

The Influence of Warm Ischemic Time on Gene Expression Profiles of Colorectal Carcinoma

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Analytical factors may affect downstream analysis

Pre-acquisition variables

1. Anesthetic administration and time
2. Surgical procedure and time
3. Blood transfusions
4. Blood clamping time

Post-acquisition variables

1. Tissue handling
2. Preservation
3. Storage conditions

Identification of molecules and molecular patterns for biospecimen integrity

- Determine general impact of surgically manipulated blood flow on gene expression
- Identify molecules and molecular patterns affected by intraoperative ischemia
- Can we use these findings to better the contribution of ischemia to molecular profiles?
- Can we use these markers to identify tissue handling artifacts?

The effects of intraoperative ischemia time on colon cancer gene expression

Seven time points: 4 tumors and 4 adjacent normal

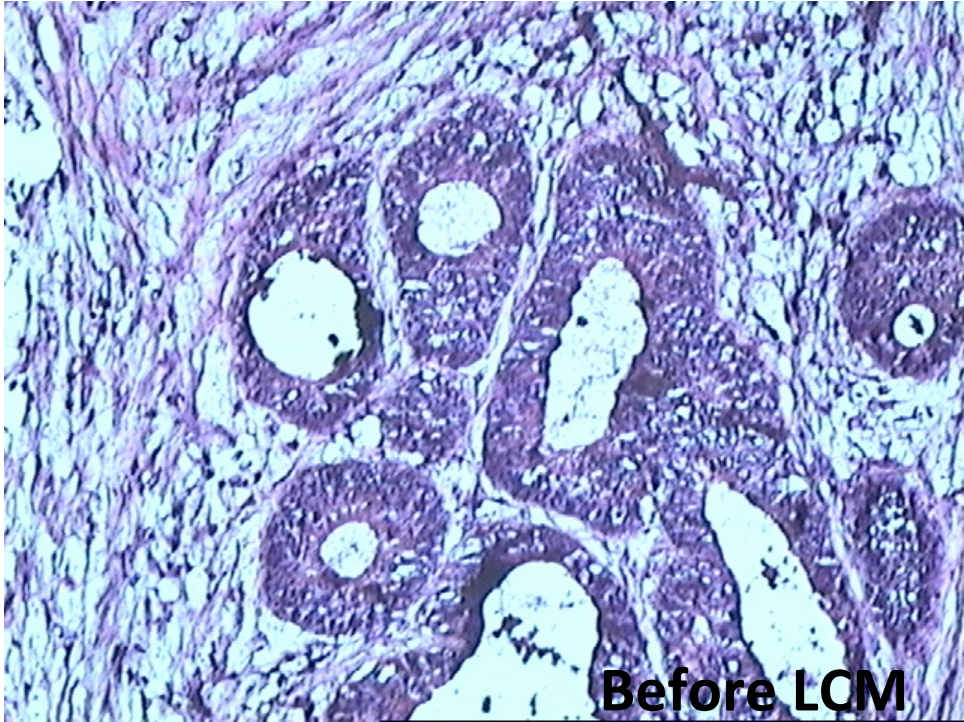
Time Points

18-20	23-25	28-30	33-35	38-40	43-45	48-50
A114 T	A156 T	A161 T	A251 T	A11 T	A24 T	A387 T
A114 N	A156 N	A161 N	A251 N	A11 N	A24 N	A387 N
A243 T	A238 T	A182 T	B128 T	A197 T	A201 T	B153 T
A243 N	A238 N	A182 N	B128 N	A197 N	A201 N	B153 N
A548 T	A511 T	A725 T	B329 T	A423 T	A304 T	B222 T
A548 N	A511 N	A725 N	B329 N	A423 N	A304 N	B222 N
A622 T	A165 T	A101 T	A76 T	A151 T	A546 T	A61 T
A622 N	A165 N	A101 N	A76 N	A151 N	A546 N	A61 N

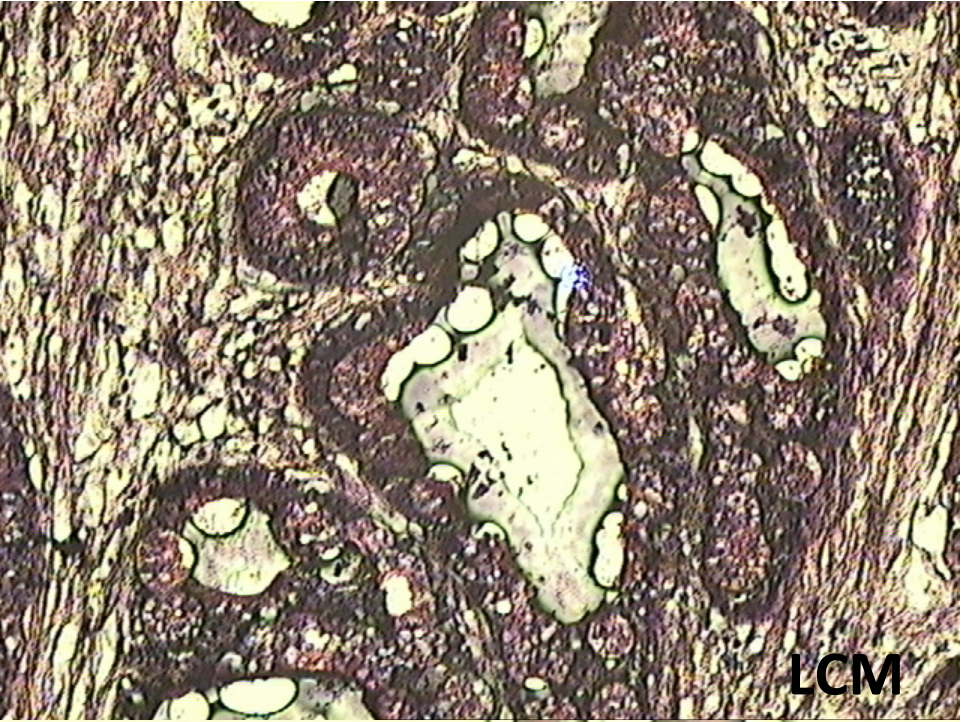
The effects of intraoperative ischemia time on colon cancer gene expression

Experimental Design:

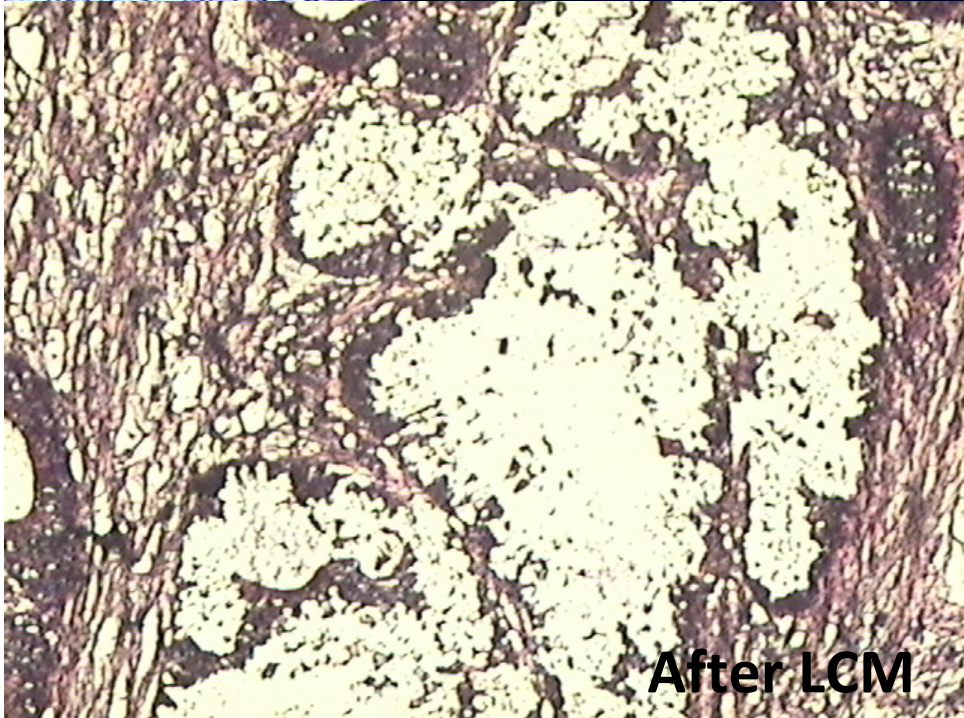
- Laser Capture Microdissection (LCM)
- Variables such as storage conditions, staining and LCM time, are controlled and annotated
- Quality control for RNA integrity
- Technical duplicates on GeneChip HU133A Plus 2.0
- Two normalization methods



