

AGENCOURT® SAMPLE SUBMISSION GUIDELINES

Agencourt strives to exceed customer expectations. In order to accomplish this goal, Agencourt has established specifications for customer sample submission. These specifications will help to improve turn around time and quality. If you have any questions about the specifications listed in the outline below, please contact customer service at 800.773.9186. For more detailed explanations and recommendations about the preparation and submission of samples, please refer to the following link: <http://www.agencourt.com/documents/services/agencourt-sample-preparation-guidelines.pdf>

For more information regarding the consumables outlined below or alternative compatible labware, please refer to the following link regarding our vendor partnerships:

<http://www.agencourt.com/documents/services/agencourt-vendor-partnerships.pdf>

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

<http://www.agencourt.com/documents/services/agencourt-shipping-specifications.pdf>

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

<https://psf.agencourt.com>

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.

Send samples NEXT DAY AIR to:

Beckman Coulter Genomics
Attention Genomic Services
36 Cherry Hill Drive
Danvers, MA 01923



High Throughput Sequencing Services

- Single Pass Sequencing (High copy plasmid and PCR¹ products)
- End Sequencing (BACs, Cosmids and Fosmids)
- SAGE™ (LSAGE/DK)

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
Agar lawns	<ul style="list-style-type: none"> • 25cm x 25cm tray <i>(ThermoFisher part #QH-2216)</i> • 200 mL LB agar, 2 – 5 mm thick 	1,500 - 2,000 colonies/lawn are required (no more than 3,000/lawn)
96-well bacterial cultures	<ul style="list-style-type: none"> • 200 µL media • 10% final glycerol concentration 	We recommend that cultures are grown in LB media and should be incubated at 37°C for 12 hours static growth. Templates containing the Zeocin-resistant gene should be grown in low salt LB media
384-well bacterial cultures	<ul style="list-style-type: none"> • 90 µL media • 10% final glycerol concentration 	We recommend that cultures are grown in LB media and should be incubated at 37°C for 12 hours static growth. Templates containing the Zeocin-resistant gene should be grown in low salt LB media
PCR amplicons	<ul style="list-style-type: none"> • 96- or 384-well full skirted plates <i>(ThermoFisher part #AB1000 or AB-111)</i> • Minimum volume of 20 µL • 15-25 ng/µL 	Normalization of samples across the plate is recommended

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

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
Bacterial transformation – glycerol for agar plating	<ul style="list-style-type: none"> • 200 µL aliquots 	2X the number of requested clones should be submitted
Primers – 2D tube format	<ul style="list-style-type: none"> • Minimum of 7.5 nanomoles for 768 reads or fewer • Minimum of 50 nanomoles for greater than 768 reads 	Primers should be ~20bp in length Primer T _m should be between 52°C and 65°C Avoid primer sequences that may self-dimerize Primers should be resuspended in dH ₂ O (not TE buffer)
Primers – plate format	<ul style="list-style-type: none"> • 25 µL of 3 µM primer/well 	Primers should be arrayed in the primer plate according to sample layout

Individual Sample Sequencing

QuickLane® Primer Walking

- All Samples for both QuickLane® and Primer Walking must be submitted in 650 µL two dimensional barcoded tubes sealed with a silicone septum (*Agencourt part #001108*) or a 96-well full skirted plate (*ThermoFisher part #AB1000*).
- For Primer Walking projects, all tubes must be labeled with a unique name. A tab-delimited file must also be submitted, which associates the unique tube name with sample/amplicon name.
- Reference sequences for Primer Walking must be submitted in fasta format.
- QuickLane® Samples submitted in plates may contain different template types. However, if the QuickLane® Samples require purification, all samples submitted within one plate must be of the same template type (i.e. high copy, low copy, PCR amplicons, etc).
- QuickLane® samples must be accompanied by a tab delimited text file that contains plate name (if applicable), 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.

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
QuickLane® - Template and Primer Separate		
High Copy Plasmids	<ul style="list-style-type: none"> • 15-25 ng/μL • 10 μL/reaction • Minimum volume of 40μL 	To determine the total volume to submit, multiply the volume of sample required (10 μL) by the number of reactions needed. Total volume must be ≥ the minimum volume. (e.g. 6rxn x 10 μL/rxn = 60 μL)
Low Copy Plasmids, Fosmids, Cosmids and BACs	<ul style="list-style-type: none"> • 15-25 ng/μL • 20 μL/reaction • Minimum volume of 40 μL 	See above
PCR Products (Purified and Unpurified)	<ul style="list-style-type: none"> • 15-25 ng/μL • 5 μL/reaction • Minimum volume of 20 μL 	See above
Difficult Templates (Containing >70% GC content or presence of secondary structures – i.e. hairpins)	<ul style="list-style-type: none"> • 15-25 ng/μL • 20 μL/reaction • Minimum volume of 40 μL 	See above
Unpurified glycerol stock	<ul style="list-style-type: none"> • 100 μL glycerol stock 	Include information on vector type and antibiotic
Primer – regardless of template type	<ul style="list-style-type: none"> • 3 μM primer/tube • 2 μL/reaction • Minimum volume of 20 μL 	See above
QuickLane® - Template and Primer Combined		
High Copy Plasmids, Low Copy Plasmids, Fosmids, Cosmids and BACs, Difficult Templates (Containing >70% GC content or presence of secondary structures – i.e. hairpins)	<ul style="list-style-type: none"> • Total amount of DNA = 0.6 μg – 1.0 μg • Total volume of sample = 40 μL • Total amount of primer = 20 pmol 	
PCR Products	<ul style="list-style-type: none"> • Total amount of DNA = 0.3 μg – 0.5 μg • Total volume of sample = 20 μL • Total amount of primer = 10 pmol 	

