Technical Training #3: Paper Overview

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How to Read & Understand a Scientific Paper

General Tips:

- Different process than reading an article / blog / newspaper
- Read sections in a different order
- Should take notes (have a highlighter & pen ready)
- Read it multiple times
- Should follow up with more research
- May take longer than you think

How to Read & Understand a Scientific Paper

Steps:

1. **SKIM** the abstract
2. Read the **INTRODUCTION**
3. Summarize the background
    a. Identify the BIG QUESTION(S) / purpose
4. Read the **RESULTS**
    a. Focus on figures → you can get a lot of information from them
5. Read the **CONCLUSION** / **discussion**
6. Read the materials & methods
7. NOW read the abstract
8. Follow-up by reading similar papers

What is the main purpose of the research?

- To find specific genetic alterations that likely cause colorectal cancer
  - Prognostic and predictive markers
    - **Prognostic-** identifies alterations and determines likelihood of developing disease, or a recurrence or progression of it.
    - **Predictive-** used in studying and determining appropriate therapeutic interventions; identifies who would have favorable or unfavorable outcomes if given a specific therapy based on biomarkers that are or are not found in others.
  - This paper focuses on prognostic markers for colorectal cancer

State of current research in the field

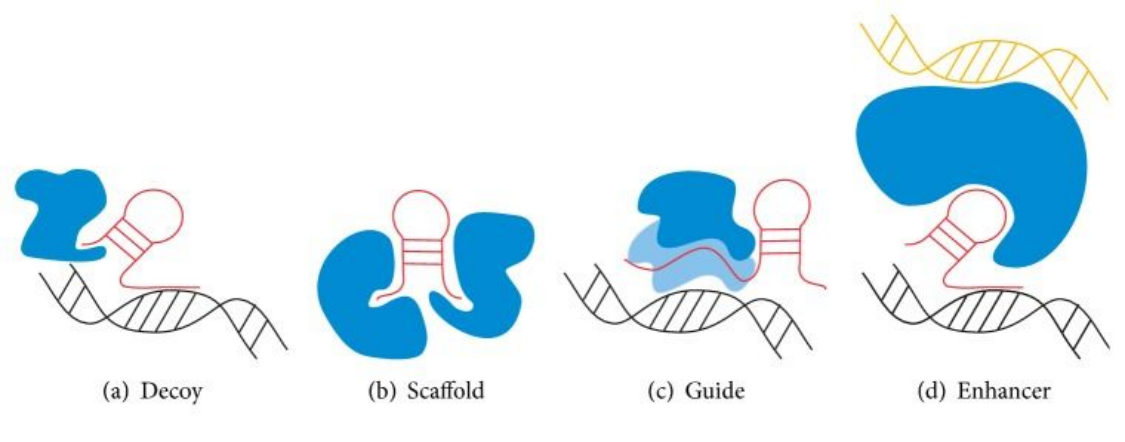
- 2019 study using stool samples for noninvasive testing of biomarkers for CRC
  - Choi HH, Cho YS, Choi JH, Kim HK, Kim SS, Chae HS. Stool-Based miR-92a and miR-144* as Noninvasive Biomarkers for Colorectal Cancer Screening. Oncology. 2019;97(3):173-179. doi:10.1159/000500639
- Using ceRNA networks to find markers for Triple-Negative breast cancer
  - Liu Z, Mi M, Li X, Zheng X, Wu G, Zhang L. lncRNA OSTN-AS1 May Represent a Novel Immune-Related Prognostic Marker for Triple-Negative Breast Cancer Based on Integrated Analysis of a ceRNA Network. Front Genet. 2019;10:850. Published 2019 Sep 13. doi:10.3389/fgene.2019.00850
- Finding lncRNAs (long noncoding RNA) associated with CRC tumorigenesis using ceRNA networks to develop outcome/risk scores for CRC
  - Yang ZD, Kang H. Exploring prognostic potential of long noncoding RNAs in colorectal cancer based on a competing endogenous RNA network. World J Gastroenterol. 2020;26(12):1298-1316. doi:10.3748/wjg.v26.i12.1298

Terminology + Scientific Background

lncRNA

Definition:

relatively well-characterized class of **noncoding RNA (ncRNA) molecules,** involved in the regulation of various cell processes, including transcription, intracellular trafficking, and chromosome remodeling



Context: Work in breakout rooms!

