

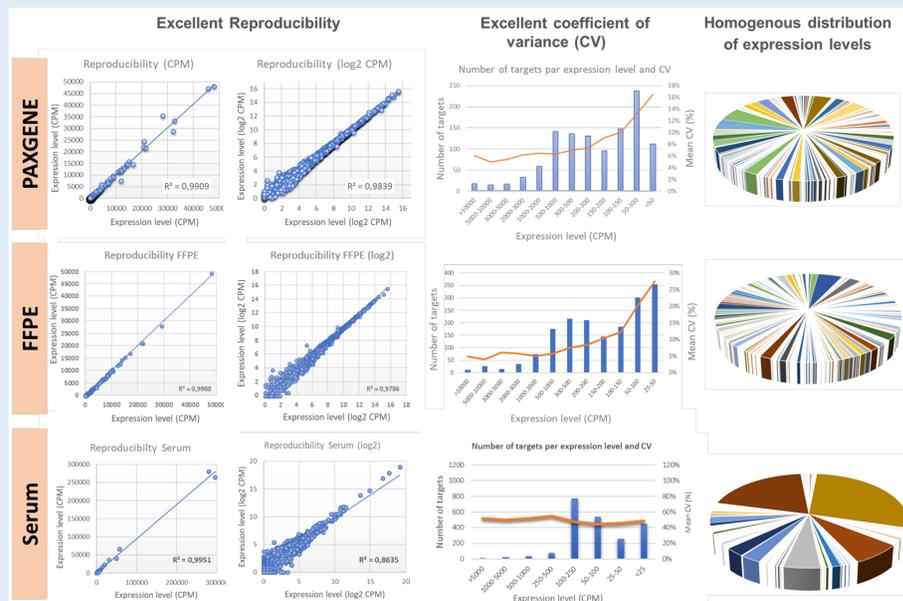
Background:

RA is a chronic, progressive, inflammatory autoimmune disease associated with articular, extra-articular and systemic effects leading to joint destruction. T cells, B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA. A better comprehension of interaction between cytokines and their signaling pathways is key for the development of new strategies with small molecules or biologicals. Today, new technologies allow the specific investigation of inflammatory pathways on mRNA level without extraction step directly from the blood. The BIOPRED panel, based on HTG EdgeSeq platform is a targeted sequencing panel with focus on specific biological pathways including 2155 mRNA from inflammatory and immune pathways. Disease activity specific gene enrichment studies in Rheumatoid Arthritis (RA) & other autoimmune-inflammatory disorders would help pharmaceutical industry tailor pathway specific therapies and would help clinicians to choose optimal & personalized therapy for their patients. Therefore, we have identified active biological pathways in RA patients associated with different disease activity status.

Objectives:

By using Firalis BIOPRED panel, an innovative targeted gene sequencing panel of 2155 mRNA targets associated with immune-inflammatory pathways, our objective is to identify active biological pathways in function to different disease activity status of RA & Healthy volunteer (HV) subjects.

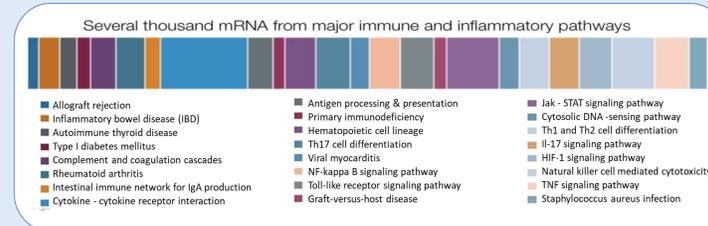
Analytical performances



BIOPRED assay

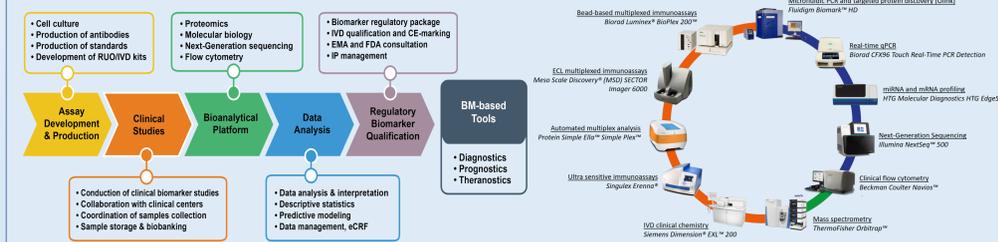
Principle

BIOPRED is intended for the investigation and identification of biomarker candidates for personalized therapy and for the support of drug development. To address these challenges, Firalis has developed a high-throughput precision medicine tool that involves targeted gene sequencing without RNA extraction.



