Simultaneous RT-qPCR measurement of 1718 long non-coding RNAs

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Background

Massively parallel RNA-sequencing revealed that the human genome is pervasively transcribed, resulting in the production of thousands of non-coding RNA transcripts.

Long non-coding RNAs (lncRNAs) are the most abundant class of ncRNA molecules and are emerging as an important regulatory layer of the transcriptome.

Currently, expression and function of lncRNAs during human disease and developement is largely unexplored.

Few platforms are available that allow sensitive and specific high-throughput lncRNA expression profiling.

Aims

1. RT-qPCR assay design for +1700 lncRNA transcripts
2. Assay validation according to MIQE-guidelines
3. High-throughput RT-qPCR platform development
4. Platform validation using biological samples

RT-qPCR assay design and validation

lncRNA transcripts obtained from

lncrna db   52 lncRNAs
www.lncRNAdb.com

Ensembl   1666 lncRNAs
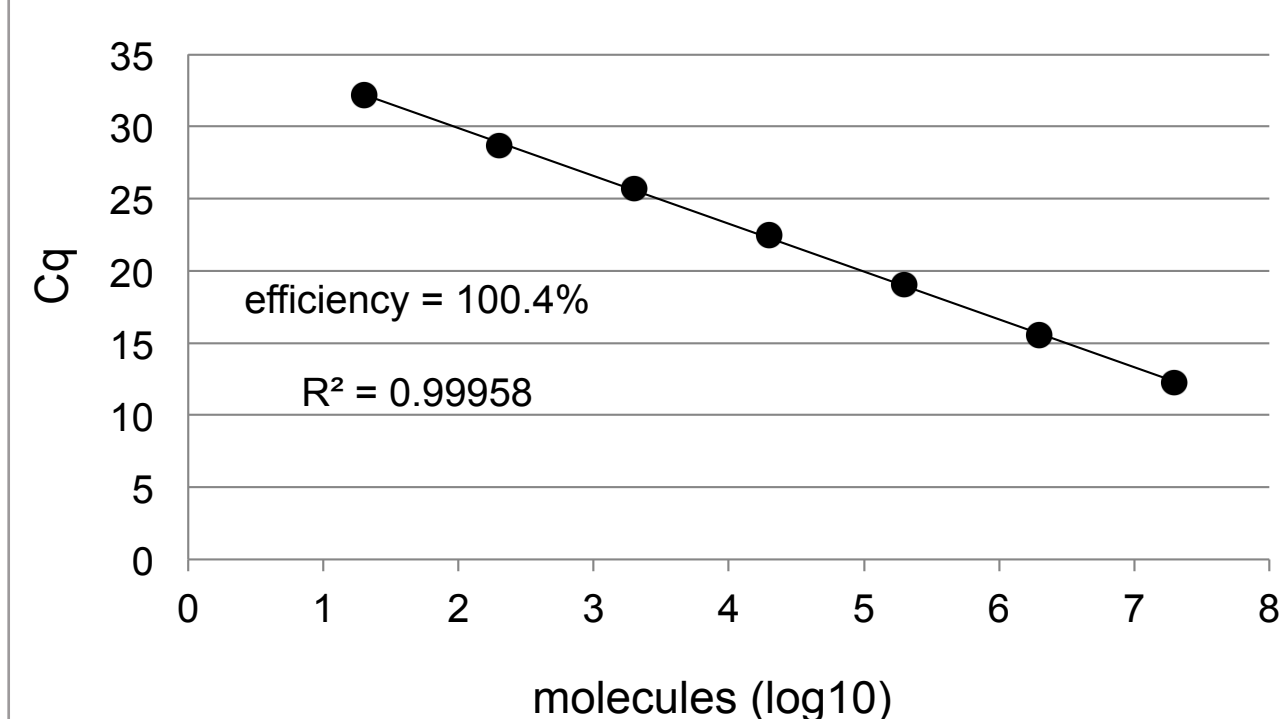www.ensembl.org

RT-qPCR assay design using

primerXL
www.primerxl.org

Primer3 primer design engine

State-of-the-art in silico quality control tools (absence of SNPs, absence of secondary structures, absolute specificity)

