
Molecular and functional integrity of cryopreserved CML CD34+ haematopoietic stem cells



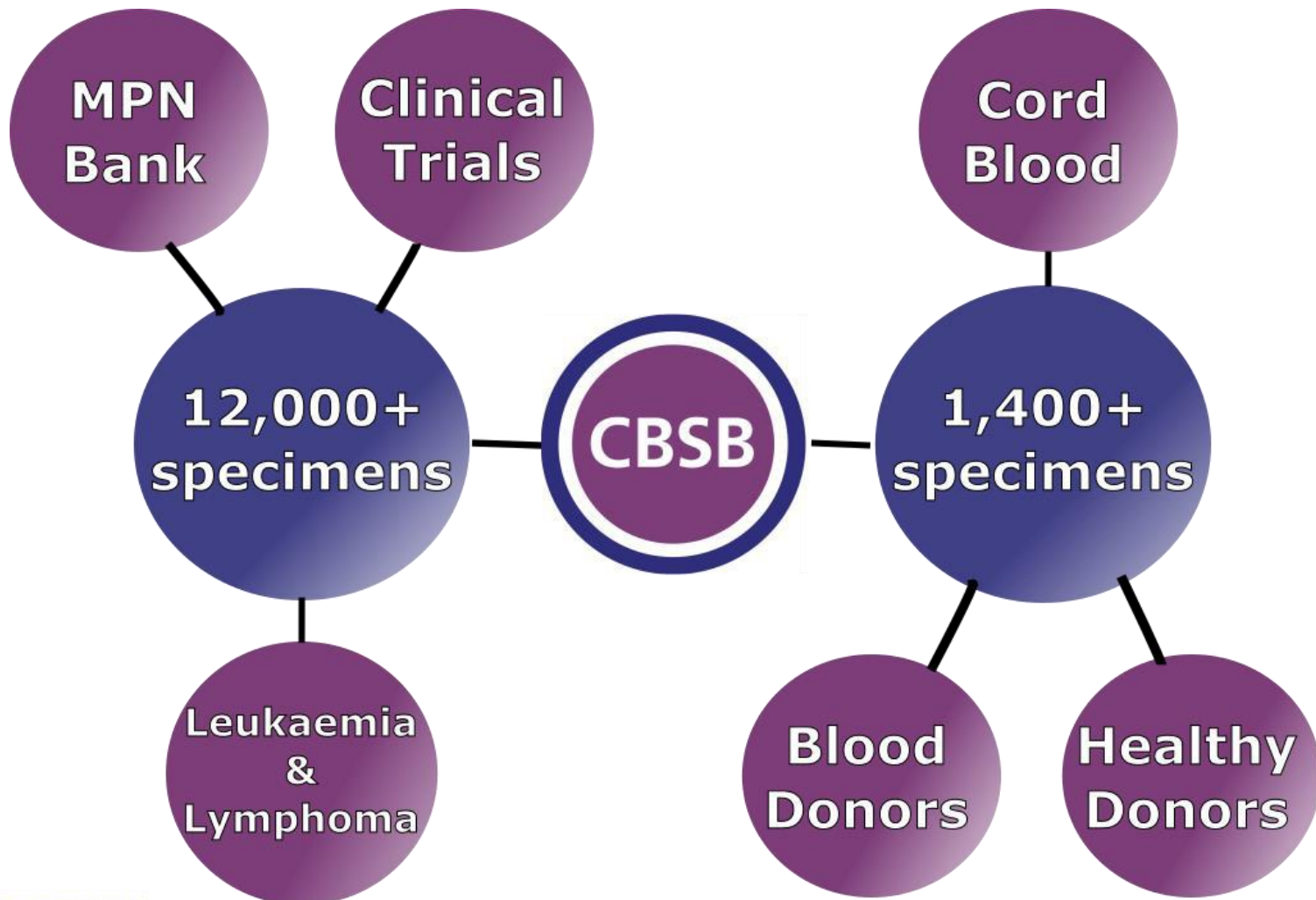
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15th September 2016



CBSB Collection



Biopreservation & Biobanking

- Access to large numbers of high quality biospecimens remains a challenge.
- Cryopreserved viable cells offer a flexible format.
- Prospectively acquired biospecimens expedite studies where access to fresh material is limited.
- Haematopoietic stem cell (**HSC**) research enhances the understanding of oncogenesis and disease progression.
- The impact of freezing and long-term storage on the quality of **CD34+ HSC** for down stream applications is under reported.