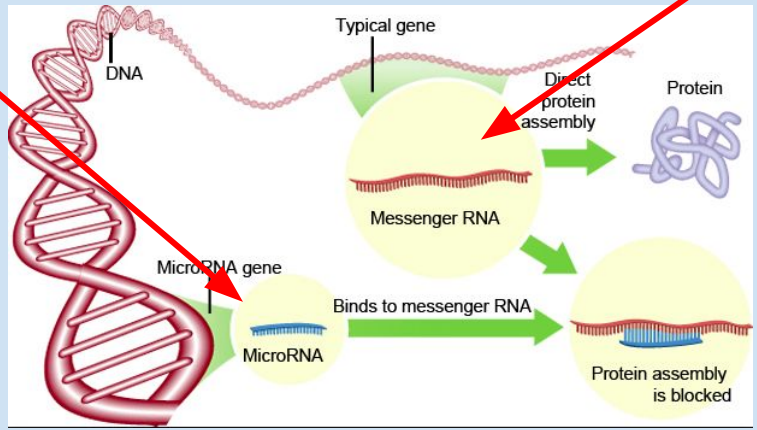
miRNA and mRNA

miRNA

Definition:

MicroRNAs (miRNAs) are small noncoding RNAs that function as major players of posttranscriptional gene regulation in diverse species.

Context: Discuss in breakout rooms!



mRNA

Definition:

Messenger RNA (mRNA) is a single-stranded RNA molecule complementary to DNA strands of gene -- DNA of gene can be transcribed into an mRNA molecule that will end up making one specific protein.

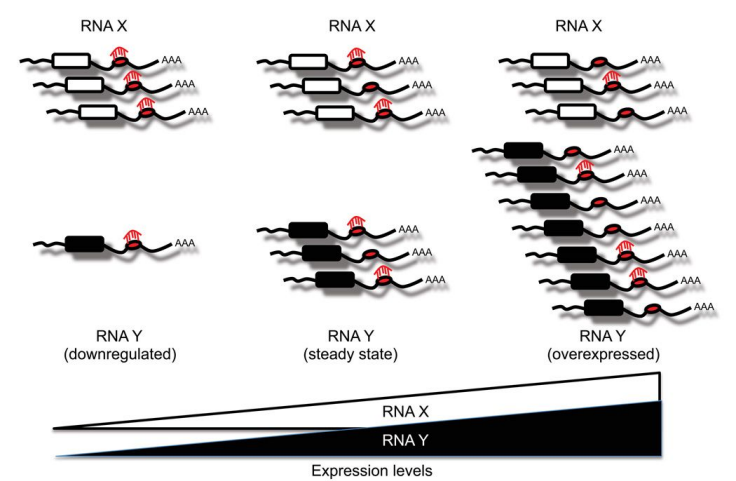
Question: Based on the paper, how do you think mRNA-miRNA interactions may indicate a prognostic marker?

