cryobio

# Additional Agreement to Use Donor CB 588

I (_____ (Recipient), and _____ (Partner, if applicable)), specifically request and accept frozen semen from Cryobio donor CB 588. I understand that this Additional Agreement is an additional part of the Sperm Use Agreement specific to donor CB 588. CB 588 had expanded genetic carrier screening to determine their carrier status for 557 recessive genetic conditions. Please note that Cryobio thoroughly evaluates each donor's results and assesses potential risks of any identified results before allowing donors to remain in our donor program.

I have reviewed genetic test results on this sperm donor, and I understand that donor CB 588 has been found to be a carrier of the following recessive genetic conditions:

**Alpha-Thalassemia (silent carrier)**
**Biotinidase Deficiency**
**Congenital Adrenal Hyperplasia, 21 Hydroxylase Deficiency (possible carrier)**

**Purpose of genetic carrier screening:** Carriers of genetic conditions have changes, called pathogenic variants or mutations, in a specific (or multiple) gene(s). Most of the genetic conditions that the Cryobio donors are tested for are inherited in an autosomal recessive pattern (see Figure 1). Typically, we all have two copies of every gene---one from the egg source and one from the sperm source. Autosomal recessive conditions require a mutation in both copies of the same gene in order for it to cause the condition. Therefore, individuals who carry just one mutation in a gene that causes recessive genetic conditions are 'carriers' of that specific condition. Carriers of most of the genetic conditions Cryobio donors are tested for do not typically show symptoms of the condition, i.e., they are asymptomatic, although there are rare exceptions. Most individuals are carriers for at least one if not multiple recessive genetic conditions.

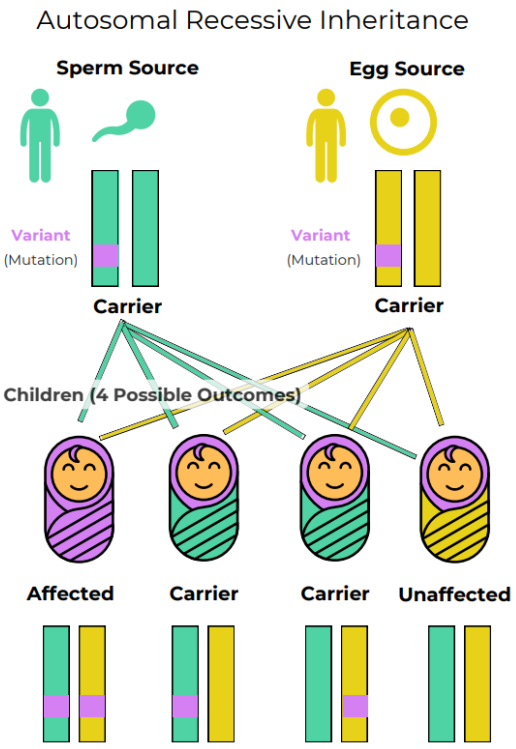



**Figure 1.** Graphic representing autosomal recessive inheritance of two carrier gametes.

Carrier status is helpful to know because if both the egg source and the sperm source are carriers for pathogenic variants or mutations in the same gene, then there is a 1 in 4 chance of the resulting child having that specific condition; a 2 in 4 chance of the resulting child being a carrier for that specific condition; and a 1 in 4 chance of the resulting child being neither a carrier or having that specific condition. Some of the conditions Cryobio donors are tested for have genotype-phenotype correlation, meaning that specific genetic pathogenic variations (the genotype) in a specific gene can be predictive of the type/specific features of a condition that may present in the individual (the phenotype), but not all do. Additionally, some of the genes can be linked to dominant conditions, meaning having a mutation in just one gene may increase the risk of a specific condition. If a specific change in a gene is linked to a dominant condition, it will be noted in this consent form.

**Alpha-thalassemia (*HBA1* and *HBA2* genes):**
Thalassemia is an autosomal recessive genetic condition that affects the hemoglobin, which is a part of the red blood cell that helps carry oxygen throughout the body. Individuals with thalassemia make less hemoglobin. Thalassemia can cause anemia (deficiency of healthy red blood cells) and prevent the body from getting enough oxygen. There are two different types of thalassemia: alpha-thalassemia and beta-thalassemia. Alpha-thalassemia is caused by mutations in the *HBA1* and *HBA2* genes, while beta-thalassemia is caused by mutations in the *HBB* gene. Donor CB 588 is a carrier for alpha-thalassemia.