assay efficiency

- 10-fold standard dilution series
- spreading 6 $\log_{10}$-units

efficiency = 100.4%
R² = 0.99958

- linear dynamic range accross 6 $\log_{10}$-units
- efficiency 90% < E < 110% for all assays

assay specificity

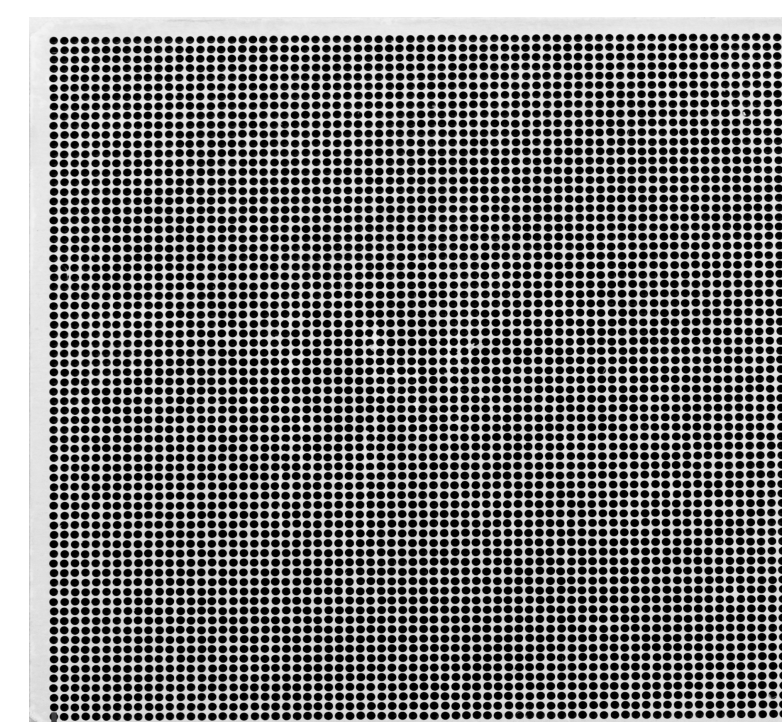- qPCR on gDNA
- amplicon sizing and sequencing

- single band detection (LabChip)
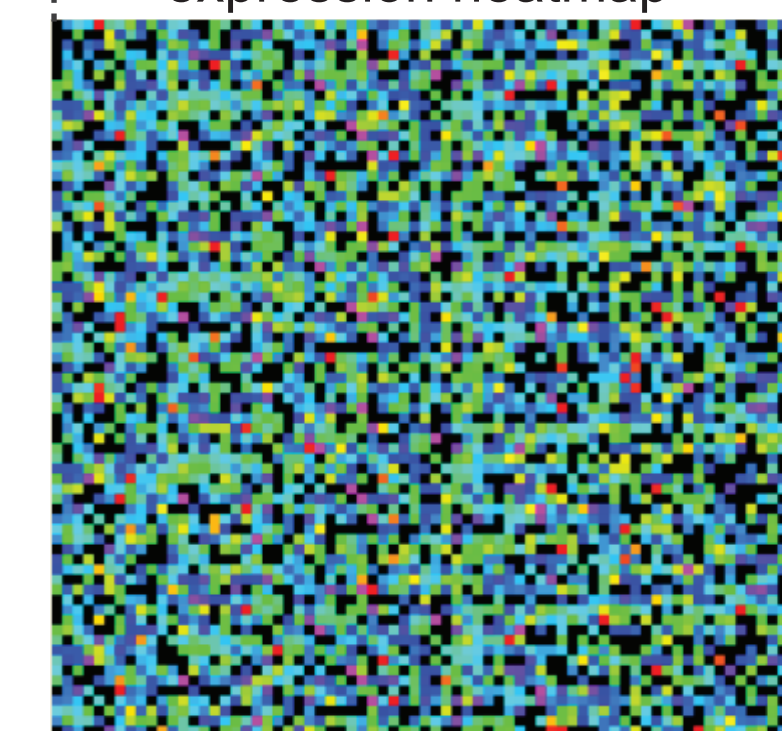- < 1% aspecific amplicons (sequencing)

Platform development

1718 MIQE-validated lncRNA RT-qPCR assays, spotted in triplicate on a SmartChip (5184 wells).

lncRNA expression is measured by RT-qPCR in 100 nl reactions.

expression heatmap

Triplicate expression values are combined based on the median Cq which is insensitive to outliers.

