In silico and RT-qPCR analysis of the 8q22.2 region in muscle invasive bladder cancer

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1 Works for me dx.doi.org/10.17504/protocols.io.bmewk3fe

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data download

1 Access cBioPortal (https://www.cbioportal.org/) and select the Bladder Cancer (TCGA, Cell 2017) study.

1.1 Select genomic profiles to putative copy-number alterations from GISTIC and mRNA expression z-Scores relative to diploid samples (z-score 1,2,3).
Select case set samples with mutation data (n=402).
Enter the genes of the 8q22.2 region: RNF19A, SPAG1, RGS22, POLR2K, FBXO43, COX6C, VPS13B, STK3, OSR2, KCNS2, RPL30, RIDA, POP1, NIPAL2, ERICH5.

1.2 Select clinical data by clicking on the pie chart symbol next to the Bladder Cancer (TCGA, Cell 2017) study.

1.3 Download data as txt. files. Import txt.files into JMP SAS 14.0 (SAS, Cary, North Carolina, USA). Merge clinical, CNA and mRNA files into a single file.

2 Open the XENA browser (https://xenabrowser.net/) and select mutations

exclusion criteria

3 Apply the following exclusion criteria to the dataset: neoadjuvant chemotherapy, unknown tumor stage (Tx) or tumor stage <T2.

Oncopront visualization

4 Visualize the data in an oncoprint from cBioPortal by copying the sample IDs and inserting them in the select patient set.

Create Kaplan Meier Curves

5 Create an Excel sheet. Copy and paste the Overall Survival (Months), the OS Zensor (1=living) and the respective amplicon column (DNA_Amplicon_Core, RNA_Amplicon_Core, DNA_Amplicon_Ext1, DNA_Amplicon_Ext2).

5.1 Now apply a filter to the amplicon columns. Select AMP to view amplified cases only. Now go to the OS Zensor column and delete the respective cases. Clear the filter.

5.2 Open GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, California, USA). Paste the Overall Survival (Months) column into the document. Now copy the OS Zensor column with the deleted AMP cases next to the Overall Survival (Months) column. Name the column DNA_NONAMP_Core.

5.3 Repeat steps 5.1 and 5.2 for the NONAMP cases.

5.4 Use the same process to create Kaplan Meier Curves for the other respective amplicon columns.

RNA Extraction

6 RNA was extracted from 10 µm thick formalin-fixed, paraffin-embedded tissue slides with the bead-based XTRAKT FFPE kit (Xtract® kit; STRATIFYER Molecular Pathology GmbH, Cologne, Germany). Further information can be found in the instructions manual (http://www.stratifyer.com/wp-content/uploads/file/2015-03-20%20Manual%20XTRAKT%20FFPE%20Kit_version2_english.pdf).

RT-qPCR

7 Relative quantification of mRNA expression of *COX6C*, *OSR2* and the reference gene *CALM2* was measured on a StepOnePlus Real-Time PCR system (Life Technologies, Karlsbad, California, USA) with the SuperScript III Platinum

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One-Step quantitative RT-PCR system (Invitrogen, Karlsruhe, Germany) using Taqman FAST advanced master Mix (Life Technologies Corporation, Austin, Texas, USA).
RT-qPCR was performed at an annealing temperature of 60°C using the Taqman FAST protocol on the StepOnePlus Real-Time PCR system with 95°C for 20 seconds, followed by 95°C for 3 seconds and finally, 60°C for 30 seconds.
Further information can be found in the respective instruction manuals:
(StepOnePlus Real-Time PCR system: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Fcms_046736.pdf&title=QUIgU3RlcE9uZSBhbmQgU3RlcE9uZVBsdXMgUmVhbC1UaW1lIFBDUiBTeXN0ZW1zIC0gUmVsYXRpdmUgU3RhbmRhcmQgQ3VydmUgYW5kIENvbXBhcmF0aXZlIENUIEV4cGVyaW1lbnRz, https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Fcms_046739.pdf&title=TWFudWFsOiBBQiBTdGVwT25lIGFuZCBTdGVwT25lUGx1cyBSZWFsLXRpbWUgUENSIFN5c3RlbXMgLSBSZWFnZW50IEd1aWRl,
SuperScript III Platinum One-Step quantitative RT-PCR system: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Fsuperscript_onestep_qrtpcr_man.pdf&title=UHJvZHVjdCBJbmZvIFNoZWV0OiBTdXBlcNjcmlwdCBJSUkgUGxhdGludW0gT25lLVN0ZXAgcVJULVBDUiBLaXQ=,
Taqman FAST advanced master Mix: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_084554.pdf).

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