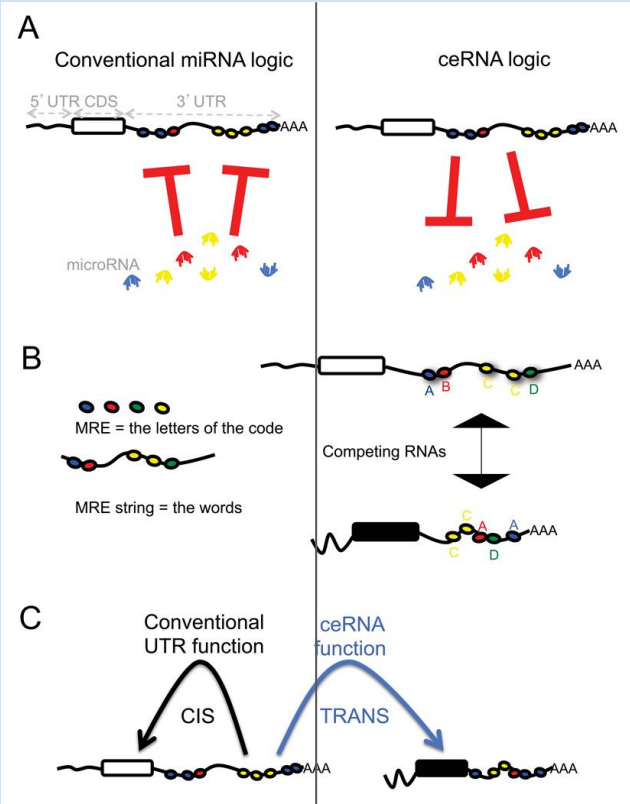
ceRNA and ceRNA Networks

Definition:

competing endogenous RNAs (***ceRNAs***) regulate other RNA transcripts by competing for shared microRNAs. Changes in the expression of one or multiple miRNA targets alter the number of unbound miRNAs and lead to observable changes in miRNA activity

ceRNA Networks: the network of different ceRNAs and how they interconnect



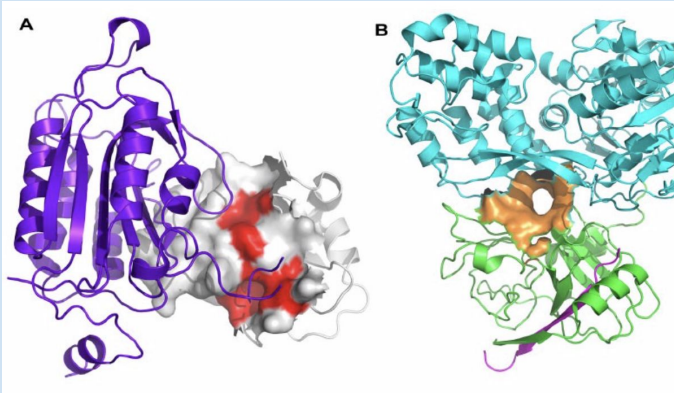


PPI Network

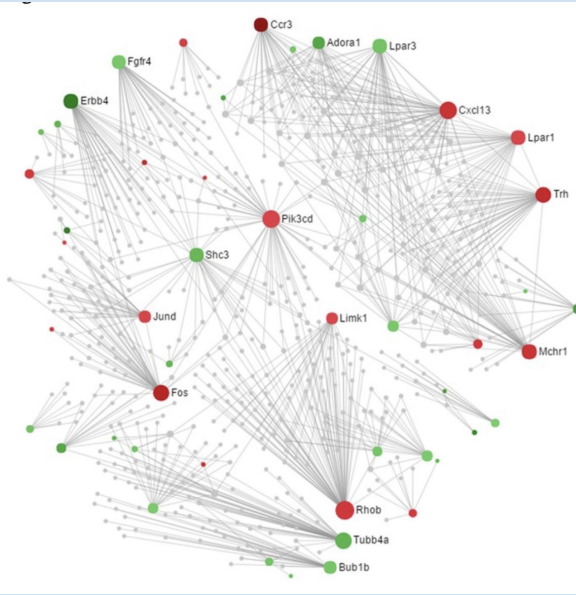
Definition:

PPI = protein-protein interaction, which are the physical contacts of high specificity established between 2+ protein molecules due to biochemical events driven by electrostatic forces, H-bonding, and hydrophobic effects

Context: Discuss in breakout rooms!



PPI



PPI Network Analysis of mRNA Expression Profile

Prognostic Marker

Definition:

Biomarkers used to measure the progress of a disease in a patient, and response to a therapeutic solution. Helpful for stratifying patients into groups. .

Context: Discuss in breakout rooms!

