QIAGEN IPA Introduction
Where does IPA fit in?

**Experimentation** → **Statistical Analysis** → **Biological Interpretation**

INGENIUTY® PATHWAY ANALYSIS
Understand How Scientists are Using IPA at Ingenuity.com

Search Page showing results for alzheimer*

Narrow Search Results:
- Clear All Filters
- Publication Date
  - All
- Research Area
  - Metabolomics
  - Methylation Profiling
  - miRNA
  - Next-Gen Sequencing
  - Proteomics Profiling
  - RNAi
- Journal Title

Results from the publications citing the use of IPA

- Graph showing the number of publications citing IPA over time, from 2003 to Dec 2014.
  - 1 publication in 2003
  - 18 publications in 2004
  - 104 publications in 2005
  - 297 publications in 2006
  - 686 publications in 2007
  - 1,306 publications in 2008
  - 2,306 publications in 2009
  - 3,786 publications in 2010
  - 5,608 publications in 2011
  - 7,704 publications in 2012
  - 9,972 publications in 2013
  - 12,513 publications thru Dec 2014
Comprehensive Curation of the Scientific Literature

For 15+ years, our MDs and PhDs have scoured the scientific literature so you don’t have to!

We document

- All known biological processes/pathways
- All known variants and their genes
- All known relationships between observed phenotype and genes
- All known relationships between known drugs and targets/pathways

THE QIAGEN KNOWLEDGE BASE
Quality, relevant content can't be spotted by an algorithm. You can't subscribe to it. You need people - actual human beings - to create or curate it.

-Christina Halvorson, CEO Brain Traffic

- Public databases are rife with errors and inconsistencies
- Voluntary submissions are sporadic or incomplete
- Quality of public content is hit/miss
Ingenuity Variant Analysis By The Numbers

5,300,000+
Expert-curated human phenotype-associated variant findings in the Ingenuity Knowledge Base

400,000+
Human samples analysed using Ingenuity Variant Analysis

25,000+
Scientific publications citing QIAGEN Bioinformatics references
## Imported annotations and findings

- Entrez Gene
- RefSeq
- OMIM
- ClinVar
- COSMIC
- GWAS Database
- Gene Ontology
- Human Metabolome Database (HMDB)
- GNF Tissue Expression Body Atlas
- NCI-60 Cell Line Expression Atlas
- BIND, DIP, MINT, MIPS, BIOGRID, INTACT, COGNIA protein-protein interactions (updated)
- TarBase
- TargetScan
- miRecords
- Clinicaltrials.gov
- Drugs@FDA.gov
- Mosby’s Drug Consult
- Goodman & Gilman’s ‘Pharmacological Basis of Therapeutics’
- DrugBank
- Hazardous Substance Database (HSDB)
- Chemical Carcinogenesis Research Information System database (CCRIS)
The QIAGEN Knowledge Base: Content & Content-aware Analytics

- Cancer Scoring
- Hereditary Disease Scoring
- Causal Network Analysis
- Druggable Pathways
- Disease Model-based Analysis

Published Biomedical Knowledge

QIAGEN Knowledge Base and Ontology

Content-Aware Analytics

Structure

Compute

Integrate

Organize

Synthesize
In 129S1/Sv * 129X1/SvJ * Swiss Webster mouse, homozygous mutant mouse **Pex2** gene (allele Pex2<sup>tm1Pfl</sup>/Pex2<sup>tm1Pfl</sup>) (knockout) increases cholestasis in mouse.


