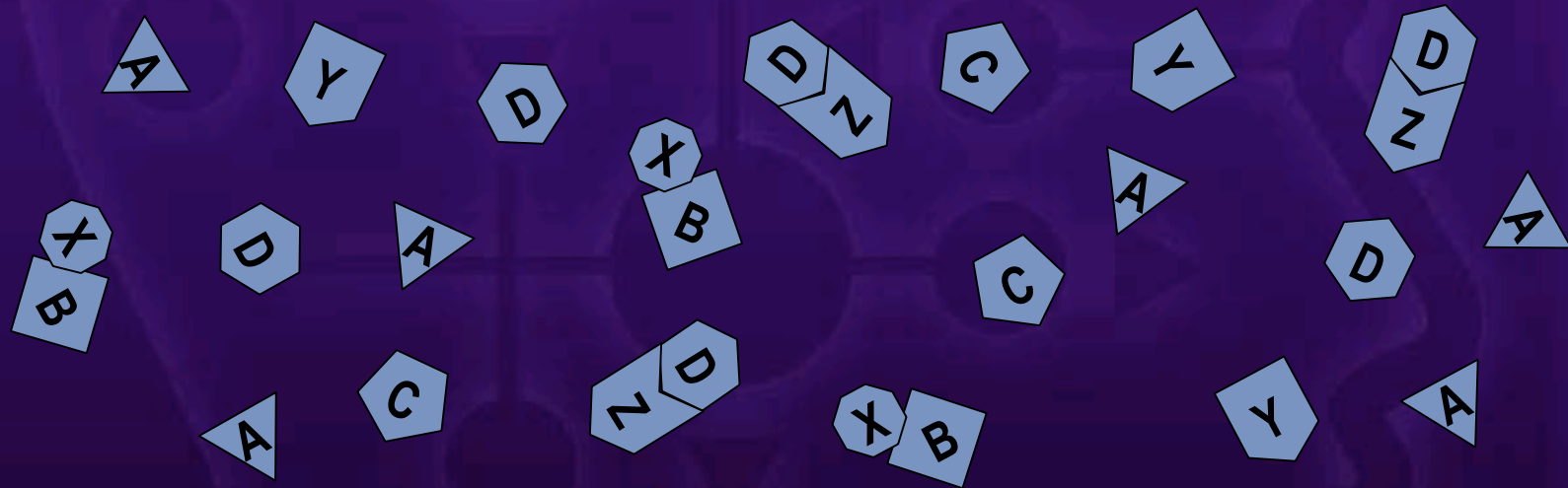
*Close Up of 1 of 16 Grids on a Kinex™ Microarray*



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# Kinex™ Antibody Microarrays



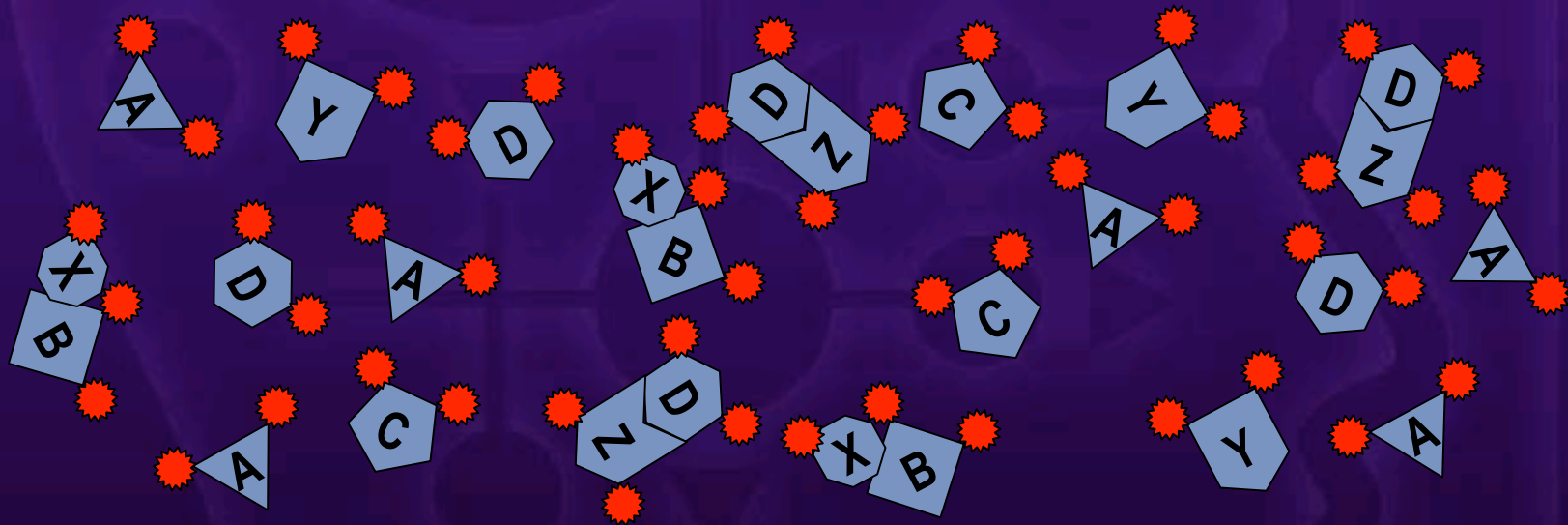
- Start with cell lysate with thousands of different proteins



