Protocol for Gel/PCR DNA Fragment Extraction Kit

PCR Clean Up Protocol

Sample Preparation

- Transfer up to 100 uL of reaction product to a 1.5 microcentrifuge tube.
- Add 5 volumes of DF Buffer to 1 volume of the same and mix by vortex.

DNA Binding

- Place a DF Column in a 2 mL Collection Tube.
- Transfer the sample mixture to the DF Column.
- Centrifuge at 14-6,000 x g for 30 seconds.
- Discard the flow-through then lace the DF Column back in the 2 mL Collection Tube.

Wash

- Add 600 uL of Wash Buffer (make sure ethanol was added) into the CENTER of the DF Column.
- Let stand for 1 minute at room temperature.
- Centrifuge at 14-6,000 x g for 30 seconds.
- Discard the flow-through then lace the DF Column back in the 2 mL Collection Tube.
- Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.

DNA Elution

- Transfer the dried DF Column to a new 1.5 mL microcentrifuge tube.
- Add 20-50 uL of Elution Buffer or TE into the CENTER of the column matrix.
- Let stand for at least 2 minutes to ensure the Elution Buffer is completely absorbed.
- Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

References

1. Gel/PCR DNA Fragment Extraction Kit. (n.d.). Retrieved October 27, 2020, from https://www.ibisci.com/products/gel-pcr-dna-fragment-extraction-kit

SDS PAGE, Coomassie Stain, and Western Blot Protocol

Cast a SDS-PAGE 12% Gel

1. Combine in a 125 mL Erlenmeyer flask
   - 4.0 mL of 30% Acrylamide/bis
   - 2.5 mL of 1.5 M Tris-HCl (pH 8.8)
   - 100 uL of 10% SDS
   - 3.35 mL deionized waterA
2. Degas the solution under a vacuum for at least 15 minutes.
3. Add to the degassed solution
   - 5 uL TEMED
   - 50 uL 10% APS
4. Combine solution and pipette into vertically-standing gel cassette (with comb in place) to about 3 centimeters below the teeth of the comb. This is the resolving gel layer.
5. Immediately overlay the gel with ethanol. the gel until it is covered.
6. Allow the gel to polymerize for 45 min to 1hr.
7. Rinse off the overlay solution with distilled water.
8. Prepare the second layer, the stacking gel, by combining:
   - 1.32 mL of 30% Acrylamide/bis
   - 2.52 mL of 0.5 M Tris-HCl (pH 6.8)
   - 100 uL of 10% SDS
   - 6 mL of Distilled deionozied water
9. Mix and degas the solution for at least 15 minutes.
10. Add to the degassed solution
    - 10 uL of TEMED
    - 50 uL 10% APS
11. Place an SDS-PAGE gel comb into the cassette at an angle, not completely in.
12. Add the solution to the cassette and cover the comb teeth. Realign the comb.
13. Allow the gel to polymerize for 30-45 minutes.

Preparing the samples and loading the gel

1. Pipet 21 uL of sample protein into a microfuge tube.

And 7 uL of 4X loading buffer in a microfuge tube and mix well.

2. Heat the samples for 5 minutes at 100 °C
3. Set up the gel unit in the chamber and fill the chamber with running buffer to the designated line.
4. Add 10 uL of the molecular weight standard in the first lane and 20 uL of sample to the following lanes.
5. Run the gel for 1 hour at 100 V.

Stain the completed SDS-PAGE gel - Coomassie Stain

1. Cover completed gel in Coomassie stain in a container.
2. Allow gel to shake on a rotating platform for about 30 minutes.
3. Remove gel from stain and rinse with distilled water.

4. Cover gel completely in destaining solution and place a few folded Kimwipes™ in the corner of the container.
5. Destain the gel on the rotating platform overnight.

Western blot

1. Soak nitrocellulose membrane, 3MM Whatman papers and sponges in 1X transfer buffer.
    - If using PVDF membrane, soak it in methanol for 5min.
2. Put the gel onto the paper and the membrane onto the gel. Mark the wells and the gel perimeter at the back of the membrane by using a ball pen. Get rid of bubbles by using a glass rod.
3. The sandwich should be: (+) sponge-paper-membrane-gel-paper-sponge (-)
4. Fill the tank with 1X transfer buffer.
5. Run: 1 hour at 100V at with a block of ice and/or in the cold room.
6. Incubate the blot in 1% casein either at +4^{o}C O/N or at RT for 1hr or at +37^{o}C for 30min.
    - 5% non-fat milk/ TBST can be used for most antibodies but, some antibodies (e.g. anti-His antibody) bind to some proteins in milk, which results in high background. Dilute these in 5% BSA in TBST. Check the manufacturers product sheet for the buffer recommendations.
7. Rinse the blot in 1X TBST for 1-2min.
8. Incubate: Primary antibody (mouse anti-His 1:2000 of stock in 1% casein) for O/N at 4^{o}C or 1hr at.
    - RT or at +37^{o}C for 30min.
9. Wash: 3-4 times 10-15min each in 1X TBST.
10. Incubate: Secondary antibody (goat anti-mouse IgG 1:5.000 of stock in 1% casein) for O/N at 4^{o}C
    - 1hr at RT or at +37^{o}C for 30min.
11. Wash: 3-4 times 10-15min each in 1X TBST.
12. Add substrate: H_2O_2 + Luminol (kit). Rock gently for 5 minutes
13. Expose using the Chemiluminescent imager.

Protocol for Expression and Purification of His-tagged Proteins

Transform expression plasmid into E.coli

1. Prepare LB Agar plates with chloramphenicol (Cm)
2. Transform plasmid (Flappase) into BL21DE3 cells and plate into Cm plates.
3. Upon successful growth on plates, use colonies to prepare 25mL LB liquid culture with Cm in a 1mL:1uL ratio.
    a. 25mL of LB media into a 125mL sterile flask.
    b. 25uL of Cm
    c. Scrape a colony from the plate with an inoculating loop and swirl loop into the flask of media.
    d. Incubate at 37°C overnight.