Table1: DNA prognostic markers for common cancer types[3]

Cancer	DNA markers
Thyroid cancer	RET-PTC, NTRK1, PTEN, TP53, PI3K, AKT, CTNNB1, PAX8, RAS, BRAF, TSHR
Bladder cancer	FGFR3, TERT, STAG2, AURKA
Ovarian cancer	TP53, WT1, Ki67, Topo-II, BRCA1, BRCA2
Cervical cancer	Ki67, MYC, p16INK4a, PTEN, Bm-3a
Breast cancer	BRCA1, BRCA2, HER-2, TP53, EGFR
Prostate cancer	GSTP1, MYC, PTEN, APC, PCA3, PSMA, AMACR, BRCA1, BRCA2
Colorectal cancer	KRAS, BRAF, PIK3CA, TP53, APC, SFRP2, ITGA4, GATA4, GATA5, OSMR

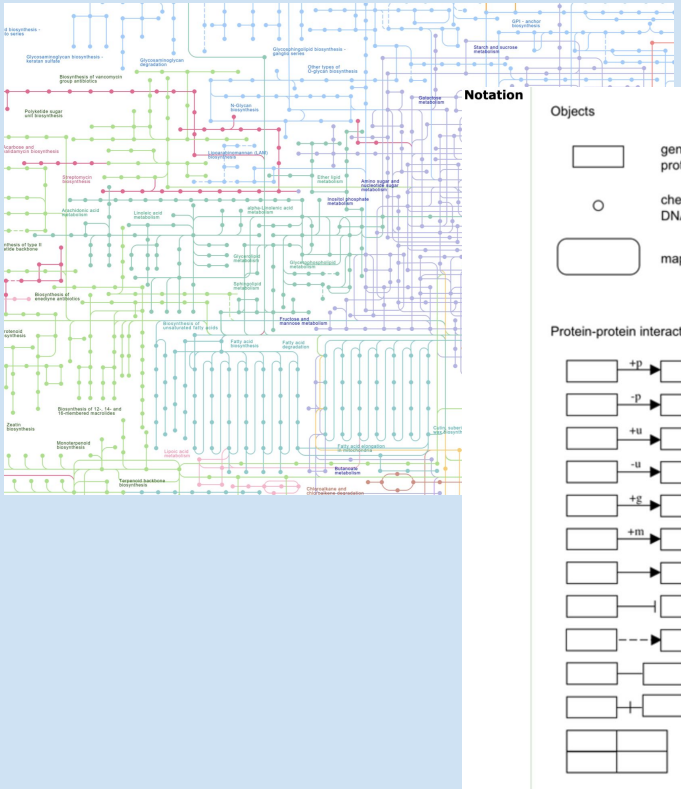
KEGG Pathway

Definition:

KEGG = Kyoto Encyclopedia of Genes and Genomes.

The KEGG PATHWAY database is a collection of manually drawn graphical diagrams (KEGG pathway maps), representing different molecular pathways for metabolism, genetic information processing, etc.

Context: Discuss in breakout rooms!



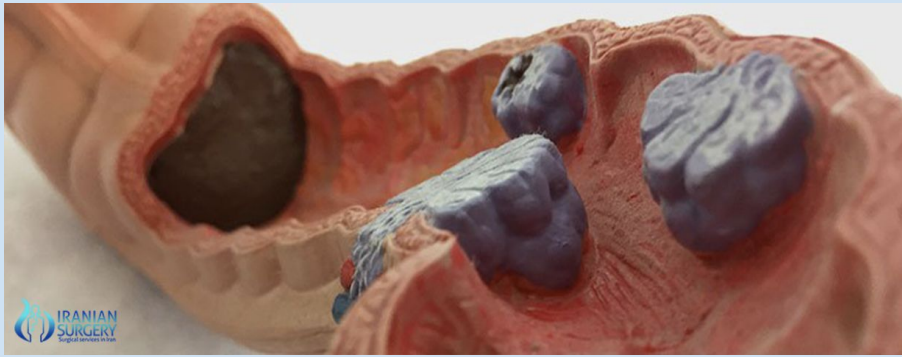
Colorectal Cancer

- 1/21 men and 1/23 women will develop it in the USA
- 2nd leading cause of cancer death in women; 3rd in men
- Death rate is decreasing with advances in screening techniques and improvements in treatment
    - Treatment includes surgery, radiotherapy, and chemo
    - It is dependent on size, location, stage of cancer, and age/overall health of patient