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# Kinex™ Antibody Microarrays



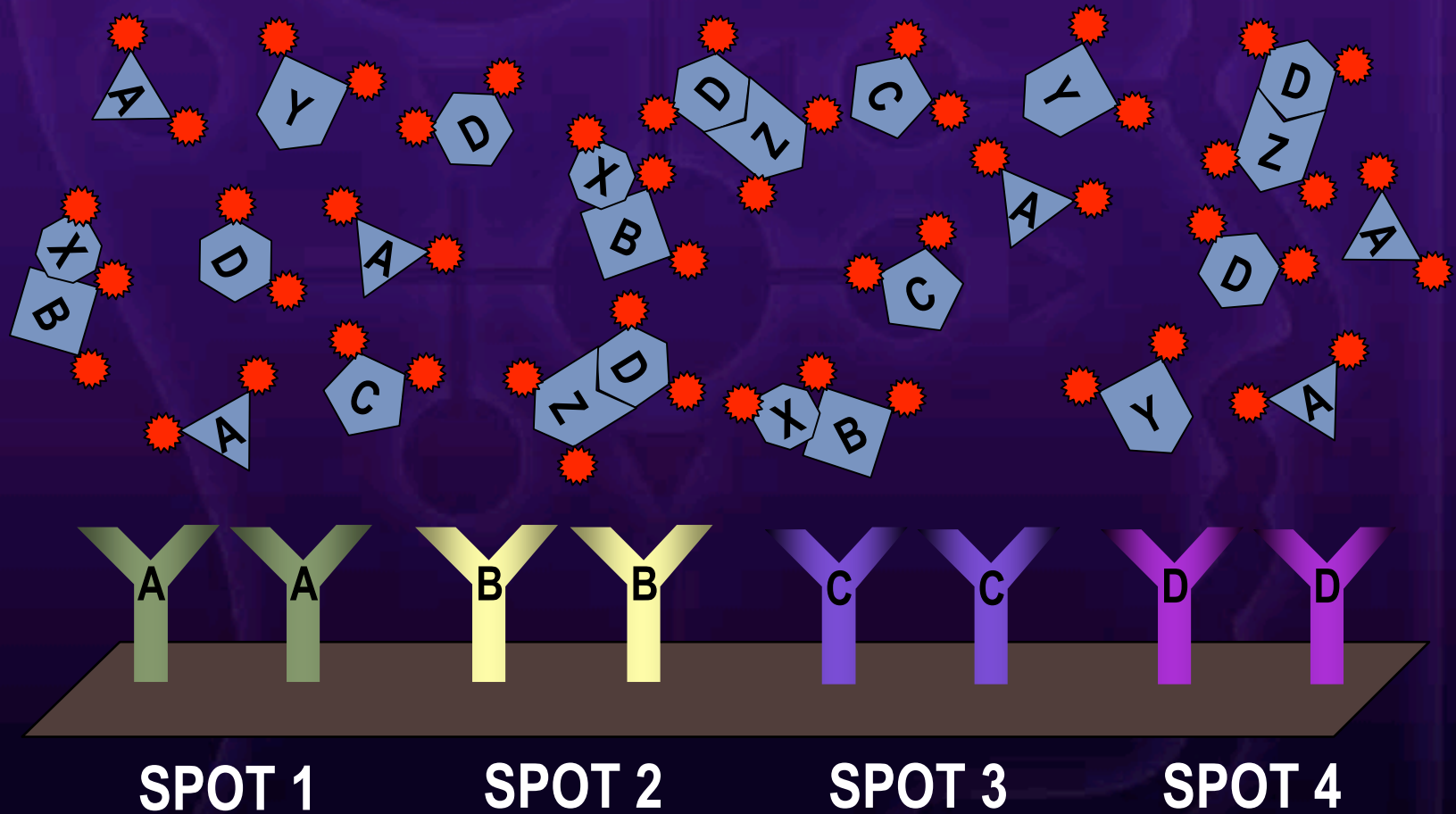
- Label cell lysate proteins with fluorescent dye