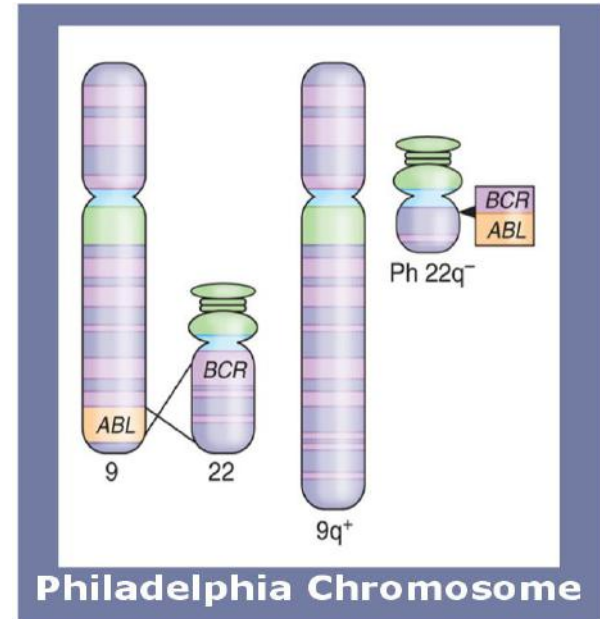
Autologous Stem Cell Harvests (SCH)

- ▶ **Acquisition of 52 autologous SCH collected between 1990-2006**
 - ▶ Storage infrastructure incompatibility
 - ▶ Inaccessible format for most end-users
 - ▶ Material quality unclear
 - ▶ Alternative product where biospecimens from rare conditions are limited
 - ▶ Potential reservoir of CD34+ HSC for research
 - ▶ Investigate the effect of freezing and thawing on biomarkers of cancer stem cells



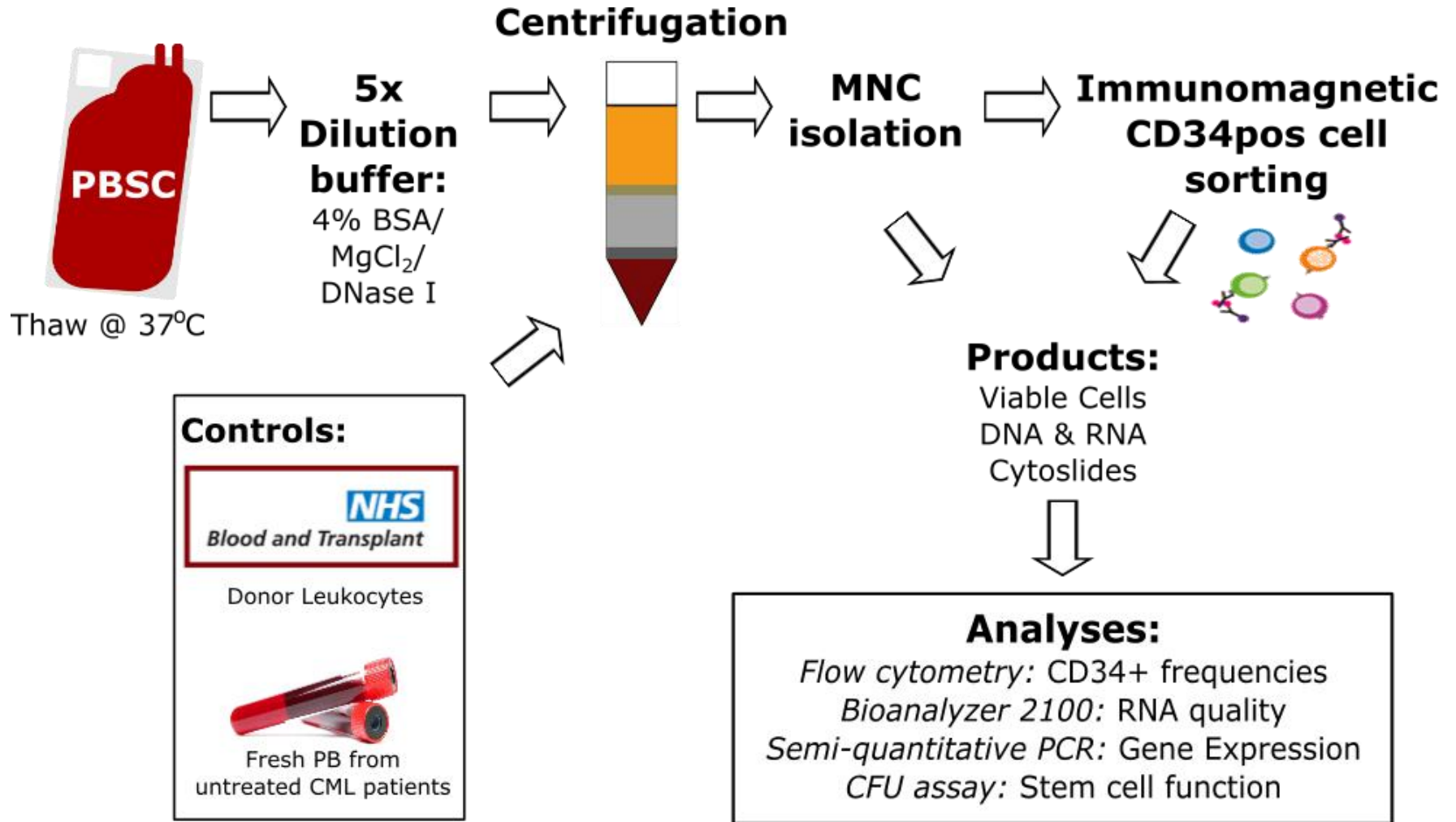
Chronic Myeloid Leukaemia: Peripheral blood stem harvests (PBSC)

