



## Overview

In this research, we optimized the scoring algorithms in PEAKS software specifically for AB SCIEX TripleTOF instruments. The algorithms were adjusted to fit the characteristics of TripleTOF spectra, and new features were incorporated to better separate the true PSMs from those that are false.

We evaluated the performance of three software packages, Mascot, PEAKS and ProteinPilot, with mass spectrometry data generated by AB SCIEX TripleTOF 5600. The optimization significantly improved PEAKS's sensitivity in peptide identification.

## Introduction

AB SCIEX TripleTOF 5600 is a powerful instrument that provides high mass accuracy and high resolution in both MS and MS/MS modes. With the ability to acquire a maximum of 50 MS/MS spectra per second, the instrument makes a great component for a proteomics research platform.

Except for AB SCIEX's ProteinPilot, most other peptide identification software tools do not provide a TripleTOF mode for data analysis at this moment, or are not fully optimized. As a compromise, TripleTOF data has to be processed in Q-TOF or TOF-TOF mode on these tools, which may diminish the improvement enabled by the instrument.

## Methods

The linear discriminative function (LDF) in PEAKS is optimized as well.

- Five new features are added into the LDF to better separate true and false PSMs.
- The LDF coefficients are specially trained for AB SCIEX TripleTOF instruments.

## Optimizations in PEAKS for TripleTOF spectra

Observation	Optimization
The instrument keeps its performance at low mass range. The y1-ions, as well as the associated neutral loss ions, are often present.	The ions in low mass range, y1, b1-ions and associated neutral loss ions, are scored in PEAKS' algorithms.
The instrument has an excellent dynamic range for detecting precursor signals. The instrument may not fragment every precursor, leaving a high precursor peak in some MS/MS spectra.	The precursor peak in MS/MS may adversely affect the database search scoring function and are removed from scoring.

	Support TripleTOF data	Support blind PTM search	Support FDR control	# of identified PSMs
Mascot 2.3 + Percolator	N	N	Y	3294 at 1% FDR
Mascot 2.3 (Error tolerance search)	N	Y	N	3598 Ion score>30
PEAKS 5.3 (Before Optimization)	N	N	Y	5154 at 1% FDR
PEAKS 6 (After Optimization)	Y	Y	Y	6884 at 1% FDR
ProteinPilot 4.0	Y	Y	Y	5349 at 1% FDR

## Results

Evaluation was performed with the ABRG iPRG 2012 dataset\* by AB SCIEX TripleTOF 5600. Each software was configured to broadly detect multiple modifications, mutations, and up to three missed cleavages were allowed for each peptide.

## Conclusion

The result at 1% estimated false discovery rate indicates:

- The optimized PEAKS 6 was able to identify twice as many PSMs as the popularly used Mascot + Percolator combination. Even compared with AB SCIEX's ProteinPilot software, PEAKS 6 identified 29% more PSMs.
- The optimization significantly improved peptide identification sensitivity using TripleTOF data, which results in significantly better FDR curves in PEAKS 6 than PEAKS 5.3.

\*The public dataset is described at [http://www.abrf.org/ResearchGroups/ProteomicsInformaticsResearchGroup/Studies/iPRG2012\\_study\\_participation.pdf](http://www.abrf.org/ResearchGroups/ProteomicsInformaticsResearchGroup/Studies/iPRG2012_study_participation.pdf)