Induction of protein expression

1. Transfer and regrowth of liquid culture into a fresh liquid culture.
    a. 25mL of fresh LB media into a 125mL flask.
    b. 1mL of previously incubated media.
    c. 25uL of Cm
2. Incubate at 37°C for 3-4 hours continuously checking the optical density at 600nm (OD600) until OD600= 0.600-0.800.
    a. Set up spectrophotometer for readings at 600nm.
    b. Prepare cuvette with liquid culture and obtain reading.
    c. If reading is not within range place back into incubator and re-check.
3. Once OD600 is within range induce protein expression with IPTG.
    a. 100mM 10uL IPTG: 1mL media ratio.
    b. Incubate while shaking for 4hrs.

Harvesting and lysing

1. Centrifugation and wash of induced cells.
    a. Transfer media into an appropriately sized conical tube.
    b. Centrifuge at 1200xg for 10 minutes
    c. Wash pellet with 20mL 1X PBS buffer and vortex.
    d. Repeat twice.
    e. Freeze pellet until ready for lysing.
2. Lysing.
    a. Prepare lyse buffer:
        i. 1.51g Tris HCl
        ii. 4.55g Sorbitol
        iii. Dissolve in 125mL of deionized water.
        iv. Check for pH 7.9 and adjust as necessary.
        v. Add deionized water to 250mL.
    b. Pellet resuspension.
        i. 1mL lysis buffer
        ii. 100uL Hatt protease inhibitor
    c. Sonication lysis.
        i. Label sonicator tubes

ii. Sonicator settings:
    1. 60% amplitude, 10:00 mins totals, 20 sec pulse, 10 seconds off

d. Store at -4°C for further analysis.

IMAC - Immobilized Metal Affinity Chromatography

1. Pack the column with Cobalt Resin.
2. Equilibrate the column by filling the column with wash buffer and inverting to mix.
3. Repeat step two 5-10 times.
4. Load sample into column and collect flowthrough.
5. Wash the column with 8 column volumes of Equilibration Buffer followed by 7 column volumes of wash buffer
6. Elute with 5-8 column volumes of elution buffer and collect 1 mL fractions.
7. Analyze these fractions using an SDS-PAGE gel.

Transformation Protocol

Resuspending DNA (if using an iGEM kit part)

1. Locate well containing the part of interest on the iGEM parts distribution plate
2. Add 10mL of nuclease free water to the well.
3. Let sit 5 minutes
4. Pipette up and down to mix
5. Transfer all 10mL to a sterile microfuge tube

Transformation into DH5a (or BL21)

1. Thaw competent DH5a cells on ice
2. Label tube of cells
3. Add 5mL of plasmid DNA and mix gently by flicking tube
4. Incubate on ice, 30 minutes
5. Heat shock at 42°C for 30 seconds
6. Return to ice for 5 minutes.
7. Add 200mL SOC
8. Incubate at 37°C for 1 hour with rotation.
9. Plate onto agar plates with Cm, or other appropriate antibiotic
    a. Plate 20mL onto one
    b. Plate the remaining 230mL onto another

Homology Model Protocol

What is a Homology Model?

A homology model, also known as a comparative model is used to create a structure for a protein that is vividly unknown. Homology models are based on the idea that if two sequences of an organism are similar then their structure are also similar. Structural biology is an area of science that focuses on the molecular structure and mechanisms of biological macromolecules, particularly proteins. The information given in the study is useful for studying how a specific protein may function, how medication may affect its stability and how structure may interact with other chemicals and protein. To create a 3D structure of the unknown protein the following steps are used:

1. Find the DNA sequence for the given target using Uniport

2. Find other organisms with the same Protein using the Protein Data Bank *make sure these organisms have a structure*
    a. Open a word document to keep track of the different organisms and their corresponding FASTA sequences. Download the PDB/FASTA file

3. Compare your sequence using BLAST search
    a. Go to Program selection: Protein-Protein BLAST
    b. Check the align two or more sequences box
    c. In the first query place in template sequence
    d. In the second query place in a different organisms sequence
    e. Create a chart that includes percent identity and expected value

Name of Organism	**Percent Identity**	**Expected Value**
…	(%)	2e-58

    f. Choose the candidate organism with the higher identity percent

4. Use SwissModel to make the homology models. The homology model is only based upon your Target it is not a
    a. Use the USER Template tab
    b. Upload your Target and Template Sequences
    c. Make note of the GMQE, QMEAN, Sequence Identities, and Ramachandran plot
        - GMQE (higher number= higher reliability)
        - QMEAN (score of <-4 indicates poor quality)
        - Sequence identities (60+ = similar to target)
        - Ramachandran plot (90 +)

Homology Models	Qualities	Oligo-state	Sequence Identity	Ligands	Ramachandran Plots
P. Furiosus	• QMEAN (-3.12) • CB (-1.14) • All Atom (-1.35) • Solvation (1.05) • Torsion (-3.16) • GMQE (0.56)	• Heterodimer	• 43.08%	• None	• MolProbity score (1.86) • Favored (90.14%) • Outliers (2.9%)
Homo Sapiens	• QMEAN (-2.48) • CB (0.54) • All Atom (-1.45) • Solvation (-0.51) • Torsion (-2.24) • GMQE (0.61)	• Monomer	• 62.31%	• 1X mg • No protein-ligand interaction detected	• MolProbity score (1.64) • Favored (92.75%) • Outliers (2.42%)

5. Upload both homology models in Chimera.
    a. Save the files to your computer
    b. Open both structures using the program
        - Open or Fetch by ID (aka. PDB code)