Colorectal Cancer

Risk factors include

- older age
- a diet that is high in animal protein, saturated fats, and calories or low in fiber
- high alcohol consumption
- breast, ovary, or uterine cancer
- a family history of colorectal cancer
- Crohn's disease, or irritable bowel disease (IBD)
- overweight and obesity
- smoking
- a lack of physical activity



Data Sources

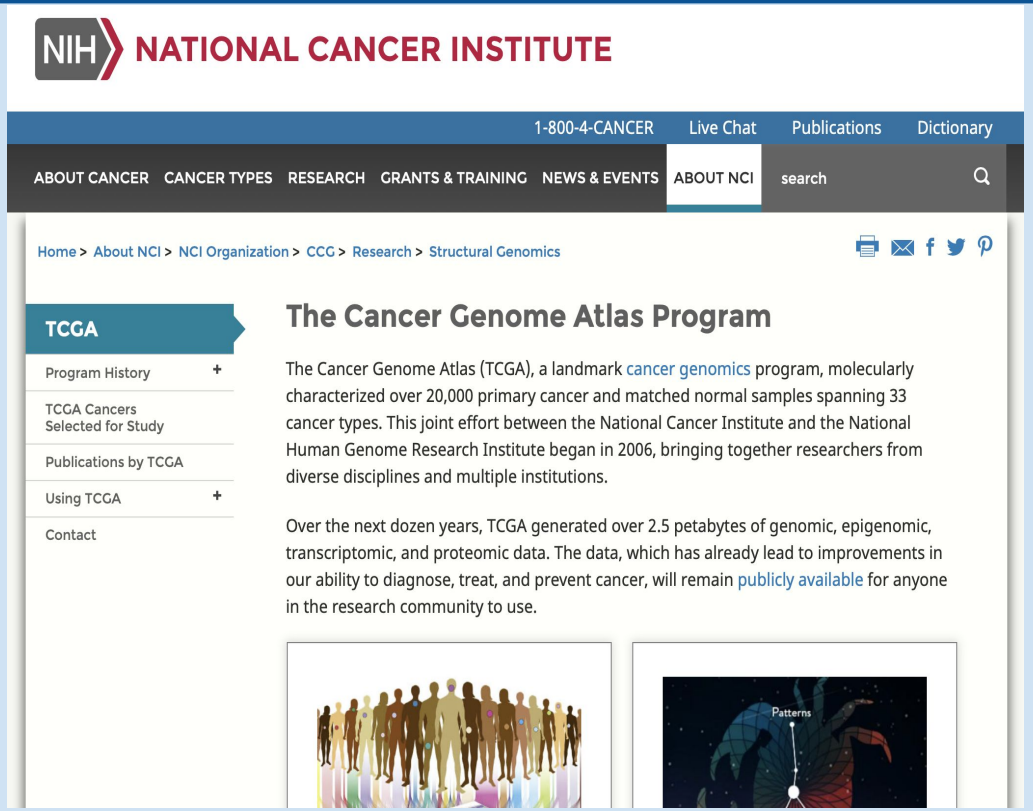
TCGA

- Catalogue genetic mutations responsible for cancer.
- Improve our ability to diagnose, treat, and prevent cancer through a better understanding of the genetic basis of this disease.

