Transcriptomics, Proteomics and Metabolic Changes in the Post-Natal Mouse Heart analyzed with QIAGEN IPA and OmicSoft

Discovery Team, QIAGEN Digital Insights
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Agenda

- QIAGEN Sample to Insight
- Highlight important results
- Processing the transcriptome, proteome and metabolome datasets
- Biological analysis of the transcriptome, proteome and metabolome of post-natal mouse cardiomyocytes
- Understand the biological results in larger context
- Conclusions
Objectives: Understand what is happening in post-natal mouse heart

• What transcriptional program underpins the development of heart postnatally?
  • Which transcription regulators are predicted to be activated or inhibited?
  • What are the significant biological processes connected to these transcription regulators?

• What hypotheses could be generated then validated in the lab?
  • Are they master regulators driving some of the post-natal mouse heart?
  • Are they therapeutically targetable or usable in biomarker application?

• Can we identify tissue-specific splicing variants of interest?
  • Are there splicing variants enriched in heart tissues?
  • What are their functions?
  • Can we identify a splicing variant for biomarker application?

• What biological information can we get by comparing our analysis to >52,000 datasets?
  • Is there a common pattern in other biological processes?
  • Can we identify common players?

• Can we establish connection between two genes in heart development?
  • What important genes are connected in heart development?
  • What correlation exist between these genes?
QIAGEN Sample to Insight

**Sample collection and stabilization**
- PAXgene® systems
- QIAcube Connect®
- QIAsymphony®
- EZ1® Advanced XL
- QIAamp® Kits
- RNeasy® Kits
- miRNeasy Kits
- exoRNeasy Kits

**Nucleic acid isolation**
- QIAseq RNA
- QIAseq DNA

**NGS library preparation**

**Sequencing**

**Illumina & Thermo Fisher sequencers**
- OmicSoft Array Suite
- CLC Genomics Workbench
- QIAGEN Microbial Genomics Module
- CLC Main Workbench
- CLC Genome Finishing Module

**Data analysis**
- Ingenuity® Pathway Analysis
- Ingenuity Variant Analysis
- HGMD®
- OmicSoft Land Explorer
- OmicSoft DiseaseLand
- OmicSoft OncoLand
- OmicSoft GeneticsLand

**Interpretation**

Transcriptomics, Proteomics and Metabolic Changes in Postnatal Mouse Heart analyzed with IPA and OmicSoft
RNAseq data analyzed using QIAGEN bioinformatics

Experimental design for the multiomics analysis of postnatal mouse hearts. Two separate sets of mouse ventricular tissue samples collected on postnatal day 1 (P01), P04, P09, and P23 were used.

<table>
<thead>
<tr>
<th>Platform</th>
<th>Omics</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA-seq</td>
<td>Transcriptomics</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Proteomics</td>
</tr>
<tr>
<td>LC-MS GCxGC-MS</td>
<td>Metabolomics</td>
</tr>
</tbody>
</table>

C57BL/6 mice

Postnatal days

Cardiomyocyte regenerative capacity

Talman V. et al. (2018)
PMID: 30371266, GSE119530
Transcriptomics, Proteomics, Metabolic Changes in Postnatal Mouse Heart

- Explore the underlying transcriptional programs (Upstream Analysis)
- Generate hypotheses to validate in the lab (Causal Network)
- Identify tissue-enriched splicing variant and its expression pattern (IsoProfiler)
- Compare our analysis to pre-computed datasets (Analysis Match – OmicSoft Lands)
- Visualize the connections of important genes in heart development (OmicSoft)
OmicSoft → Ingenuity Pathway Analysis (IPA)

RNA-seq FASTQ files

Sample metadata

IMPORT

Your expression data

OmicSoft Lands

Expression data
Upload dataset to IPA

OS-IPA integration: Analyzed dataset in AS is sent to IPA via Plugin
Auto-submit IPA core analysis from Array Studio dataset

The dataset will be automatically analyzed in IPA with the supplied cutoffs
Summary of the Core Analysis: mRNA day 23 vs day 1

