cryobio

Additional Agreement to Use Donor CB 556

I, (_____ (Recipient), and _____ (Partner, if applicable)), specifically request and accept frozen semen from Cryobio donor CB 556. I understand that this Additional Agreement is an additional part of the Sperm Use Agreement specific to donor CB 556. CB 556 had expanded carrier screening to determine their carrier status for 283 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 556 has been found to be a carrier of the following recessive genetic conditions:

Beta-globin related hemoglobinopathies;
Congenital adrenal hyperplasia due to 21-hydroxylase deficiency;
Familial Mediterranean fever; and
Phenylalanine hydroxylase deficiency.

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.

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.

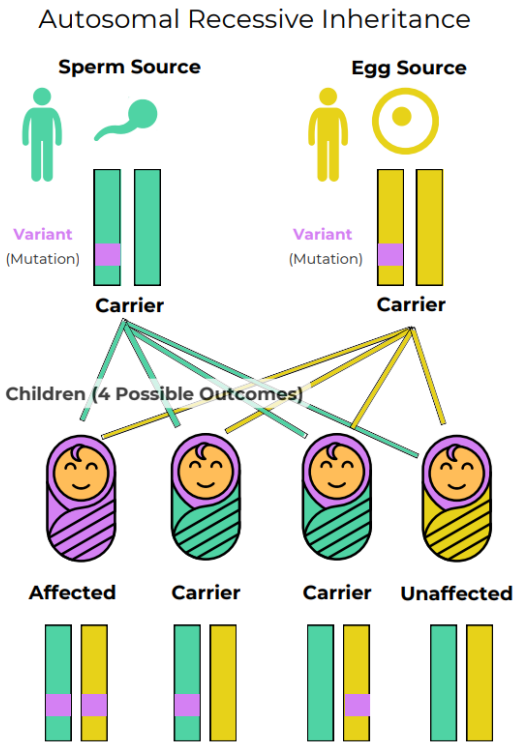

Figure 1. Graphic representing autosomal recessive inheritance of two carrier gametes.

Beta-globin related hemoglobinopathies (*HBB* gene): Pathogenic variants in the beta-globin gene (*HBB* gene) cause a variety of autosomal recessive conditions of atypical hemoglobin, the protein that carries oxygen in the blood. Diseases of abnormal hemoglobin are called hemoglobinopathies. The most frequent hemoglobinopathies are beta-thalassemia, sickle cell disease, and HbC disease. However- different combinations of different *HBB* variants can cause a specific "type" of hemoglobinopathy. With donor CB 556's particular variant, the following conditions should be considered for appropriate carrier screening of the recipient (or the egg source if different) to best understand the risks for beta-globin related hemoglobinopathies:

- ***Beta-thalassemia:*** In individuals with beta-thalassemia, hemoglobin is not properly made and results in small red blood cells (RBCs). RBCs are important for carrying oxygen throughout the body, so abnormalities in RBCs can cause inefficient amounts of oxygen in the body. Individuals with severe beta-thalassemia require life-long blood transfusions and chelation therapy (which is a therapy that removes the extra iron in the blood that results from blood transfusions). Individuals with milder forms of beta-thalassemia may not require transfusions. Life expectancy may be shortened due to cardiac complications of iron overload. It has been reported that individuals who carry one Hb City of Hope variant (like donor CB 556) on one *HBB* gene and one beta zero pathogenic variant on the other *HBB* gene have presented with beta-thalassemia type clinical disease. Beta zero variant means a genetic variant that results in no protein being produced. This is in contrast to a beta plus variant where there is some quantity of protein produced, but not the "normal" amount.