- ▶ Rare condition
- ▶ CML is a clonal disorder originating from a mutated CD34+ HSC
- ▶ 95% of CML patients positive for the Philadelphia (Ph) chromosome:
 - ▶ translocation between chromosomes 9 and 22
 $t(9;22)(q34;q11)$
- ▶ Chimeric oncogene (**BCR-ABL1**) and fusion protein p210

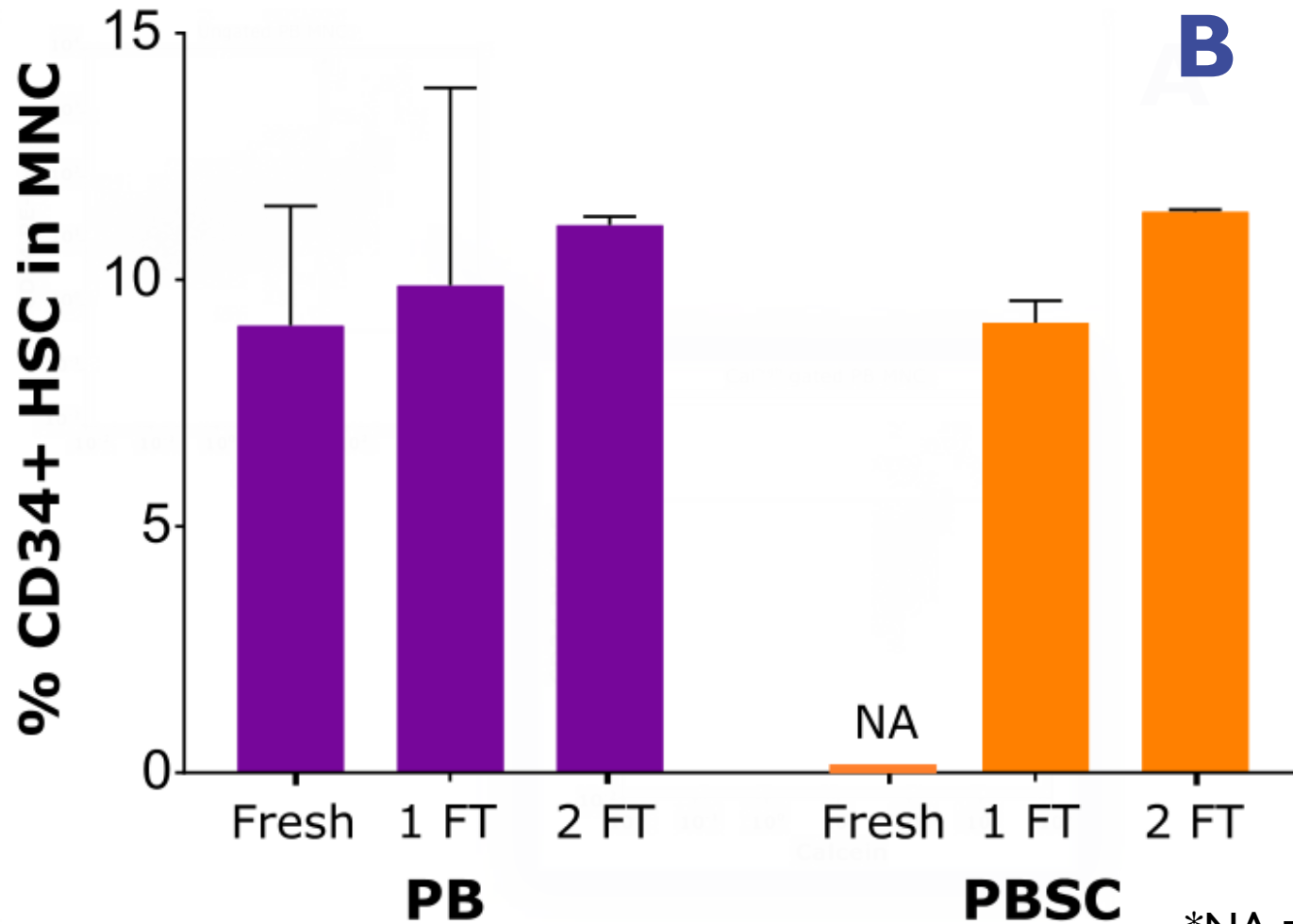


- ▶ Marker of disease burden and treatment response
- ▶ Trackable target for study

Sample Processing Workflow

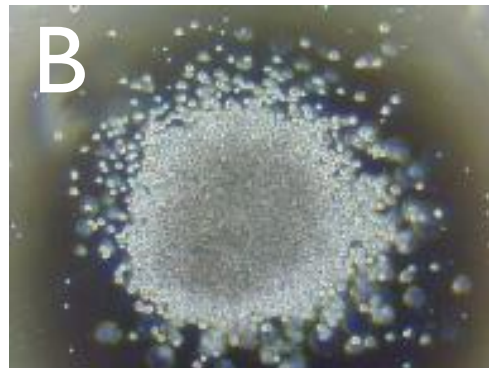
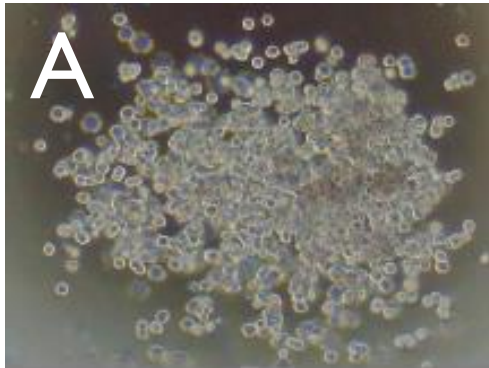


Frequency of CD34+ HSC in CML MNC fraction remains stable with repeated freeze-thaw (FT)

