

DANVERS SAMPLE PREPARATION GUIDELINES

This document supplements the Danvers Sample Submission Guidelines:

http://www.beckmangenomics.com/documents/services/danvers_sample_submission_guidelines.pdf

Please see below for more details and recommendations regarding sample preparation and submission.

If you have any questions about the specifications listed in the outline below, please contact customer service at 800.361.7780.

When shipping samples on dry ice, be sure to comply with the terms of your specific courier and refer to MSDS instructions when handling or shipping any chemical hazards.

For information regarding shipping specifications, please refer to the following link:

http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

All customers submitting samples must also complete the project submission form, located at:

<https://psf.agencourt.com>



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High Throughput Sequencing Services

Beckman Coulter Genomics strongly recommends that international customers submit frozen transformations for plating or frozen glycerol plates.

Submitting Transformations for Plating and Picking

1. Determine titer of transformation before submitting samples
2. It is important to determine the titer of samples that have been frozen and thawed as efficiencies will inevitably drop once samples have been frozen
3. 2X the number of requested clones should be submitted
4. Beckman Coulter Genomics recommends the procedure below to determine the titer:
 - a) Add glycerol to a final concentration of 20% to samples.
 - b) Freeze samples overnight in 200 μ L aliquots in a -80°C freezer
 - c) Thaw one 200 μ L aliquot on ice
 - d) Plate 0.1, 1.0, and 10 μ L on three separate LB agar plates with the appropriate antibiotic
 - e) Grow overnight at 37°C
 - f) Determine the average titer obtained from the three plates in cfu/mL (colony forming units/milliliter). This titer should be the same for the other aliquots still remaining in the freezer that have not yet endured their first thaw
 - g) Place remaining aliquots (while still frozen) in plastic bags
 - h) Ship the samples according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Submitting Agar Lawns for Picking

1. All lawns must be in 25 cm x 25 cm Assay Trays (ThermoFisher part # QH-2216)
2. All agar dishes must be filled with 200 mL of LB Agar or the equivalent volume of agar to have a thickness of 5 to 7 mm. If the agar is too thick or thin, it will prevent the automated picking machine from accurately identifying the colonies. The formula for 1 L of LB Agar is as follows:
 - a) 25 g Luria Broth, Miller (American Bioanalytical Part # AB01201-00500)
 - b) 15 g Bacteriological Agar (American Bioanalytical Part # AB01185-00500)
 - c) Add antibiotic to the concentrations below:

Table 1 – Antibiotic Concentrations

Antibiotic	Final Concentration in LB with Glycerol
Chloramphenicol	12.5 µg/mL
Carbenicillin/Ampicillin	50 µg/mL
Kanamycin	50 µg/mL
Zeocin	50 µg/mL

- d) Templates containing the zeocin-resistant gene should be grown in low salt LB media
3. Agar lawns should be allowed to cool on a level surface. The agar in the dish must be level for the automated colony pickers to work efficiently
 4. Agar should not have clumps in it
 5. All agar dishes should contain 1500-2000 pickable colonies (highest efficiency is at 2000 colonies), but no more than 3000 total colonies per plate. Customers wanting fewer than 1000 samples should still submit colonies on bioassay trays
 6. Colonies on agar lawns should be between 1.5 mm and 2 mm in diameter
 7. Spacing between colonies must be ≥ 2 mm
 8. Beckman Coulter Genomics recommends making 15 to 20% more plates than you expect will be needed because automated colony pickers can not physically pick colonies less than one half inch from the edge of the bioassay tray. Plating more ligation on a single agar plate will lower picker efficiency; if there are more than 5000 colonies on the plate, the colonies will be too close together for accurate picking
 9. Do NOT use Petri dishes
 10. Do NOT stamp colonies onto agar lawns
 11. Make sure there is no condensation on the lid of the bioassay tray
 12. Blue/white selection is available at no extra charge
 13. Ship the lawns according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Submitting Glycerol Stock Plates for Purification