- ***Sickle cell disease:*** Sickle cell disease is typically caused by the inheritance of two copies of Hemoglobin S (HbS), which is caused by a specific *HBB* variant. Symptoms typically first present in infancy or childhood and include chronic anemia (deficient amount of RBCs), pain and/or swelling in the hands and feet, episodes of severe pain, and infections. The clinical presentation is highly variable between affected individuals. The life expectancy for individuals with sickle cell disease may be shortened. HbS can also cause related diseases if inherited along with a different type of variant in *HBB*. It has been reported that individuals who carry one Hb City of Hope variant (like donor CB 556) on one HBB gene and one HbS variant on the other *HBB* gene have presented with disease.

Variants causing beta-thalassemia are most prevalent in Mediterranean and South-East Asian populations, whereas HbS is most common in people of African, Mediterranean, Middle Eastern, and Indian ancestry. However, individuals of any ancestry can be carriers of *HBB* variants. Hemoglobinopathies are typically screened for on the newborn blood screen, so most individuals with a hemoglobinopathy are diagnosed at birth.

Important Note: Donor CB 556's specific Hb City of Hope variant has been reported to cause disease when found in combination with specific beta zero variants, as well as HbS variants. Therefore, it is recommended the recipient, or egg source if different, be screened for beta-globin related hemoglobinopathies via molecular testing (such as this type of carrier screening), as well as undergo a complete blood count (CBC) and hemoglobin electrophoresis screen. This is especially important if the egg source is of Asian, African, Hispanic, or Mediterranean ancestry. This ensures the most comprehensive type of screening and helps further reduce the possibility of carrier status that could be missed by molecular screening alone. This recommendation is consistent with the American College of Obstetricians and Gynecologists as well as the American College of Medical Genetics. We understand that genetic carrier testing can be confusing, and especially in the case of inherited hemoglobinopathies. ***We highly encourage you to review these results in detail with your providers to ensure you the recipient, or egg source if different, receive the appropriate carrier screening and interpretation of***

results prior to pregnancy. As always, Cryobio has genetic counseling services available for any additional questions.

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency, non-classic form (*CYP21A2* gene): Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder which results from a deficiency in enzymes involved in cortisol production (a steroid hormone naturally produced by the body). Approximately 95% of cases of CAH are caused by defects in the *CYP21A2* gene, which leads to a deficiency of the steroid 21-hydroxylating enzyme. Approximately 1 in 12 individuals is a carrier of CAH. 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 and presents with infant's external genitalia not appearing to be clearly male or female (ambiguous genitalia), puberty earlier than typical (precocious puberty), excessive facial hair, and includes inadequate adrenal aldosterone secretion that can result in the body not being able to retain enough salt which can result in a fatal health event called a "salt-wasting crises".
- 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 precociously in a boy), ambiguous genitalia, precocious puberty, and excessive facial hair, 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 may present with too much testosterone in the body (hyperandrogenism). Hyperandrogenism may cause abnormal growth of hair on the face and body, especially on a woman (hirsutism), 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, affected individuals typically have good health and normal lifespans. Often, individuals with non-classic CAH require no treatment at all. CAH is typically included on the newborn blood screen (check with your state/delivering hospital to be sure), so most individuals who have it will be diagnosed shortly after birth.

Of note: Cryobio donor CB 556 tested positive as a carrier for a pathogenic variant associated with the non-classic type of CAH. This means that even when paired with another *CYP21A2* pathogenic variant associated with any type, the offspring would only be at risk for the mild, non-classic form of CAH.

Familial Mediterranean fever (*MEFV* gene): Familial Mediterranean fever (FMF) is an autosomal recessive disorder caused by pathogenic variants in the gene *MEFV*. It is primarily characterized by recurrent fevers, abdominal and thoracic pain, joint pain and inflammation, myalgia (muscle pain), and amyloidosis in the kidneys (buildup of proteins in the kidneys causing it not to work right). It is particularly common in the Middle Eastern and Mediterranean populations, as well as in individuals of Ashkenazi or Sephardic Jewish ancestry. Clinical symptoms are variable, and some patients have mild forms that never require clinical attention.