6. Taking Pictures and Extra Settings to complete your model
    a. Selecting A Specific Chain: Select, Chain, Chain Type
    b. Align: Tool, Structure Comparison , Match Maker (Select Reference and Structure)
    c. Changing Model Color: Select, Chain, Chain Type, Action, Color
    d. Hiding Backbones/Atoms/Ribbons: Select, Chain, Chain Type, Action, Atom/Ribbon, Hide
    e. Finding RMSD: Tools, Volume Data, Volume Mean/SD/RMS
    f. Regions Of Complementarity: Tools, Structure Comparison, Match Align, Select Chain, Apply
    g. Easy Access Command Line: General Control, Command Line
        - Type in “Focus” to Recenters View
        - Type in “ Del” or “Delete” to Remove Whatever Is Selected
        - Type in “~Select” to Unselect
    h. Pictures and Fixing Lighting: Tools, =1Viewing Controls, Camera, Side View, Rotation, Effects, Lighting

7. Verify model vitality using a simulation (CHARMN or AMBER)

Resources and Links

- https://swissmodel.expasy.org
- https://www.rcsb.org
- https://www.uniprot.org

- https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome
- https://www.youtube.com/watch?v=YJo2-1PDJtI&list=PL9hsubGPISJFDk_YFRr65rtLjsXvd0-2r
- https://www.cgl.ucsf.edu/chimera/current/docs/UsersGuide/

Antibiotic Stock Protocol- (1000x 1µL/mL Media)- 10mL solutions

Ampicillin

1. Dissolve 1.0g of Sodium Ampicillin (100mg/mL) in MilliQ water up to a volume of 10mL.
2. Once dissolved, filter by washing through a 0.2 um syringe filter into a new sterilized test tube.
3. Store the ampicillin in aliquots of 1000uL at -20℃.

Chloramphenicol

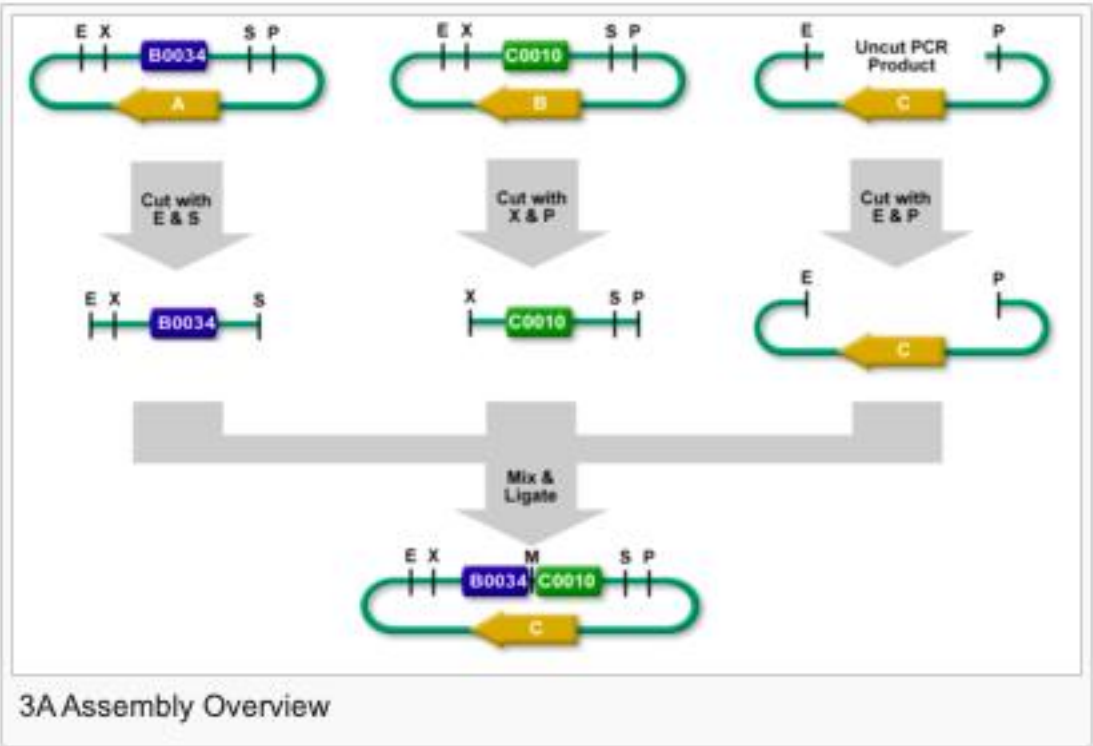
4. Dissolve 0.25g of Chloramphenicol (25mg/mL) in MilliQ water up to a volume of 10mL.
5. Once dissolved, filter by washing through a 0.2 um syringe filter into a new sterilized test tube.
6. Store the chloramphenicol in aliquots of 1000uL at -20℃.

Kanamycin

7. Dissolve 0.50g of Kanamycin sulfate (50mg/mL) in MilliQ water up to a volume of 10mL.
8. Once dissolved, filter by washing through a 0.2 um syringe filter into a new sterilized test tube.
9. Store the kanamycin in aliquots of 1000uL at -20℃.

Overview – 3A assembly method

Method



3A Assembly Overview

- Restriction Digests
    a. The left part sample is cut out with EcoRI and SpeI.
    b. The right part sample is cut out with XbaI and PstI.
    c. The linearized plasmid backbone is a linear piece of DNA. It has a few bases beyond the EcoRI and PstI restriction sites. It is cut with EcoRI and PstI.
- All 3 restriction digests are heated to heat kill all the restriction enzymes.
- An equimolar quantity of all 3 restriction digest products are combined in a ligation reaction.
- The desired result is the left part sample's SpeI overhang ligated with the right part sample's XbaI overhang resulting in a scar that cannot be cut with any of our enzymes.
- The new composite part sample is ligated into the construction plasmid backbone at the EcoRI and PstI sites.
- When the ligation is transformed into cells and grown on plates with antibiotic C, only colonies with the correct construction survive.

http://parts.igem.org/Help:Assembly/3A_Assembly

Restriction Digest

This protocol can be used for 3A assembly, or as a recipe for any restriction digestion.

