

In vitro models of erythropoiesis - Transcriptional analysis

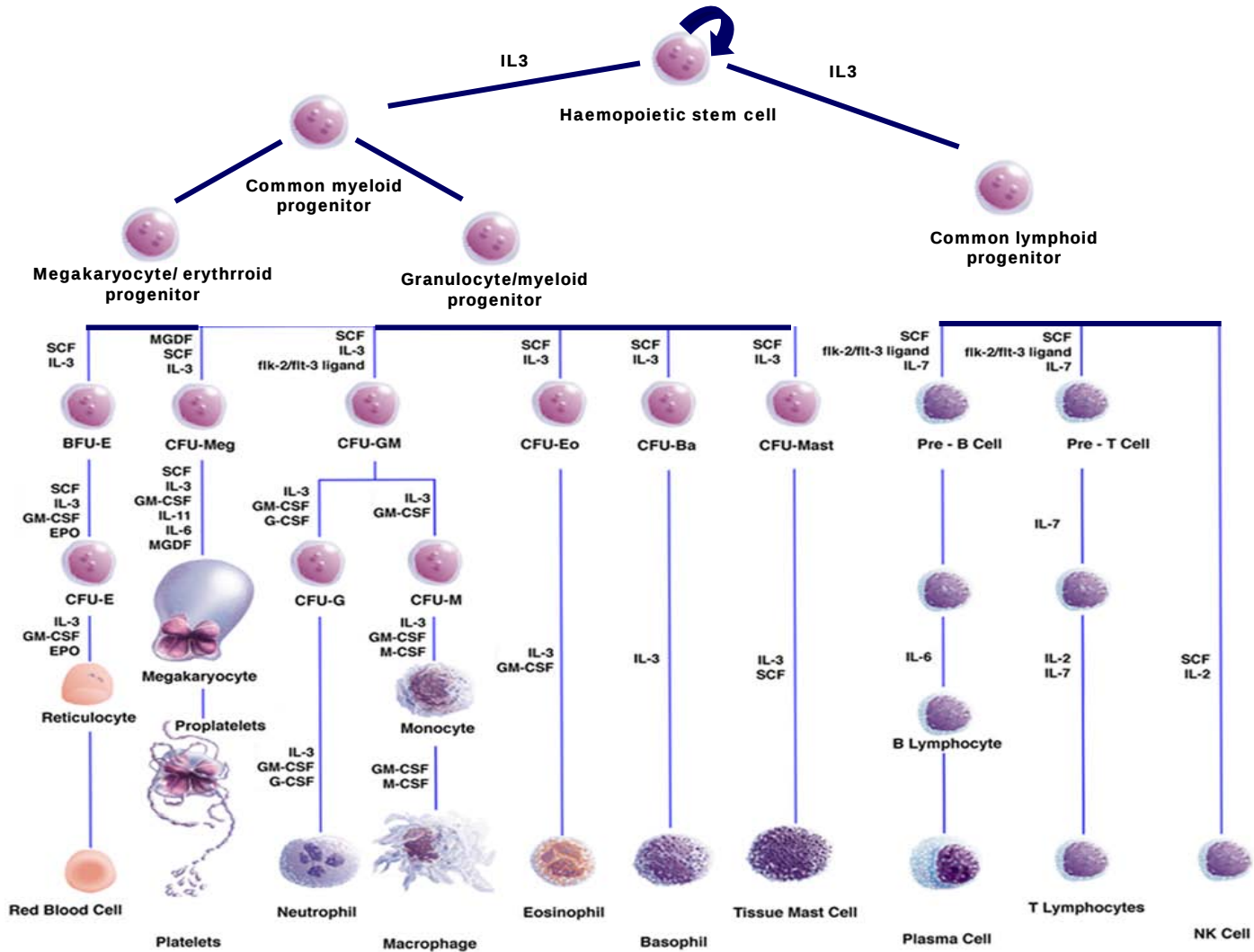
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Western Australian Institute for Medical Research
Perth, Australia