1. Media for glycerol stocks should be LB with 10% glycerol (American Bioanalytical Part # CU08048-01000)
 - a) LB with 10% glycerol can be made with the following formula:

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For 1L of LB with 10% glycerol add 10 g Tryptone (American Bioanalytical Part # AB02031-00500), 5 g yeast extract (American Bioanalytical Part # AB01208-00500), 10 g NaCl (American Bioanalytical Part # AB13214-00500) **OR** 25 g LB broth, Miller (American Bioanalytical Part # AB01201-0500). Add 100 mL anhydrous glycerol (American Bioanalytical Part # AB00750-00500) to a 1 L bottle. Fill to 1 L with dH₂O and sterilize.

b) Add antibiotic to the concentrations below:

Table 2 – Antibiotic Concentrations

Antibiotic	Final Concentration in LB with Glycerol
Chloramphenicol	12.5 µg/mL
Carbenicillin/Ampicillin	50 µg/mL
Kanamycin	35 µg/mL
Zeocin	25 µg/mL
Other	Please Contact Agencourt

- c) Templates containing the zeocin-resistant gene should be grown in low salt LB media
- Colonies should be picked into 384-well Greiner plates containing approximately 90 µL of media (PCG Scientific # 7-81101) or 96-well Costar Round Bottom Plates (Fisher Part # 07-200-105) containing approximately 200 µL of media.
 - Cover the plates with a lid and wrap loosely with cellophane or plastic wrap to minimize evaporation.
 - Glycerol plates should be grown statically (i.e. no shaking) for exactly 12 hours at 37°C. If glycerol plates are grown for longer than 12 hours, the cells in plate may not be viable and sequencing quality will suffer. Extensive growth curves for optimal sequencing results and cell viability have been done at Beckman Coulter Genomics showing that 12 hours of growth is optimal for glycerol plates. Overgrowing glycerol plates may also make cells vulnerable to multiple freeze thaw cycles.
 - Plates should be checked after growth. Wells should be slightly cloudy with no more than 38 wells out of 384 or 9 wells out of 96 with no growth.
 - Seal plates with a clear polystyrene heat seal (ThermoFisher Part # AB-3797), if possible. This heat seal requires the use of an ALPS-300 automated plate sealing machine. Beckman Coulter Genomics recognizes that not every lab has access to this type of sealer. In such cases, aluminum adhesive seals (ThermoFisher Part # AB-0626) are recommended. When using aluminum adhesive seals, be sure to tightly press the seal around each well to ensure the best possible seal.
 - Immediately freeze all glycerols at -80°C.

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8. It is recommended that all glycerols submitted to Beckman Coulter Genomics have a “CODE128” or equivalent barcode attached as an identifier. If barcode labels are unavailable, each plate should have either a handwritten or computer printed label affixed to one of the long sides of the plate as an identifier. All plates must have a unique identifier so that data can be packaged and returned to the customer at the end of the project. If requested, Beckman Coulter Genomics can supply barcode labels for use on plates that are being submitted.
9. Ship the glycerol plates according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Note: All cells delivered to Agencourt should be T1 resistant.

Submitting PCR¹ Amplicons for Single Pass Sequencing to Beckman Coulter Genomics

1. PCR products should be submitted in either 96-well full skirted plates (ThermoFisher Part # AB1000) or 384-well full skirted plates (ThermoFisher Part # AB0937)
2. All DNA samples should be submitted with a minimum volume of 20 µL and at a minimum concentration of 15-25 ng/µL. For optimal results all DNA samples should be normalized across the plate.
3. Seal plates with a clear polystyrene heat seal (ThermoFisher Part # AB-3797), if possible. This heat seal requires the use of an ALPS-300 automated plate sealing machine. Beckman Coulter Genomics recognizes that not every lab has access to this type of sealer. In such cases, aluminum adhesive seals (ThermoFisher Part # AB-0626) are recommended. When using aluminum adhesive seals, be sure to tightly press the seal around each well to ensure the best possible seal.
4. Ship the plates according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Sequencing Primer Submissions

1. Agencourt offers a number of universal primers for sequencing, See Table 2. Customers can submit their own primers for sequencing, if desired. Primers for sequencing can be submitted in tubes or in 96-well skirted PCR plates (ThermoFisher Part # AB-0100)

¹ *The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*

Table 3 - Available universal primers for custom sequencing.