Materials

- Your two part Samples, **A** and **B**: Miniprepped DNA (in BioBrick RFC [10] plasmid backbones), or Synthetic DNA With prefix and Suffix sequences added
- Linearized **Plasmid Backbone** (with a different resistance to the plasmid backbones containing your part samples)
- EcoRI, XbaI, SpeI, PstI, DpnI
- NEB 10X Cutsmart Buffer
- BSA
- DH20

Reactions

Enzyme Master Mix for **Plasmid Backbone** (25ul total)

- 5 ul Cutsmart buffer
- 0.5 ul EcoRI-HF
- 0.5 ul PstI
- 0.5 ul DpnI (Used to digest any template DNA from production)
- 100 ng linear plasmid
- Nuclease free water to a final volume of 25 ul

Enzyme Master Mix for **Part A** (25ul total)

- 5 ul Cutsmart buffer
- 0.5 ul EcoRI-HF
- 0.5 ul SpeI
- 100 ng Part A
- Nuclease free water to a final volume of 25 ul

Enzyme Master Mix for **Part B** (25ul total)

- 5 ul Cutsmart buffer
- 0.5 ul XbaI
- 0.5 ul PstI
- Nuclease free water to a final volume of 25 ul

Digest all three reactions at 37°C for 30 min, heat kill at 80 °C for 20 min. This step is only necessary if you will perform a ligation using the reactions.

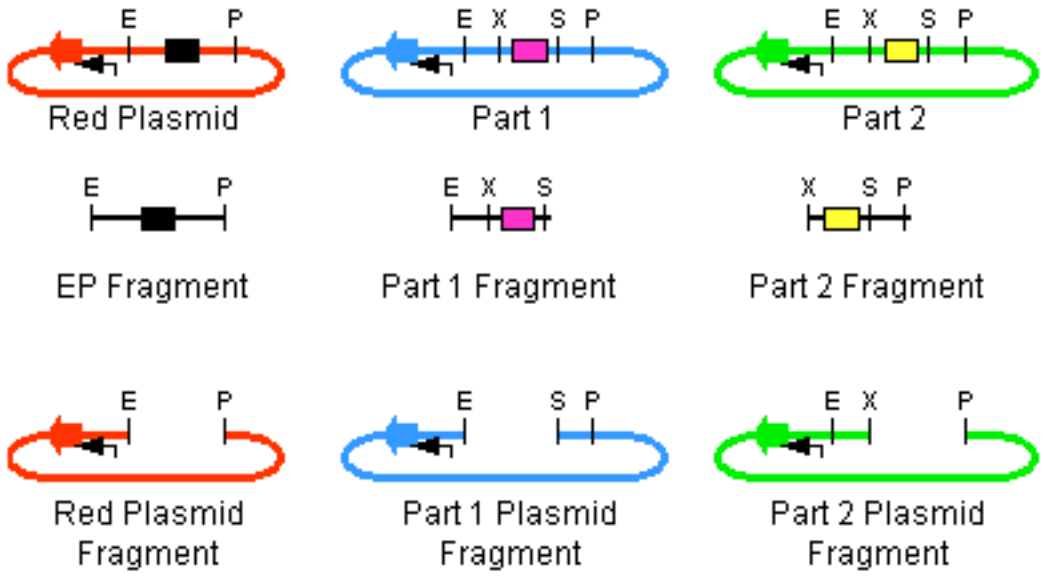
Ligation

- Add 2ul of digested **Plasmid Backbone** (25 ng)
- Add equimolar amount of **Part A** (EcoRI-HF SpeI digested) fragment (< 3 ul)
- Add equimolar amount of **Part B** (XbaI PstI digested fragment) (< 3 ul)
  - **Note:** use NEB Cloner to assist with these calculations.
- Add 1 ul T4 DNA ligase buffer. **Note:** Do not use quick ligase
- Add 0.5 ul T4 DNA ligase

- Add nuclease free water to 10 ul
- Ligate 16 °C for 30 min. Heat kill at 80 °C for 20 min
- Transform with 5 ul of product

Note: For linearized plasmid backbones provided by iGEM HQ, a plasmid backbone with an insert of BBa_J04450 was used as template. As a result, any red colonies that appear during your ligation may be due to the template as a background. Digesting with Dpn1 before use should reduce this occurrence.

Analysis Example:



Schematic of linear DNA fragments present in ligation reaction

https://parts.igem.org/Help:Protocols/3A_Assembly

Agarose Gel electrophoresis (for a 0.8% gel)

1. Obtain 0.4 g of agarose powder and transport into 125 mL Erlenmeyer flask
2. Add 50 mL of 1X TBE or TAE buffer to the flask.
3. Heat solution in microwave in 15 second intervals, stirring gently between intervals, until the agarose has completely dissolved (do not overheat solution!).
4. Allow the solution to cool. This can be sped up by running the outside of the flask under cold water. Do not run under water for more than a few seconds or the gel may solidify in the flask.
5. Add 2 uL of Ethidium Bromide to the flask and swirl gently to mix thoroughly.
6. In a gel-cast with a comb in place, slowly pour the solution into the cast and allow to set for a few minutes until firm.
7. Rotate gel so that the wells of the comb are at the negative end of the electrophoresis apparatus.
8. Pour enough 1X TBE or TAE buffer in the electrophoresis apparatus so that it covers the agarose gel completely.
9. Gently remove the comb from the gel.
10. Load 10 uL of molecular weight marker into the first lane.
11. Load the remaining samples mixed with sample loading buffer (1 uL of the 6X stock for each 5 uL of sample).
12. Run the gel at 80 – 120 V until the sample has migrated at least half way through the gel.

Note: The percentage of the gel can be changed depending on the predicted size of the bands. A higher percent gel (1.3% (0.65 g agarose) is recommended for products less than 500 bp).