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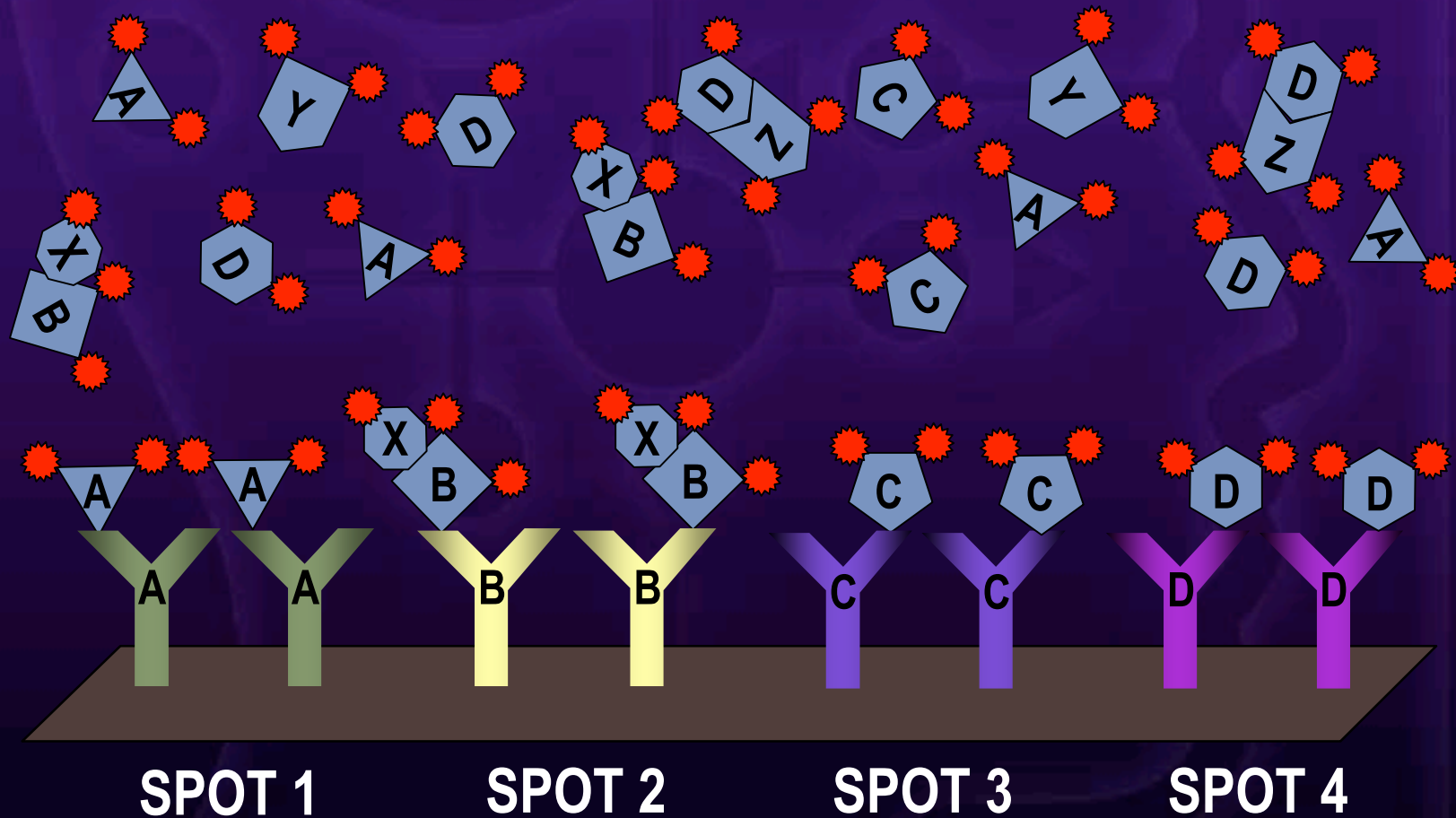
# Kinex™ Antibody Microarrays