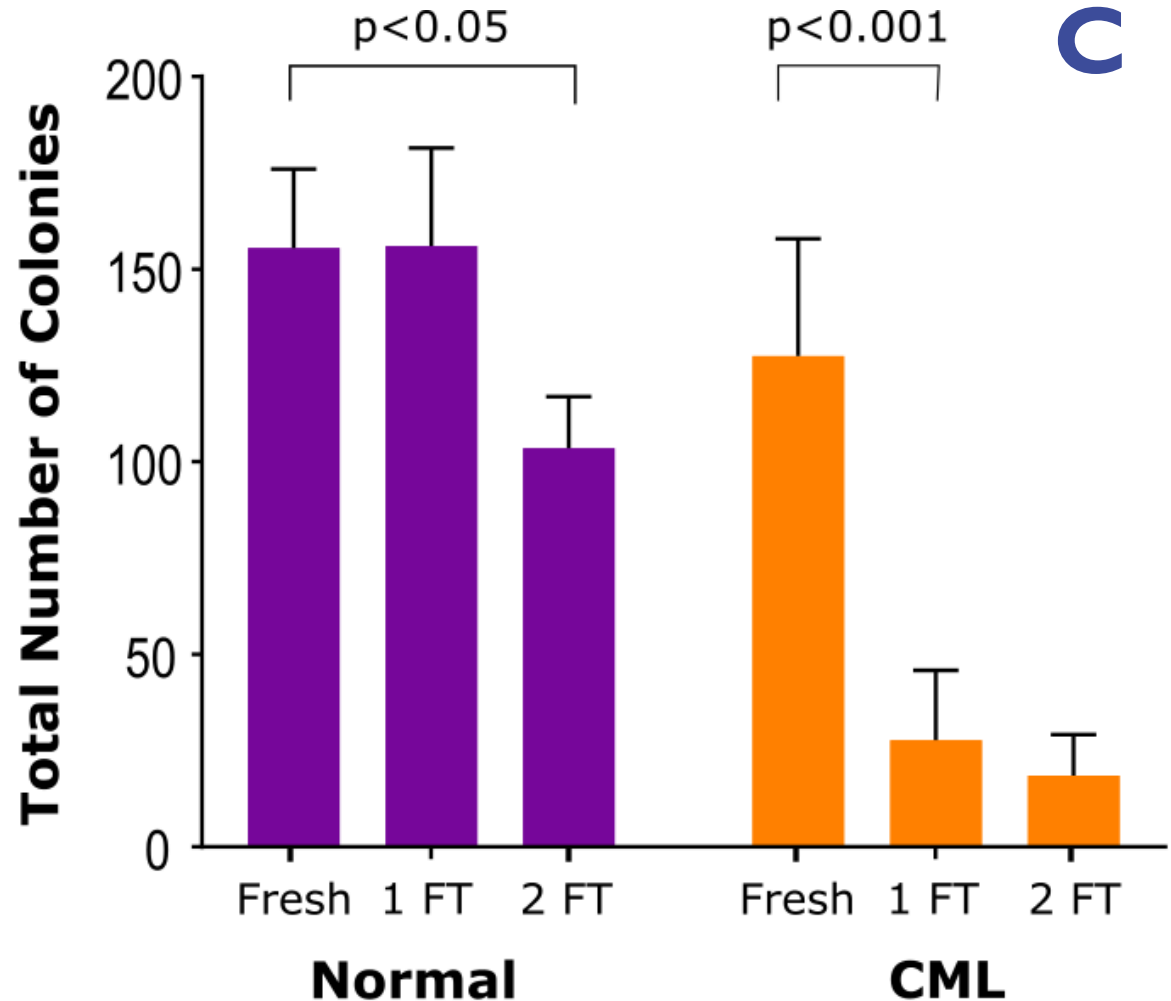


*NA = not available

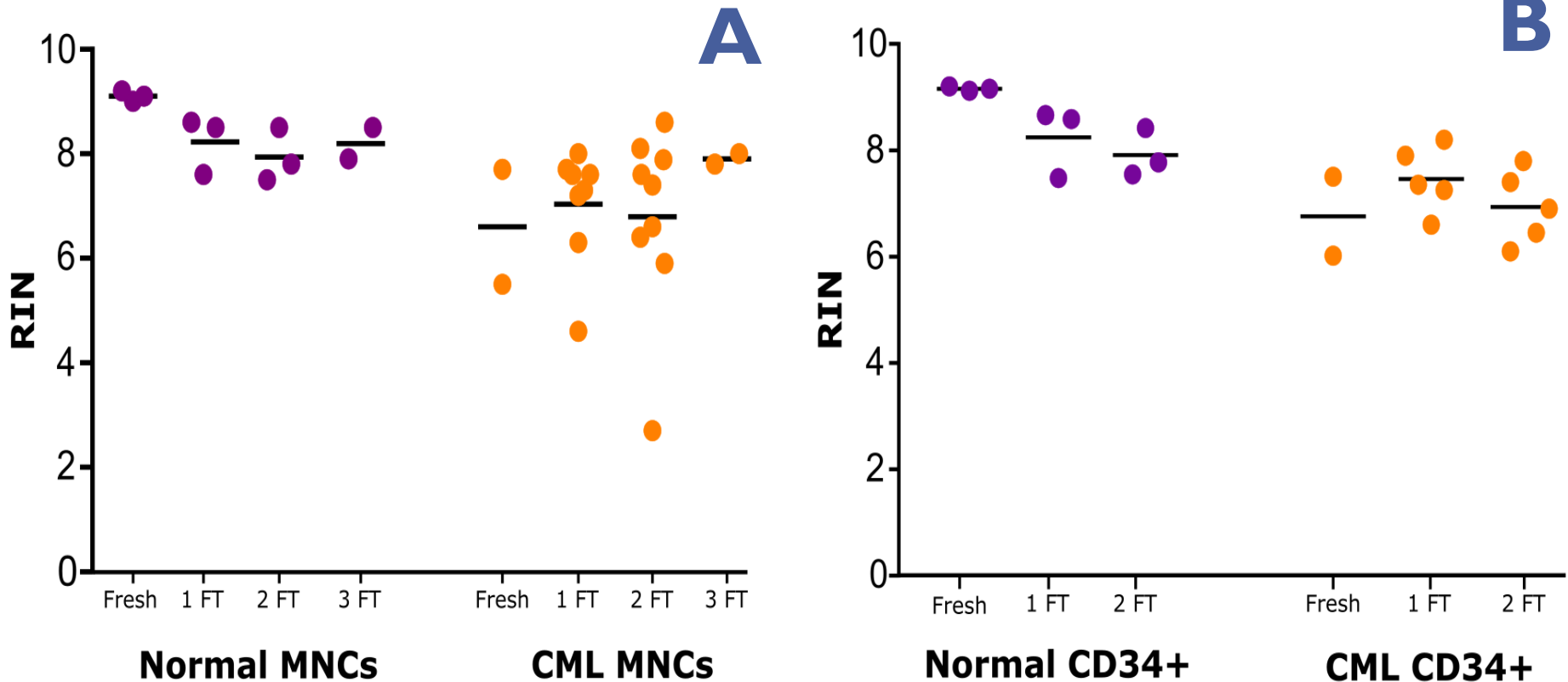
Reduced colony forming potential indicates loss of stem cell function with repeated FT