Haemopoiesis

PROGENITOR

COMMITTED



PROGENITOR

COMMITTED

Myc
Myb
Fos
Jun

Gata2
Fli1
NfκB
Notch
Runx1
LMO2
Cbfb



Haemopoietic stem cell



Multipotent progenitor



Common myeloid progenitor

IL1
IL3
ILSCF
GM-CSF

Megakaryocyte/ erythroid
progenitor

SCF
IL-3

BFU-E

SCF
IL-3
GM-CSF
EPO

CFU-E

IL-3
GM-CSF
EPO

Reticulocyte

Red Blood Cell

Epor
Flt3
Foxo3
Gata1
Klf1
Nfe2

Fog
CBP
BRG1

Heme enzymes
Tfra
Globins

Ankyrin
Gypa
specktrin

EPO

Granulocyte/myeloid
progenitor

SCF
IL-3

flk-2/flt-3 ligand

CFU-GM

IL-3
GM-CSF
G-CSF

CFU-G

IL-3
GM-CSF
G-CSF

Neutrophil

IL-3
GM-CSF

CFU-M

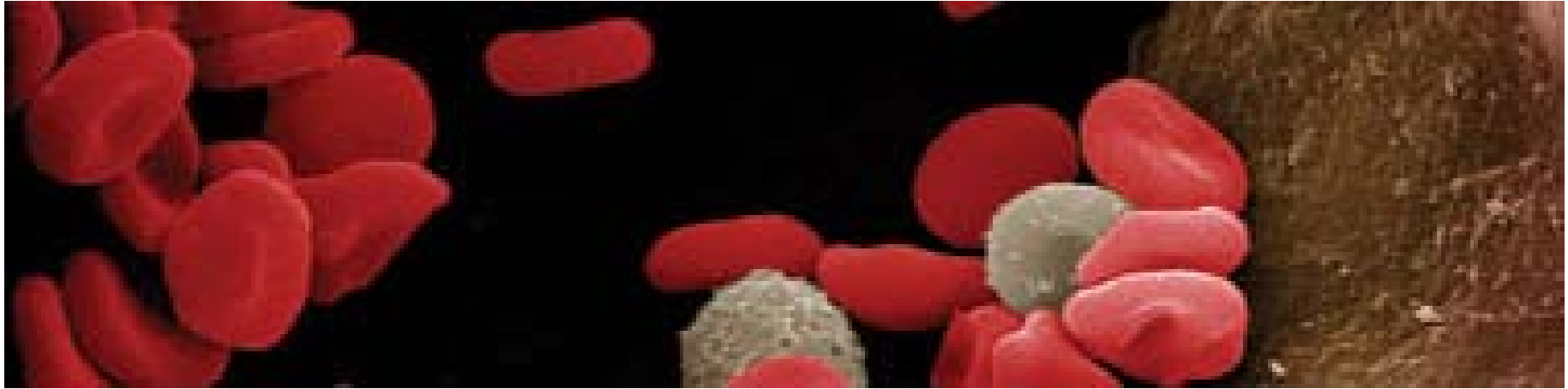
IL-3
GM-CSF
M-CSF

Monocyte

GM-CSF
M-CSF

Macrophage