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
Primer Walking		
Purified plasmid or PCR product	<ul style="list-style-type: none"> 2 µg/1kb of each purified template at a concentration of 25 ng/µL 	
Unpurified glycerol stock	<ul style="list-style-type: none"> 100 µL glycerol stock 	Include information on vector type and antibiotic

Large Insert Sequencing

Purified and unpurified BAC, fosmid, or cosmid template

- All Samples must be submitted in 650 µL two dimensional barcoded tubes sealed with a silicone septum (*Agencourt part #001108*) or a 96-well full skirted plate (*ThermoFisher part #AB1000*).
- All tubes must be labeled with a unique name.

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
Unpurified glycerol stock	<ul style="list-style-type: none"> 100 µL glycerol stock 	Include information on vector type and antibiotic
Purified template	<ul style="list-style-type: none"> 50 µg of purified template DNA 	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.

SNP Discovery

- Customer provided mRNA sequences must be submitted in FASTA file format, preferably with GenBank Accession Number reference.
- Customers who have designed their own primers must submit primer sequences in tab delimited form, associating primer pairs and nested primer pairs properly.

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
Genomic DNA	<ul style="list-style-type: none"> • Purified DNA minimum concentration of 5.0 ng/μL • Amount of DNA required for each individual specimen: ((40 ng x X amplicons) + 15 μL for dead volume) 	<p>DNA should be standardized as much as reasonably possible across all specimens</p> <p>2 wells should be left blank per submitted plate for Agencourt positive and negative controls</p>

Whole Genome Sequencing

Library Type	Sample Requirements
High Copy Plasmid Library	<ul style="list-style-type: none"> • Minimum 10 μg of purified genomic DNA
Fosmid Library	<ul style="list-style-type: none"> • Minimum of 100 μg of purified genomic DNA
454 Fragment Library	<ul style="list-style-type: none"> • Minimum of 20 μg of purified genomic DNA
454 Mate Pair Library	<ul style="list-style-type: none"> • Minimum of 20 μg of purified genomic DNA
SOLiD Fragment Library	<ul style="list-style-type: none"> • Minimum of 20 μg of purified genomic DNA
SOLiD Mate Pair Library	<ul style="list-style-type: none"> • Minimum of 60 μg of purified genomic DNA
Genomic DNA for draft sequencing and finishing	<ul style="list-style-type: none"> • Minimum 300 - 400 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. Since OD260 readings can at times result in vast overestimation of actual DNA sample concentrations Agencourt strongly recommends the use of flourometric 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 flourometry 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

- Standard
- Normalized
- Microquantity
- Nanoquantity

Sample Type/Format	Sample Requirements	Comments/Additional Requirements
Tissue	<ul style="list-style-type: none"> • For standard/normalized/subtracted libraries: 1 g • For microquantity libraries: 1 mg • For nanoquantity libraries: 250 µg 	Tissues or cells must be harvested as quickly as possible and flash-frozen in liquid nitrogen
Cells	<ul style="list-style-type: none"> • For standard/normalized/subtracted libraries: 1×10^8 • For microquantity libraries: 1×10^7 • For nanoquantity libraries: 25,000 	See above
mRNA	<ul style="list-style-type: none"> • For standard/normalized/subtracted libraries: ≥ 5 µg • For microquantity libraries: ≥ 500 ng • For nanoquantity libraries: ≥ 5 ng 	
Total RNA	<ul style="list-style-type: none"> • For standard/normalized/subtracted libraries: 1 mg • For microquantity libraries: 50 µg 	Total RNA will need to be isolated from tissue or cells using commercial kits or equivalent methods
Total RNA	<ul style="list-style-type: none"> • For nanoquantity libraries: 250 ng • Specifications of the RNA must be as follows: <ul style="list-style-type: none"> ▪ From 2 µg to 50 µg of total RNA, concentration of 0.5 µg/µL ▪ From 1 µg to 2 µg of total RNA, volume of no more than 4 µL ▪ From 250 ng to 1 µg of total RNA, volume of no more than 3 µL 	See above