*Colonies counted: BFU-E, CFU-E, CFU-G, CFU-M, CFU-GM, CFU-GEMM



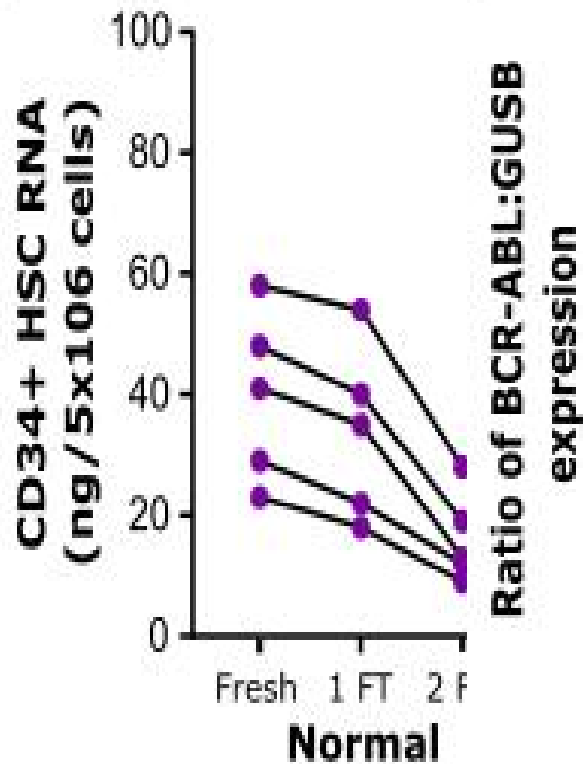
No significant impact of repeated FT on RNA quality



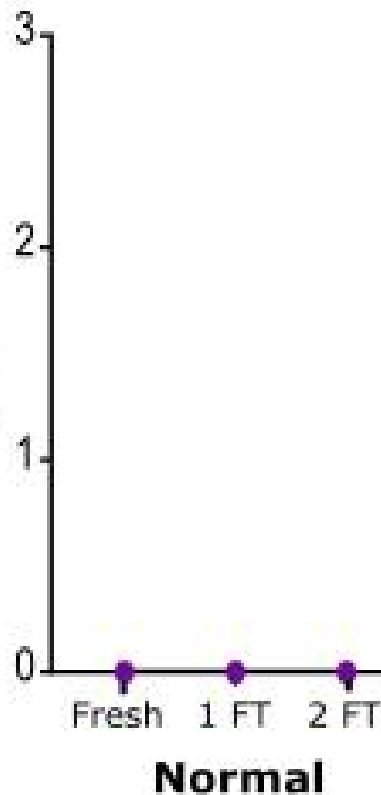
- Normal MNC ($p < 0.05$) and CD34+ HSC ($p < 0.05$) of significantly higher quality than MNC and CD34+ HSC derived from CML patients.
- No significant difference in RNA quality observed between MNC and CD34+ HSC.