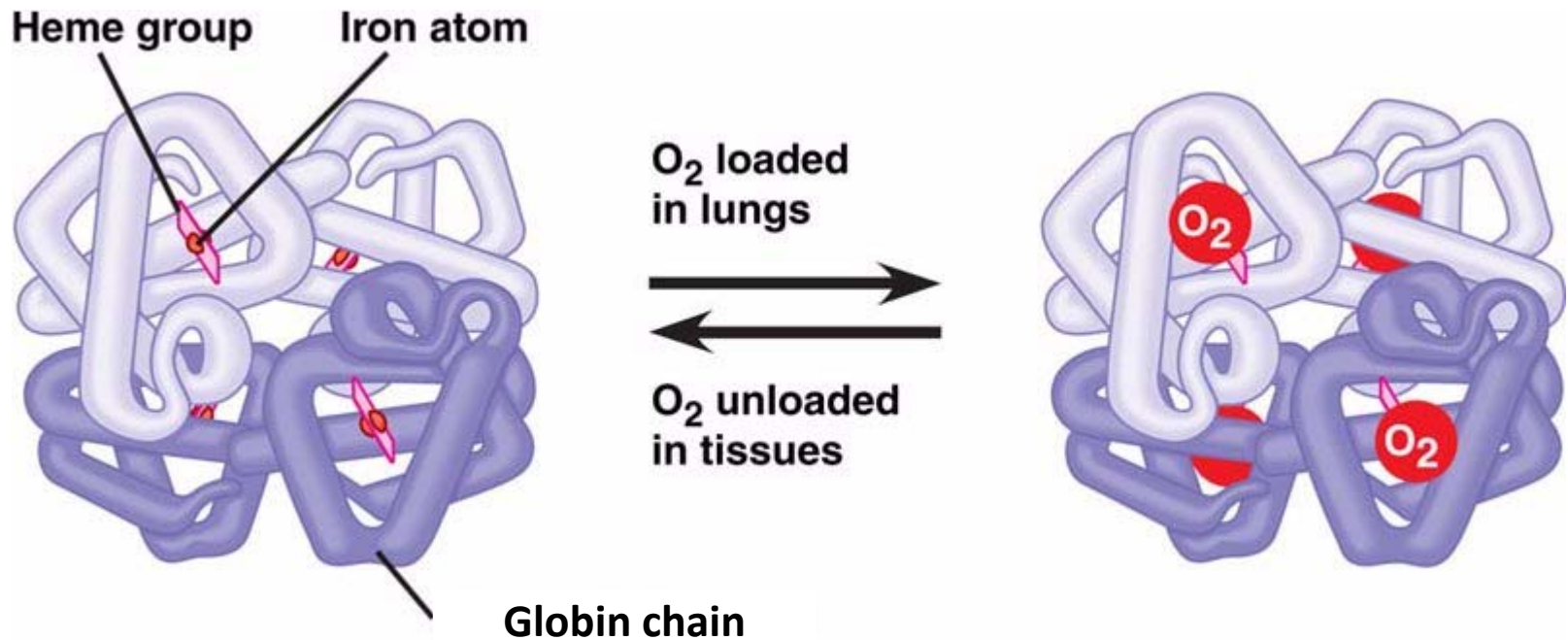
Key features of red blood cells



Red blood cells

- must make haemoglobin
- Must undergo significant cytoskeletal changes

The haemoglobin molecule



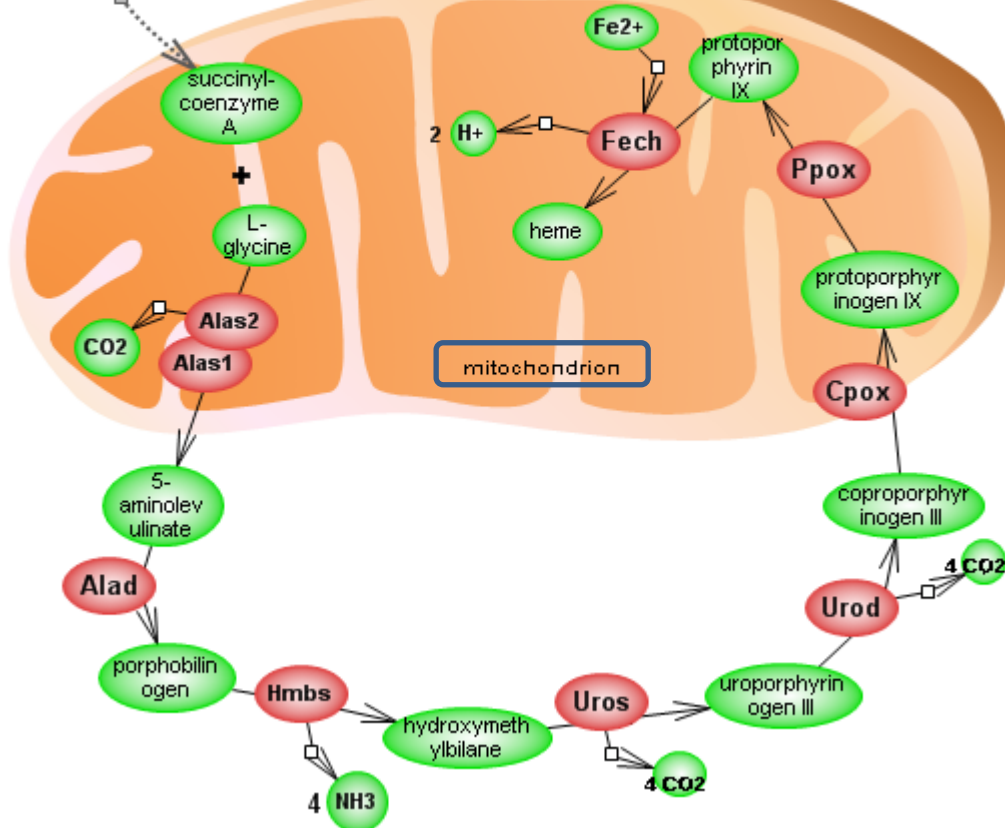
Heme biosynthesis

cytosol

citrate cycle pathway

mitochondrion

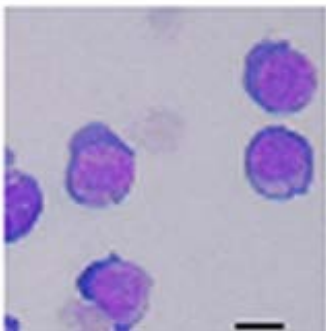
Alas1 - aminolevulinate, delta-, synthase 1
 Alas2 - aminolevulinate, delta-, synthase 2
 Alad - aminolevulinate, delta-, dehydratase
 Hmbs - hydroxymethylbilane synthase
 Uros - uroporphyrinogen III synthase
 Urod - uroporphyrinogen decarboxylase
 Cpox - coproporphyrinogen oxidase
 Ppox - protoporphyrinogen oxidase
 Fech - ferrochetalase