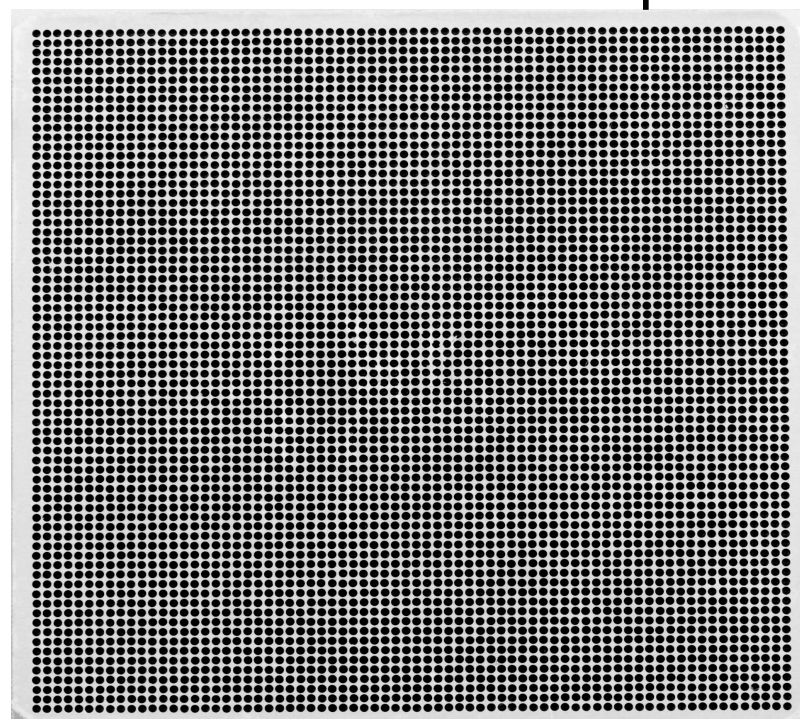lncRNA expression data is normalized using the global mean (qbase^PLUS).

Platform validation

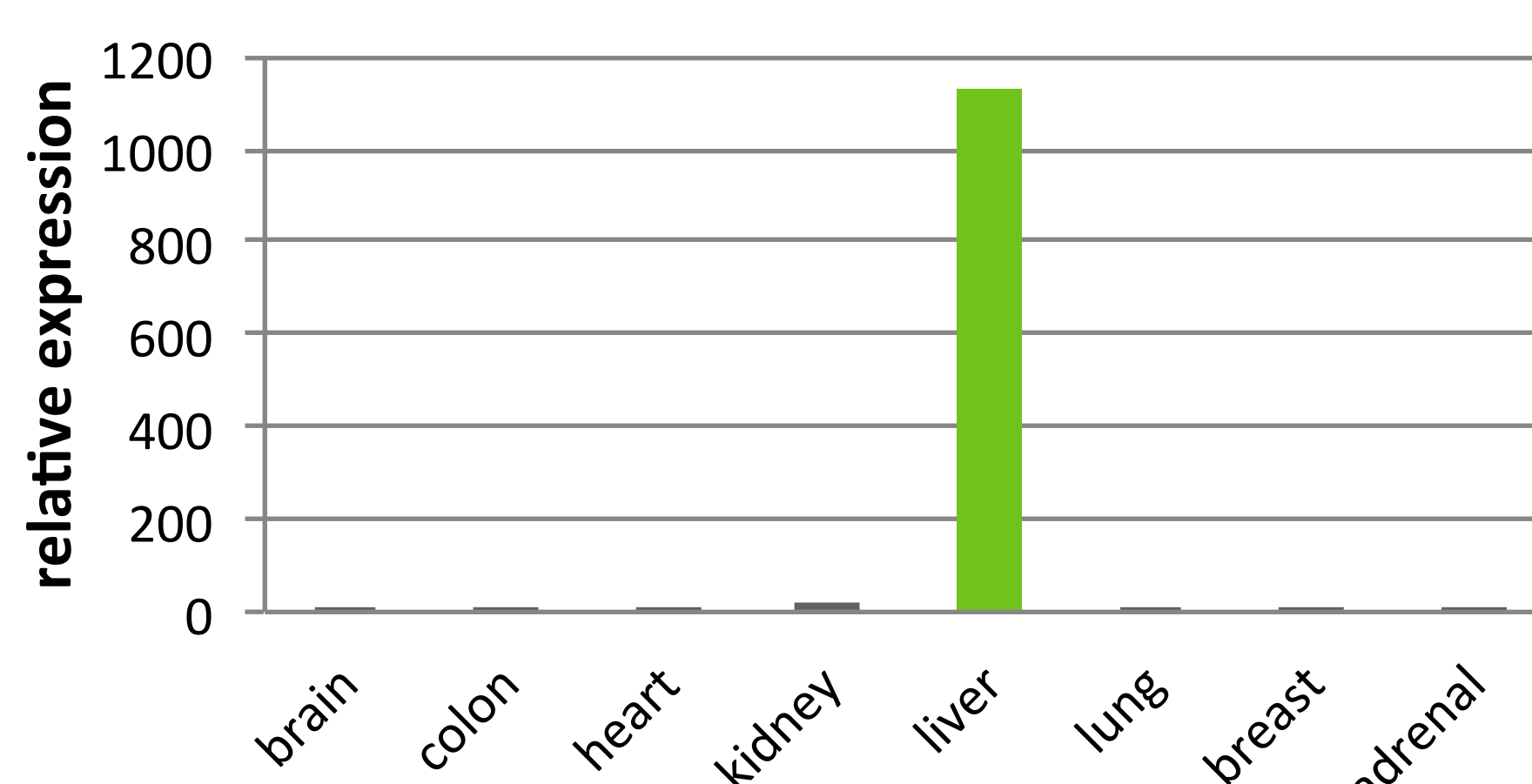lncRNA expression profiling of normal human tissues identifies tissue specific lncRNAs associated with tissue function

