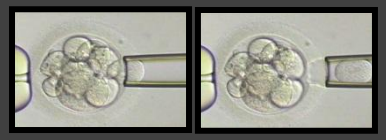
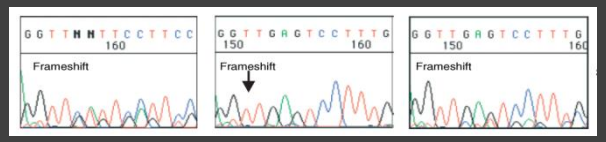
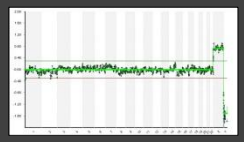
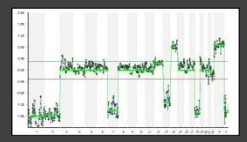
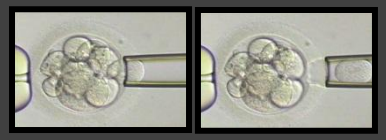
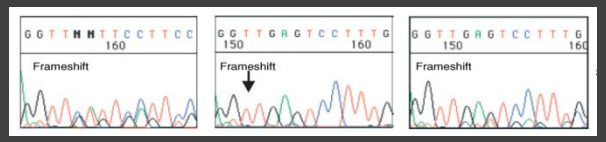
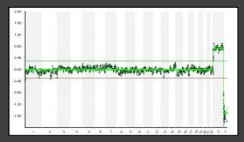
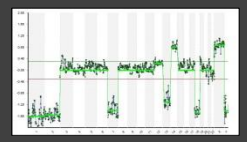



PGD of alpha-Thal^{-SEA} : 1st cycle

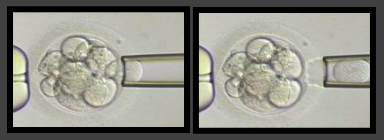
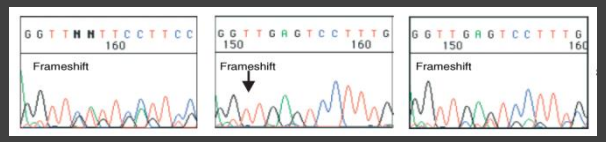
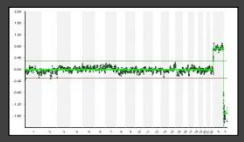
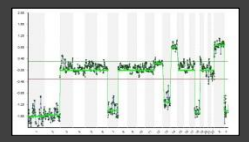
- 14 oocytes collected
- 14 embryos biopsied
- Results:
  - 4 normal (2 suggestive of Ht by LA1)
  - 2 heterozygous (Ht)
  - 6 affected (1 suggestive of Ht by LA1)
  - 2 with no result
- 1 normal + 2 heterozygous - ET
- no pregnancy resulted

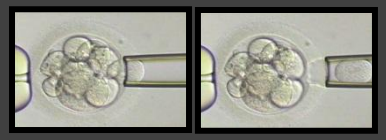
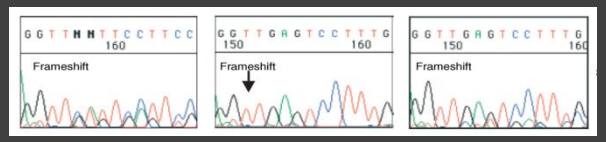
PGD of alpha-Thal^{-SEA} : 2nd cycle

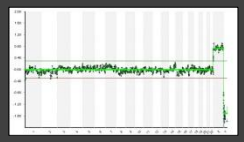
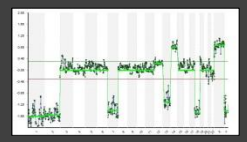
- 10 oocytes collected
- 8 embryos biopsied
- Results:
  - 1 heterozygous (Ht)
  - 3 affected (1 suggestive of Ht by LA1)
  - 4 with no result
- 1 heterozygous – ET on Day 6
- Baby boy – 3,280gm – 2 October 2008