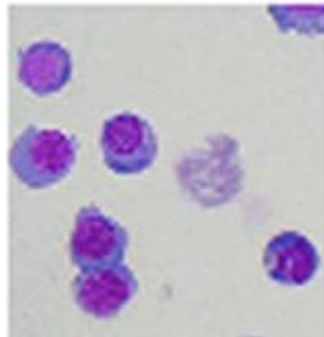
In vitro models of erythropoiesis

J2E

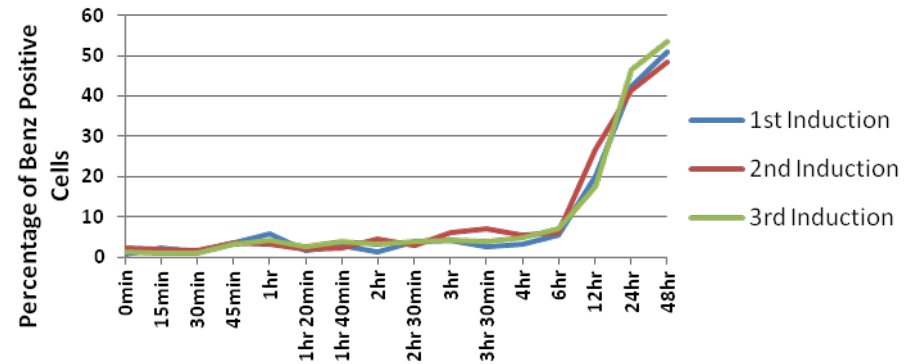
Unstimulated



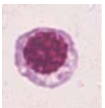
+ Erythropoietin



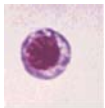
J2E Inductions



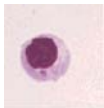
Pro
erythroblast



Basophilic
erythroblast



Chromatophilic
erythroblast



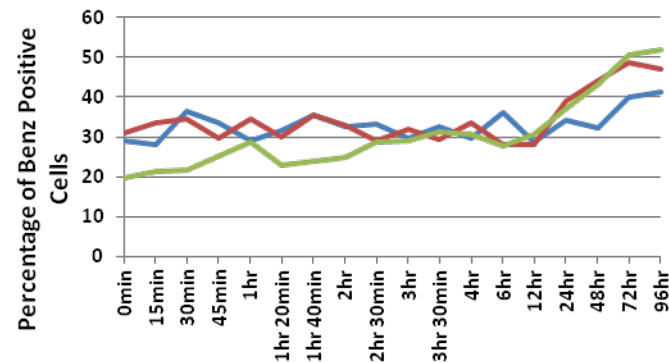
Ortho
chromatophilic
erythroblast



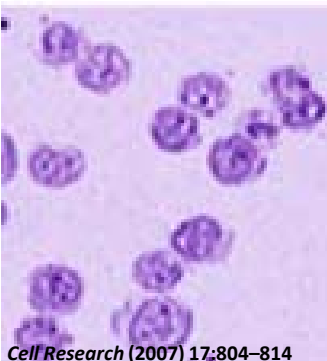
enucleation



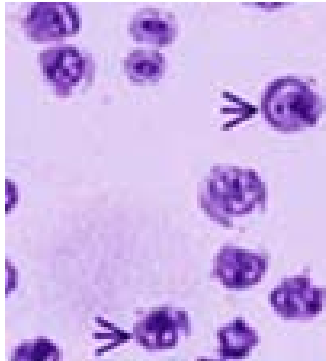
K562 Inductions



Unstimulated



+ Hemin

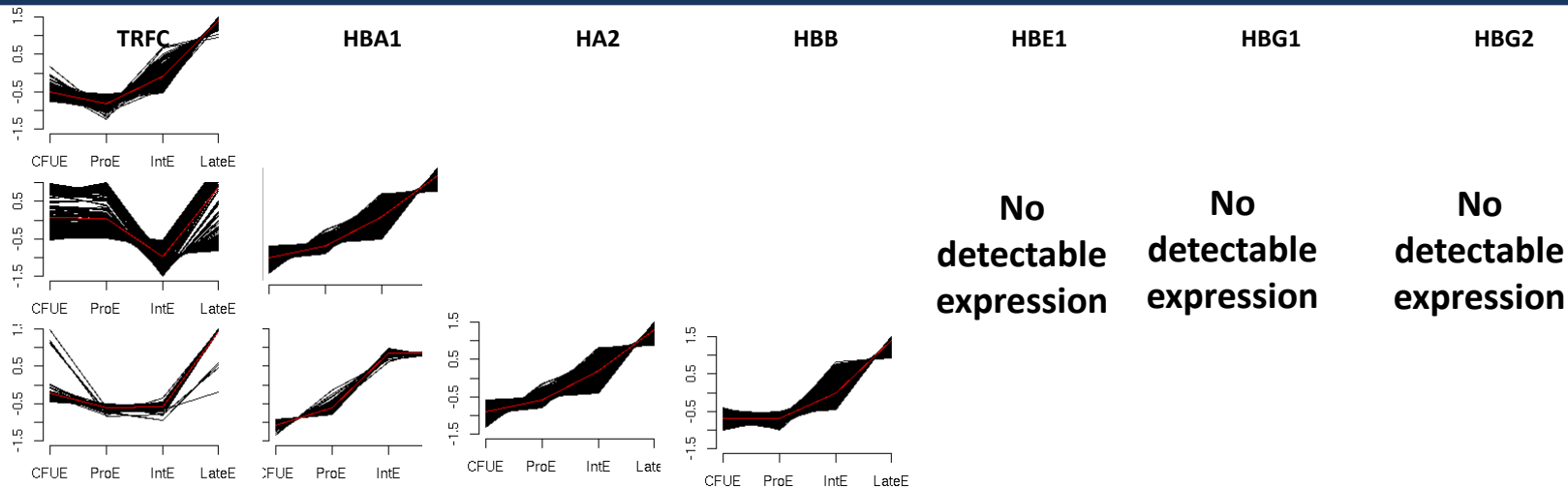


K562

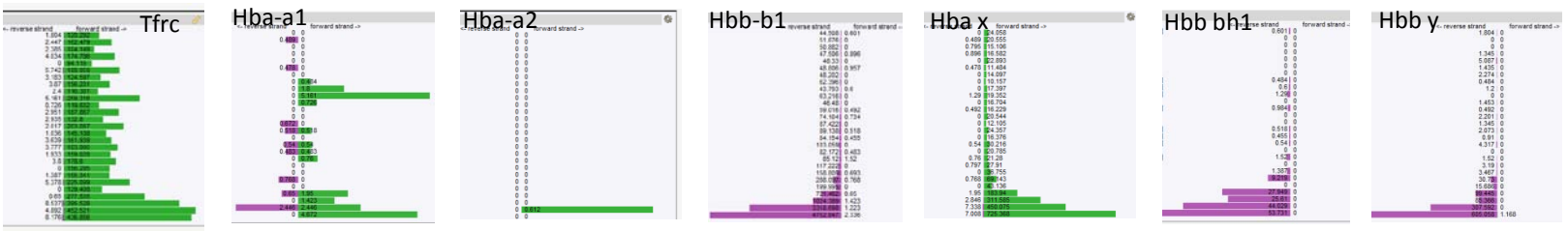