**Disease/phenotype:**
- **Species:**
- **Strain:**
- **Gene:**
- **Zygosity:**
- **Mutation type:**

**Direction of effect on disease/phenotype:**

**Activity of the molecule in this finding (decreased):**

This structure powers the algorithms for causal analysis.
Entry Points in IPA

Biological Questions

Search

Experimental Data

Custom Pathway

BioProfile

Bio/Tox Functions
Diseases / Disorders
Canonical Pathways
Upstream Regulators
Mechanistic / Causal Networks
Interaction Networks

Expression Arrays
Mass Spec
Protein Arrays
2D Gel Electrophoresis

Comparison Analysis

Communicate & Collaborate
IPA: A Unique Resource for Biological Analysis and Interpretation

Gene View, Chem View, and Disease/Function View

Gene View: CASP8 (Mammalian) > Interaction Network > View Reagents (226)

Provide Feedback | Live Support

Summary: Human Mouse Rat

Member Of: caspase, Caspase 8/10
Entrez Gene Name: caspase 8, apoptosis-related cysteine peptidase
Syonym(s): ALPS2B, CASP4, CASPASE-8, FLICE, MACH, MCHS, PROCASP8
NCBI CDI Domains (Superfamilies / Multi-Domains):
- CASc: The Death Domain Superfamily of protein-protein interaction domains
- Active site, apoptosis activation domain, caspase homology domain, catalytic domain, Ced3-homology domain, cleavage site, caspase endopeptidase, death effector domain-interacting domain, FADD-like prodomain, identical protein binding, peptidase domain, prodomain, protease domain, protein binding, tumor necrosis factor receptor binding
- Subcellular Location: cellular membrane, centrosome, Cytosol, cytoplasmic fraction, cytoskeleton, cytosol, cytosolic fraction, membrane raft, mitochondria, mitochondrial inner membrane, mitochondrial intermembrane space, mitochondrial matrix, mitochondrial outer membrane, neurite, Nucleus, perinuclear, Plasma Membrane
- Canonical Pathway:
  - Apoptosis Signaling: CD27 Signaling in Lymphocytes; Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells; Death Receptor Signaling; Erodothelin-1 Signaling; eNOS Signaling; Granzyme B Signaling; Huntington’s Disease Signaling; Induction of Apoptosis by HIV1; Mitochondrial Dysfunction; Molecular Mechanisms of Cancer; Myc Mediated Apoptosis Signaling; NF-kB Signaling; Retinoic Acid and Mediated Apoptosis Signaling; Role of PKR in Interferon Induction and Antiviral Response; Role of ROS-like Receptors in Antiviral Immune Response; Sphingosine-1-phosphate Signaling; TNFR1 Signaling; Tumoral Function of Hepatic Natural Killer Cells; TH17MA Signaling; Type 1 Diabetes Mellitus Signaling
- Targeted By mRNA Function
  - miR-103-5p, miR-1224-3p, miR-1220-3p, miR-1231, miR-1270, miR-128-3p, miR-1287, miR-1290, miR-142-5p/miR-3390-3p, miR-143-3p/miR-4770/miR-143, miR-17-5p/miR-20b-5p/miR-93-3p (includes others), miR-187-5p/miR-187, miR-19b-3p/miR-19b-5p/miR-10a-3p, miR-301a/miR-30b-5p/miR-138a-3p (includes others), miR-339-5p/miR-3586-5p, miR-512a-3p/miR-512c-3p, miR-518a-3p/miR-518b-3p (includes others), miR-548b-3p, miR-548b-5p/miR-3579/miR-3586-5p
- Cluster
  - miR-548b/miR-548av-5p, miR-548p, miR-576-5p, miR-590-3p/miR-590-5p/miR-21-5p/miR-21, miR-607 (Human), miR-644-3p/miR-579, miR-709/miR-1827, miR-989

Top Findings from Ingenuity Knowledge Base (show all 6916 categorized literature findings)

regulates: CASP3, BID, HTT, CASP7, PARP1, CASP8, NFkB, CASR6, CASP9 (includes ES:100140945), APP, STRK4, PAK2, MCL1, NUP351, RB1
regulated by: FAS, TUNSF10, TNF, FADD, FASLO, CFLR, CASP8, duxoxibulin, etoposide, BCL2, GLMV, CYC5, cyclin, hexokinase, staurosporine, IFN3 (includes ES:15978)
binds: FADD, FAS, CFLR, RBPK1, TUNSF10, CASP8, CASP10, TUNSF10A, TRAF2, FASLG, NOL3, BID, TRADD, CASPR2, HET
role in cell: apoptosis, cell death, activation in, molecular cleavage in, proliferation, transmembrane potential, cleavage in, necrosis, blabbing, formation in
IPA: A Unique Resource for Biological Analysis and Interpretation