T7	5' TAA TAC GAC TCA CTA TAG GG 3'
M13-FWD	5' GTA AAA CGA CGG CCA GT 3'
M13-REV	5' CAG GAA ACA GCT ATG ACC 3'
SP6	5' ATT TAG GTG ACA CTA TAG 3'
T3	5' AAT TAA CCC TCA CTA AAG G 3'
KBR/TJ	5' CTG GCC GTC GAC ATT TAG G 3'
2BP Clamp	5' TTT TTT TTT TTT TTT TTT TTT VN 3'
SeqL-A	5' TCG CGT TAA CGC TAG CAT GGA TCT C 3'
SeqL-B	5' GTA ACA TCA GAG ATT TTG AGA CAC 3'
T7 Terminator	5' TAG TTA TTG CTC AGC GGT GG 3'
CMV Forward	5' CGC AAA TGG GCG GTA GGC GTG 3'
BGH Reverse	5' TAG AAG GCA CAG TCG AGG3'

2. The quantity of primer submitted in a tube should be a minimum of 7.5 nm for 768 reads or fewer; minimum of 50 nm for greater than 768 reads.
3. Primers submitted in plates should have a volume of 25 µL of 3 µM primer per well and be arrayed according to clone layout.
4. Ship the primers according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Rearray Projects

1. See above for submitting glycerol or primer stock plates
2. The worklist format should be as follows:
 - a) Three comma separated values with the following meaning:
 - b) Source Barcode/Plate Name, Well Position, Sample Name²

Source Barcode/Plate Name, Well Position, Sample Name
00000012345, A01, Plate1_SampleA01
00000012345, G12, Plate1_SampleG12
00000012345, E05, Plate1_SampleE05
00000012345, G10, Plate1_SampleG10

² The sample names must not be longer than 64 characters as anything beyond 64 characters will be truncated.

Individual Sample Sequencing

Submitting Research Samples with Primer and Template Mixed

1. Samples submitted for QuickLane® services can be purified DNA mixed with the appropriate sequencing primers. If the sample is to be sequenced in two directions, two tubes or plates containing the same DNA and different primers should be submitted.
2. Individual samples, submitted in tube format, should be submitted in 650 µL two dimensional barcoded tubes sealed with a silicone septum (Agencourt Part # 001108).
3. Samples submitted in plates must be submitted in 96-well ThermoFisher full skirted cycling plates (ThermoFisher Part # AB-1000). Each plate must contain samples that are all the same template type (i.e. high copy, low copy, PCR amplicons, etc).
4. Samples submitted in plates must be accompanied by a tab delimited text file that contains plate name, well location, and clone name. If clone name is not supplied, samples will be identified as plate_well at the time of packaging and data delivery.
5. All samples should contain a final volume of 40 µL for plasmids and 20 µL for PCR amplicons. Table 3 below indicates sample volumes required:

Table 4 - Concentrations and volumes of template and primer necessary for QuickLane submission (template and primer mixed)

Template	Total Amount of DNA	Total Amount of Primer	Total volume of Sample
High Copy Plasmids	0.6 µg - 1.0 µg	20 pmol	40 µL
Low Copy Plasmids, Fosmids, Cosmids	0.6 µg - 1.0 µg	20 pmol	40 µL
BACs	0.6 µg - 1.0 µg	20 pmol	40 µL
PCR Products (indicate size)	0.3 µg - 0.5 µg	10 pmol	20 µL
Difficult Templates (containing >70% GC content or presence of secondary structures – i.e. hairpins)	0.6 µg - 1.0 µg	20 pmol	40 µL

6. The method of DNA preparation (AMPure®, CosMCPrep®, or other method) must be indicated on the project submission form.
7. All tubes submitted to Beckman Coulter Genomics should be labeled with a unique name. The two dimensional barcodes on the bottom of each tube may be used as the unique tube name. Well position is not a unique name and is not permanently linked to the two dimensional barcode.