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# Kinex™ Antibody Microarrays

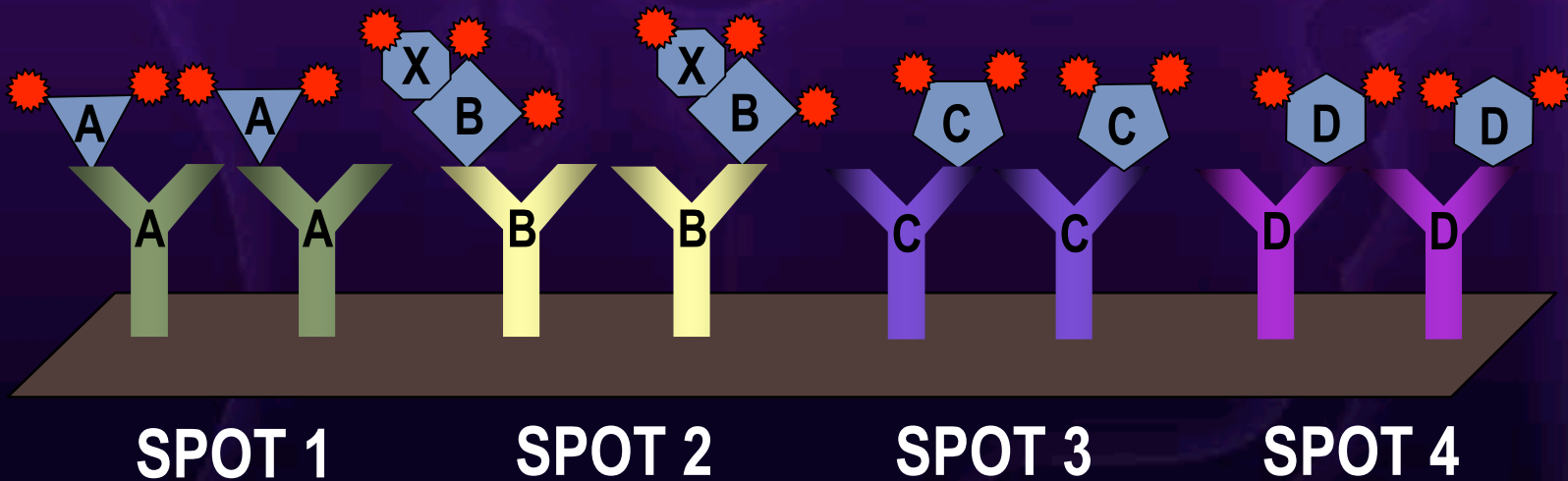


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# Kinex™ Antibody Microarrays