Cell Research (2007) 17:804-814

Globin gene transcripts

A M-C Blood 2011



J2E

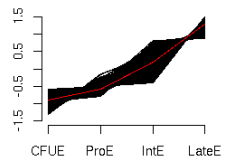


K562

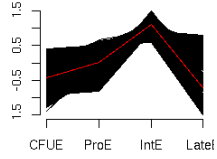


Heme enzymes

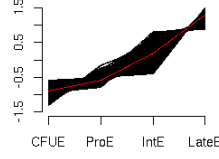
ALAS2



ALAD



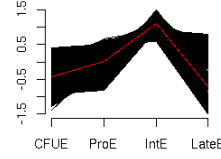
HMBS



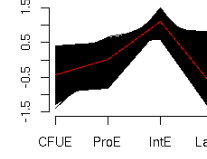
UROS

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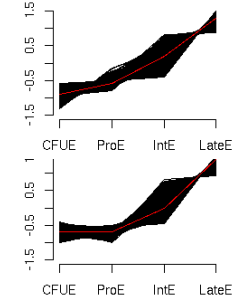
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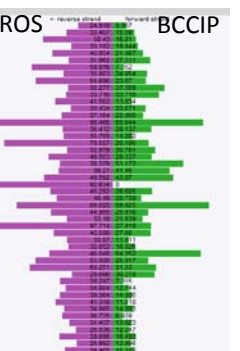
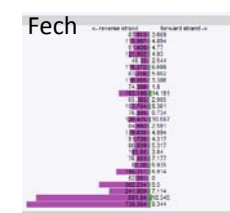
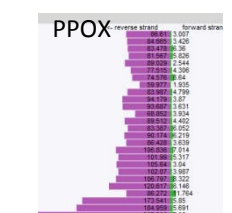
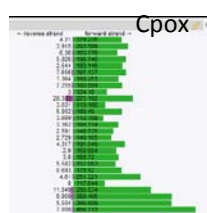
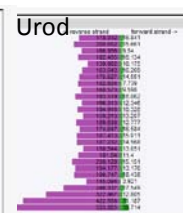