For CRC:

- over 500 individuals
- 480 tumor and 41 normal samples

NOTE: As advised by the mentors, we won't be working with TCGA



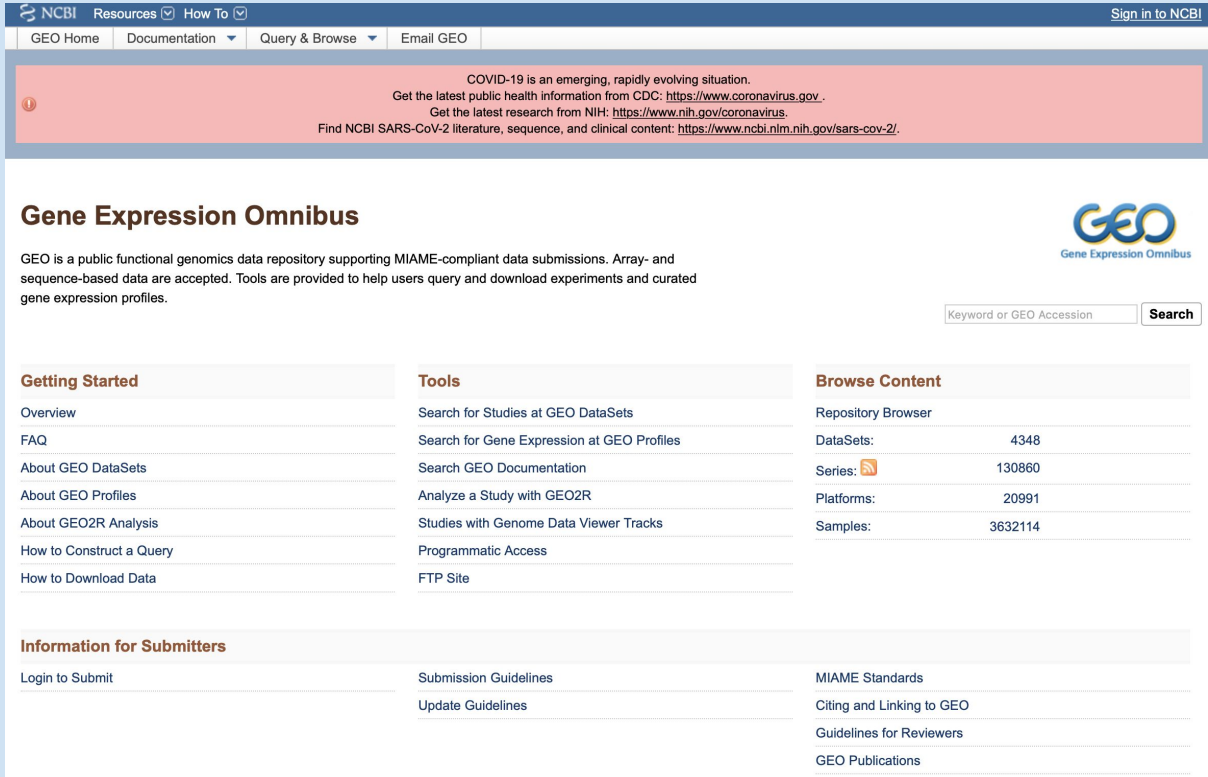
Data Sources

- The largest fully public repository for high-throughput molecular abundance data
- The database has a flexible and open design that allows the submission, storage and retrieval of many data types.