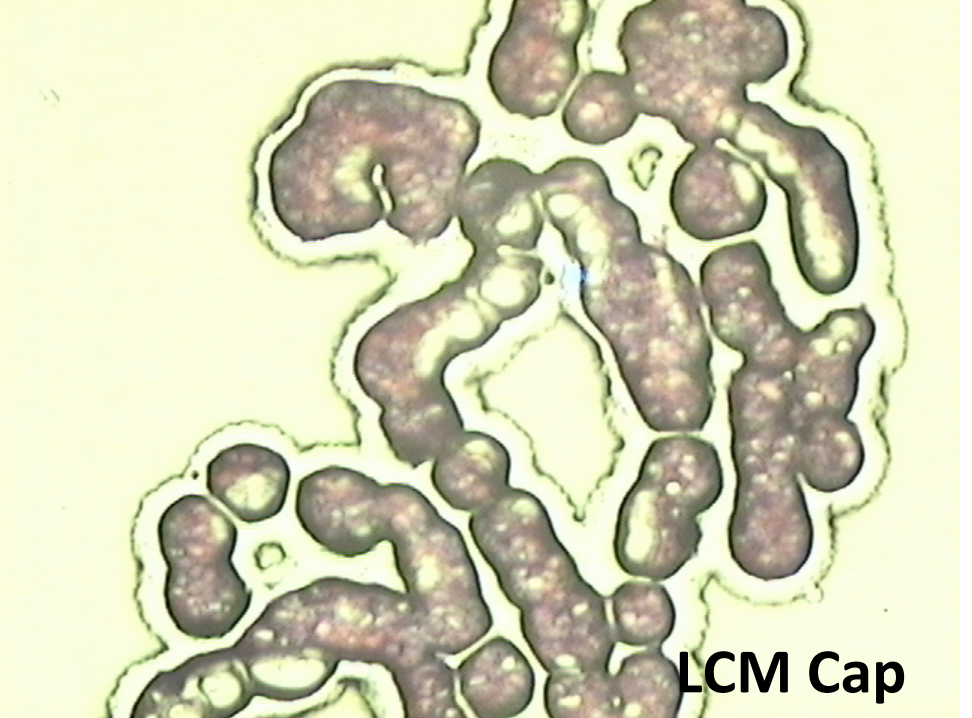
Before LCM



LCM

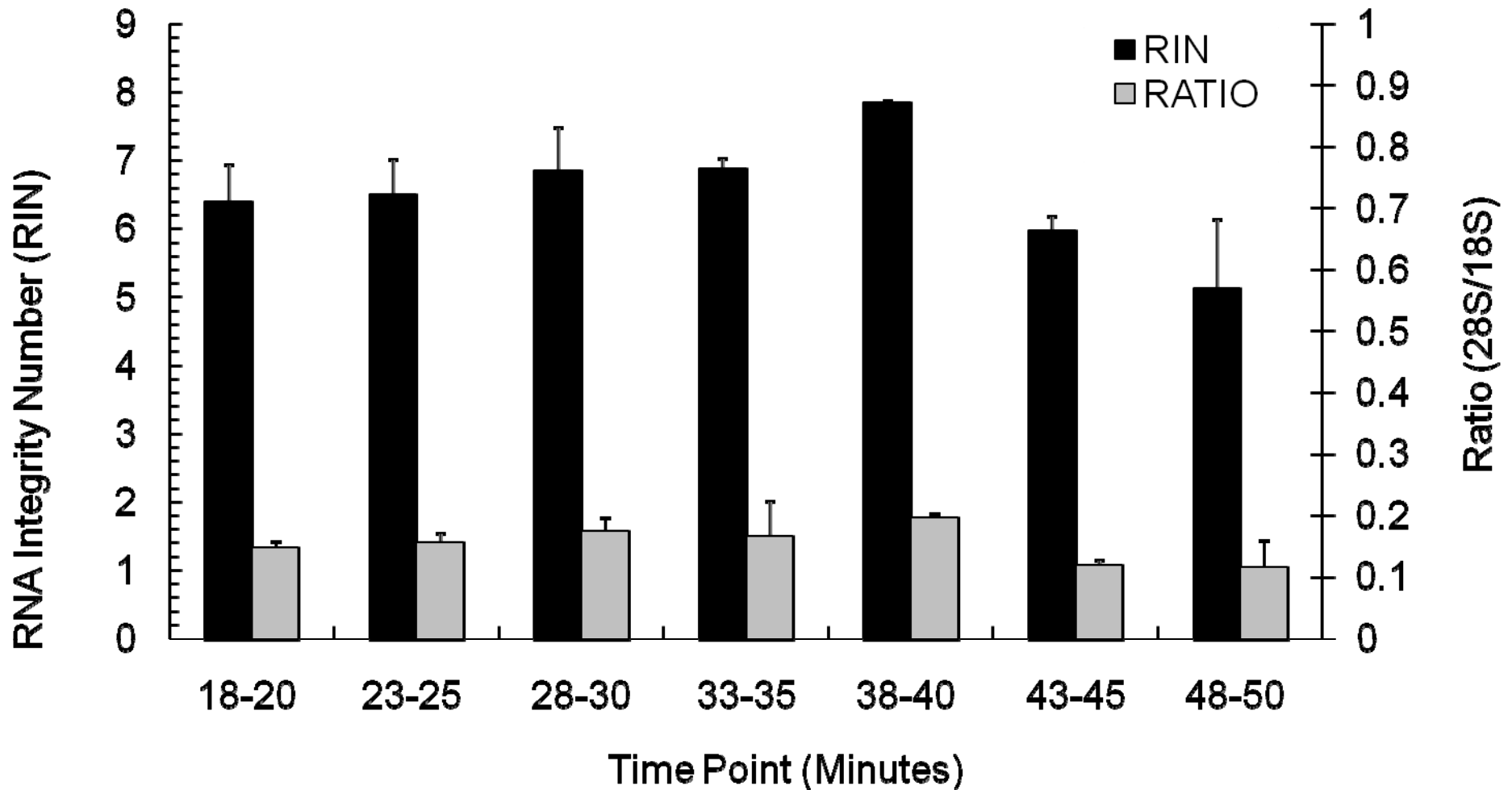


After LCM

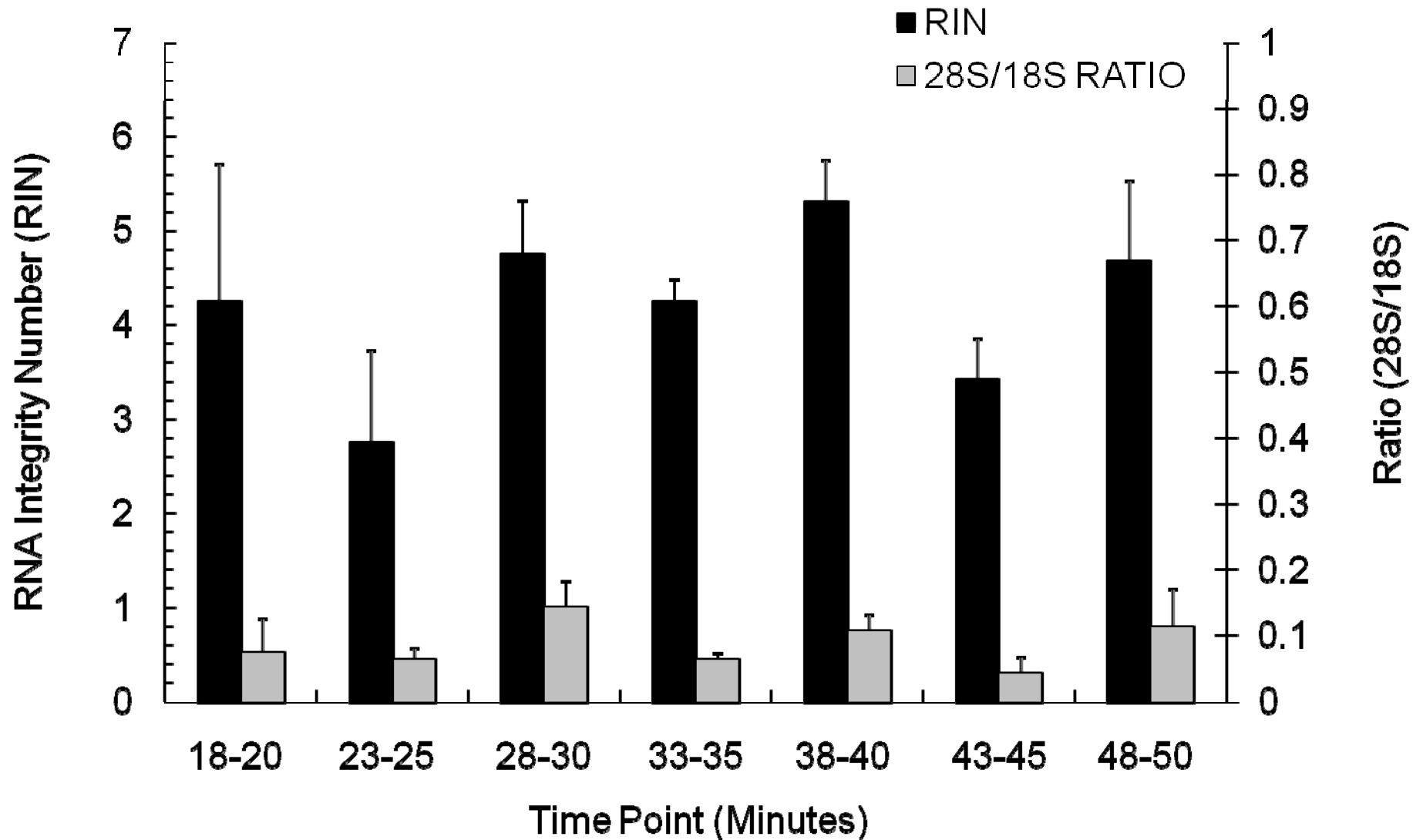