High Speed Mini Prep Protocol (IBI Scientific)

Materials

- Autoclaved Eppendorf tubes
- Bacteria from which the plasmids are to be extracted (culture tubes)
- Pipettes and tips
- High Speed mini prep kit
- Tabletop Centrifuge

Procedure

1. Centrifuge down the bacteria in the culture tubes for 10 minutes at 4,000 rpm (Tabletop Centrifuge). Make sure the tubes are well balanced.
2. Discard the supernatant in the bacterial waste
3. Add 200 µl of buffer PD1 to resuspend the pellets, do this by pipetting the suspension up and down or vortexing
4. Transfer the suspension to an Eppendorf tube
5. Add 200 µl of buffer PD2 (make sure this buffer is not precipitated) to the mixture.
6. Invert the Eppendorf tube 4-6x to mix the solution. Do not vortex
7. After 2-5 minutes, add 300 µl of buffer PD3 and invert the solution 10X (do not vortex), allowing the solution to become clear and a 'cloud' of cell debris to form
8. Centrifuge the mixture for 3 minutes at 13,000 rpm (MiniSpin Centrifuge). A pellet of the cell debris will form.
9. Pipette the supernatant off the pellets (carefully; do not transfer any pellet) and transfer the supernatant to a new Eppendorf tube.
10. Centrifuge the mixture again for 5 minutes at 13,400 rpm (MiniSpin Centrifuge). A pellet of the cell debris will form
11. Transfer the supernatant into a spin column in a collection tube
12. Centrifuge the column for 1 minute at 13,000 rpm. DNA will be bound to the column, discard the flow-through.
13. Wash the column by loading 400 µl buffer W1 and subsequent centrifugation for 1 minute at 13,000 rpm – **This step is optional, but recommended if the DNA will be sequenced.**
14. Wash the column by loading 600 µl Wash bufferand subsequent centrifugation for 1 minute at 13,000 rpm.
15. Dry spin the column again for 3 minute at 13,000 rpm.
16. Transfer the column from the collection tube to a new Eppendorf tube. Cut the lid off the tube, but save it.
17. Load 30 - 50 µl of autoclaved H2O or elution buffer on the column (pipette drops in the middle of the membrane, do not touch the membrane). Incubate for 2 minutes and centrifuge for 2 minute at 13,000 rpm.
18. The resulting elution product will contain the DNA

Colony PCR Protocol

Note – this protocol is for NEB One Taq DNA Polymerase, but can be adapted for others. The volumes of each component may change.

Note – keep the master mix and reactions on ice until ready to begin thermocycling.

1. Prepare Master mix using the recipe below. Make enough master mix for the number of colonies to be screened, plus a negative control, plus one or two additional (for waste). Mix each component before adding to the master mix. Mix the master mix well before proceeding to step 2.

Component	**For 1 reaction (50uL)**
5x OneTaq Buffer	10 uL
10 mM dNTPs	1 uL
10 uM VF (or other F primer)	1 uL
10 uM VR (or other R primer	1 uL
OneTaq DNA Polymerase	0.25 uL
Nuclease Free Water	To 50 uL

2. Add 50 uL of the master mix to PCR tubes. Do an additional tube for the negative control.
3. Circle and label (A,B, C… or 1,2,3….) the colonies you will screen.
4. Take a sterile 200 uL pipet tip or toothpick and poke a colony. Swirl the toothpick in one PCR tube. Label the tube with the colony number.
5. Repeat step 4 for each colony. For the negative control, do not poke a colony.
6. Begin thermocycling.

Thermocycling conditions.

Step	**Temperature ℃**	**Time**
Initial Denaturation	94	30 seconds
30 Cycles	94	20 seconds
	55	30 seconds
	68	1 minute/kb
Final Extension	68	5 minutes
Hold	6	∞

Preparing Agar Plates

1. Gather sterile plates and pipette tips, and Bunsen burner

2. Prepare 1L of LB agar using the recipe below:
Weigh pre mixed 40g of LB Agar.
Alternatively, LB can be made without agar or from the following components

Tryptone	10 g
NaCl	10 g
Yeast extract	5 g

Resuspend in 1L of water.
Stir until dissolved (note: the agar will not dissolve, only the rest of the LB components will)

3. Autoclave the LB agar to sterilize. Use the “Liquid 45” cycle.

4. Remove the media and place in a 50-55°C waterbath to cool.

5. Place 1 mL/L of antibiotics, either Chloramphenicol (Cm), Ampicillin (Amp), or Kanamycin (Kan) 1000X stock solutions into each of the Agar Media

6. Stir the Agar Media using the stir plate until well mixed

7. Light the Bunsen burner and mark your plates using the Antibiotic Color Code

8. Pour the media next to the Bunsen burner to ensure that there is a low chance of contamination

9. Stack approximately 5 plates together, next to the burner, while leaving the top plate slightly open (this reduces condensation)

Primer-BLAST Results

[?]