The *HBA1* and *HBA2* genes are responsible for making a part of the hemoglobin called alpha globin. Generally, we have four functioning copies of the alpha globin genes (two copies of *HBA1* and two copies of *HBA2*). One copy of the *HBA1* gene and *HBA2* gene is inherited from the egg and the other copy of the *HBA1* gene and *HBA2* gene is inherited from the sperm. This is often written as aa/aa. There are four types of alpha-thalassemia, and the type of disease varies based on how many alpha genes are not working:

- Alpha-thalassemia silent carrier (aa/a-): Individuals have one alpha gene that is not working. Individuals are typically asymptomatic.

- Alpha-thalassemia trait (a-/a-) or (aa/--): Individuals have two alpha genes that are not working. They can inherit two non-working alpha genes from either the egg or sperm source (aa/--), or one non-working alpha gene from both the egg and the sperm source (a-/a-). Individuals with alpha-thalassemia trait may have mild anemia, but many are still asymptomatic.

- Hemoglobin H disease (a-/--): Individuals have three alpha genes that are not working. Individuals may have moderate to severe anemia, an enlarged spleen, and mild jaundice (yellow discoloration of the eyes, skin, etc.). Some individuals may require blood transfusions.

- Hemoglobin Bart hydrops fetalis (Hb Bart) syndrome (--/--): Individuals have all four alpha genes that are not working. This is the most severe form and usually presents with symptoms prenatally. This is a fatal condition resulting in severe fetal anemia, hydrops fetalis (fluid buildup around the baby in utero), and often stillbirth or death shortly after birth.

**Of note:** Donor CB 588 was identified to have one non-working copy of the *HBA2* gene. This means donor CB 588 is a silent carrier (aa/a-) for alpha-thalassemia. Depending on the carrier status of the egg source, offspring of donor CB 588 could be a non-carrier (have all working alpha genes), be a silent carrier (have one non-working alpha gene), have alpha-thalassemia trait (have two non-working alpha genes), or Hemoglobin H disease (have three non-working alpha genes).

**Biotinidase deficiency (*BTD* gene):** Biotinidase deficiency is an autosomal recessive disorder caused by pathogenic variants in the gene *BTD*. Individuals with biotinidase deficiency are unable to effectively "reuse" a vitamin, called biotin, in their body. Biotin is important in helping the body break down proteins, fats, and carbohydrates. If individuals with biotinidase deficiency are identified prior to developing symptoms; they typically remain asymptomatic if appropriate biotin therapy is started early (i.e. taking extra biotin for the body to use). However, some symptoms such as hearing loss and vision problems may still occur. Biotin supplements need to be continued throughout the individual's life.

If left untreated, biotinidase deficiency affects individuals within the first few months of life or in childhood. Severe forms of the disorder cause children to experience neurological abnormalities such as seizures, decreased muscle tone, developmental delay, vision problems, and even death. Other symptoms include hearing loss, respiratory problems, and abnormalities related to or affecting the skin such as rash

and alopecia (hair loss). While effective treatment is available, symptoms such as vision problems, hearing loss, and developmental delay are irreversible once they have appeared. Carriers are not expected to show symptoms of this disease. Biotinidase deficiency is recommended as part of the newborn screen in the United States, therefore most babies are screened for and diagnosed with the disease at birth.

**Of note**: Donor CB 588 tested positive for two copies of the c.1330G>C mutation in the *BTD* gene. In other words, both copies of donor CB 588's *BTD* gene carries a genetic mutation. This means that all children born from donor CB 588 will inherit one *BTD* c.1330G>C mutation from the donor.

The c.1330G>C mutation is mild and does not cause biotinidase deficiency, in individuals with two copies, like this donor. Because this donor will pass on a *BTD* mutation in every pregnancy, if the egg source is a carrier of a severe *BTD* mutation, the offspring would have a 50% risk rather than the typical recessive inheritance risk.

**Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CYP21A2 gene):**
Congenital adrenal hyperplasia (CAH) is an autosomal recessive condition which results from a deficiency in enzymes involved in cortisol production (a steroid hormone naturally produced by the adrenal glands). These hormones produced by the adrenal glands help regulate many essential functions in the body, including sexual development and maturation. There are several types of CAH, which can be caused by changes in different genes, but approximately 95% of cases of CAH are caused by changes in the *CYP21A2* gene which leads to a deficiency of the steroid 21-hydroxylating enzyme. Symptoms of CAH vary based on the form of CAH, the age of diagnosis, and the sex of the individual.