LCM Cap

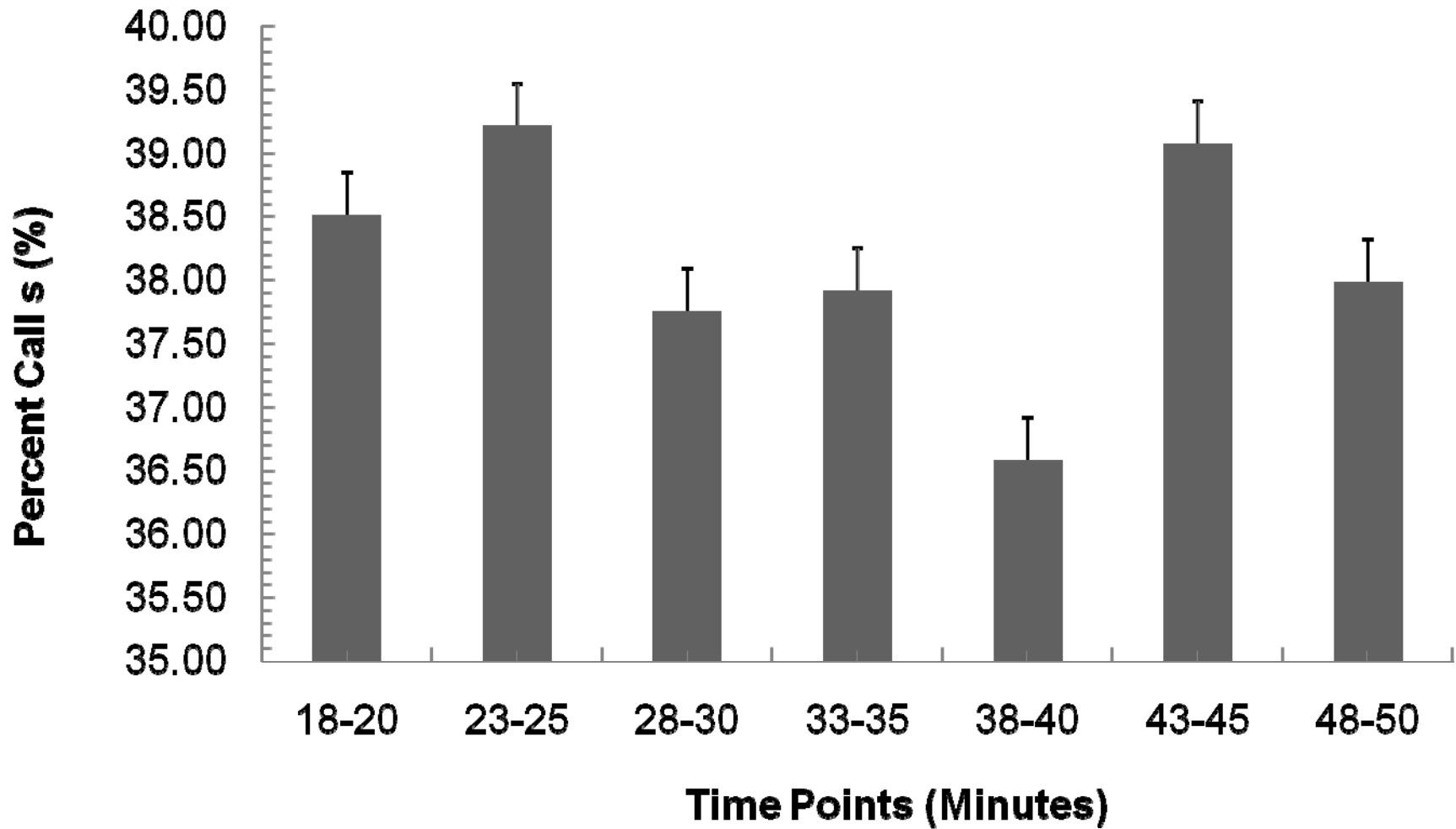
Laser microdissected samples of tumor show high quality RNA integrity