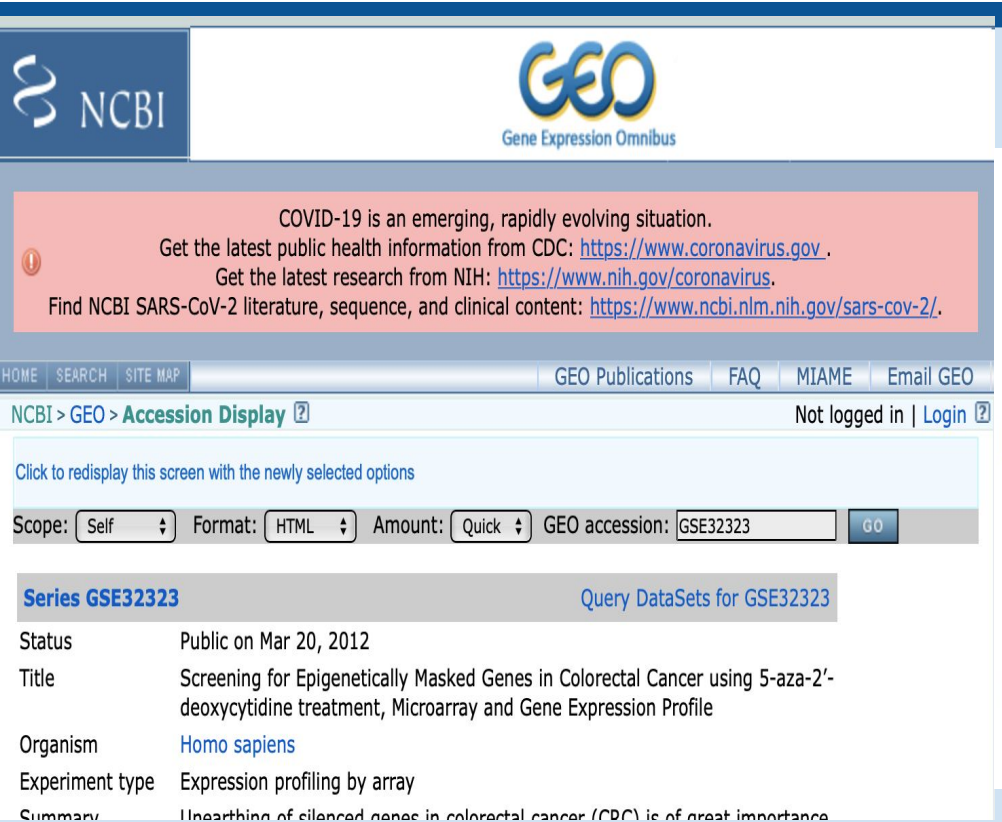
GSE32323 : 17 normal and 17 tumor samples

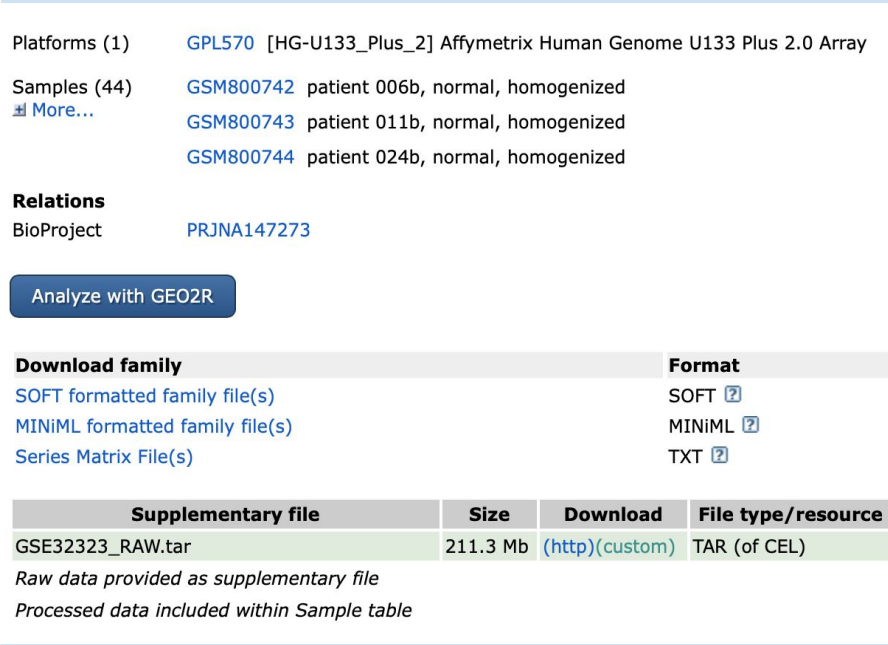
GSE21510 : 44 normal and 104 tumor samples

GSE8671 : 32 normal and 32 tumor samples



Example file Images





MATERIALS AND METHODS

Screening Potential Hub Genes

DAVID

(The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery)

It provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes

Input: You can either load a gene list from a file or paste a gene list to the text box.

Output: It depends on the type of analysis you're doing. In most of the cases you're result is going to be a table.