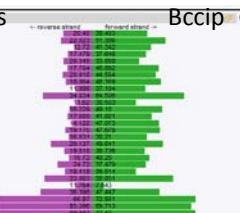
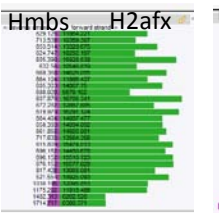
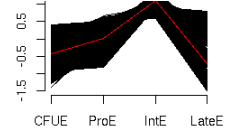
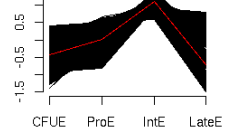
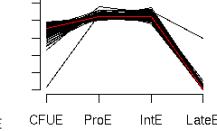
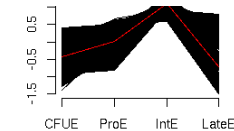
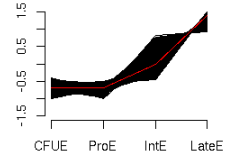
CPOX



PPOX

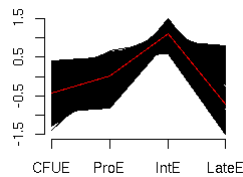


FECH

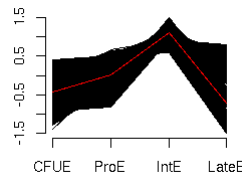


Genes that affect RBC cytoskeleton

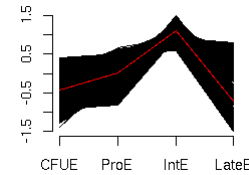
ANK1



GYP A



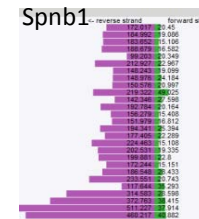
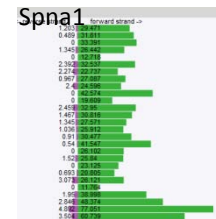
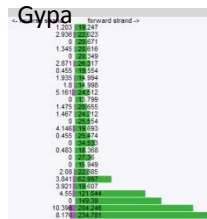
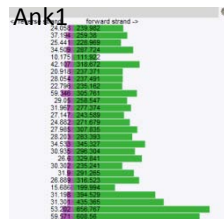
SPTA1