Three different forms of CAH have been reported: a classic salt wasting form, a classic simple virilizing form, and a non-classic form.

- The classic salt wasting form of CAH is the most severe. Individuals with the salt-wasting form lose large amounts of sodium (salt) in the urine, which can lead to "salt-wasting crises" that can be life-threatening in early infancy. Female infants with the classic type usually have external genitalia that do not look clearly male or female (ambiguous genitalia), while males typically have normal genitalia, although their testes may be smaller than typical. Individuals with the classic form may also have decreased fertility.
- The classic simple virilizing form involves prenatal virilization (the development of male physical characteristics such as muscle bulk, body hair, and deep voice in a female or earlier than expected in a boy), ambiguous genitalia, and excessive facial hair, and decreased fertility, but does not include the life-threatening salt-wasting crises.
- The mildest form, non-classic CAH, is often not diagnosed until later in childhood or adulthood. Individuals with the non-classic form typically have typical external genitalia but may present with hyperandrogenism (too much testosterone in the body), hirsutism (abnormal growth of hair on the face and body, especially on a woman), male pattern baldness, delayed menarche (the first period for females), and infertility.

Treatment for CAH usually includes steroids to replace the low hormones. The long-term prognosis for individuals with CAH is usually favorable, and with lifelong treatment, individuals with CAH typically have good health and normal lifespans. Oftentimes individuals with non-classic CAH require no treatment at all. CAH is typically included on the newborn screen (check with your state/delivering hospital to be sure), so most individuals who have it will be diagnosed shortly after birth.

**Special note specific to CB 588's *CYP21A2* carrier status:** Donor CB 588 was identified to be at an "increased risk" of being a carrier of CAH. An increased carrier risk test result suggests the donor might carry CAH, but the test does not provide definite confirmation of carrier status. Donor CB 588's test

identified that he has one extra copy (duplication) of the *CYP21A2* gene than the average person, and at least one of the copies contains a mutation. Of note, it is very common to have a duplication, and duplications are not disease-causing.

In this case, it is not possible to determine which of donor CB 588's *CYP21A2* gene copies contains the mutation. If the copy of the *CYP21A2* gene with a mutation is with the extra copy, the donor would still always pass on a working copy of the *CYP21A2* gene and therefore would not be a carrier of CAH. However, if the copy of the *CYP21A2* gene with a mutation is by itself, the donor would be a carrier of CAH, and typical autosomal recessive reproductive risks would apply.

At most, donor CB 588 is a carrier of CAH, but because we cannot fully determine donor CB 588's carrier status, he should be treated as a carrier of CAH. Carriers are not expected to have symptoms of the condition. This means that we strongly recommend the egg source be tested for CAH carrier status to better understand the risks of the condition to future children.

**Carrier status frequency:** Carrier status frequency is the chance of an individual being a carrier for a genetic condition based on general population risks, or ethnicity, prior to any genetic screening. If an individual tests negative as a carrier for a condition or conditions, then the chance of being a carrier is significantly decreased. There is still remaining risk called residual risk. Residual risk means the risk of being a carrier even after negative genetic testing for a condition. Residual risk data on the conditions the donor tested negative for can be requested from Cryobio. The carrier frequency provided is from the test provider. As with all genetic information, these carrier frequency numbers may change over time, and may slightly vary from lab to lab depending on the data used to curate them. Therefore, the carrier frequencies from this additional agreement are based on the numbers available from the performing laboratory on the date the donor's test results were reviewed by the lab.