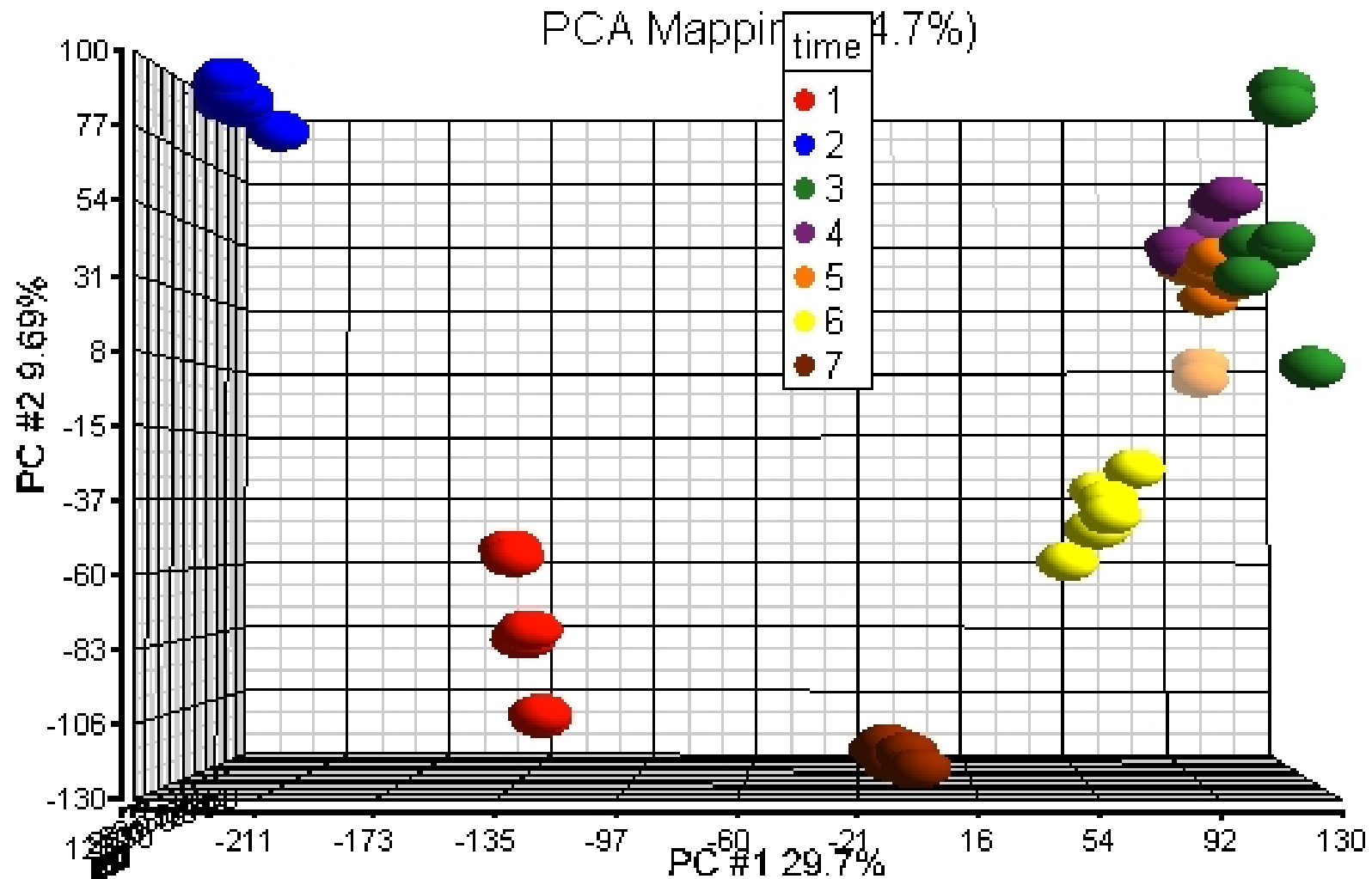
Laser microdissected samples of normal show low quality RNA integrity



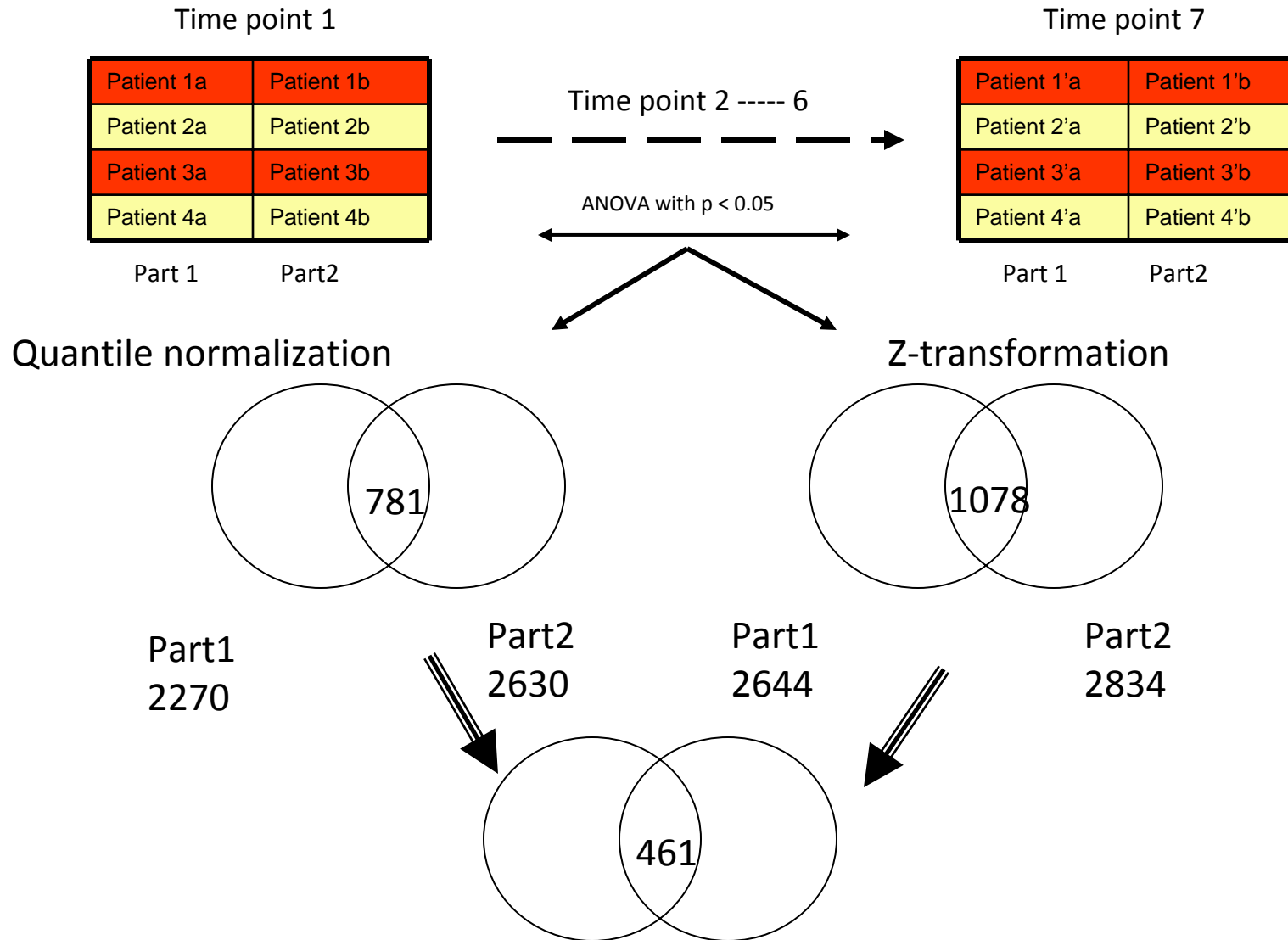
Quantitative control of percent calls showed high quality



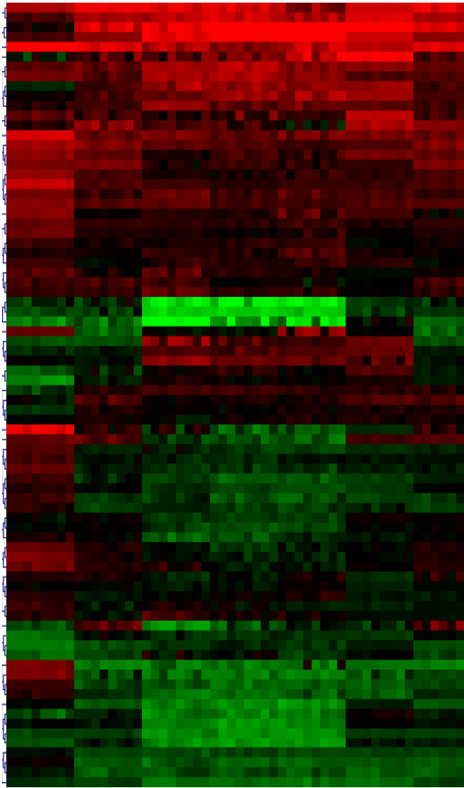
Principal Component Analysis show genes cluster according to time point



Two normalization methods were performed to generate one gene list of 461 genes



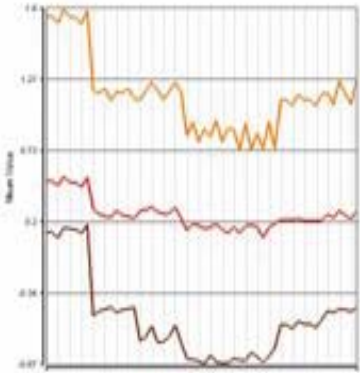
Analysis show three different trends of change over ischemic time