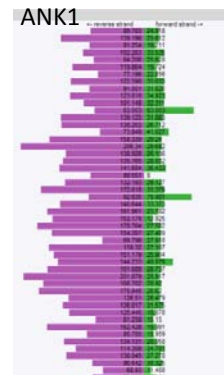
SPTB1

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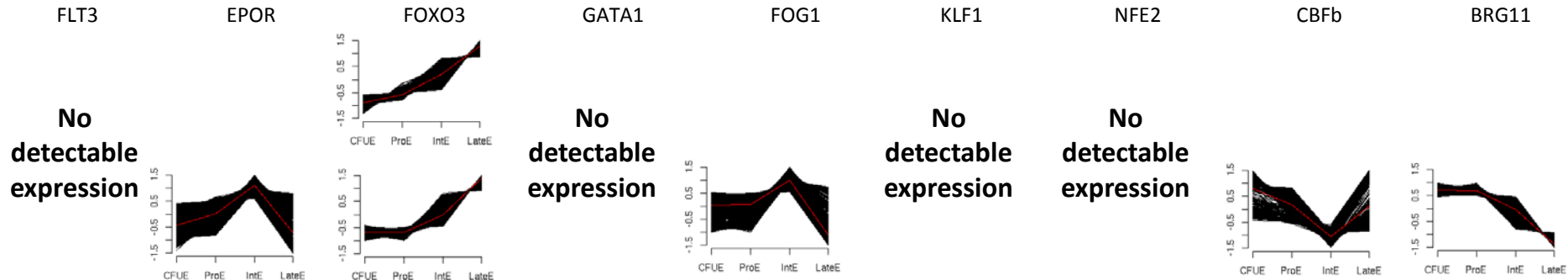
J2E



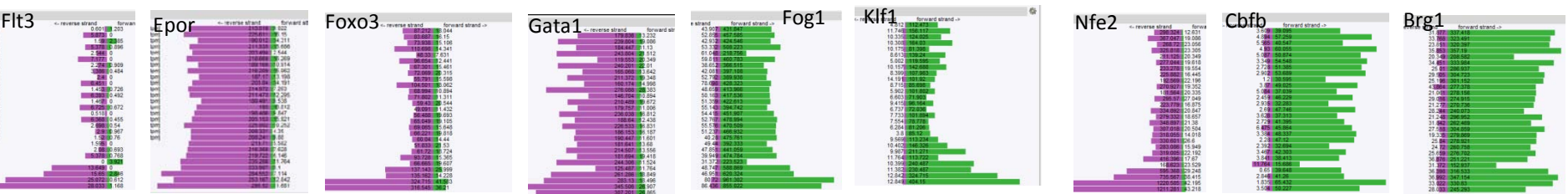
K562



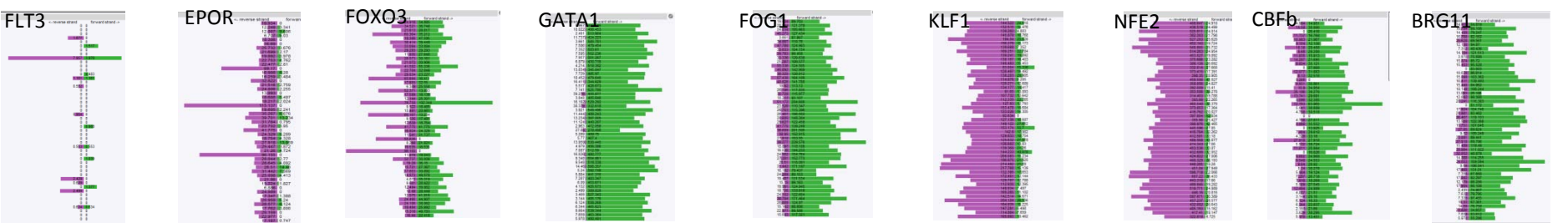
Erythroid transcription factors



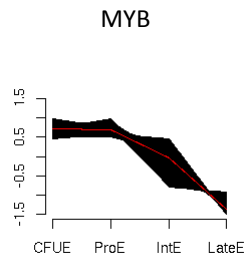
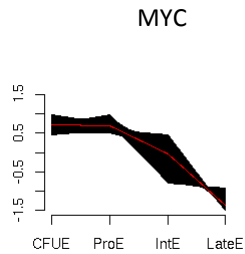
J2E



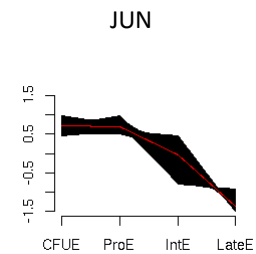
K562



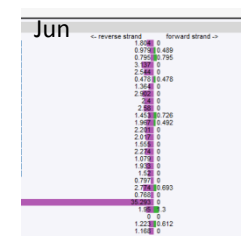
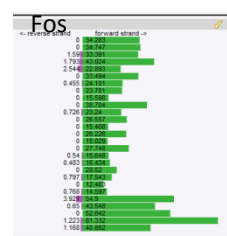
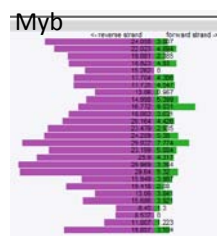
Immediate early genes