<table>
<thead>
<tr>
<th>Pathway</th>
<th>p-value</th>
<th>Overlap</th>
<th>p-value</th>
<th>Overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetochore Metaphase Signaling Pathway</td>
<td>3.79E-21</td>
<td>59.4%</td>
<td>6.04E-01</td>
<td>21.05%</td>
</tr>
<tr>
<td>Oxidative Phosphorylation</td>
<td>7.24E-19</td>
<td>55.0%</td>
<td>6.04E-01</td>
<td>21.05%</td>
</tr>
<tr>
<td>Mitochondrial Dysfunction</td>
<td>1.17E-17</td>
<td>45.6%</td>
<td>7.84E-01</td>
<td>17.17%</td>
</tr>
<tr>
<td>Hepatic Fibrosis / Hepatic Stellate Cell Activation</td>
<td>4.91E-13</td>
<td>39.8%</td>
<td>7.45E-01</td>
<td>18.69%</td>
</tr>
<tr>
<td>Sirtuin Signaling Pathway</td>
<td>1.39E-11</td>
<td>33.7%</td>
<td>9.82E-01</td>
<td>29.12%</td>
</tr>
</tbody>
</table>

Summary at the gene level:
- |fold change|>1.5
- q<0.05
- min counts >10 in day 23 or day 1
Core Analysis: day 4 vs day 1 (example)

Experiment Metadata

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<thead>
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<th>VALUE</th>
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<tbody>
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<tr>
<td>case.animalstrain</td>
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</tr>
<tr>
<td>case.celltype</td>
<td>cardiomyocyte</td>
</tr>
<tr>
<td>case.tissuedescription</td>
<td>heart</td>
</tr>
<tr>
<td>case.treattime[days]</td>
<td>Day4</td>
</tr>
<tr>
<td>comparisoncategory</td>
<td>Other comparisons</td>
</tr>
<tr>
<td>comparisoncontrast</td>
<td>Day4 vs Day1</td>
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<tr>
<td>control.animalstrain</td>
<td>C57BL/6JOlahsd</td>
</tr>
<tr>
<td>control.treattime</td>
<td>Day1</td>
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<tr>
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<td>Hg38 Ensembl92</td>
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<tr>
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<td>mus musculus</td>
</tr>
<tr>
<td>projectname</td>
<td>GSE119530</td>
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</table>
Transcriptomics changes in post-natal mouse heart

Canonical Pathways comparison indicate switch in energy metabolism and changes in cell cycle

- **Cell Cycle decreased**
- **Cholesterol synthesis decreased**
- **Oxidative phosphorylation increased**
- **Cell cycle checkpoint increased**
- **TCA cycle increased**

Post-natal cardiomyocytes arrest cell cycle progression and increase ox. phos. starting at day 9 after birth.
Oxidative phosphorylation is predicted to be activated at day 9 and day 23. Comparison of transcriptomics analysis indicates that oxidative phosphorylation pathway is activated from day 9 on. Post-natal mouse cardiomyocytes switch to oxidative phosphorylation for efficient ATP production starting at day 9 after birth.
Proteomics analysis shows energy switch in post-natal cardiomyocytes

Proteomics indicate major switch in energy metabolism and energy substrates after birth.

Post-natal mouse cardiomyocytes switch from glycolysis to oxidative phosphorylation and increase fatty acid β-oxidation and branched-chain amino-acid degradation.
Explore the underlying transcriptional programs

Upstream Analysis
Multi-omics analysis indicate similar transcriptional drivers

Transcriptomic

Proteomic

Metabolomic

Upstream Regulators Analysis of transcriptomics, proteomics and metabolomics show induction of fatty oxidation regulation by PPARG coactivators.
Multi-omics analysis indicate similar transcriptional drivers

Transcriptomic

Proteomic

Metabolomic

Upstream Regulators Analysis of transcriptomics, proteomics and metabolomics show induction of fatty oxidation regulation by PPARG coactivators.
PPARGC1A is predicted to induce ATP synthesis

At day 23 post-birth, PPARGC1A predicted to be activated and drives ATP synthesis and metabolism of ROS through increase of fatty acid oxidation (transcriptomics).