normal human tissues

brain
colon
heart
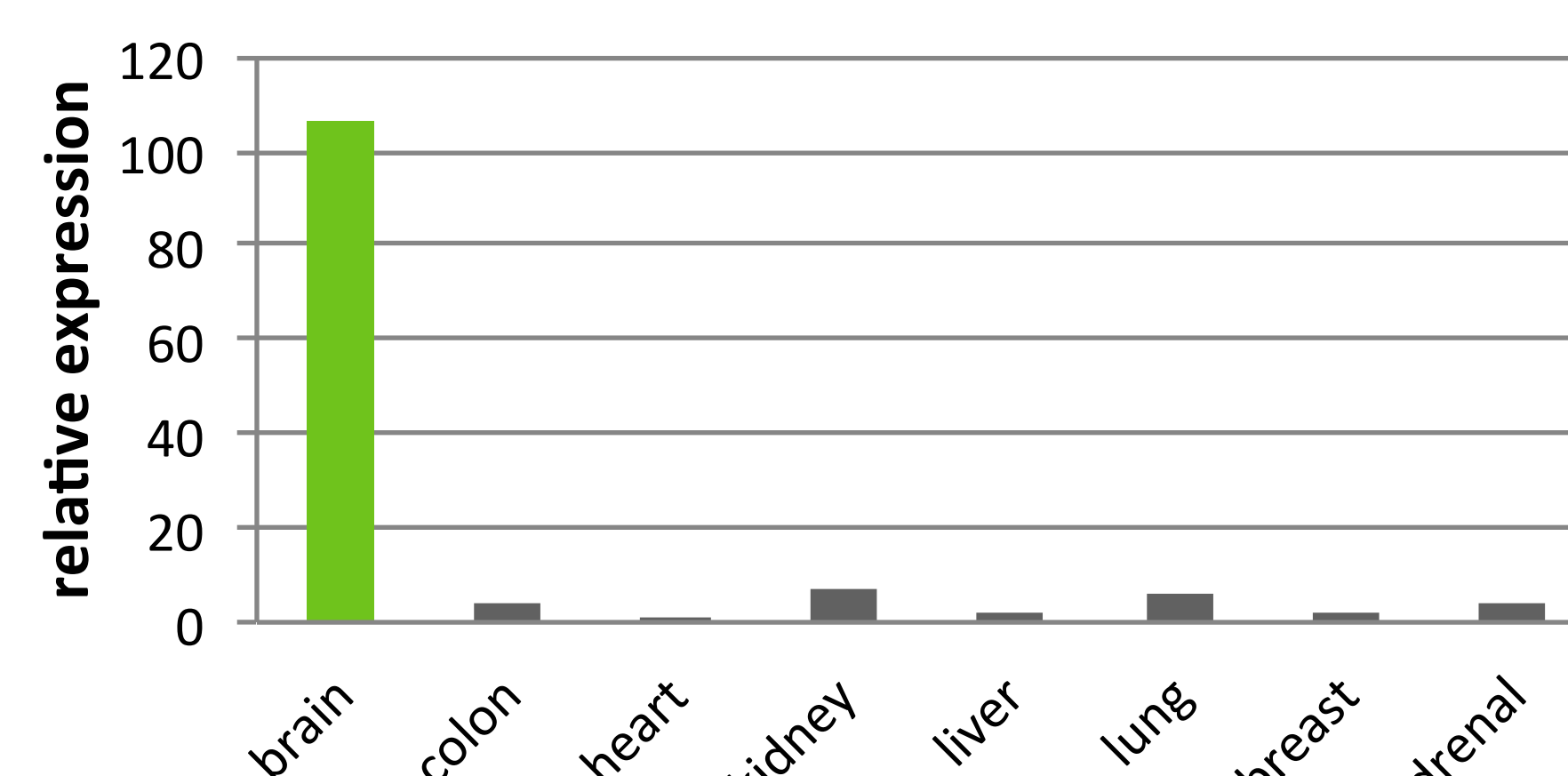kidney
liver
lung
breast
adrenal