461

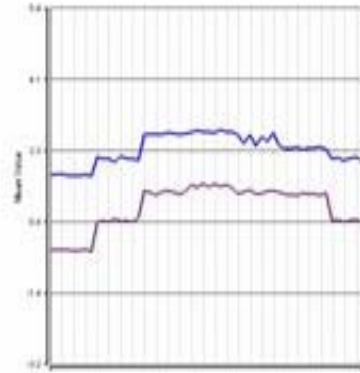


Pattern 1



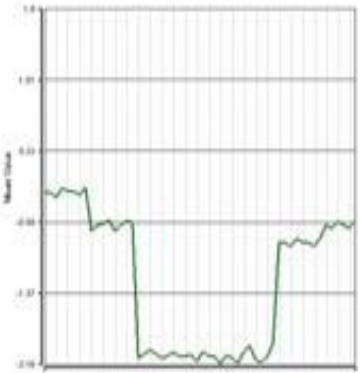
313

Pattern 2



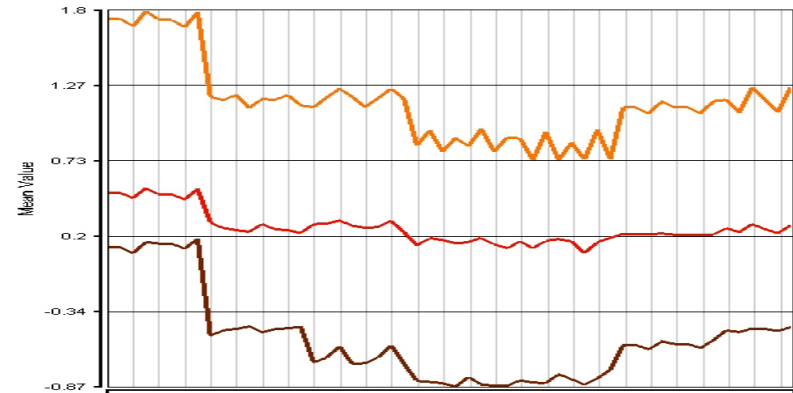
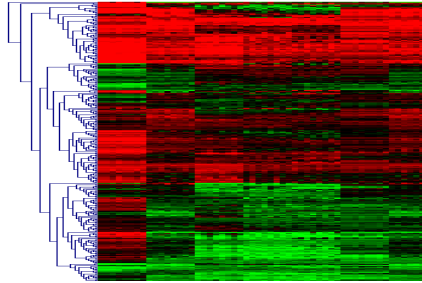
94

Pattern 3

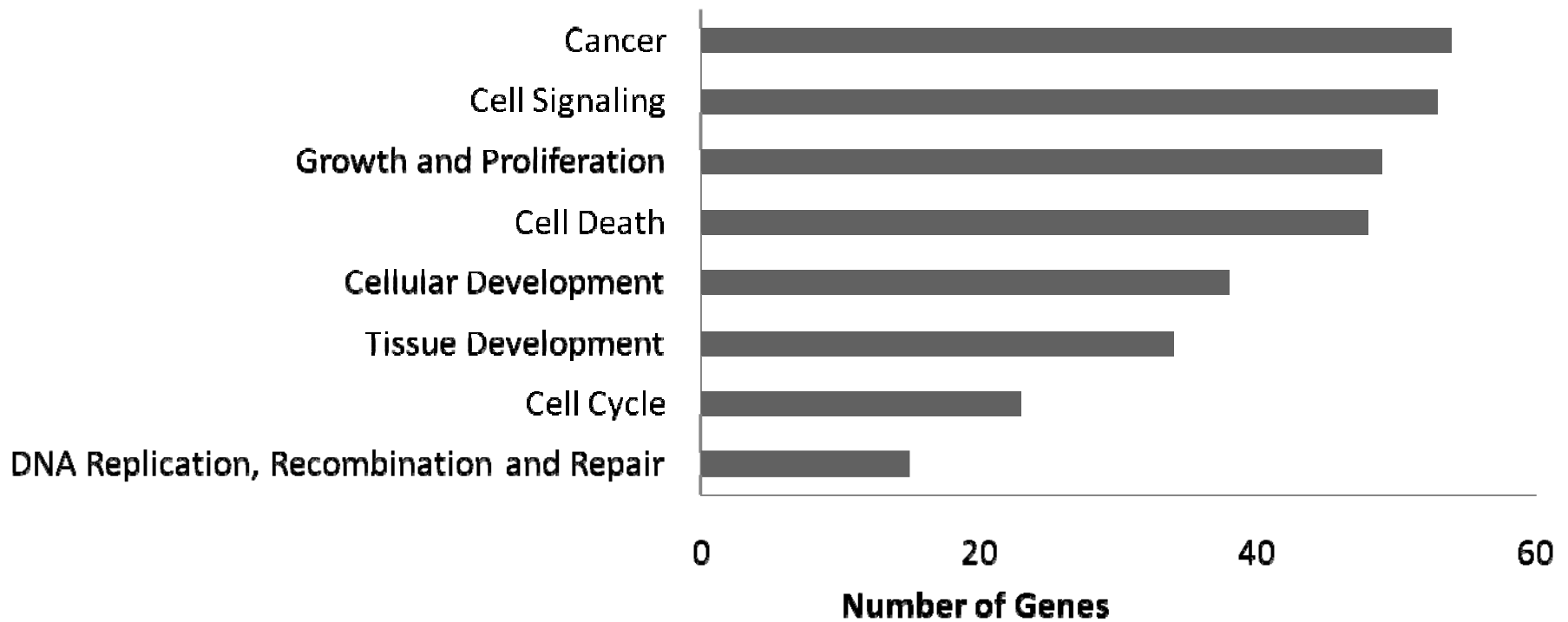


54

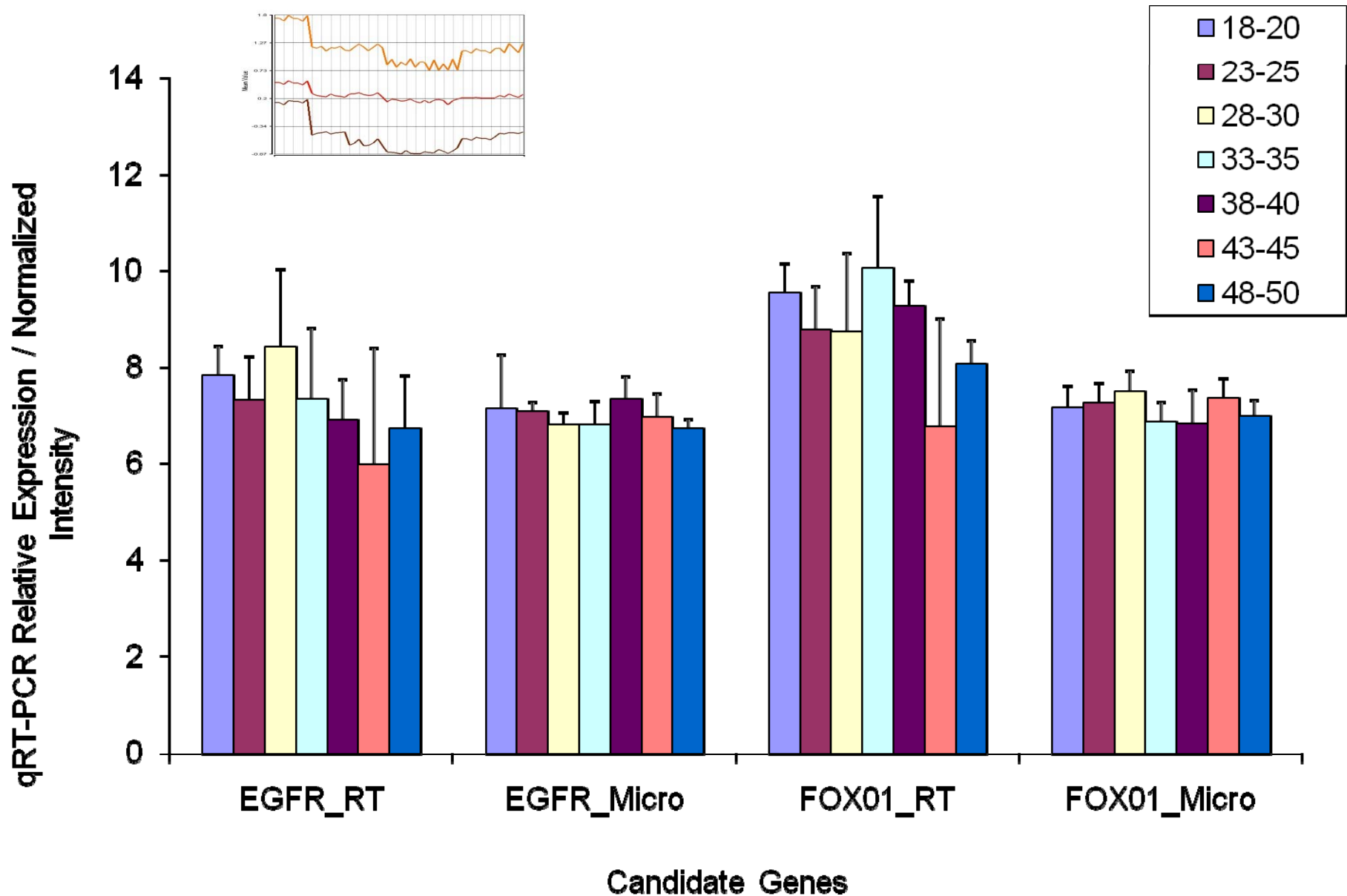
Differential gene expression show UP,Down regulation of 313 of genes