Two main forms of the condition exist:

- Type 1: Type 1 is characterized by recurrent bouts of fever and inflammation and pain in the abdomen or joints. Depending on the individual these bouts may occur often or rarely. Each episode typically lasts about 3 days. Some patients have symptoms of discomfort before an episode begins.

- Type 2: In type 2, some patients who do not experience fever episodes may develop a buildup of proteins called amyloids in the kidneys. This can lead to kidney damage and end-stage renal disease, requiring dialysis or kidney transplant. Life expectancy is not reduced, except in untreated patients with severe kidney manifestations. Certain variants are associated with more severe disease, development of amyloidosis, and earlier onset of symptoms.

Important Note: Carriers of FMF most often are not expected to have symptoms of the disease. **However, there are reports of carriers of a single *MEFV* variant exhibiting mild to severe symptoms of FMF.** There are multiple possible explanations for why some individuals who are carriers may exhibit disease while others may not. Multiple studies have suggested that an increasingly plausible explanation as to why having only one *MEFV* mutation results in a FMF clinical picture for some individuals is the presence of one or more "modifying alleles" in other related genes. This means that variants in other genes in combination with the *MEFV* variant is what would actually be causing disease. If this is the case, it is important to consider ethnicities where FMF is more prevalent, as individuals with those ethnic backgrounds may carry more of these additional "modifying alleles". Again-FMF is particularly present in individuals of Middle Eastern and Mediterranean populations, as well as in individuals of Ashkenazi or Sephardic Jewish ancestry. Therefore-it is important to consider if the recipient, or egg source if different, identifies any of these in their own ethnic background.

Researchers have also suggested the presence of environmental factors (such as stress) that may contribute to disease in single *MEFV* mutation carriers. Others have indicated that some variants may be enough to cause FMF alone, but that there is reduced penetrance (i.e., not everyone with a particular mutation always develops disease).

Individuals who have a diagnosis of FMF but are carriers of only one *MEFV* variant have been associated with a milder form of the disease. However, it is important to reiterate that still most carriers of *MEFV* variants will NOT exhibit signs or symptoms of FMF. One study suggests that less than 1% of all *MEFV* mutation carriers would develop FMF. It is also important to note that donor CB 556's particular variant (c.2177>T) is most often associated with mild disease. Additionally, donor CB 556 did not report having a personal or any family history of FMF-nor did he report any family members with symptoms such as recurrent fevers or auto-inflammatory conditions.

Phenylalanine hydroxylase deficiency (*PAH* gene): Phenylalanine hydroxylase deficiency is an autosomal recessive disorder caused by pathogenic variants in the gene *PAH*. While found in many different ethnicities, it is particularly prevalent in Sephardic Jewish, Sicilian, Irish, Turkish and Caucasian individuals. Pathogenic *PAH* variants result in loss of function of the phenylalanine hydroxylase enzyme, which breaks down one of the building blocks that help make up protein called phenylalanine. Phenylalanine is found in most protein containing food, including meat, eggs, cheese, and fish. It is also found in things like aspartame, an artificial sweetener.

The severity of the condition varies based on the level of deficiency of the phenylalanine hydroxylase enzyme. The most severe form of the condition is known as phenylketonuria, or PKU. When untreated, buildup of phenylalanine will result in irreversible brain damage and severe intellectual disability. However, even with strict adherence to the treatment, some neurologic deficiencies have been noticed in individuals long-term. Psychological problems, including anxiety, depression, phobias, and panic attacks may occur in adults who do not comply well to their treatment.

Treatment involves the removal of phenylalanine from the diet, and in some cases medications/supplements to assist with the breakdown of phenylalanine. Some patients have a milder form of hyperphenylalaninemia (too much phenylalanine) and may tolerate higher levels of phenylalanine in their diet than others. Depending on the genotype (the specific pathogenic variants in the *PAH* gene),

patients may be responsive to medications and dietary supplement, which can direct their treatment. However, it is not always possible to predict the severity of the disease based on genotype. Phenylalanine hydroxylase deficiency is screened for on the newborn blood screen, so most individuals who are affected are now diagnosed at birth.