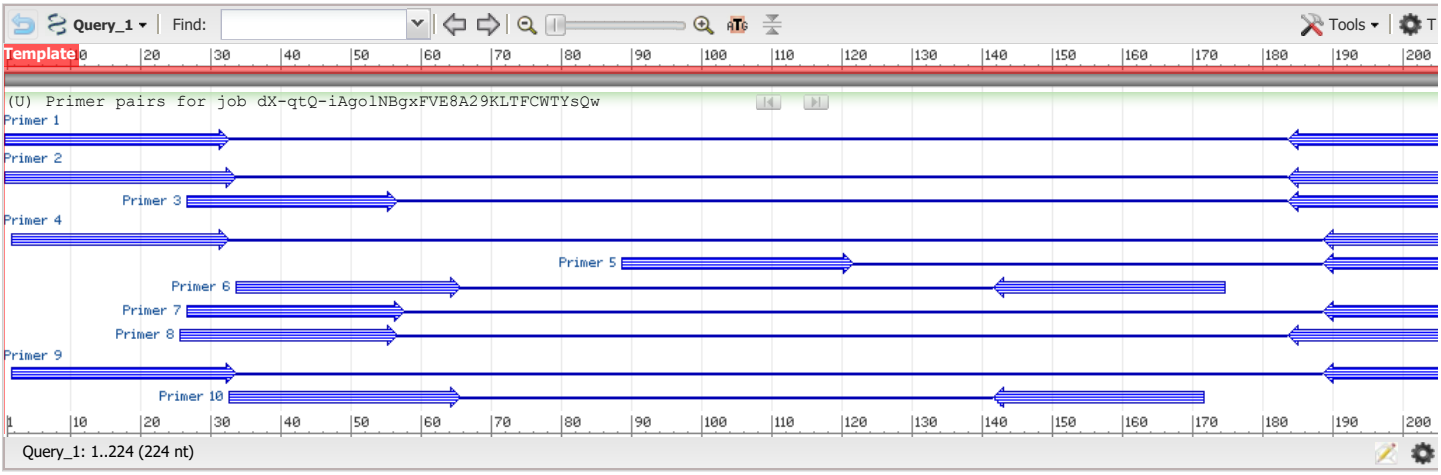
Input PCR template
lcl|Query_1

Range
1 - 224

Specificity of primers
Primer pairs are specific to input template as no other targets were found in selected database: Genome database (reference assembly only) for selected species (Organism limited to Bos taurus)

Other reports
Search Summary

Graphical view of primer pairs



Detailed primer reports

You can re-search for specific primers by accepting some of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers Submit

[?]

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGAACTCCAGGATAAAATCCACCCCTTTGC	Plus	32	1	32	67.25	46.88	5.00	2.00
Reverse primer	ATATTTAGGGAAGGGCATTTCTTTGTGCTTAGG	Minus	33	216	184	65.38	39.39	4.00	2.00
Internal oligo		Plus							

Product length	216
Product Tm	
Product Tm - min(OLIGO Tm)	
Exon junction	
Total intron size	

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 216
Forward primer  1         GATGAACTCCAGGATAAAATCCACCCCTTTGC  32
Template        85451371  ................................  85451340

Reverse primer  1         ATATTTAGGGAAGGGCATTTCTTTGTGCTTAGG  33
Template        85451156  ................................  85451188
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGAACTCCAGGATAAAATCCACCCCTTTGCC	Plus	33	1	33	68.58	48.48	5.00	1.00
Reverse primer	TATTTAGGGAAGGGCATTTCTTTGTGCTTAGG	Minus	32	215	184	65.28	40.62	4.00	2.00
Internal oligo		Plus							
Product length	215								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 215
Forward primer  1         GATGAACTCCAGGATAAAATCCACCCCTTTGCC  33
Template        85451371  ................................  85451339

Reverse primer  1         TATTTAGGGAAGGGCATTTCTTTGTGCTTAGG  32
Template        85451157  ................................  85451188
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTTTGCCCAGACACAGTCTCTAGTCTATCC	Plus	30	27	56	65.80	50.00	7.00	3.00
Reverse primer	GATATTTAGGGAAGGGCATTTCTTTGTGCTTAGG	Minus	34	217	184	65.79	41.18	4.00	2.00
Internal oligo		Plus							
Product length	191								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 191
Forward primer  1         CTTTGCCCAGACACAGTCTCTAGTCTATCC  30
Template        85451345  ..............................  85451316

Reverse primer  1         GATATTTAGGGAAGGGCATTTCTTTGTGCTTAGG  34
Template        85451155  ..................................  85451188
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGAACTCCAGGATAAAATCCACCCCTTTGC	Plus	31	2	32	66.87	45.16	5.00	2.00
Reverse primer	ACTGGATATTTAGGGAAGGGCATTTCTTTGTGC	Minus	33	221	189	67.05	42.42	4.00	2.00
Internal oligo		Plus							
Product length	220								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 220
Forward primer  1         ATGAACTCCAGGATAAAATCCACCCCTTTGC  31
Template        85451370  ..............................  85451340

Reverse primer  1         ACTGGATATTTAGGGAAGGGCATTTCTTTGTGC  33
Template        85451151  ................................  85451183
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAAACATCCCTCCTCTTACTCAAACCCCTGTGG	Plus	33	89	121	69.07	48.48	3.00	3.00
Reverse primer	CTGGATATTTAGGGAAGGGCATTTCTTTGTGC	Minus	32	220	189	66.09	43.75	4.00	2.00
Internal oligo		Plus							
Product length	132								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 132
Forward primer  1         AAAACATCCCTCCTCTTACTCAAACCCCTGTGG  33
Template        85451283  ................................  85451251

Reverse primer  1         CTGGATATTTAGGGAAGGGCATTTCTTTGTGC  32
Template        85451152  ................................  85451183
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CAGACACAGTCTCTAGTCTATCCCTTCCCTGG	Plus	32	34	65	67.87	53.12	7.00	3.00
Reverse primer	CTCCTTCACTTTGGAGACTCCCATTACTTCAGG	Minus	33	174	142	67.55	48.48	7.00	3.00
Internal oligo		Plus							
Product length	141								

Product Tm	
Product Tm - min(OLIGO Tm)	
Exon junction	
Total intron size	

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 141
Forward primer  1         CAGACACAGTCTCTAGTCTATCCCTTCCCTGG  32
Template        85451338  ................................  85451307

Reverse primer  1         CTCCTTCACTTTGGAGACTCCCATTACTTCAGG  33
Template        85451198  ................................  85451230
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTTTGCCCAGACACAGTCTCTAGTCTATCCC	Plus	31	27	57	67.24	51.61	7.00	2.00
Reverse primer	GGATATTTAGGGAAGGGCATTTCTTTGTGC	Minus	30	218	189	64.61	43.33	4.00	2.00
Internal oligo		Plus							
Product length	192								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 192
Forward primer  1         CTTTGCCCAGACACAGTCTCTAGTCTATCCC  31
Template        85451345  ...............................  85451315