Biological Functions

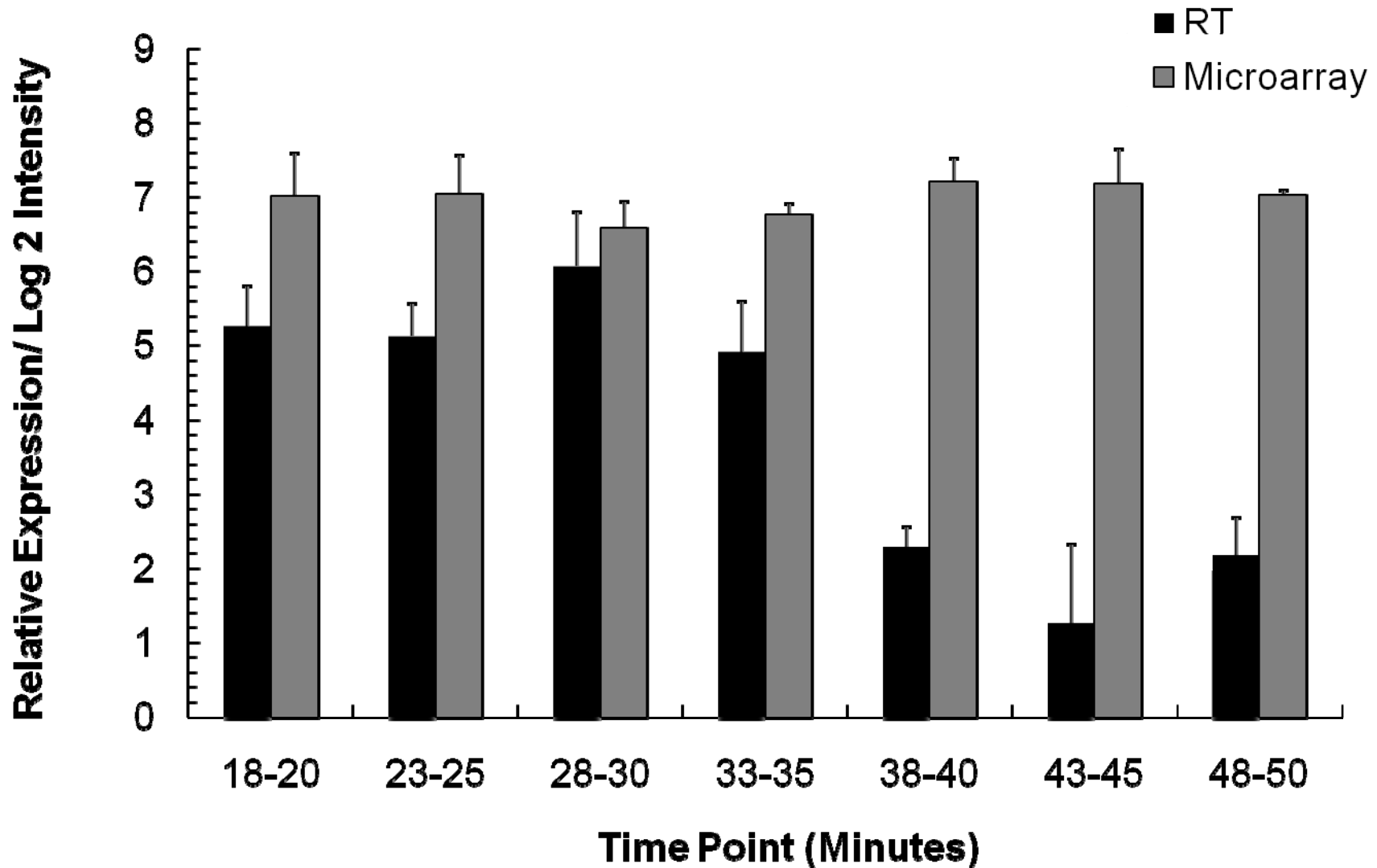


RT-PCR Data showing similarities to Pattern 1

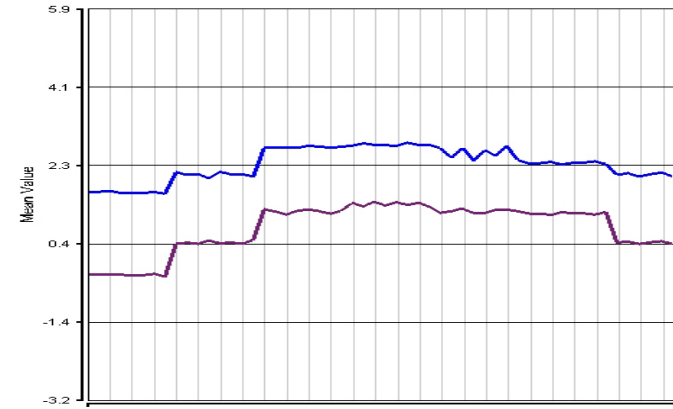
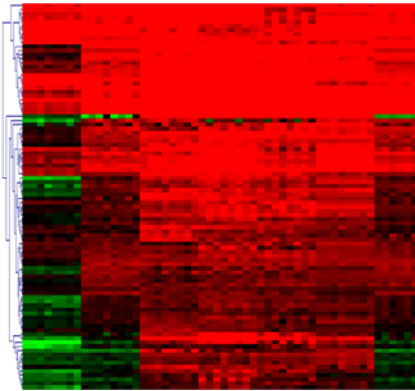


Real time –PCR show candidate gene Superoxide Dismutase 2 (SOD2) follow gene expression pattern