PGD of alpha-Thalassemia^{-SEA}

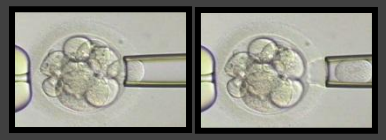
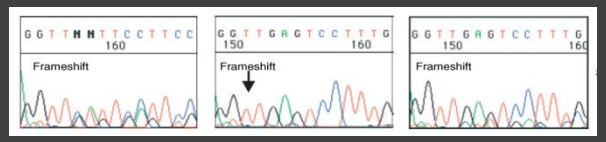
- New set of primers was developed and tested for alpha-thalassemia^{-SEA}
- Primers for internal normal control fragment was also included
- Linked marker was used for back-up linkage analysis

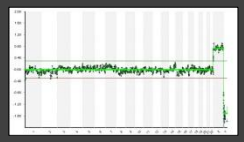
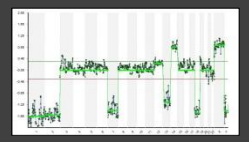



PGD of alpha-Thalassemia^{-SEA}

- Multiplex PCR using 4 sets of primers amplifying 5 fragments in a heterozygote sample was successfully done on single cells
- Clinical PGD cycles for alpha-thalassemia^{-SEA} were performed
- First birth following PGD of alpha-thal^{-SEA} in Thailand

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- Eisai (Thailand) Co., Ltd.

PREGNANCY FOLLOWING PREIMPLANTATION GENETIC DIAGNOSIS OF ALPHA-THALASSEMIA

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GENETICS

Preimplantation genetic diagnosis of alpha-thalassemia^{-SEA} using novel multiplex fluorescent PCR

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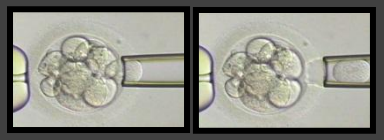
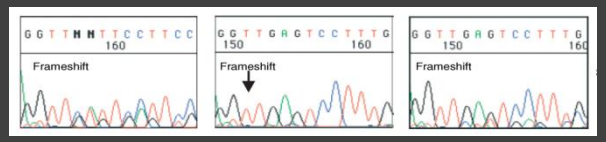
Abstract
Purpose Preimplantation genetic diagnosis (PGD) is an alternative to prenatal diagnosis (PND) giving couples at risk a chance to start a pregnancy with a disease-free baby. This study aimed to develop a new PGD protocol for alpha-thalassemia^{-SEA} mutation, the commonest Mendelian disorder.
Patients and methods Multiplex fluorescent PCR was employed for mutation, contamination and linkage analysis. A couple experienced termination of pregnancy following positive PND decided to join the project.
Results Novel primers for alpha-thalassemia^{-SEA} mutation widely applicable. Interestingly, a potential effect of alpha-thalassemia^{-SEA} mutation on preimplantation embryonic development was noticed.

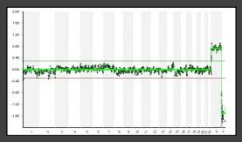
Keywords Embryo selection · Multiplex fluorescent single cell polymerase chain reaction (PCR) · Preimplantation genetic diagnosis (PGD) · Alpha-thalassemia