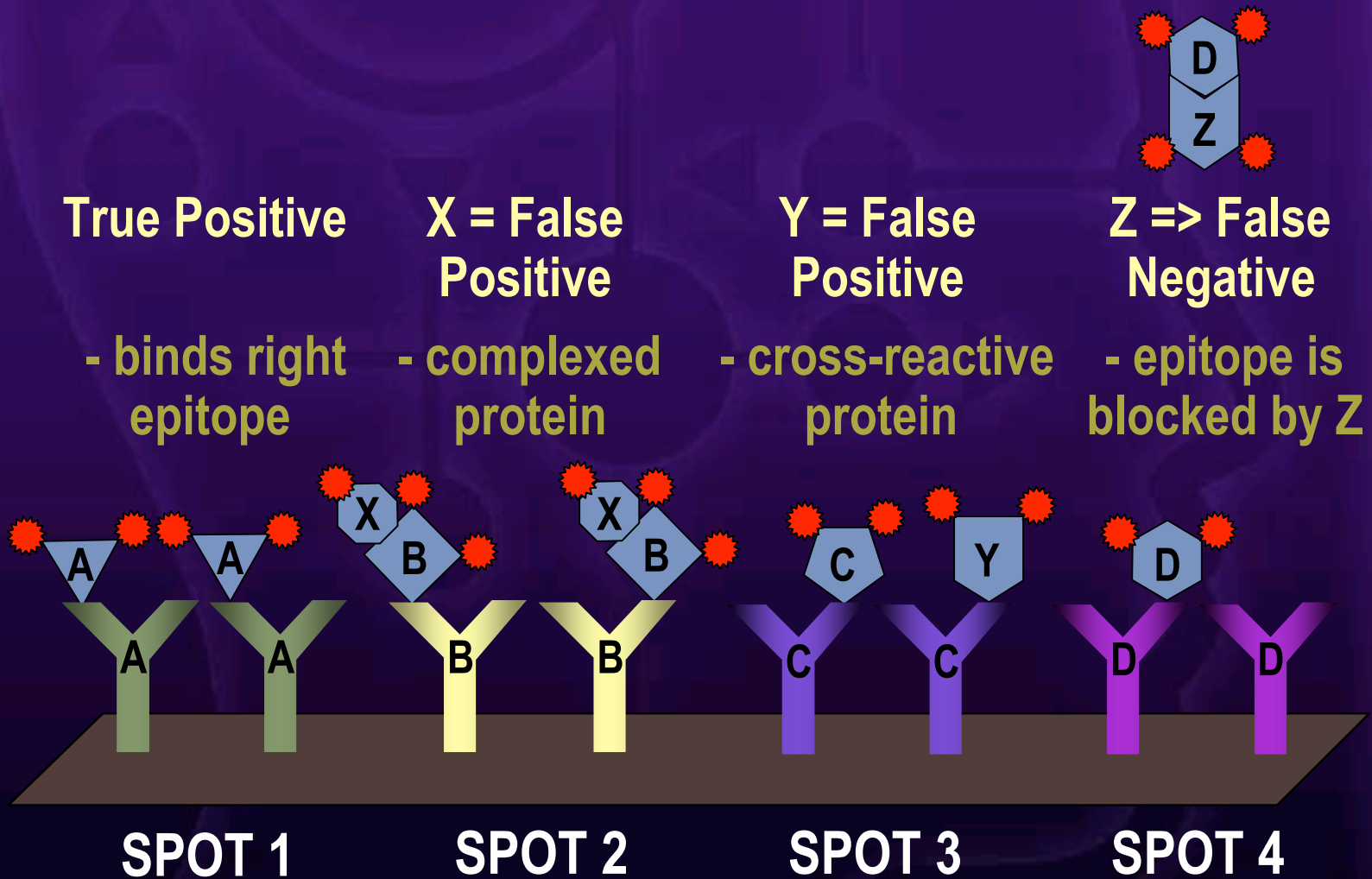
- Wash away unbound dye-labeled lysate proteins and quantify captured proteins



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# Kinex™ Antibody Microarrays



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# Kinex™ Antibody Microarrays

## Spot Accuracy

Analysis of A431 cells  
treated with EGF for 5 min

### Discovery

Antibodies tested = 603

Pan Ab increases = 3%

Pan Ab decreases = 5%

P.S. Ab increases = 3%

P.S. Ab decreases = 1%

≥ 25% CFC

### Immunoblot Validation

Phospho-site antibodies tested = 154

Positive correlations = 41%

Negative correlations = 42%

No detection by immunoblotting = 17%

True positives on array = 44%

False positives on array = 41%

False negatives on array = 85%

True negatives on array = 80%



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# Protein Kinase Dimerization

## Group

## Examples of Dimerized Human Protein Kinases

|                 |                                                                                 |
|-----------------|---------------------------------------------------------------------------------|
| <b>AGC</b>      | DMPK1, GRPK6, MSK1+2, PKA, PKB, PKG, ROCK2, RSK1-4,                             |
| <b>ATYPICAL</b> | ATM, ATR, DNAPK                                                                 |
| <b>CAMK</b>     | CHK2, DAPK1-3, PHK $\gamma$ , smMLCK                                            |
| <b>CK1</b>      | Not reported                                                                    |
| <b>CMGC</b>     | ERK1, ERK2, ERK3, JNK, p38                                                      |
| <b>OTHER</b>    | CK2, GCN2, HRI, IKK $\alpha+\beta$ , IRAK, IRE1+2, NEK2, PEK, PKR, Plk4, SgK069 |
| <b>RGC</b>      | ANPa+b, CYGD+F, HSER                                                            |
| <b>STE</b>      | ASK1, MEKK2+4, MST1+2, PAK1, SLK, TAO1-3,                                       |
| <b>TK</b>       | All 58 receptor-tyrosine kinases, JAK1-3, Tyk, Abl, FAK, Bmx, Btk               |
| <b>TKL</b>      | All 12 receptor-serine kinases, DLK, Ksr1, LZK, MLK3, Raf1, RafA, RafB          |
| <b>Total</b>    | <b>Over 145 reported human protein kinases</b>                                  |

- Most protein kinases may be dimerized.
- Notable exceptions appear to be the Src, PKC and CDK families.