Human Isoform Views

Human Isoforms From RefSeq

CASP8 Chromosome: 2
Location: 2q33-q34

Domains

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<tr>
<th>Gene</th>
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IPA: A Unique Resource for Biological Analysis and Interpretation
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<th>Predicted Effect</th>
<th>Activation z-score</th>
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Regulator Effects

contractility of cardiac muscle

function of heart

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IPA: A Unique Resource for Biological Analysis and Interpretation

microRNA tools

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<tr>
<th>ID</th>
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<th>Symbol</th>
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<th>Disease</th>
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<td>increases</td>
<td>flow of bile</td>
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<td>1</td>
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</table>
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Interaction Networks, Build and Overlay tools
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IPA: A Unique Resource for Biological Analysis and Interpretation
Getting Started

System/Software Requirements Supported Operating Systems:
- PC: Windows XP (SP2 or later), Windows 7, Windows 8
- Mac: Mac OS X Mavericks, Lion, Mountain Lion

Supported Browsers:
- PC: Internet Explorer (IE6 or later), Firefox (FF5 or later), Chrome 10 or later
- Mac: Safari (5.0.5 or later)

Supported Java Versions:
- 1.6.0_xx, 1.7.0_xx

There are known issues with Java that conflict with IPA. Contact support for the specific issues.

IPA Login Steps and Troubleshooting
http://www.ingenuity.com/customer-support/system-requirements
http://www.ingenuity.com/customer-support/login-troubleshooting

Java 1.6.0_45 version from

Java 1.7.0_xx version from
java.com/en/download/manual.jsp

To set-up correctly Java on Mac
http://ingenuity.force.com/ipa/IPATutorials?id=kA250000000TNeuCAG


https://analysis.ingenuity.com/pa/installer/select
IPA training videos: Search & explore

IPA search and explore series videos:

- The Ingenuity Knowledge Base for IPA  http://youtu.be/4lFxsfMkpQg
- Searching and accessing the Knowledge Base  http://youtu.be/iU9ihqzfeEY
- Building a pathway: Filtering and growing  http://youtu.be/8rYEs8F0Cws
- Building a pathway: Exploring the path of interaction  http://youtu.be/---TRmuMVP9E
- Overlay contextual information  http://www.youtube.com/watch?v=rSp8X6Y6Wlc
- Editing a pathway for publication  http://youtu.be/yEJjqIUM4So
IPA data analysis series videos:

- Data analysis : Part 1 (Data upload)  http://youtu.be/XrdMN9eGWjg
- Data analysis : Part 2 (Results interpretation)  http://youtu.be/PfF_Ru73-1o
- Comparison analyses  http://youtu.be/JCanWpyfvQE
- Analysis results  http://youtu.be/rrppI9OGPUY
- Statistical calculation  http://www.youtube.com/watch?v=0oxCQ9dOQIE
- Canonical pathways  http://youtu.be/6iZdD9Ojll0
- Network Analysis  http://youtu.be/eReZrNE2bWY
- Downstream effects analysis  http://youtu.be/CYMrhwuvVKs
- Upstream regulator analysis  http://www.youtube.com/watch?v=X2bStYNJXm4
- Human isoforms  http://youtu.be/Po07vk3pOVE
- Molecular toxicology  http://youtu.be/m1nYDFdY_Zg
- Biomarker filter and comparison analysis  http://youtu.be/XQFUy0s6wCU
- MicroRNA target filter  http://www.youtube.com/embed/06xoKQL9-KA
Epithelial-to-Mesenchymal Transition

- best prognosis of all intrinsic subtypes
- responds to endocrine therapy
- corresponds to invasive lobular breast cancer