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- Please complete the sample submission form located at <https://psf.agencourt.com> and enclose it with the samples.
- Ship the templates according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Submitting Research Samples with Primer and Template Unmixed (Purified Plasmid and Purified/Unpurified PCR Products)

- Individual samples, submitted in tube format, should be submitted in 650 μ L two dimensional barcoded tubes sealed with a silicone septum (Agencourt Part # 001108).
- Samples submitted in plates must be submitted in 96-well ThermoFisher full skirted cycling plates (ThermoFisher Part # AB-1000). Each plate must contain samples that are all the same template type (i.e. high copy, low copy, PCR amplicons, etc).
- Samples submitted in plates must be accompanied by a tab delimited text file that contains plate name, well location, and clone name. If clone name is not supplied, samples will be identified as plate_well at the time of packaging and data delivery.
- Individual primers being submitted should follow the same guidelines as stated in steps 1 and 2 of the previous section.
- If primers are being ordered through Beckman Coulter Genomics, primer sequences must be submitted in FASTA format as one file. Primer/template associations must be established in the sample submission template as described in step 6a.
- Template and primer concentrations and volumes must be submitted according to the table below:

Table 5 - Concentration and volumes of DNA template and primer needed for non-mixed samples

Template	Template Concentration	Volume of Sample per Reaction**	Minimum Sample Volume
High Copy Plasmids	15-25 ng/ μ L	10 μ L	40 μ L
Low Copy Plasmids, Fosmids, Cosmids	15-25 ng/ μ L	20 μ L	40 μ L
BACs	15-25 ng/ μ L	20 μ L	40 μ L
PCR Products (indicate size)	15-25 ng/ μ L	5 μ L	20 μ L
Difficult Templates (containing >70% GC content or presence of secondary structures – i.e. hairpins)	15-25 ng/ μ L	20 μ L	40 μ L

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Primer (regardless of template)	3.2 pmol/ μ L	2 μ L	20 μ L
<i>**To determine the total volume to submit, multiply the volume of sample by the number of reactions needed. Total volume must be \geq the minimum volume. Eg 6 rxn x 10 μL/rxn = 60 μL</i>			

- a) All individual primers should be submitted in 650 μ L two dimensional barcoded tubes sealed with a silicone septum seal (Beckman Coulter Genomics part #001108).
- b) The primers in the following table are offered by Beckman Coulter Genomics as “universal primers.” These primers can be chosen on the project submission form.

Table 6 - Available universal primers for custom sequencing.

T7	5' TAA TAC GAC TCA CTA TAG GG 3'
M13-FWD	5' GTA AAA CGA CGG CCA GT 3'
M13-REV	5' CAG GAA ACA GCT ATG ACC 3'
SP6	5' ATT TAG GTG ACA CTA TAG 3'
T3	5' AAT TAA CCC TCA CTA AAG G 3'
KBR/TJ	5' CTG GCC GTC GAC ATT TAG G 3'
2BP Clamp	5' TTT TTT TTT TTT TTT TTT TTT VN 3'
SeqL-A	5' TCG CGT TAA CGC TAG CAT GGA TCT C 3'
SeqL-B	5' GTA ACA TCA GAG ATT TTG AGA CAC 3'

7. If one primer is submitted for multiple DNA samples, then a text file matching the DNA sample identification (tube label, clone name, or well address) with the primer(s) to be used, must be supplied. Please complete the sample submission template form located at <https://psf.agencourt.com> and enclose it with the samples.
8. Ship the templates according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Submitting Glycerol Stocks for QuickLane Sequencing and Primer Walking