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 based on 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.

Beta-globin-related hemoglobinopathies (*HBB* gene) carrier status frequency in different ethnicities, from SEMA4:

Worldwide	1 in 81
African	1 in 97
Ashkenazi Jewish	1 in 28
East Asian	1 in 87
Finnish	1 in 1901
European (Non-Finnish)	1 in 214
Native American	1 in 438
South Asian	1 in 25

HbS Variant (*HBB* gene) carrier status frequency in different ethnicities, from SEMA4:

Worldwide	1 in 115
African	1 in 11
European (Non-Finnish)	1 in 7903
Native American	1 in 232
South Asian	1 in 810

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (non-classic) (*CYP21A2* gene) carrier status frequency in different ethnicities, from SEMA4:

Worldwide	1 in 16
Ashkenazi Jewish	1 in 7
European (Non-Finnish)	1 in 11

Familial Mediterranean Fever (*MEFV* gene) carrier status frequency in different ethnicities, from SEMA4:

Worldwide	1 in 40
African	1 in 230
Ashkenazi Jewish	1 in 8
East Asian	1 in 141
Finnish	1 in 29
European (Non-Finnish)	1 in 40
Native American	1 in 74
South Asian	1 in 56

Phenylalanine hydroxylase deficiency (*PAH* gene) carrier status frequency in different ethnicities, from Sema4's website:

Worldwide	1 in 50
African	1 in 143
Ashkenazi Jewish	1 in 17
East Asian	1 in 68
Finnish	1 in 158
European (Non-Finnish)	1 in 37
Native American	1 in 70
South Asian	1 in 121

Recommendation: Cryobio recommends that the recipient, or egg source if different than recipient, be tested for beta-globin related hemoglobinopathies (*HBB* gene); congenital adrenal hyperplasia due to 21-hydroxylase deficiency (*CYP21A2* gene); familial Mediterranean fever (*MEFV* gene); and phenylalanine hydroxylase deficiency (*PAH* gene) carrier status and consider genetic counseling. **Important Note:** To ensure the recipient, or egg source if different than recipient, is thoroughly screened for all beta-globin related hemoglobinopathies, it is the gold standard to do the *HBB* gene molecular testing, as well as a CBC (complete blood count), and hemoglobin electrophoresis. This testing will ensure the most comprehensive screening to best define risks to a pregnancy for hemoglobinopathy diseases.

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 283 genes. These results indicate that the donor is a carrier for beta-globin related hemoglobinopathies; congenital adrenal hyperplasia due to 21-hydroxylase deficiency; familial Mediterranean fever; and phenylalanine 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 beta-globin related hemoglobinopathies; congenital adrenal hyperplasia due to 21-hydroxylase deficiency; familial Mediterranean fever; and phenylalanine 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 beta-globin related hemoglobinopathies (*HBB* gene), congenital adrenal hyperplasia due to 21-hydroxylase deficiency (*CYP21A2* gene), familial Mediterranean fever (*MEFV* gene), and phenylalanine hydroxylase deficiency (*PAH* gene).	Initials: _____ Initials: _____
In rare cases, carriers of familial Mediterranean fever (FMF) may present with the disease. Based on donor CB 556's carrier status for FMF, there is a 50% chance of any resulting child also being a carrier, and therefore inheriting an increased risk of FMF.	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 SEMA4 in 2021. My health care provider may recommend an expanded carrier screen that includes/included more than the 283 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 genetic conditions. I am making the choice to use donor sperm from donor CB 556 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 556.

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 556.

Recipient	Date	Email

Partner, if applicable	Date	Email

William C. Baird, PhD, HCLD	05-25-2022
Cryobio	Date