- Synthesis of ATP (p-value 8.01E-15)
- Oxidation of Fatty acid (p-value 4.61E-16)
- Metabolism of ROS (p-value 7.65E-12)
- Cardiogenesis (P-value 4.03E-10)
Generate hypotheses to validate in the lab

Causal Network
Causal Network Analysis of transcriptomics in post-natal mouse heart

Comparison of Causal Network at day 4 and day 23, switch in usage of PTGER2 and PTGER1.
Regeneration of heart is predicted to be increased at day 4 post-birth

PTGER2 is predicted to be activated and may promote the regeneration of heart at day 4 post-birth.
Regeneration of heart is predicted to be decreased at day 23 post-birth

PTGER2 is predicted to be inhibited and may inhibit the regeneration of heart at day 23 post-birth.
Regulator Effects predicts ICMT as a player in post-natal mouse heart

Comparison of metabolomics analysis predicts that ICMT (Isoprenylcysteine carboxyl methyltransferase) increases O2 consumption and oxidative phosphorylation at day 23 in post-natal mouse heart.
Identify tissue-enriched splicing variant and its expression pattern

IsoProfiler
Isoforms differentially expressed observed in post-natal mouse heart

At q<0.05, 2256, 2965 and 6639 differentially expressed isoforms are found at day 4, day 9 and day 23 post-birth, respectively.

<table>
<thead>
<tr>
<th>Sy...</th>
<th>Molecule</th>
<th>Gene-level Disease or Func...</th>
<th>Gene-level Function</th>
<th>Express...</th>
<th>Max Ex...</th>
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<tbody>
<tr>
<td>ABCC9</td>
<td>ion channel</td>
<td>Abnormal ST segment, Antivir... all 67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>2</td>
<td>3 more</td>
<td>1.340</td>
<td>1.711</td>
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<tr>
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<td>transporter</td>
<td>Abnormal conduction by nerves,...all 74</td>
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<tr>
<td>383</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1.471</td>
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<td>2.602</td>
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<td>Abnormal composition of bile,...all 24</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>1</td>
<td>--- ---</td>
<td>-1.990</td>
<td></td>
<td></td>
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<td>ABCE1</td>
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<td>Antiviral response,Apoptosis o......all 10</td>
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<tr>
<td>30</td>
<td>1</td>
<td>- -</td>
<td>1.457</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.691</td>
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</table>
IsoProfiler to filter transcripts from post-natal mouse cardiomyocytes

<table>
<thead>
<tr>
<th>Index</th>
<th>Name</th>
<th>Fold Chan.</th>
<th>p-value</th>
<th>p-value</th>
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<td>1</td>
<td>transcripts day4 vs day1</td>
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<td>✔</td>
<td>✔</td>
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<td></td>
</tr>
<tr>
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<td>✔</td>
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</tr>
<tr>
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<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
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</table>

**APPRIS (principal splice isoforms)**

- Select all
- PRINCIPAL:1
- PRINCIPAL:2
- PRINCIPAL:3
- PRINCIPAL:4
- PRINCIPAL:5
- ALTERNATIVE:1
- ALTERNATIVE:2

**Gene-level Disease or Function**

- cardiomyocytes
- Oxidative stress response of cardiomyocytes
- Polyploidization of cardiomyocytes
- Polyploidy of cardiomyocytes
- Proliferation of cardiomyocytes
- Quantity of apoptotic cardiomyocytes
- Quantity of cardiomyocytes
- Recruitment of cardiomyocytes
## Isoforms involved in proliferation of cardiomyocytes

Principal isoforms of 4 genes of 21 after filtering are inversely regulated at day 4 and day 23 post-birth.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Role</th>
<th>Abnormality</th>
<th>D4</th>
<th>D9</th>
<th>D23</th>
<th>Fold Change</th>
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<td>ALDH1A2</td>
<td>enzyme</td>
<td>Abnormal morphology of at……all 93</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>↑1.636</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>×</td>
<td>×</td>
<td></td>
<td>↓2.084</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>BIRC5</td>
<td>other</td>
<td>Accumulation of breast ca……all 297</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>×</td>
<td>×</td>
<td></td>
<td>↓1.702</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>↓12.801</td>
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<tr>
<td>CCNA2</td>
<td>other</td>
<td>Activation of R… Acute my….all 74</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>↑1.975</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>↓9.772</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>E2F2</td>
<td>transcription reg.</td>
<td>Abnormal function of immu….all 96</td>
<td>☐</td>
<td>-</td>
<td>-</td>
<td>↑1.698</td>
</tr>
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<td></td>
<td></td>
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<td>-</td>
<td></td>
<td>↓1.972</td>
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<td>☐</td>
<td>☐</td>
<td>☐</td>
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</tr>
</tbody>
</table>
Four isoforms are differentially expressed between day 4 and day 23