lncRNA SmartChip

HULC

HULC (Highly Upregulated in Liver Cancer) is specifically expressed in human liver suggesting HULC is a potential lineage specific oncogene.

GOMAFU

GOMAFU is a lncRNA with known functions in neuronal glial fate specification and oligodendrocyte lineage maturation. Its expression is restricted to brain tissue.

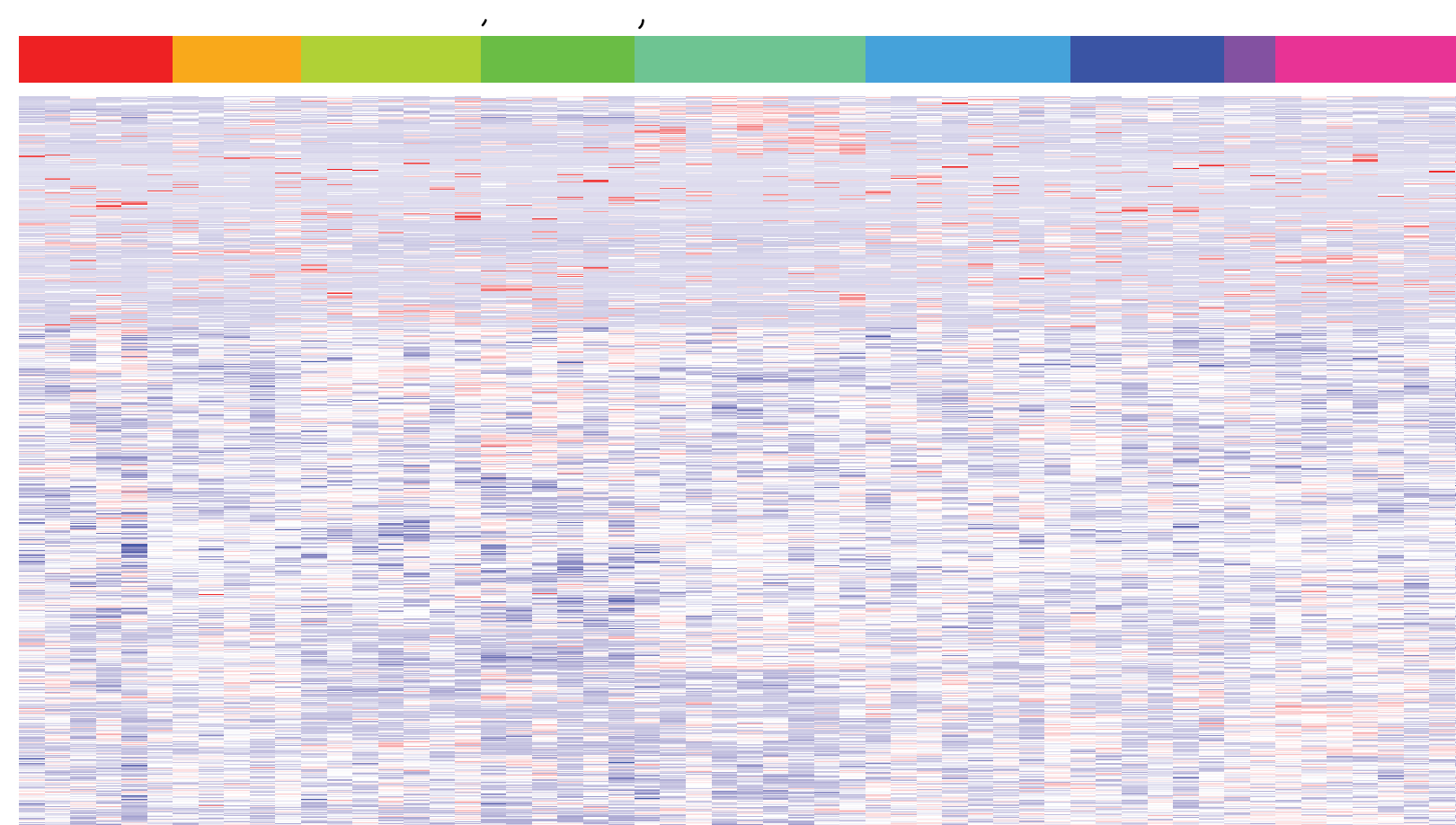lncRNA expression profiling of the NCI60 cancer cell line panel reveals cancer-specific lncRNA expression profiles