1. All samples need to be identified by DNA Name, vector type, and insert size.
2. Media for glycerol stocks should be LB with 10% glycerol (American Bioanalytical Part # CU08048-01000)
 - a) LB with 10% Glycerol can be made with the following formula:
For 1L of LB with 10% Glycerol add 10 g Tryptone (American Bioanalytical Part # AB02031-00500), 5 g Yeast Extract (American Bioanalytical Part # AB01208-00500), 10 g NaCl (American Bioanalytical Part # AB13214-00500) **OR** 25 g LB Broth, Miller

(American Bioanalytical Part # AB01201-0500). Add 100 mL Anhydrous Glycerol (American Bioanalytical Part # AB00750-00500) to a 1 L bottle. Fill to 1 L with dH₂O and sterilize.

- b) Add antibiotic to the concentrations below:

Table 7 - Antibiotic Concentrations.

Antibiotic	Final Concentration in LB with Glycerol
Chloramphenicol	12.5 µg/mL
Carbenicillin/Ampicillin	50 µg/mL
Kanamycin	35 µg/mL
Zeocin	25 µg/mL
Other	Please Contact Agencourt

- c) Templates containing the zeocin-resistant gene should be grown in low salt LB media
3. Colonies should be picked into tubes containing approximately 250 µL of media.
 4. Glycerol stocks should be grown statically for exactly 12 hours at 37°C. If grown for longer than 12 hours, the cells may not be viable and sequencing quality will suffer. Extensive growth curves for optimal sequencing results and cell viability have been done showing that 12 hours of growth is optimal for glycerol stocks. Overgrowing glycerol stocks may also make cells vulnerable to multiple freeze thaw cycles.
 5. Glycerol stock should be checked after growth. The tube should be slightly cloudy.
 6. Transfer 100 µL of glycerol stock to an Agencourt 2D barcoded tube. 2D barcoded tubes can be purchased directly from Beckman Coulter Genomics (Part # 001108)
 - Part # 001108/Ea (0.650 mL 2D tube with split septum)
 - Part # 001108/Cs (0.650 mL 2D tubes with split septum in lockable rack, 10 racks of 96 per case)
 - Part #001108/RK (0.650 mL 2D tubes with split septum in lockable rack, 1 rack of 96).
 7. Immediately freeze all glycerols at -80°C.

Submitting Purified DNA for Primer Walking of PCR or Plasmid Samples

1. All samples need to be identified by DNA Name, vector type, and insert size.
2. All samples should have a minimum concentration of 25 ng/µL.
3. All samples should have a minimum total amount of 2 µg/1kb of insert.

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4. The method of DNA preparation (AMPure[®], CosMCPrep[®], or other method) must be indicated on the project submission form.

Note: Individual samples can be submitted in 0.650 mL 2D barcoded tubes sealed with a silicone septum seal. (2D barcoded tubes can be purchased directly from Agencourt (Part # 001108).

5. Samples submitted in plates must be submitted in 96-well ThermoFisher full skirted cycling plates (ThermoFisher Part # AB-1000). Each plate must contain samples that are all the same template type (i.e. high copy, low copy, PCR amplicons, etc).
6. Samples submitted in plates must be accompanied by a tab delimited text file that contains plate name, well location, and clone name. If clone name is not supplied, samples will be identified as plate_well at the time of packaging and data delivery. All tubes submitted to Agencourt should be labeled with a unique name. The two dimensional barcodes on the bottom of each tube may be used as the unique tube name. Well position is not a unique name and is not permanently linked to the two dimensional barcode.
7. All samples submitted in tubes should be submitted with a tab-delimited text file that lists the unique tube.
8. Ship the samples according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Large Insert Sequencing

Submitting BAC/Fosmid/Cosmid Glycerol Stocks

1. The customer must submit 100 µL of glycerol stock.
2. Media for glycerol stocks should be LB with 10% glycerol (American Bioanalytical Part # CU08048-01000)

a) LB with 10% Glycerol can be made with the following formula:

For 1L of LB with 10% Glycerol add 10g Tryptone (American Bioanalytical Part # AB02031-00500), 5g Yeast Extract (American Bioanalytical Part # AB01208-00500), 10g NaCl (American Bioanalytical Part # AB13214-00500) **OR** 25 g LB Broth, Miller (American Bioanalytical Part # AB01201-0500). Add 100 mL Anhydrous Glycerol (American Bioanalytical Part # AB00750-00500) to a 1L bottle. Fill to 1L with dH₂O and sterilize.

b) Add antibiotic to the concentrations below:

Table 8 - Antibiotic Concentrations.