STRING database

It is a database of known and predicted protein-protein interactions.

The interactions include direct (physical) and indirect (functional) associations.

Input: You can either load a gene list from a file or paste a gene list to the text box.

Output: You can export graphs as images, simple tabular text files or even protein sequence.

MATERIALS AND METHODS

Potential Prognostic Values of Candidate Genes

GEPIA database

- Interactive web server for analyzing the RNA sequencing expression data
- Facilitate data mining in wide research areas, scientific discussion and the therapeutic discovery process.

Input: gene symbols or Ensemble ID

Output: It depends on the type of analysis you're doing. In most of the cases you're result is going to be a table.

StarBase database

- Facilitate the comprehensive exploration of miRNA–target interaction maps
- Useful to assigning miRNAs to their regulatory target genes

Input: Nucleotides of a mature sequence or a mature miRNA sequence

Output: Information about the target gene and visual sequence alignments matched to a specific CLIP-Seq

MATERIALS AND METHODS

Screening Related miRNAs and lncRNAs Based on the Hub mRNAs

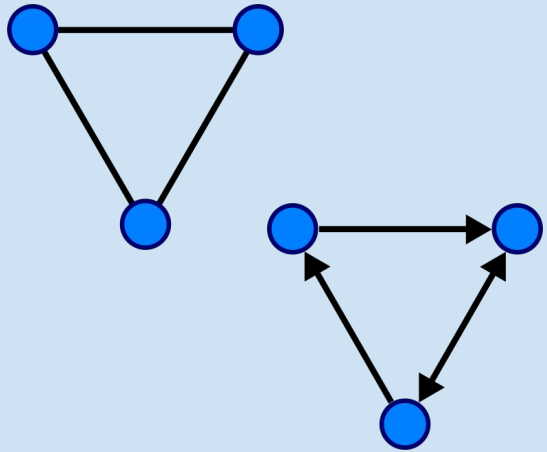
MiRNet

An easy-to-use web-based tool designed for creation, customization, visual exploration and functional interpretation of miRNA-target interaction networks.

Input: a list of miRNAs,genes, small molecules,etc.; or a data table microarray or RNAseq

Output: a graph → What is a graph?

graphs are mathematical structures used to model pairwise relations between objects.



MATERIALS AND METHODS

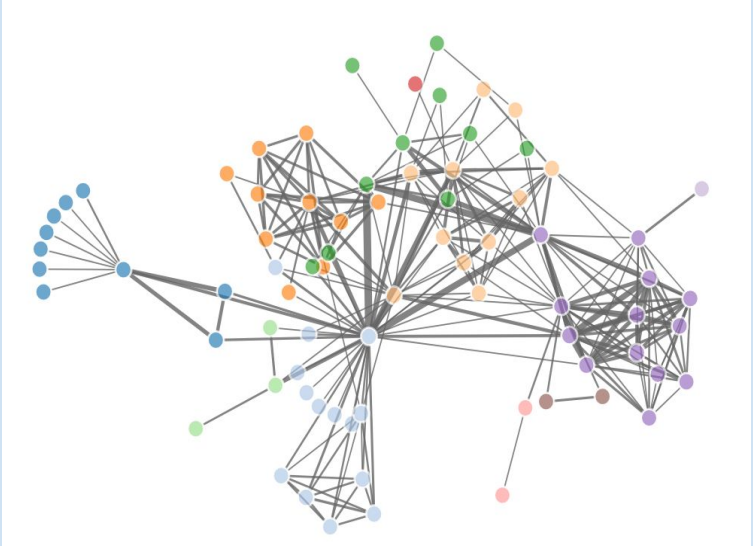
Construction and In-Depth Analysis of the ceRNA Network

Network D3

It makes easy to create these network graphs from R. It is designed to take a simple data frame that has two columns specifying the *sources* and *targets* of the nodes in a network and turn it into a graph. You can easily customise the look and feel of the graph.

Input: Dataframe

Output: Graph



MATERIALS AND METHODS

Statistical Analysis and Network Visualization