**Carrier status frequency (as reported by Natera):**

Alpha-Thalassemia (*HBA1/HBA2* genes):
- General Population carrier frequency: 1 in 25
- African American ethnicity: 1 in 30
- Asian ethnicity: 1 in 20
- Caucasian ethnicity: <1 in 500

Biotinidase Deficiency (*BTD* gene):
- General Population carrier frequency: 1 in 120
- African American ethnicity: 1 in 30
- Caucasian ethnicity: 1 in 12
- Hispanic ethnicity: 1 in 30

Congenital Adrenal Hyperplasia, 21-Hydroxylase Deficiency (*CYP21A2* gene), including non-classical type:
- General Population carrier frequency: 1 in 17
- Ashkenazi Jewish ethnicity: 1 in 7
- Caucasian ethnicity: 1 in 11

**Recommendation:** Cryobio recommends that the recipient, or egg source if different than recipient, be tested for alpha-thalassemia, biotinidase deficiency, and congenital adrenal hyperplasia, 21 hydroxylase deficiency carrier status and consider genetic counseling. Please contact Cryobio with any questions or to arrange genetic counseling. Genetic counseling services can also be found through the National Society of Genetic Counselors. We also strongly recommend that you discuss the donor's genetic carrier status results with your health care provider. Finally, we recommend that any future child be notified of this

donor's carrier status once they are of reproductive age, as even if they do not have a recessive disease, they could be a carrier, and their carrier status could help them identify risks related to their own reproductive future.

| **Cryobio has advised me of the following:** | **Please initial to show your understanding and agreement:** |
|---|---|
| The donor I have chosen has positive results from genetic testing looking at carrier status for 557 genes. These results indicate that the donor is a carrier for alpha-thalassemia, biotinidase deficiency, and congenital adrenal hyperplasia, 21 hydroxylase deficiency. | Initials: _____ Initials: _____ |
| The genetic conditions tested for are inherited as recessive patterns. This means that if both the egg source and the sperm source are carriers for the same condition, there is a significantly higher chance of the resulting child having that genetic condition. | Initials: _____ Initials: _____ |
| By the donor testing positive for carrier status for alpha-thalassemia, biotinidase deficiency, and congenital adrenal hyperplasia, 21 hydroxylase deficiency the risk to a resulting child would now be higher than that of the general population. | Initials: _____ Initials: _____ |
| When an individual tests negative for carrier status, it does not completely eliminate their chance of being a carrier for that condition, however their remaining risk is greatly reduced. This remaining risk is called residual risk, and the residual risk can vary significantly from person to person. For more detailed information regarding the sensitivity of testing and remaining risk after negative screening, please contact Cryobio. | Initials: _____ Initials: _____ |
| As genetic testing evolves and more data becomes available, I understand that there is the possibility of updated genetic information that may be uncovered for this donor for a variety of reasons. It is my responsibility to check back with Cryobio to see if any new genetic information is available for this donor. | Initials: _____ Initials: _____ |
| Genetic testing for me (or the egg source, if different) can also be done to better understand and further reduce the risk to offspring. | Initials: _____ Initials: _____ |
| Genetic testing is *strongly recommended* for me (or the egg source, if different) to see if I am a carrier for alpha-thalassemia, biotinidase deficiency, and congenital adrenal hyperplasia, 21 hydroxylase deficiency. | Initials: _____ Initials: _____ |
| Expanded genetic carrier screening is continuing to evolve, and at the time this donor entered the program this was the screening available. This donor had genetic testing with Natera in 2024. My health care provider may recommend an expanded carrier screen that includes/included more than the 557 genes screened for in this donor. It is my responsibility to share this information with my health care provider and review the risks and benefits of being screened for more (or fewer) genetic conditions. | Initials: _____ Initials: _____ |

| | |
|---|---|
| The genetic testing done on the donor does *not* screen for all known genetic conditions. | Initials: _____ Initials: _____ |
| While genetic testing can lower the likelihood for certain genetic conditions, no amount of genetic testing can guarantee that a child will be free of all genetic conditions. | Initials: _____ Initials: _____ |
| Genetic counseling is available to me if I have additional questions regarding these test results and potential risks. | Initials: _____ Initials: _____ |
| Both the donor's carrier status and whether the donor is acceptable for my use should be discussed with my health care provider. | Initials: _____ Initials: _____ |

***I have read the above material and assume the risk of using donor sperm from a donor who has been found to be a carrier of a genetic condition or genetic conditions. I am making the choice to use donor sperm from donor CB 588 willingly and agree to release any legal claims, including negligence, that may arise from or are related to insemination or assisted reproduction using donor sperm from donor CB 588.***

I have read and had the chance to ask questions, and I understand and agree to the terms of this Additional Agreement to use donor CB 588.

_____

Recipient Date Email

_____

Partner, if applicable Date Email

William C. Baird, PhD, HCLD 01-20-2026

Cryobio Date