

DepleteX™ Globin Depletion Kit

Remove contaminating globin fragments for RNA-Seq

Introduction

Globin mRNA dominates the mRNA content of red blood cells and whole blood. Three globin genes (HBA1, HBA2 and HBB) are highly expressed and together can constitute 30% or more of total mRNA from blood. Jumpcode has developed the DepleteX Globin Depletion Kit that removes abundant globin sequences from libraries to improve RNA-Seq from blood samples.

Methods and Results

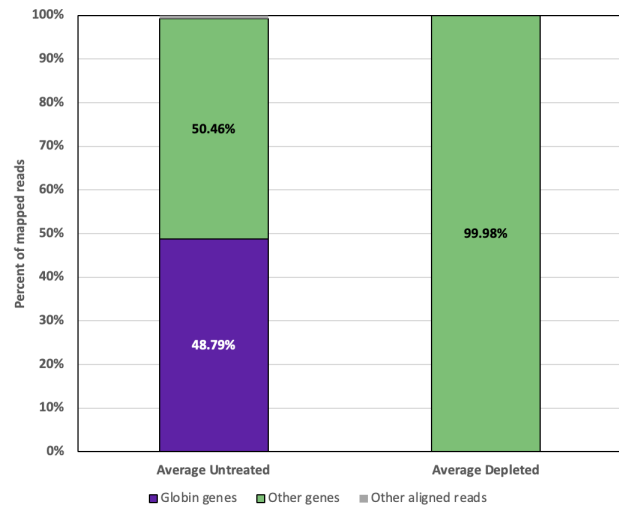
CRISPR guides were designed to target four human globin genes (HBA1, HBA2, HBB, HBD) using Jumpcode’s proprietary guide design pipeline.

Total RNA from whole blood was kindly provided by Stan Nelson (University of California, Los Angeles, CA). Libraries were prepared with the NEBNext Ultra II Directional RNA Library Prep kit (New England BioLabs, Ipswich, MA). The library prep workflow involves reverse transcription with random hexamers, second strand synthesis with dUTPs, DNA end repair and adapter ligation, PCR amplification and SPRI bead-based cleanup. Depletion was performed on fully prepared libraries. Cas9 and CRISPR guides were combined to create ribonucleoprotein complexes, which were subsequently incubated with 10 ng of NGS library for 1 hour at 37 °C. CRISPR-mediated cleavage was followed by bead-based size selection to remove fragmented DNA and PCR amplification to enrich for uncleaved fragments. Depleted and non-depleted libraries were generated in triplicate.

Sequencing was performed on a NextSeq 2000 instrument using a P2 flow cell in 2 x 150 bp format (Illumina, San Diego, CA). Reads were downsampled to 40 million per sample.

Highlights

- Simple unbiased depletion of human globin from RNA-Seq libraries
- Achieve >99% depletion of contaminating globin
- Quick one hour depletion incubation



Effective globin depletion. Comparison of reads aligning to protein coding genes with and without depletion. Sequencing downsampled to 40M clusters per sample. DepleteX effectively removed >99% of globin resulting in less than 0.02% of reads after depletion.

Ordering information

Catalog	Product name	Samples
KIT1024	DepleteX Globin Depletion Kit	24