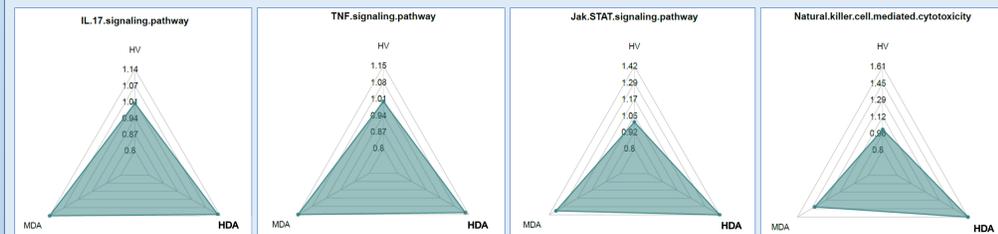
Company overview:

Firalis group company aims to discover, validate and qualify biomarkers. All competences, from assay development, biomarkers measurement, clinical studies and biobanking to the submission to regulatory authorities, are performed within a high quality multi ISO environment (ISO9001, 13485, 17025 and NFS96-900). We are a biotech but also a CRO to help other company reaching their goals.



Results:

After transformation and normalization of the gene expression data, 22 mRNA genes are found to be significantly upregulated in RA (p -value < 0.005, fold change > 2) as compared to HV group. After one-way ANOVA analysis on three groups as stated above, 351 mRNA targets are significantly regulated (p -value < 0.05). Pathway analysis based on protein-protein interaction from Biogrid, String and Intact database was used and score were generated based on fold change for 22 pathways to assess modification in HDA and MDA groups versus HV group. Various pathways including JAK/STAT pathway; IL17 signaling pathway; TNF Signaling pathway and Natural killer cell mediated cytotoxicity (KEGG) pathway were shown to be significantly upregulated.



Pathway score	HV	MDA	HDA
Natural.killer.cell.mediated.cytotoxicity	1	1,41	1,61
Cytosolic.DNA.sensing.pathway	1	1,44	1,51
Autoimmune.thyroid.disease	1	1,38	1,46
Antigen.processing.and.presentation	1	1,27	1,45
Jak.STAT.signaling.pathway	1	1,36	1,42
Toll.like.receptor.signaling.pathway	1	1,35	1,37
Cytokine.cytokine.receptor.interaction	1	1,26	1,29
TNF.signaling.pathway	1	1,15	1,14
IL.17.signaling.pathway	1	1,14	1,13
Inflammatory.bowel.disease..IBD.	1	1,10	1,12
Complement.and.coagulation.cascades	1	1,08	1,10

Table: pathways score for healthy volunteer (HV), Mild Disease Activity (MDA) and High Disease Activity (HDA). Pathways identified as upregulated are pathways targeted by anti Rheumatoid Arthritis drugs. BIOPRED panel can be useful to identify potential therapeutic targets and for monitoring treatment response.

Methods:

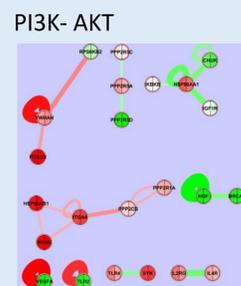
PAXgene samples of active RA patients with DAS28>3.2 (n= 178) and HV (n= 25) are directly profiled without RNA extraction with BIOPRED panel on HTG EdgeSeq platform, a combination of a nuclease protection assay & next generation sequencing (NGS). Subjects are categorized into three groups; High disease activity (HDA) group with DAS28 >5.1, Moderate disease activity (MDA) group with DAS28 between 3.2 and 5.1, and Healthy volunteer (HEV) group.

A second analysis using several databases for interaction as BioGrid, IntAct and String was used to define pathways in BIOPRED panel. Construction of networks were performed using NetworkMaker and Bisogenet. As a result, 1726 nodes and 7798 interactions were define. Data integration was performed using Cytoscape and ExprEssence plugin (Warsow et al., 2010). This tool allows the visualization of pathways that are differentially regulated.

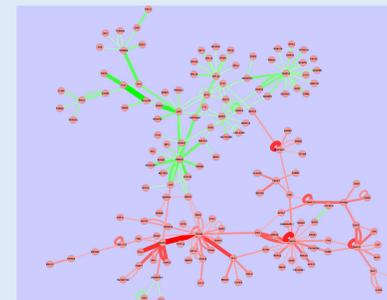
Results:

Cytoscape and ExprEssence visualization of differentially regulated targets (Healthy vs RA) in global network and PI3K-AKT and JAK/STAT networks

Example of condensed network



Global condensed network Healthy vs RA



Conclusion:

Our results identify a list of mRNAs relevant to RA pathology and pathways and have the potential to be candidate biomarkers/therapeutic targets. Moreover, BIOPRED panel accurately measures 2155 mRNA from inflammatory and immune pathways and can be further used to study pathway analysis in autoimmune-inflammatory disorders such as RA.