**ALDH1A2** (retinoic acid producing enzyme) is necessary during the epicardial development.

**BIRC5** controls cardiomyocytes number in heart development, its overexpression promotes cell cycle progression. Its downregulation contributes to cell cycle arrest during postnatal cardiac development in a mouse model.

**CCNA2** is silenced after birth in the mammalian heart and its constitutive expression enhances cardiomyocyte proliferation resulting in cardiac hyperplasia.

**E2F2** has been shown to promote adult cardiomyocyte proliferation.
Compare your analysis to pre-computed datasets

Analysis Match – OmicSoft Lands
Analysis Match: Postnatal mouse heart day 23 vs. precomputed datasets

Looking for a similar pattern in

- CP (Canonical Pathways)
- UR (Upstream Regulators)
- DE (Downstream Effects)
- CN (Causal Networks)
Analysis Match: Postnatal mouse heart day 23 vs. precomputed datasets

Filtering with unique criteria on overall Z-score indicating highest similar pattern possible between day 23 vs day 1 and others precomputed analyses.

Z-score % > 60
Analysis Match: Postnatal mouse heart day 23 vs. precomputed datasets

Highest similarity at Canonical Pathways, Upstream Regulators, Causal Networks and Diseases & Functions is found with a cancer dataset.

Acute Myeloid Leukemia

CARM1 shRNA vs control shRNA

GSE103528
What we know about CARM1...

CARM1 is an important regulator in embryonic development and cellular differentiation.

- CARM1 is “Co-activator-associated arginine methyltransferase 1”
- CARM1 adds asymmetric demethylation to arginine residues in histones, with specificity for H3R17 and H3R26 and other protein substrates (RUNX1, and members of the SWI/SNF...).
- CARM1 regulates critical cellular processes such as RNA splicing and autophagy.
- In solid tumors, overexpression of CARM1 correlates with cancer cell proliferation, metastasis, and poor survival outcomes.
Unique analysis sharing similar pattern with mRNA day 23 is GS103528

GSE103528: CARM1 is essential for myeloid leukemogenesis but dispensable for normal hematopoiesis

- 3 leukemia cell lines treated with short hairpin inhibition of CARM1 or short hairpin scramble control.
- Knockdown of CARM1 impairs cell cycle progression, induces apoptosis and downregulated E2F target genes in leukemia cell lines

Hypothesis: CARM1 may be involved as well in the post-natal mouse heart biology
Knockdown of CARM1 induces a similar program to day 23 post-natal heart

Upstream Regulators

Canonical Pathways

Diseases & Functions
Upstream Regulator Analysis indicates inhibition of cell cycle progression

All upstream regulators (only transcription factors) predicted to be inhibited and activated at day 23 vs day 1

Cell cycle progression decreased
CARM1 itself is downregulated in post-natal mouse heart at day 23

CARM1 (down-regulated) is connected to transcription regulators and induces a decrease of cycle progression at day 23
Activation of CARM1 may allow cell cycle to progress again

CARM1 is upregulated at day 4 and is driving increase of cell cycle progression

Hypothesis: activating CARM1 at day 23 would re-initiate cell cycle progress
Visualize the connections of important genes in fetal heart and post-natal mouse heart

OmicSoft
Expression of important genes in GTEX and connections to predictions
CARM1, PPARGC1A, and PTGER2 expression profile in normal heart tissue or in blood

PPARGC1A is enriched in heart and predicted to be activated at day 23
CARM1A is not enriched in heart and down-regulated at day 23
PTGER2 is not enriched in heart and predicted to be inhibited at day 23
Dynamic correlation with CARM1 in fetal heart

COX5B is positively correlated with CARM1 and FTX is negatively correlated with CARM1.