NCI60 cancer cell line panel

breast (6)   cns (6)   colon (7)
leukemia (6)   melanoma (9)   lung (9)
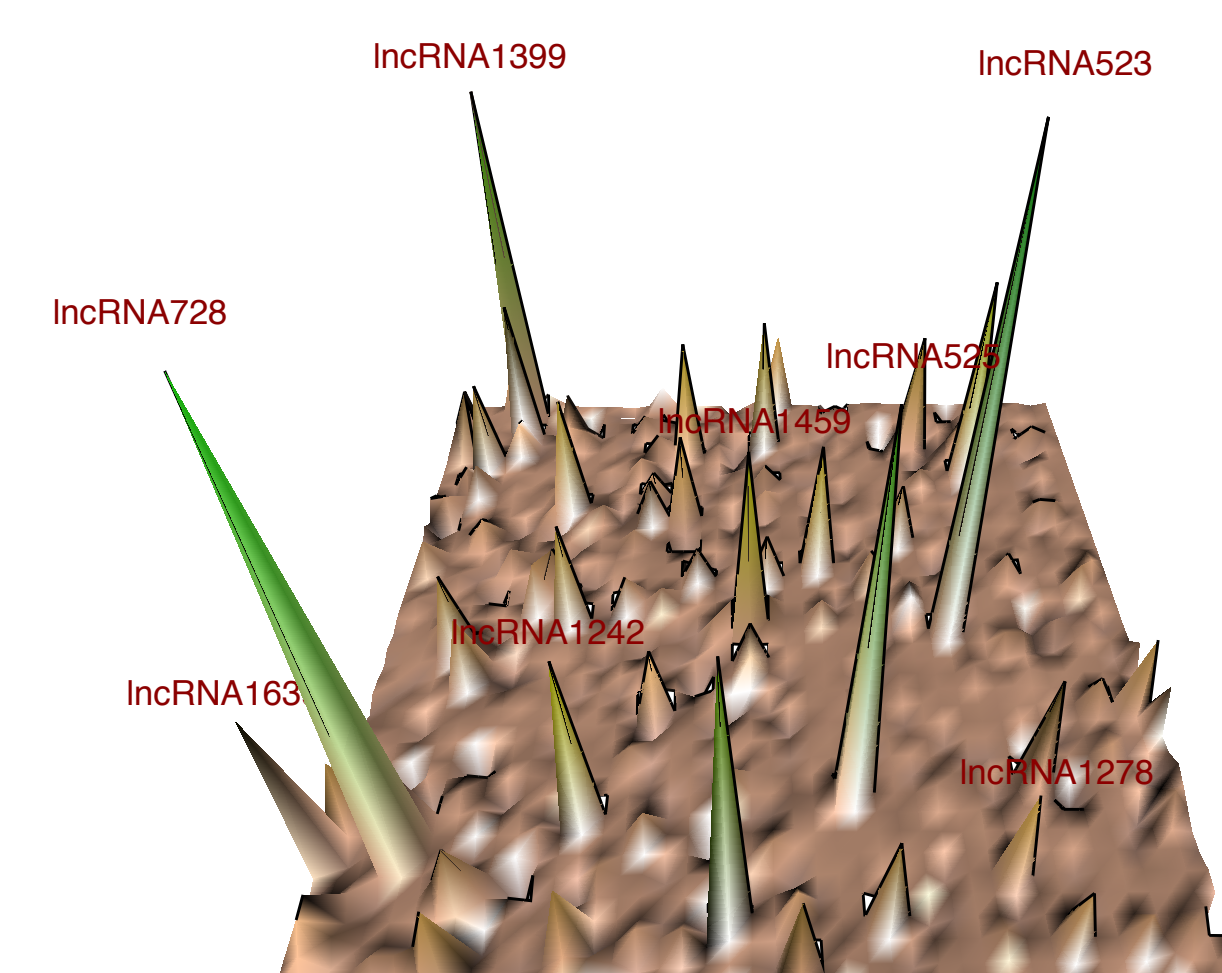ovarian (7)   prostate (2)   renal (8)