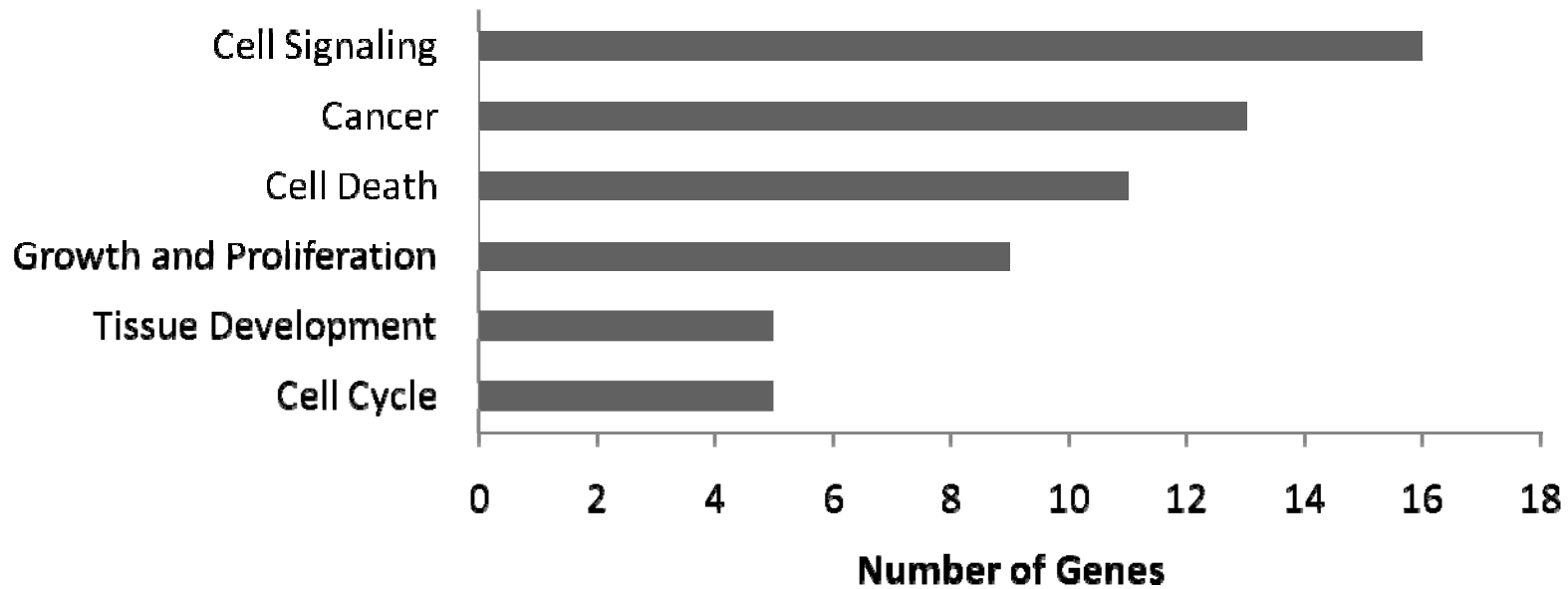
1



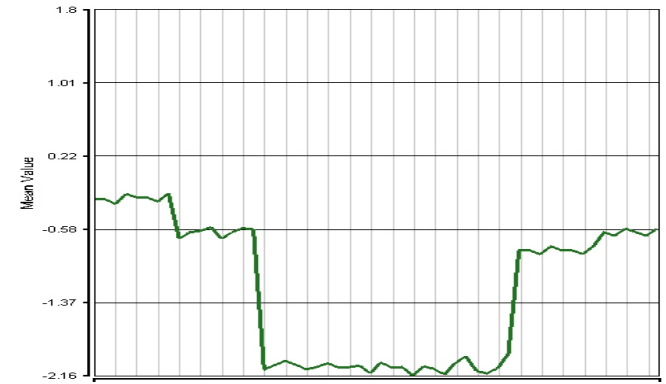
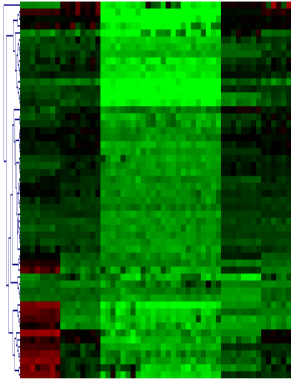
Differential gene expression pattern 2 show Down-UP-Down Trend of 94 genes