Antibiotic	Final Concentration in LB with Glycerol
Chloramphenicol	12.5 µg/mL
Carbenicillin/Ampicillin	50 µg/mL
Kanamycin	35 µg/mL
Zeocin	25 µg/mL
Other	Please Contact Agencourt

- c) Templates containing the zeocin-resistant gene should be grown in low salt LB media.
3. Glycerol stocks must be submitted in Eppendorf tubes. All tubes must be labeled with a unique name (ie. #1, #2, or barcode can be used if legible). A tab-delimited file must also be submitted, which associates the unique tube name with the sample name.
 4. The customer must indicate the vector, growth conditions, and any special requirements for each submission.
 5. Reference sequence for each sample must be submitted in FASTA format if available.
 6. Ship the samples according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Submitting Purified BAC/Fosmid/Cosmid

1. Agencourt recommends purifying DNA using the Qiagen Large Construct Kit
2. Submit a minimum of 50 µg of purified template DNA
3. The DNA must be of high molecular weight and can be submitted as a pellet or resuspended in water or EtOH. The customer must indicate the weight/concentration of the DNA submitted.
4. To determine the weight/concentration of the DNA, Agencourt recommends a PicoGreen reading
5. Note that the minimum quantity of template DNA is the amount required once **purified**. If the amount of DNA totals 50 µg before purification, the purification process will reduce the total amount of DNA available.
6. Ship the samples according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

SNP Discovery

1. For SNP discovery services, mRNA sequences must be submitted as 1 FASTA file
2. Purified DNA samples should be pre-arrayed and submitted in 96-well full skirted ThermoFisher plates (ThermoFisher Part # AB1000). The minimum concentration should be 5.0 ng/ μ l. The DNA should be standardized as much as reasonably possible across all specimens. The minimum amount of DNA required for each individual specimen is: ((40 ng x ___ amplicons) + 15 μ L for dead volume).
3. 2 wells should be left blank per submitted plate for positive and negative controls.
4. An example grid for genomic DNA requirements for a variety of assays per sample is shown below:

Number of Amplicons	Genomic DNA requirement
5	200 ng
10	400 ng
25	1,000 ng
50	2,000 ng
100	4,000 ng

5. Instead of submitting genomic DNA, Beckman Coulter Genomics offers a Coriell panel. Please specify use of the Coriell panel for the project on the project submission form. For more information on the Coriell panel please call 1 800 773 9186.
6. If it is required that Agencourt keep track of the names of your DNA samples through our LIMS (Laboratory Information Management System), please submit a DNA submission worklist electronically. This worklist will contain three columns in tab-delimited text format (DNA plate ID, DNA Plate Well, and DNA Sample ID).
7. Seal plates with a clear polystyrene heat seal (ThermoFisher Part # AB-3797), if possible. This heat seal requires the use of an ALPS-300 automated plate sealing machine. Agencourt recognizes that not every lab has access to this type of sealer. In such cases, aluminum adhesive seals (ThermoFisher Part # AB-0626) are recommended. When using aluminum adhesive seals, be sure to tightly press the seal around each well to ensure the best possible seal.
8. Ship the samples according to the shipping guidelines:
http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

Whole Genome Sequencing

High Copy Plasmid Library:

Agencourt will require a minimum of 10 µg of purified genomic DNA.

Fosmid Library:

Agencourt will require a minimum of 100 µg of purified genomic DNA.