lncRNA SmartChip
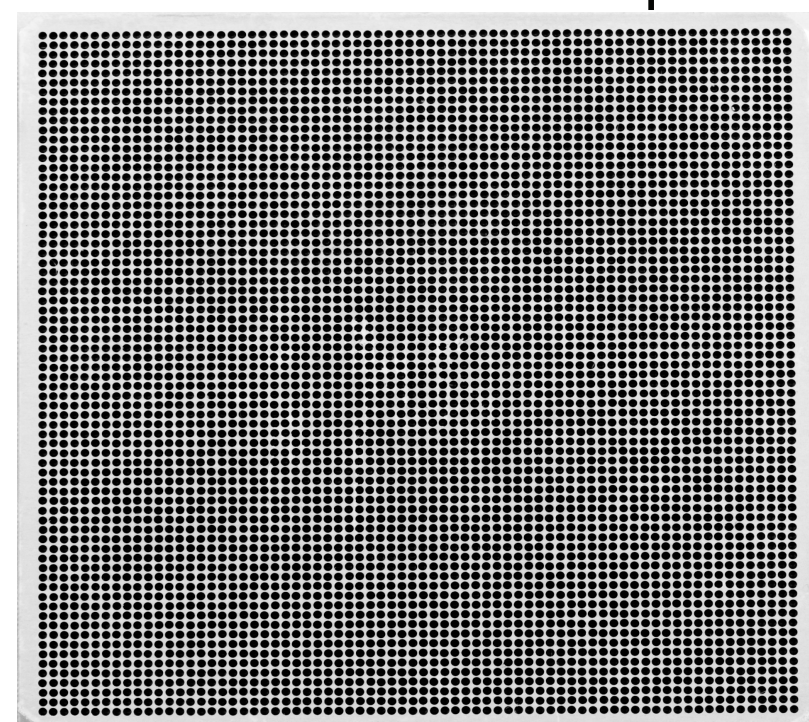
lncRNA expression landscape of NCI60 panel

melanoma-specific lncRNA landscape

Conclusion

1. Biogazelle developed a high-throughput RT-qPCR lncRNA expression profiling platform capable of measuring 1718 human lncRNAs.
2. LncRNA assays were validated according to MIQE-standards and spotted in triplicate on 5184-well SmartChips (Wafergen).
3. LncRNA expression profiling of normal human tissues reveals tissue specific lncRNAs.
4. LncRNA expression profiling of 60 cancer cell lines representing 9 different tumour entities reveals cancer-specific lncRNA expression profiles. The resulting lncRNA expression landscapes provide novel candidate cancer genes.

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