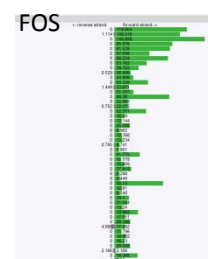
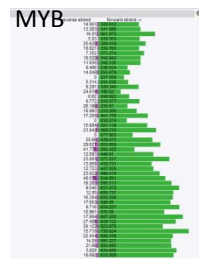
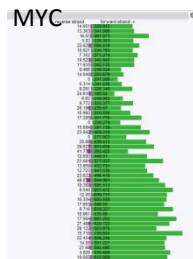
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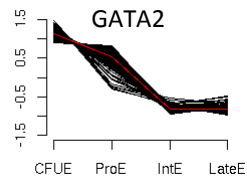
J2E



K562



Genes that regulate stem /progenitor cells



FLI1

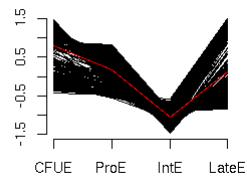
NFKb

NOTCH

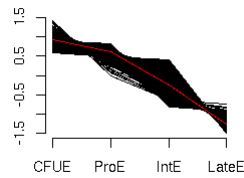
RUNX1

LMO2

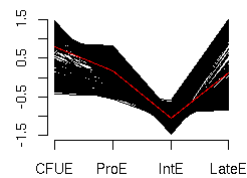
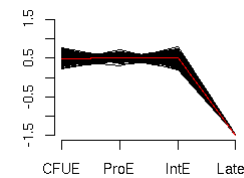
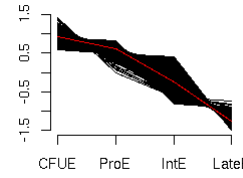
CBFb



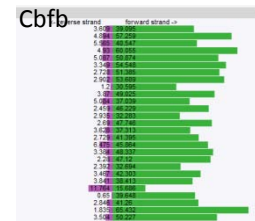
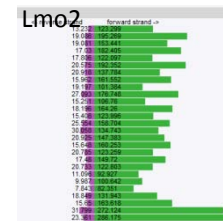
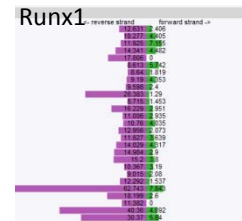
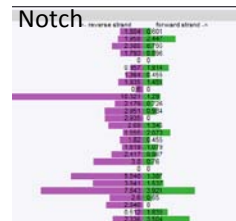
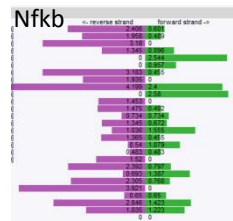
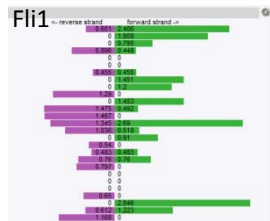
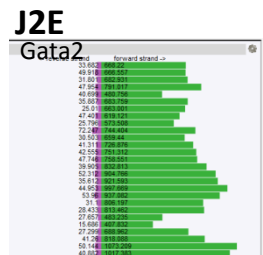
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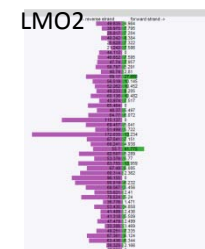
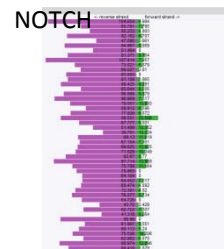
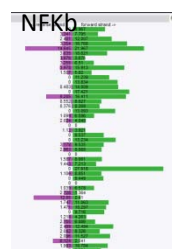
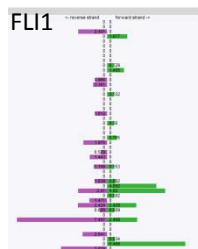
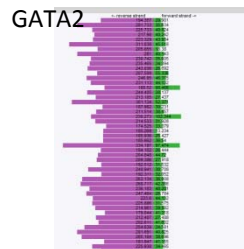
**No
detectable
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CBFb



K562



Ideas for paper

- K562 model for haemoglobin synthesis
- J2E model of Epo induced RBC differentiation
- Define TSS for erythroid differentiation for two models
- Compare gene expression
- TSS usage / Gene expression embryogenesis vs adult erythropoiesis
- Compare to human normal cells (PBM, MSC)
- Differences in TSS *in vitro* vs *in vivo*
- Can we define haemopoiesis by dynamic changes in TSS across different lineages/within a lineage.

Acknowledgements

Perth, WA

Jennifer Beaumont
Simon Kobelke

Japan

Riken OSC
Fantom5