Reverse primer  1         GGATATTTAGGGAAGGGCATTTCTTTGTGC  30
Template        85451154  ...............................  85451183
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTTTGCCCAGACACAGTCTCTAGTCTATCC	Plus	31	26	56	67.24	51.61	7.00	3.00
Reverse primer	ATTTAGGGAAGGGCATTTCTTTGTGCTTAGG	Minus	31	214	184	65.61	41.94	4.00	2.00
Internal oligo		Plus							
Product length	189								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 189
Forward primer  1         CCTTTGCCCAGACACAGTCTCTAGTCTATCC  31
Template        85451346  ..............................  85451316

Reverse primer  1         ATTTAGGGAAGGGCATTTCTTTGTGCTTAGG  31
Template        85451158  ..............................  85451188
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGAACTCCAGGATAAAATCCACCCCTTTGCC	Plus	32	2	33	68.26	46.88	5.00	1.00
Reverse primer	TGGATATTTAGGGAAGGGCATTTCTTTGTGC	Minus	31	219	189	65.65	41.94	4.00	2.00
Internal oligo		Plus							
Product length	218								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 218
Forward primer  1         ATGAACTCCAGGATAAAATCCACCCCTTTGCC  32
Template        85451370  ..............................  85451339

Reverse primer  1         TGGATATTTAGGGAAGGGCATTTCTTTGTGC  31
Template        85451153  ..............................  85451183
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCAGACACAGTCTCTAGTCTATCCCTTCCCTGG	Plus	33	33	65	69.19	54.55	7.00	3.00
Reverse primer	CTTCACTTTGGAGACTCCCATTACTTCAGG	Minus	30	171	142	64.90	46.67	7.00	1.00
Internal oligo		Plus							
Product length	139								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 139
Forward primer  1         CCAGACACAGTCTCTAGTCTATCCCTTCCCTGG  33
Template        85451339  .................................  85451307

Reverse primer  1         CTTCACTTTGGAGACTCCCATTACTTCAGG  30
Template        85451201  ..............................  85451230
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

If you want to allow any of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers

Submit

[?]

Primer-BLAST Results [?]

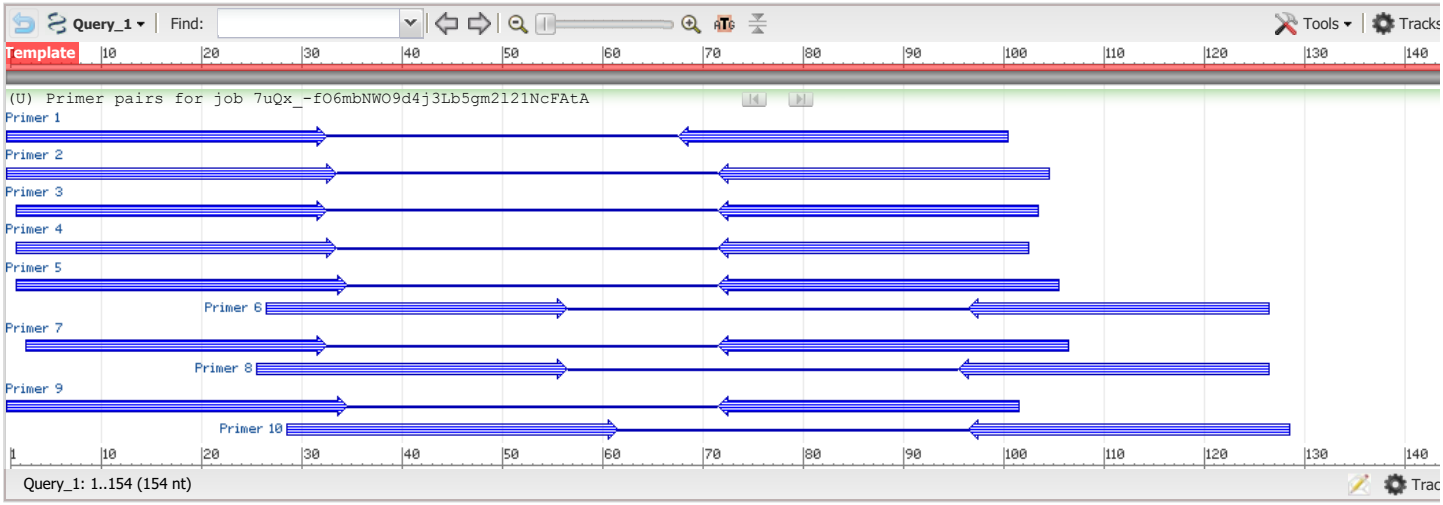
Input PCR template
lcl|Query_1

Range
1 - 154

Specificity of primers
Primer pairs are specific to input template as no other targets were found in selected database: Genome database (reference assembly only) for selected species (Organism limited to Bos taurus)

Other reports
Search Summary

Graphical view of primer pairs



Detailed primer reports

You can re-search for specific primers by accepting some of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers Submit [?]

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGAACTCCAGGATAAAATCCACCCCTTTGC	Plus	32	1	32	67.25	46.88	5.00	2.00
Reverse primer	GAGGGATGTTTTGTGGGAGGCTGTTATGGATGG	Minus	33	100	68	69.89	51.52	3.00	0.00
Internal oligo		Plus							

Product length	100
Product Tm	
Product Tm - min(OLIGO Tm)	
Exon junction	
Total intron size	

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 100
Forward primer  1         GATGAACTCCAGGATAAAATCCACCCCTTTGC  32
Template        85451371  ................................  85451340

Reverse primer  1         GAGGGATGTTTTGTGGGAGGCTGTTATGGATGG  33
Template        85451272  ................................  85451304
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGAACTCCAGGATAAAATCCACCCCTTTGCC	Plus	33	1	33	68.58	48.48	5.00	1.00
Reverse primer	AGAGGAGGGATGTTTTGTGGGAGGCTGTTATGG	Minus	33	104	72	70.49	51.52	3.00	0.00
Internal oligo		Plus							
Product length	104								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 104
Forward primer  1         GATGAACTCCAGGATAAAATCCACCCCTTTGCC  33
Template        85451371  ................................  85451339