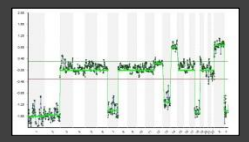
Introduction

alpha-Thalassemia is the most common single gene

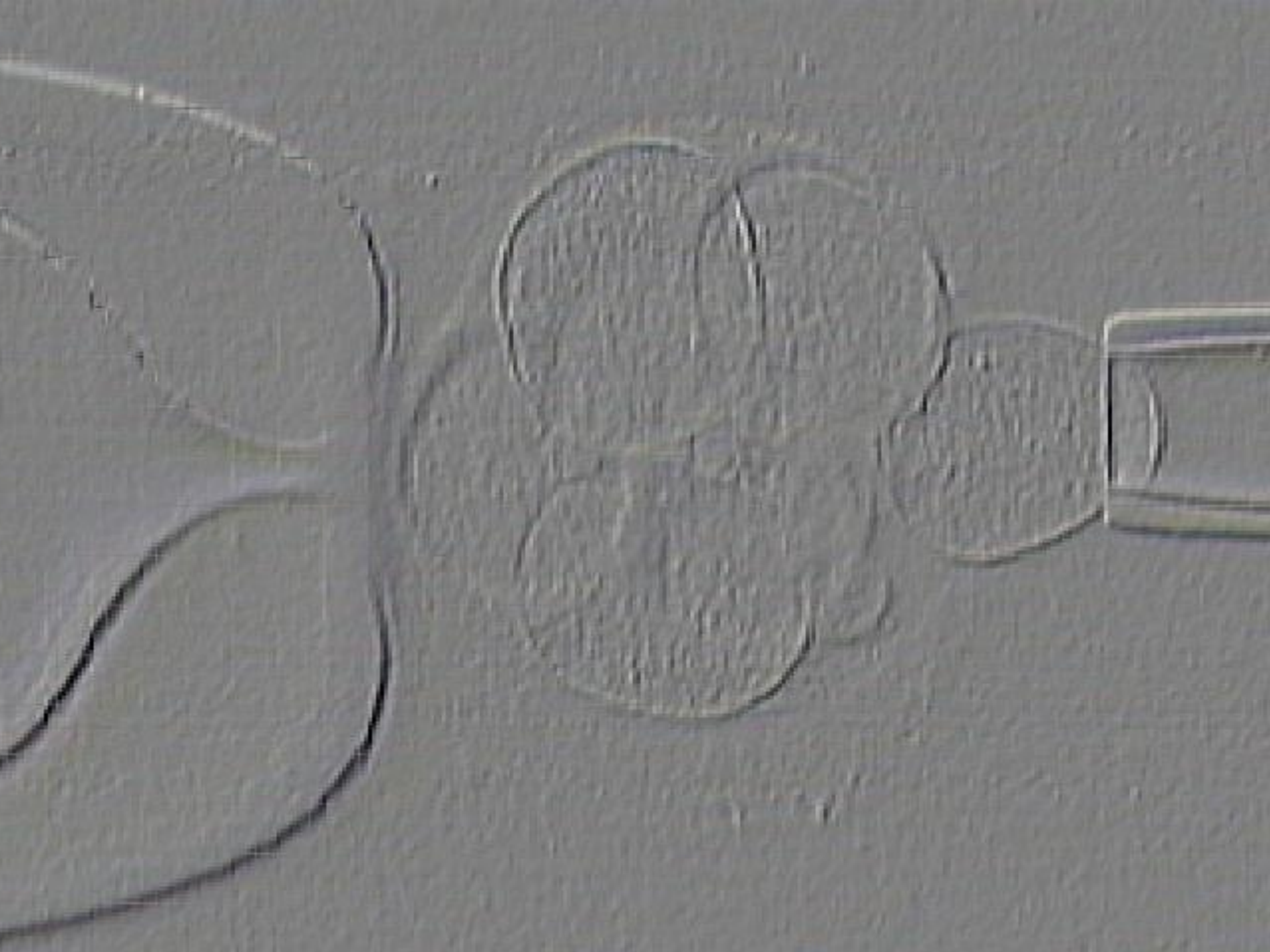


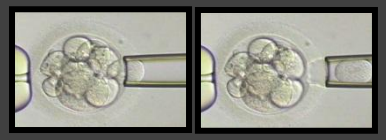
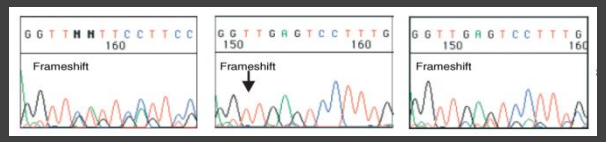


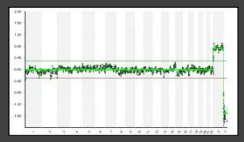
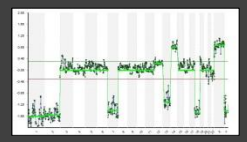




alpha-Thal PGD Baby

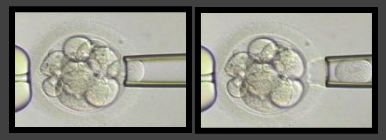


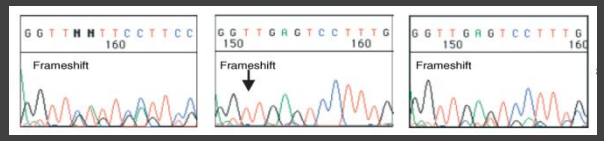



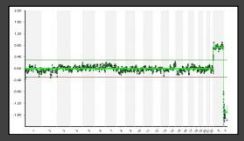



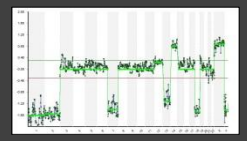
Factors Influencing Single Cell PCR

- Freezing/thawing, quality of cells
- Number of cells
- Multiplexing
- Thermal cycle programs
- Fragment lengths
- GC contents
- Cell lysis protocols