Cryopreservation associated with global down-regulation of gene expression in CD34+ HSC

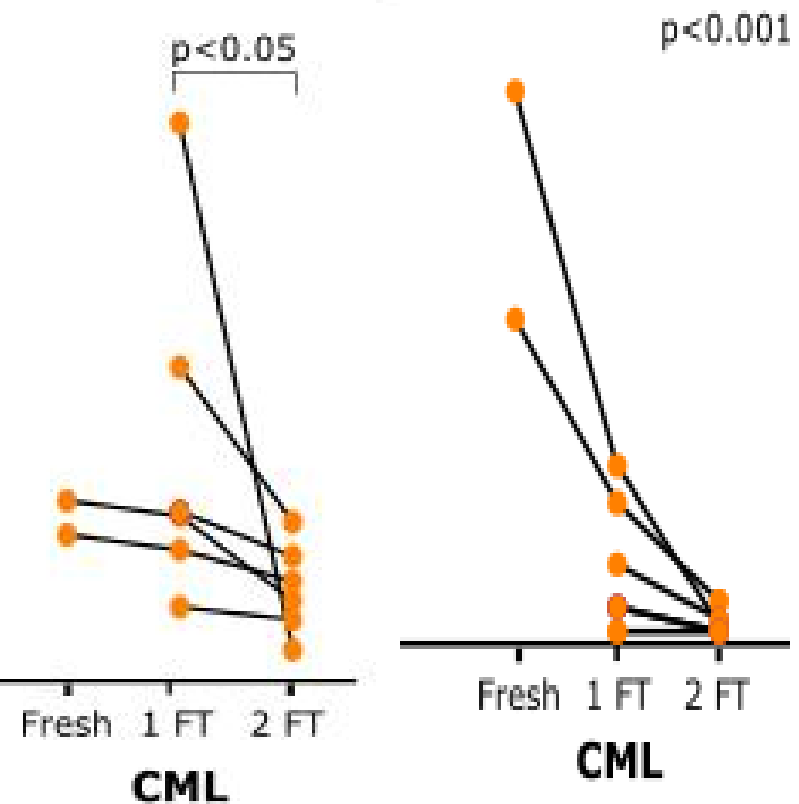
A



C



B



Summary

- ▶ **Cryopreservation of CD34+ HSC:**
 - ▶ Sufficiently robust to survive
 - ▶ Reduction in stem cell function
 - ▶ Down-regulation of gene expression
 - ▶ Persistence of malignant HSC
 - ▶ High quality RNA obtainable

Impact on Biobanking Practice

- ▶ Meaningful analysis where fresh material is limited.
- ▶ Quality assessed using technologies of interest to end users.
- ▶ Standardisation of bioprocess.
- ▶ Selection of like-for-like material for biological comparisons.

- ▶ To date, CML PBSC samples been successfully used in method development assays for antibody screening and assay validation.

Acknowledgements

▶ **CBSB Lab Team**

- ▶ Joanna Baxter (Lead Scientist)
- ▶ David Sewell
- ▶ Jayne Downes
- ▶ Heather McMurray
- ▶ Krishna Vaghela
- ▶ Rachel Glover
- ▶ Nicla Manes
- ▶ James Roberts
- ▶ Hayley Protheroe
- ▶ Sweta Chandel
- ▶ Chris Watt

▶ **CBSB Nurses & Midwives**

- ▶ Cat Evans
- ▶ Anne O'Maolain
- ▶ Lesley Dark
- ▶ Myrna Maquinana
- ▶ Emma Burhan

▶ **NHS Stem Cell Lab, NHSBT**

- ▶ Kevin Jestice
- ▶ Karen Richardson

