

# cAMP signaling and differentiation state in BRAF-mutant melanoma

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## Introduction

Melanocyte differentiation depends on cAMP: MC1R ->  $G_{\alpha s}$  -> AC -> cAMP -> PKA -> CREB -> MITF.

## Workflow

- Data access
  - Tsoi et al. GREIN database
  - CCLE gene counts: DepMap portal
  - both resources provide length-scaled gene counts processed in similar pipelines
- Data integration
  - Join gene counts tables
  - Combat-Seq batch correction: “negative binomial regression model that retains the integer nature of count data in RNA-seq studies, making the batch-adjusted data compatible with common differential expression software packages that require integer counts”
- Integrated data processing
  - DESeq2 variance stabilizing transformation: “log-space transformation to decouple per-gene variance from mean expression level (alternative/preferred vs. TPM)”
  - Gene-wise z-scores across all samples
- Dimensionality reduction
  - PCA for gene-wise z-scores across all samples
- Linear regression
  - mean z-score for designated genesets: Are mean z-scores for CREB family transcription factor targets correlated to mean z-scores for differentiation state signatures?
- Expression levels of individual cAMP/CREB pathway components across differentiation states (per-gene ANOVA)

## Tidy data & annotations

1. Tidy length-scaled gene expression counts data from Tsoi et al. (2018) and CCLE (downloaded from GREIN and DepMap, respectively)
2. Tidy manually generated annotation tables and gene lists (downloaded from Harmonizome, BioPlanet, and GO)
3. Aggregate annotations into combined tables

```
source("scripts/1_tidy_data.R")
source("scripts/2_tidy_annotations.R")
source("scripts/3_aggregate_annotations.R")
```

## Integrate gene expression counts data and get z-scores

1. Batch correct for data source using CombatSeq
2. Use DESeq2 Variance Stabilizing Transformation (log-space transformation that decouples per-gene variance from mean expression level; a preferred alternative to TPM)
3. Calculate gene-wise z-scores on VST values
4. Calculate mean z-scores for gene lists

```
source("scripts/4_get_integrated_vst_zscores.R")
source("scripts/5_get_mean_zscores.R")
```

## Analysis

1. Demonstrate batch correction