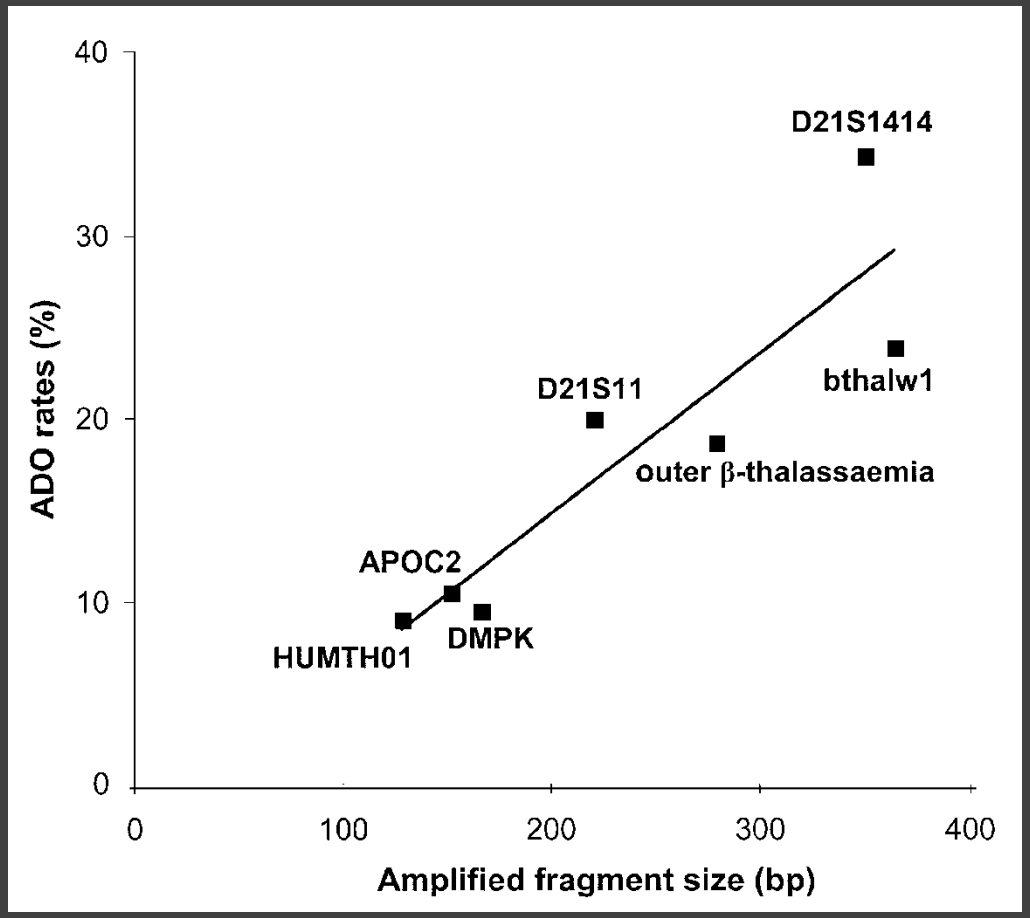


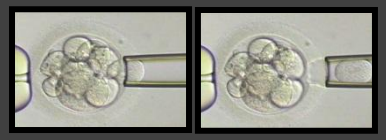


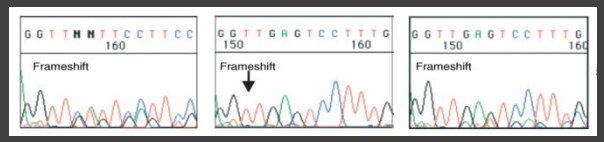


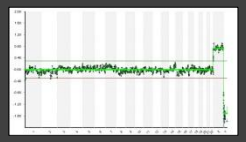


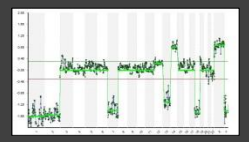
Factors Influencing Single Cell PCR











Factors Influencing Single Cell PCR