Reverse primer  1         AGAGGAGGGATGTTTTGTGGGAGGCTGTTATGG  33
Template        85451268  ................................  85451300
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGAACTCCAGGATAAAATCCACCCCTTTGC	Plus	31	2	32	66.87	45.16	5.00	2.00
Reverse primer	GAGGAGGGATGTTTTGTGGGAGGCTGTTATGG	Minus	32	103	72	69.77	53.12	3.00	0.00
Internal oligo		Plus							
Product length	102								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 102
Forward primer  1         ATGAACTCCAGGATAAAATCCACCCCTTTGC  31
Template        85451370  ..............................  85451340

Reverse primer  1         GAGGAGGGATGTTTTGTGGGAGGCTGTTATGG  32
Template        85451269  ..............................  85451300
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGAACTCCAGGATAAAATCCACCCCTTTGCC	Plus	32	2	33	68.26	46.88	5.00	1.00
Reverse primer	AGGAGGGATGTTTTGTGGGAGGCTGTTATGG	Minus	31	102	72	69.49	51.61	3.00	0.00
Internal oligo		Plus							
Product length	101								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 101
Forward primer  1         ATGAACTCCAGGATAAAATCCACCCCTTTGCC  32
Template        85451370  ..............................  85451339

Reverse primer  1         AGGAGGGATGTTTTGTGGGAGGCTGTTATGG  31
Template        85451270  ..............................  85451300
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGAACTCCAGGATAAAATCCACCCCTTTGCCC	Plus	33	2	34	69.58	48.48	5.00	0.00
Reverse primer	AAGAGGAGGGATGTTTTGTGGGAGGCTGTTATGG	Minus	34	105	72	70.54	50.00	3.00	0.00
Internal oligo		Plus							
Product length	104								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 104
Forward primer  1         ATGAACTCCAGGATAAAATCCACCCCTTTGCCC  33
Template        85451370  .................................  85451338

Reverse primer  1         AAGAGGAGGGATGTTTTGTGGGAGGCTGTTATGG  34
Template        85451267  .................................  85451300
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTTTGCCCAGACACAGTCTCTAGTCTATCC	Plus	30	27	56	65.80	50.00	7.00	3.00
Reverse primer	CACCACCACAGGGGTTTGAGTAAGAGGAGG	Minus	30	126	97	69.44	56.67	5.00	0.00
Internal oligo		Plus							
Product length	100								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 100
Forward primer  1         CTTTGCCCAGACACAGTCTCTAGTCTATCC  30
Template        85451345  ..............................  85451316

Reverse primer  1         CACCACCACAGGGGTTTGAGTAAGAGGAGG  30
Template        85451246  ..............................  85451275
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGAACTCCAGGATAAAATCCACCCCTTTGC	Plus	30	3	32	66.81	46.67	5.00	2.00
Reverse primer	TAAGAGGAGGGATGTTTTGTGGGAGGCTGTTATGG	Minus	35	106	72	70.10	48.57	4.00	0.00
Internal oligo		Plus							
Product length	104								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 104
Forward primer  1         TGAACTCCAGGATAAAATCCACCCCTTTGC  30
Template        85451369  ..............................  85451340

Reverse primer  1         TAAGAGGAGGGATGTTTTGTGGGAGGCTGTTATGG  35
Template        85451266  ....................................  85451300
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTTTGCCCAGACACAGTCTCTAGTCTATCC	Plus	31	26	56	67.24	51.61	7.00	3.00
Reverse primer	CACCACCACAGGGGTTTGAGTAAGAGGAGGG	Minus	31	126	96	70.79	58.06	5.00	0.00
Internal oligo		Plus							
Product length	101								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 101
Forward primer  1         CCTTTGCCCAGACACAGTCTCTAGTCTATCC  31
Template        85451346  ..............................  85451316

Reverse primer  1         CACCACCACAGGGGTTTGAGTAAGAGGAGGG  31
Template        85451246  ..............................  85451276
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGAACTCCAGGATAAAATCCACCCCTTTGCCC	Plus	34	1	34	69.84	50.00	5.00	0.00
Reverse primer	GGAGGGATGTTTTGTGGGAGGCTGTTATGG	Minus	30	101	72	68.71	53.33	3.00	0.00
Internal oligo		Plus							
Product length	101								
Product Tm									
Product Tm - min(OLIGO Tm)									
Exon junction									
Total intron size									

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 101
Forward primer  1         GATGAACTCCAGGATAAAATCCACCCCTTTGCCC  34
Template        85451371  ..................................  85451338

Reverse primer  1         GGAGGGATGTTTTGTGGGAGGCTGTTATGG  30
Template        85451271  ..............................  85451300
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTGCCCAGACACAGTCTCTAGTCTATCCCTTCC	Plus	33	29	61	69.34	51.52	7.00	0.00
Reverse primer	GGCACCACCACAGGGGTTTGAGTAAGAGGAGG	Minus	32	128	97	72.41	59.38	5.00	0.00
Internal oligo		Plus							
Product length	100								
Product Tm									

Product Tm - min(OLIGO Tm)

Exon junction

Total intron size

Products on intended targets

>NC_037333.1 Bos taurus isolate L1 Dominette 01449 registration number 42190680 breed Hereford chromosome 6, ARS-UCD1.2

```
product length = 100
Forward primer  1         TTGCCCAGACACAGTCTCTAGTCTATCCCTTCC  33
Template        85451343  .................................  85451311

Reverse primer  1         GGCACCACCACAGGGGTTTGAGTAAGAGGAGG  32
Template        85451244  .................................  85451275
```

Products on allowed targets

Products on allowed transcript variants

Products on potentially unintended templates

Products on target templates

If you want to allow any of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers

Submit

[?]