COX5B is correlated with CARM1 in fetal heart and is the terminal enzyme in the mitochondrial respiratory chain. FTX is a long non-coding RNA is involved in cardiomyocyte apoptosis and is inversely correlated with CARM1.
Dynamic correlation with CARM1 in post-natal heart

Laminin A is correlated positively with CARM1, TUG1 is negatively correlated with CARM1 in adult heart.

LMNA is correlated with CARM1 in adult heart and is important in structural scaffolding of nuclear lamina. TUG1 is a long-non-coding RNA and is participating in hypoxia mechanism in myocardial injury involving WNT pathway essential in heart development.
Conclusion: Multi-omics analyses in postnatal mouse heart

- A potential transcriptional program with TFs (PPARGC1A, PPARGC1B, etc.) is detected and drives the metabolism switch in post-natal heart.

- One master regulator, PTGER2, is predicted to be inhibited at day 23, its activation could revert the arrest of cell cycle in post-natal heart.

- Four isoforms connected to heart development are specifically down-regulated in post-natal heart (ALDH1A2-201, BIRC5-201, CCNA2-201, E2F2-201).

- A common signature between postnatal mouse heart and AML was detected, this signature indicates CARM1 as a major player in cell cycle progression in post-natal heart.

- CARM1 is correlated with important genes involved in myocardial function or structure (COX5B, FTX, LMNA, TUG1).
Conclusion

Secondary analysis in Array Studio of RNAseq data
- Find differentially-expressed genes/transcripts
- Send the data to IPA

Biological interpretation of the whole transcriptome, proteome, and metabolome
- Identify significantly differentially expressed isoforms and their association to post-natal mouse heart
- Generate novel regulatory networks as hypotheses suggesting drivers of the expression changes observed in postnatal mouse heart.
- Compare this analysis across a repository of processed datasets from OmicSoft Lands (Analysis Match)
- Visualize a specific gene of interest in OmicSoft Lands
Customer support and additional resources

Contact

Contact us via email or telephone

Tel:
- Global: +1 (650) 381-5111
- US toll free: +1 (866) 464-3684
- Denmark toll free: +45 80 82 01 67
- German toll: +49 (0)341 33975301

Email:
AdvancedGenomicsSupport@qiagen.com

Reply

A response within ONE business day

Hours

08:00 - 17:00 Pacific
08:00 - 13:00 GMT

Websites:
www.qiagenbioinformatics.com
http://tv.qiagenbioinformatics.com

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Sample to Insight
Resources

QIAGEN IPA


• IPA Analysis Match: https://tv.qiagenbioinformatics.com/video/37242337/exploring-ipas-analysis-match-an


• Coronavirus Network Explorer: https://digitalinsights.qiagen.com/coronavirus-network-explorer/

QIAGEN OmicSoft:


QIAGEN CLC Genomics

QIAGEN expands integrated coronavirus NGS and software solutions to accelerate COVID-19 research

- **QIAseq SARS-CoV-2 Primer Panel converts viral RNA samples into libraries ready for sequencing**

- **QIAGEN Digital Insights solutions support COVID-19 drug, vaccine and epidemiology research**


- To explore QIAGEN’s NGS-specific solutions for COVID-19 research, please visit [https://go.qiagen.com/CoronavirusNGS](https://go.qiagen.com/CoronavirusNGS)

- For details of QIAGEN’s SARS-CoV-2 Whole Genome Sequencing Service, please visit [https://www.qiagen.com/applications/genomic-services/sars-cov-2-whole-genome-sequencing-services](https://www.qiagen.com/applications/genomic-services/sars-cov-2-whole-genome-sequencing-services)
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Contact us via email or telephone

Telephone:
• Global: +1 (650) 381-5111
• US toll free: +1 (866) 464-3684
• Denmark toll free: +45 80 82 01 67
• German toll: +49 (0)341 33975301

Email:
sts-bioinformatics@qiagen.com

Websites:
https://digitalinsights.qiagen.com/
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08:00 - 17:00 Pacific
08:00 - 13:00 GMT

Hours
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