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# Functional Proteomics Database

Internet-Accessible at [www.kinexus.ca/kinet](http://www.kinexus.ca/kinet)

**KINET**™

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**KINEXUS BIOINFORMATICS**

Search by

**PROTEIN TARGET**

**PROTEIN COMPARISON**

**TREATMENT**

**MODEL SYSTEM**

**KINETWORKS™ SCREEN**

**ORDER NUMBER**

**PhosphoNET**

**Change Password**

Welcome to your KiNET workspace, Bob Smith

Search data, perform analysis, and generate reports using the navigation buttons.

**KINET Statistics**

|                                 |        |
|---------------------------------|--------|
| Number of Protein Measurements: | 207413 |
| Number of Protein Bands:        | 1674   |
| Number of Treatments:           | 2099   |
| Number of Immunoblots:          | 6306   |
| Number of Samples:              | 5765   |
| Number of Orders:               | 886    |

# Functional Proteomics Database

Internet-Accessible at [www.kinexus.ca/kinet](http://www.kinexus.ca/kinet)



## SCREEN

### ORDER

### SAMPLE

### MEASURED PROTEINS

PROTEIN PROFILE

SPOT COMPARISON

PROTEIN DETAILS

### TREATMENT

### Band Search

Screen

Band #1

Band #2

SEARCH

### Sample Filter Search

Organ

Control

Sex

Treatment

Species

Tissue Source

Tissue

Disease

Number of Results:

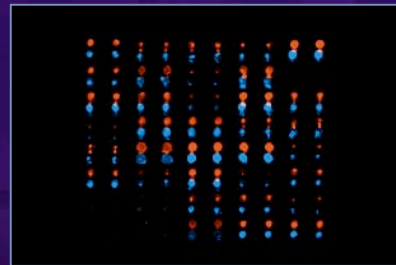
| Name                     | Band #1 PCFC | Band #2 PCFC | Organ    | Sex  | Species | Disease | Tissue source       | Tissue     | Treatment                              | Co  |
|--------------------------|--------------|--------------|----------|------|---------|---------|---------------------|------------|----------------------------------------|-----|
| <a href="#">2-vec-tb</a> | (2.54%)      | (53.01%)     | prostate | Male | human   |         | prostate epithelial | epithelial | t-butyl hydroperoxide 150 uM for 6 hrs | FAI |
| <a href="#">1-vec-un</a> | 0.00%        | 0.00%        | prostate | Male | human   |         | prostate epithelial | epithelial | -- for --                              | TRI |
| <a href="#">3-p75-un</a> | 36.59%       | 2.41%        | prostate | Male | human   |         | prostate epithelial | epithelial | -- for --                              | FAI |
| <a href="#">4-p75-tb</a> | (39.22%)     | (67.47%)     | prostate | Male | human   |         | prostate epithelial | epithelial | t-butyl hydroperoxide                  | FAI |

# Discovery Engine for Biomarker and Drug Target Discovery

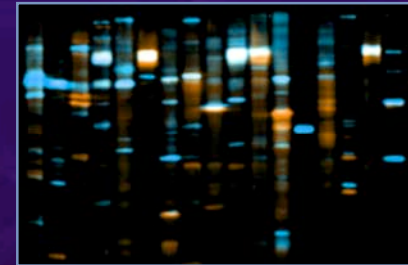
Drug/Cell  
Service



Kinex™ Ab  
Microarrays



Kinetworks™  
Immunoblots

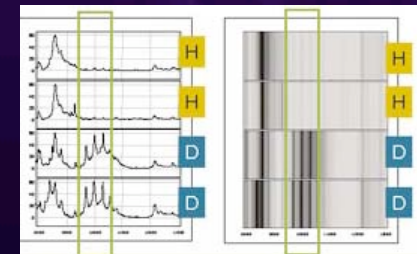


Dx

Patient  
Profiling



KiNET Internet  
Database



Mass  
Spectrometry



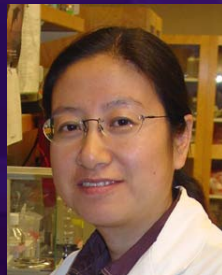
KINEXUS

UBC-VCH Brain Research Centre

## Credits



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