HCC1428
MCF-7
MDA-MB-361
T47D
ZR-75-1

• poor prognosis
• invasive ductal carcinomas
• high frequency of metaplastic and medullary differentiation

BT-549
HS578T
MDS-MB-231
SUM1315-PT
SU159-PT

Breast development

Luminal cells in ducts
Luminal progenitors
MaSC (stem cell)

Luminal Breast cancer
HER2-enriched
Basal
Mesenchymal

Ratio Claudin-low to Luminal
5 vs 5 cell lines, RNA-Seq data

Claudin-low cell lines

New IPA Client Installer

https://analysis.ingenuity.com/pa/installer/select
The summary of analysis is accessible while rest of results load into IPA.

Graphs have been added near the right side of each section to graphically depict the results (p-values). Distribution of p-values for functions within the category is visualized (median).
Cell Movement and EMT are significantly increased in Claudin-low Subtype
How EMT is predicted being increased in Claudin-low breast cancer cells?

Yellow glow surrounds genes that have multiple isoforms in the dataset.
Regulation of CD44 alternative splicing contributes to EMT

- CD44 protein isoforms are involved in proliferation, adhesion, and migration.
- CD44 variant 4 / CD44s accelerates EMT and breast cancer progression; overexpressed in tumors
Do other regulated isoforms in Claudin-low cells affect cell movement and invasion?

IsoProfiler (Fall release 2015)

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<tr>
<th>Molecule</th>
<th>Symbol</th>
<th>Tissue</th>
<th>Protein</th>
<th>Function</th>
<th>Evidence</th>
<th>Disease or Function</th>
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Human PKM2 protein increases migration of Dld 1 cells in cell culture.

Experiment Type: wound closure assay


Source: Ingenuity Expert Findings
What are the underlying transcriptional programs for EMT in Claudin-low cells?

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<th>Upstream Regulator</th>
<th>Fold Change</th>
<th>Molecule Type</th>
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<th>Activation z-score</th>
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Transcriptional Program in Claudin-low Breast cancer cells

Add EMT to the upstream regulator network and predict the effect on the function activity (MAP)

Use PathTracer to highlight gene targets and transcription factors involved with EMT
How might TWIST1 drive the expression changes in Claudin-low subtype?
SB203580 might be able to reverse EMT activation and migration in Claudin-low cells

Assess hypotheses for how predicted regulators impact phenotype, function or disease (Regulator Effects)

Z-score = -4.353

Add a compound can reverse the phenotype (MAP)
Which significant pathways tend to be activated or inhibited in Claudin-low cells?

PTEN signaling is predicted inhibited and PI3K/AKT signaling is predicted activated.
PTEN1 Signaling Pathway

Overlay: Expected Activation State

Overlay: Claudin-low vs. Luminal

Overall Activation Score of Eligible Pathway

\[ z = \frac{x}{\sigma_x} = \frac{\sum x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}} = -1.732 \]
Overlay: Expected Activation State

Z = 1.941

vs. Luminal

n-low
The Claudin-low-regulated gene set shows a strong increased for cell movement and EMT activation compared to luminal breast cancer cells.

Several isoforms regulated in claudin-low subtypes may contribute to cell movement and increased invasion-related processes.

Upstream analysis and mechanistic networks can identify causal paths from transcription factors (TWIST1, SNAI1, ZEB1) to activated EMT, pro-metastasis function and increased invasion in claudin-low cells.

Regulator Effects can help identify novel drugs or drug candidates targeting key gene targets involved in reactivation of EMT program and cell invasiveness.

Reorganization in actin cytoskeleton and cell migration-associated molecular interactions are highlighted in significant canonical pathways.

Interestingly, PTEN signaling pathway is predicted to be inhibited in claudin-low cells with PTEN1, a tumor suppressor gene known to be frequently inactivated in cancers. Conversely, PI3K/AKT signaling pathway is predicted to be activated in claudin-low cells. PIK3 activity is negatively regulated by PTEN1 activity.