About the InnoSIGN Portal

The InnoSIGN Portal uses Comprehensive Pathway Analysis (CPA) to determine the activity of signal transduction pathways by interpreting gene expression values from target genes of the pathway transcription factors with computational pathway models. CPA enables functional and quantifiable assessment of signal transduction pathway activity in tissue samples, such as tumor samples. The expression levels of selected target genes of these pathways are measured by the customer using RNASeq; our sophisticated computational models interpret the data and indicate the activity scores of the different pathways via the InnoSIGN Portal.

Intended Use

CPA quantitatively measures (on a scale from 0-100) defined functional activity for the Estrogen Receptor (ER) cell signaling pathway, Androgen Receptor (AR) pathway, PI3Kinase (PI3K)[1] pathway, MAPKinase (MAPK)[2] pathway, Hedgehog (Hh) pathway, Notch pathway, TGFβ pathway, NF-κB pathway, JAK-STAT1/2 pathway and WNT pathway in disease tissue.

- CPA is available for Research Use Only (RUO) and cannot be used for diagnostic decisions.
- Please be aware that the biological range per pathway may differ among tissue types.
- For the interpretation of the results a reference is recommended.

[1] The PI3K reading is derived from the inverse activity reading of the FOXO transcription factor.
[2] The MAPK reading is derived from the activity reading of the AP1 transcription factor.

Limitations for Use

WARNING: Do not use CPA for any purpose other than those for which it is intended.

WARNING: CPA has been validated using gene expression values obtained as explicitly prescribed in these Instructions for Use. When using other protocols, tools and algorithms than described, reliable results cannot be guaranteed.

Compliance

CPA complies with relevant international and national standards and laws. Information on compliance is provided on request.

Training

Typical users of CPA are bio-informatics professionals skilled in performing genomics analysis. Reading our Instructions for Use should be sufficient to perform CPA. However, if needed, and depending on the experience of the user, training can be provided.

Preparing data for processing in the InnoSIGN Portal

This section provides instructions for using the generating the data required as input for CPA.

RNASeq library preparation requirements

This section describes the requirements of the sample and laboratory protocols for performing RNASeq. Whole transcriptome bulk RNA sequencing should be done and the instructions of the manufacturer should always be followed. The minimal amount of reads per sample type are listed in the table below.

Sample type	Minimal amount of reads (fixed minimum)
FFPE RNA	100 Million
FF RNA (RIN<7)	50 Million
FF RNA (RIN>7)	25 Million

Table 1 Preferred sequencing depth for various sample types

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Sequencer

All commonly used sequencers are supported (e.g. Illumina or BGI). Please contact us in case of using another sequencer.

Sequencing

Paired-end; 2x50bp / 2x75bp / 2x100bp / 2x125bp /2x150bp, or similar. Single-end sequencing is also possible.

Bioinformatics pipeline requirements

FASTQ files obtained as output of the RNASeq process should be processed using a typical bioinformatics pipeline.

Read Trimming

Read/adapter trimming should be performed using commonly used tools, such as *Trim Galore.*

Read Alignment and quantification of gene expression

CPA is validated using RSEM+STAR, but any aligner providing similar results should work. Please refer to the RSEM Documentation for more information.

The hg38 reference genome (GRCh38 assembly) and transcriptome should be used for alignment and quantification.

Quality control

In the InnoSIGN Portal gene expression values are used to perform CPA. Before a result is presented to the user, several Quality Control steps are performed.

However, since typical RNASeq Quality Control is also performed on the raw FASTQ input data, it is recommended that users conduct such QC themselves before submitting any data to the InnoSIGN Portal. Commonly used tools for this purpose include FastQC and RSeQC.

InnoSIGN Portal input requirements

The InnoSIGN Portal only accepts text-based tab-separated values (ending on .txt or .tsv). The file should be in the following format:

- Column 1: Title 'gene_id'; Contents: Ensembl ID values for all genes
- Column 2 – end: Title: Sample ID; Contents: Gene expression values as TPM (Transcripts Per Million) values.

An example of an input file is shown in Figure 2.

gene_id	Breast_2	Lung_3	Prostate_1	Breast_1	Colon_3	Blood_3
ENSG00000000003	15.12	47.94	23.67	49.9	46.8	62.94
ENSG00000000005	0	0.63	0.02	0.04	0	0
ENSG00000000419	52.7	52.18	30.04	30.74	61.61	33.02
ENSG00000000457	8.23	7.97	3.45	16.7	8.36	5.83
ENSG00000000460	7.02	6.43	4.62	6.31	14.78	3.11
ENSG00000000938	16.77	10.37	7.58	13.77	10.75	9.35
ENSG00000000971	16.71	59.62	5.35	11.15	55.64	72.54
ENSG00000001036	32	36.18	25.32	22.5	32.96	25.34
ENSG00000001084	126.94	13.34	79.02	23.91	21.96	32.96
ENSG00000001167	16.51	24.11	7.15	19.31	20.91	17.28
ENSG00000001460	8.68	13.72	6.03	19.13	13.3	9.13
ENSG00000001461	26.21	19.87	4.67	52.63	17.51	20.85

Figure 1 Example of a tab-separated values input file. Only the top rows are shown.

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