454 Fragment and Mate-paired Libraries:

Agencourt will require a minimum of 20 µg of purified genomic DNA.

SOLiD Fragment Library:

Agencourt will require a minimum of 20 µg of purified genomic DNA.

SOLiD Mate Pair Library:

Agencourt will require a minimum of 60 µg of purified genomic DNA.

Comments/Additional Requirements

The DNA must be of high molecular weight and can be submitted as a pellet or resuspended in water or EtOH. The customer must indicate the weight/concentration of the DNA submitted.

In addition the customer must supply Information on the DNA purification and quantification methods used. As OD260 readings can at times result in vast overestimation of actual DNA sample concentrations Agencourt strongly recommends the use of fluorometric measures (Picogreen) when available. Agencourt will QC the DNA for quantity, purity and quality. Samples will be analyzed with a gel QC and OD260 reading, and/or fluorometry reading. Within 15 working days Agencourt will provide written notice to the client if the DNA is not of sufficient quality or quantity.

cDNA Library Construction

Submission of Tissue or Cells

Beckman Coulter Genomics requires a minimum of 1g of tissue or 1×10^8 cells for the preparation of each standard or subtracted cDNA library, 1 mg of tissue or 1×10^7 cells for the construction of each microquantity library, and 250 µg of tissue or 25,000 cells for the construction of each nanoquantity library. Isolations from root tissue may require the submission of additional material. Tissues or cells will need to be harvested as quickly as possible and flash-frozen in

liquid nitrogen at the time of harvesting, maintained at -80°C and shipped in a large quantity of dry ice to preserve the genetic make-up of the tissue or cells.

Submission of Total RNA

Beckman Coulter Genomics requires a minimum of 1 mg of total RNA for the construction of each standard or subtracted cDNA library, 50 μg of total RNA for the construction of each microquantity cDNA library, and 250 ng of total RNA for the construction of each nanoquantity cDNA library. Total RNA will need to be isolated from tissue or cells using commercial kits or by equivalent methods, resuspended in nuclease free water (no DEPC), stored at -80°C and shipped in a large quantity of dry ice to preserve the integrity of the RNA. Total RNA quality should be verified by the client and A260/A280 readings and gel photo should be included in the shipment.

For nanoquantity libraries, the concentration of the DNA must be as follows:

From 2 μg to 50 μg of total RNA, a concentration of 0.5 $\mu\text{g}/\mu\text{L}$

From 1 μg to 2 μg of total RNA, a volume of no more than 4 μL

From 250 ng to 1 μg of total RNA, a volume of no more than 3 μL

Also for microquantity libraries, a Bioanalyzer trace is a suitable substitute for a gel photo.

Upon receipt, Beckman Coulter Genomics will QC the total RNA sample to ensure that the A260/A280 is at least 1.8 and that the upper ribosomal RNA band is approximately twice the intensity of the lower ribosomal RNA band. The client should supply any information that may aid in the construction of each library.

Submission of mRNA

Beckman Coulter Genomics requires a minimum of 5 μg of mRNA for the construction of each standard or subtracted cDNA library, ≥ 500 ng of mRNA for the construction of each microquantity cDNA library, and ≥ 5 ng of mRNA for the construction of each nanoquantity cDNA library. mRNA will need to be isolated from tissue or cells using commercial kits or by equivalent methods. mRNA should be dissolved in nuclease-free water (no DEPC) at a concentration of ≥ 1 $\mu\text{g}/\mu\text{L}$. The A260/A280 ratio must be at least 1.8. RNA should be stored at -80°C and shipped packed in a large quantity of dry ice. Total RNA quality should be verified by the client and A260/A280 readings and gel photo should be included in the shipment.

For microquantity libraries, a Bioanalyzer trace is a suitable substitute for a gel photo.

Submission of Custom Vector

The customer must submit 100 µg of purified super-coiled plasmid in TE buffer. The sample must be frozen.

Ship the samples according to the shipping guidelines:

http://www.beckmangenomics.com/documents/services/danvers_shipping_specifications.pdf