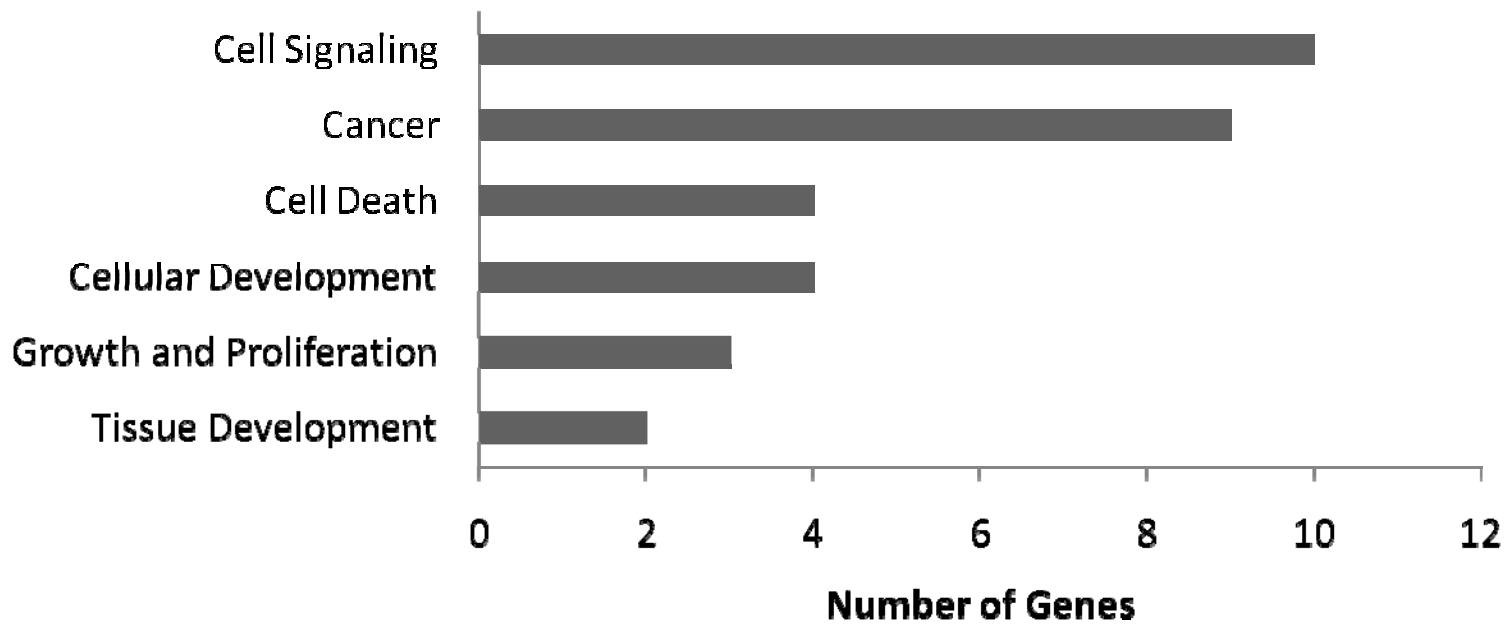
Pattern 2: Biological Functions



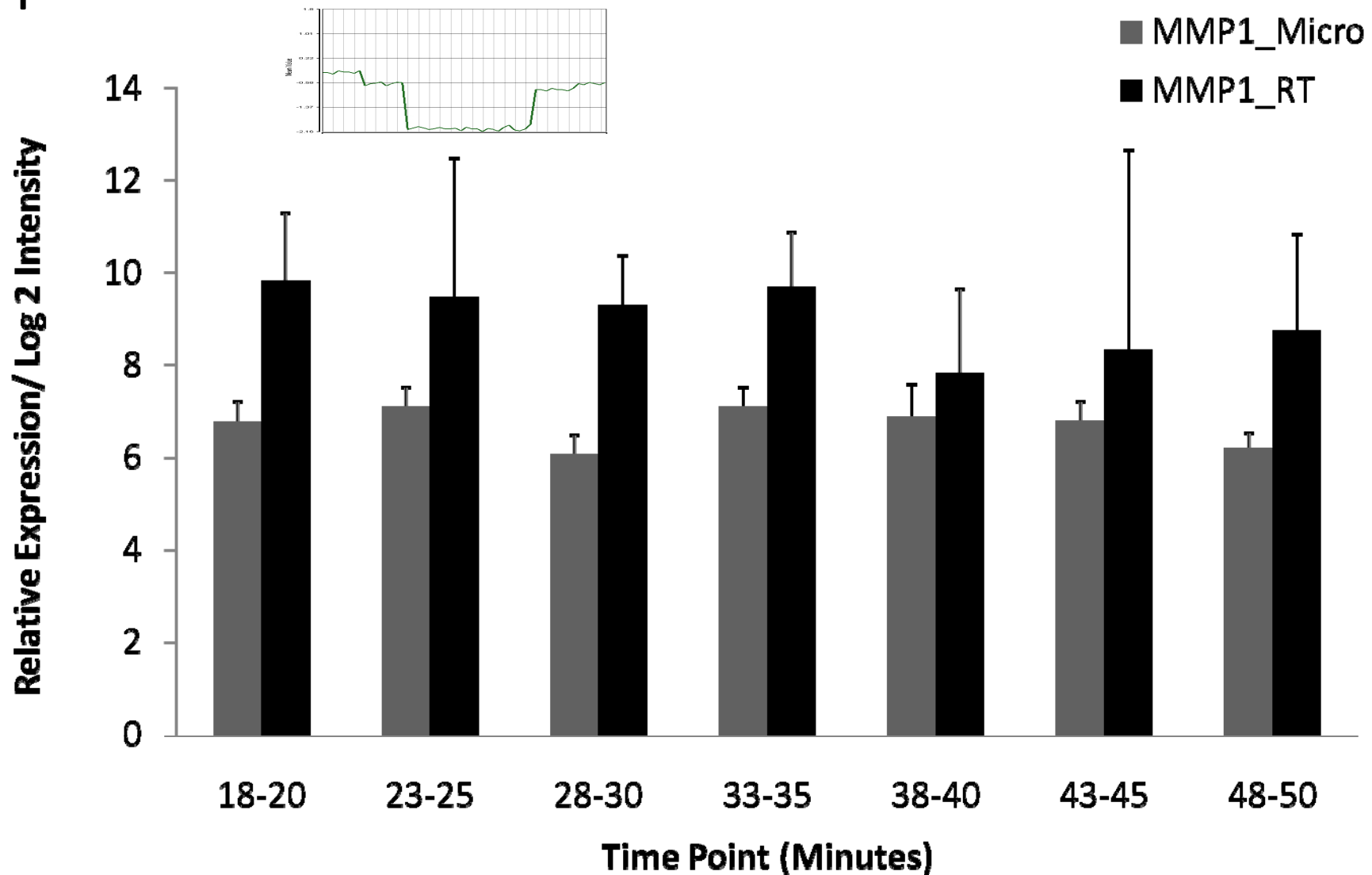
Differential gene expression pattern 2 show UP-Down-UP Trend of 54 genes



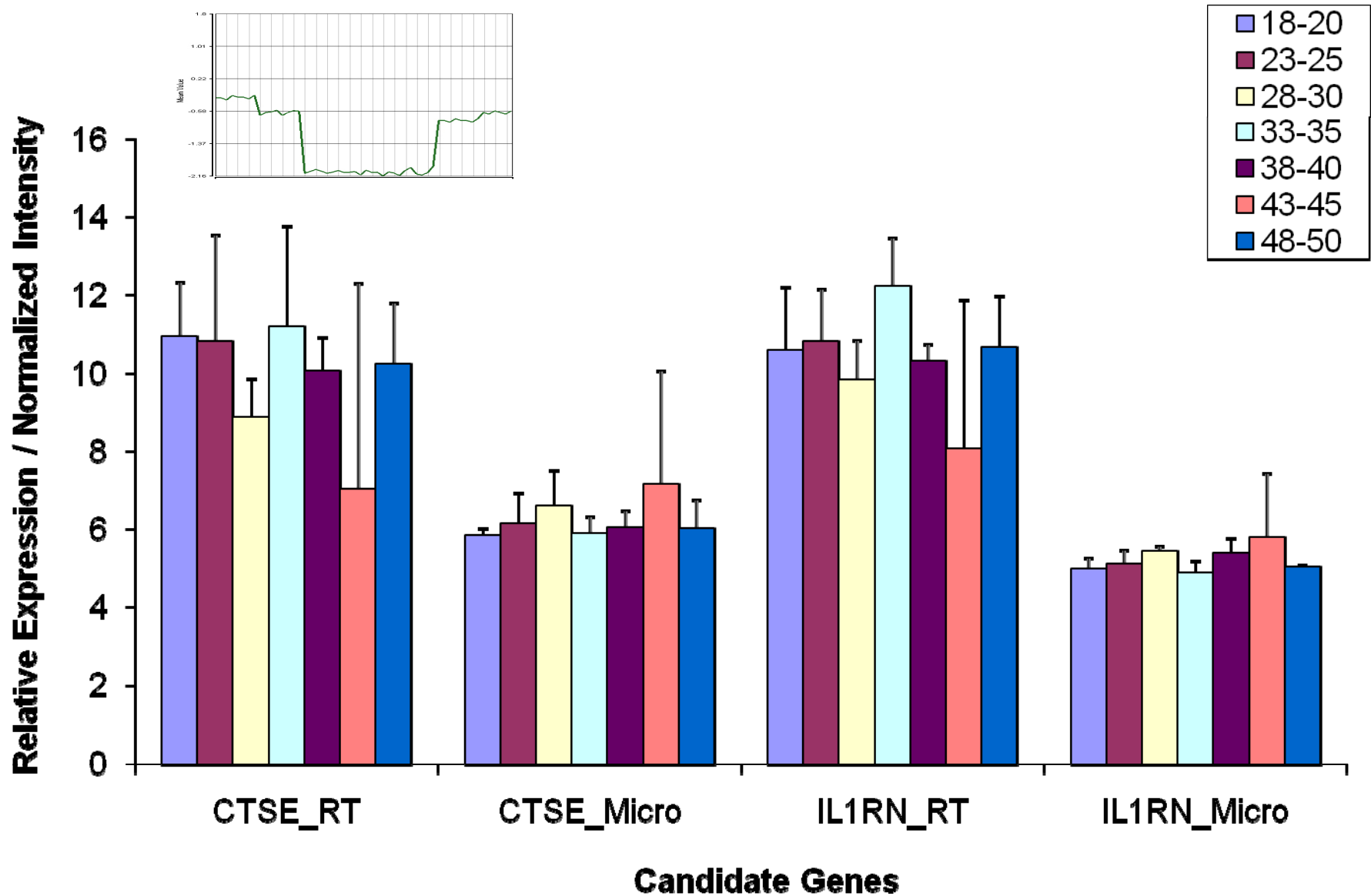
Pattern 3: Biological Functions



Real time –PCR show candidate gene matrix metallopeptidase 1(MMP1) follow gene expression pattern 2



Pattern 3 RT-PCR data of candidate genes mimic trend



Candidate Gene List show changes of clinically relevant genes

Gene	Description	Therapeutic Drugs Associated with Gene	Gene Exp
CA12	Carbonic anhydrase XII	Methazolamide, hydrochlorothiazide, acetazolamide, te	Pattern 1; U,D
MAOB	Monoamine oxidase B	Safinamide, ladostigil, rasagiline, selegiline, dextroamphetamine	Pattern 1; U,D
BCR	Breakpoint cluster region	Imatinib	Pattern 1; U,D
CDK2	Cyclin-dependent kinase 2	BMS-387032, flavopiridol	Pattern 1; U,D
EGFR	Epidermal growth factor receptor	Cetuximab, panitumumab, canertinib, gefitinib, lapatinib, vandetanib,	Pattern 1; U,D
LCK	Lymphocyte-specific protein tyrosine kinase	Dasatinib	Pattern 1; U,D
PPP3CA	Protein phosphatase 3 catalytic subunit, alpha	ISAtx-247, tacrolimus, pimecrolimus, cyclosporin A	Pattern 1; U,D
PML	Promyelocytic leukemia	Arsenic trioxide	Pattern 1; U,D
IL2RG	Interleukin 2 receptor, gamma	Aldesleukin, denileukin diftitox	Pattern 1; U,D
FGB	Fibrinogen beta chain	Thrombin	Pattern 2; D,U,D
GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3	Methohexital, aspirin/butalbital/caffeine, aspirin/butalbital/caffeine/codeine, acetaminophen/butalbital/caffeine,	Pattern 3; U,D,U
GABRP	gamma-aminobutyric acid (GABA) A receptor, pi	Alphadolone, sevoflurane, isoflurane, isoniazid, felbamate, etomidate,	Pattern 3; U,D,U

Summary Slide

- High quality gene expression data was produced from high quality samples that were carefully annotated and controlled
- Gene expression analysis shows intraoperative ischemia time affects gene expression profiles
- SOD2 showed significant changes in gene expression over ischemic time
- Gene expression profiles of clinically relevant genes can be affected by intraoperative ischemia time

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