



# ISSUE NUMBER 8 FEBRUARY 2006

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# COMMUNITY NEWS



# Antirrhinum and the Solanaceae

Contributed by Brendan Davies, Zsuzsanna Schwarz-Sommer, Andrew Hudson

Legend tells that Antirrhinums protect from witchcraft and the plant is sometimes recommended for medical use. Beyond these scientifically unconfirmed applications, *Antirrhinum majus* has attracted gardeners as an ornamental crop (Fig. 1) with current annual sales of about \$20M per year in

the US alone – and a scientific community since the birth of genetic research. For modern molecular genetics *Antirrhinum* excelled as a model plant, primarily because the presence of active transposons provided a tool for gene identification and because collections of classical and transposon-induced mutants facilitated access to various areas of developmental biology. These and other aspects still provide good

reasons and resources for work with Antirrhinum majus; one publicly less recognized utility of the Antirrhinum species lies in its advantages to study molecular genetic mechanisms underlying species diversity.

The species group comprises about 20 interfertile Antirrhinum species (each with 8 chromosomes) that grow in Southern Europe under ecologically different, sometimes extreme, conditions and display broad phenotypic diversity (Fig.2). Combined with the available research resources (arrayed EST collection with ~12,000 unigenes, BAC library, normalised cDNA libraries, genetic and physical maps, mutant collections, reverse genetics and transposonmutagenised populations) these features can be utilised to molecularly approach mechanisms of diversification and adaptation.

Phylogenetically, within the Euasterid clade, Antirrhinum is a member of the family of Scrophulariaceae (Lamiales), closely related to the Solanaceae (Solanales) – with obvious consequences for the mutual exploitation of 'genomics resources'. In fact, preliminary analyses of synteny between tomato and Antirrhinum



diversity

reveal a considerable level of gene-to gene accordance (Fig. 3). This can become the basis for corroborating ancestral gene orders (when compared to gene orders determined within the Rosid clade), and can also serve as a guide for comparative mapping approaches, gene isolation and genome sequencing.

Upon invitation, the Antirrhinum community is ready to share information and resources with the Solanaceae community, and hopes to gain by obtaining support for



ongoing and future work in this large community. The first step of joining will be the establishment of links to DragonDB (www.antirrhinum.net), the database that contains almost all *Antirrhinum*-relevant information, followed by merging sequence and other data into the SGN database. In the long term, we hope that interesting common research aspects will emerge, such as comparative analyses on common and distinct aspects of evolution of traits due to domestication (e.g. in potato or tomato) and natural selection (e.g. snapdragon).

## Lat-SOL Network

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## Provided by Fernando Carrari

The Lat-Sol initiative is an international and integrative research network of Latin America laboratories working in *Solanaceae*. This plant family comprises more than 3,000 species and is well represented in South America growing in diverse habitats. Large collections of native *Solanaceae* plant germplasm are preserved at several public Institutions of South America. The goals of the network are as follows: 1) Joining efforts and promoting information and resource flow between labs working in basic and applied aspects of *Solanaceae* species. 2) Coordinating research initiatives for establishing postgenomic technologies. 3) Integrating Latin America research within the existing SOL and EU-SOL programs.

The newly formed network currently involves 10 affiliated labs with more than 70 people including scientists, technicians, students, and various associated non-members. A website (http://cnia.inta.gov.ar/lat-sol/) has been launched recently to serve as a platform of information exchange and to present Lat-SOL to all the scientific community. The site contains more detailed information about the network, updates on upcoming events, job announcements, resources and facilities, relevant publications, and partnership opportunities.

## Micro-Tom cDNA Clones Available

## Províded by Koh Aokí

Kazusa DNA Research Institute started accepting requests for the Micro-Tom full-length cDNA clones on February 1, 2006. The new releases include 8,418 5'-end-sequencing clones (3,808 contigs) from our Micro-Tom fruit full-length cDNA library. For the detailed information of the clones, see Tsugane et al., 2005, Plant Biotechnology, 22: 161-165. For the clone search, please visit our database MiBASE (<http://www.kazusa.or.jp/jsol/microtom/>http://www.kazusa.or.jp/jsol/microtom/). Find "Clone Request" in the top page of MiBASE for clone request. Contact address: Koh Aoki (kaoki@kazusa.or.jp).

# **EU-SOL Update**

# Contributed by René Klein-Lankhorst

As of February 1, 2006, René Klein-Lankhorst will have a new position as Head of the EU-SOL Management Office. Up until that point, René was a principal investigator for the Dutch tomato chromosome 6 sequencing project, which will now be taken over by Roeland van Ham. René will be responsible for setting up and implementing all management structures in EU-SOL and for scientific and financial reporting towards the EUheadquarters. In addition, he will be responsible for the sequencing-workpackage in EU-SOL. The content of the EU-SOL program itself is formulated as follows: "EU-SOL is a network of plant scientists from universities, research institutes and industry within the EU, its partner countries Bulgaria, Israel and USA and INCO countries Westbank, Argentina and South-Africa. EU-SOL focuses on the development of high quality and healthy tomato and potato varieties with improved consumer-, processor- and producer-directed traits. The consortium brings together expertise across a wide variety of disciplines across the EU - from taxonomy to molecular biology to consumer integration. Quality and wholesomeness of food and food products are two issues addressed prominently by society especially in relation to obesity (and atherosclerosis), the most important cause of cardiovascular disease, age-related diseases, such as cancer and diabetes and to the increasing preference of consumers for 'regional' and 'niche' food specialties. Additionally producers are challenged by constraints in plant architecture and development such as fruit set and tuberisation". The total run-time of EU-SOL will be 5 years with a total budget of approximately \$40M. Approximately 500 people will be involved in EU-SOL.

# TOMATO SEQUENCING UPDATES

#### Chromosomes 1, 10, 11 (US)

#### Contact: Joyce Van Eck (jv27@cornell.edu)

Fourteen BACs have been sequenced, and we are in the process of selecting additional BACs for sequencing.

Since our last report on FISH using tomato SC spreads, an additional 17 BACs have been localized. This represents seven BACs on chr 1, two on chr 7, seven on chr 9, and one on chr 11. These BACs are identified in the table below, and examples of some of the micrographs used to obtain the data follow at the end of this update.

Chromosome Arm	BAC ID	Chromosome Arm	BAC ID
1P	130112	9P	026I24
1Q	008L19		116C14
	155M04		168F14
	054N01		203J14
	245N21	9Q	109D11
	108J06		165P17
	329A12		278J12
7Q	167K07	11Q	323E19
	215P04		

The top fluorescence micrograph illustrates the positions of two BACs, 245N21 (green) and 130I12 (red) on chr 1. The bottom micrograph illustrates the locations of three BACs, 109D11 (green), 165P17 (red)





and 116C14 (green) on chr 9.

## Chromosome 2 (Korea)

#### Contact: Sanghyeob Lee (sol6793@kribb.re.kr)

To date, we have completed the sequence for 39 BACs. After the first round of BAC extension, we have found that 67% of the seed BACs can be extended, and the average overlap length is 18 kb. Currently, we are focusing on BAC extension using the BAC end sequences and doing Fiber FISH for confirmation of the extended BACs.

## Chromosome 3 (China)

### Contact: Chuanyou Li (cyli@genetics.ac.cn)

To facilitate BAC-by-BAC based sequencing, we are constructing a genome-wide physical map of the tomato genome. First, we have made extensive manual editing of FPC (Fingerprinted Contigs) physical map based on fingerprinting of the Hind III BAC library of Heinz 1706 developed by Arizona Genomics Institute. The contig number has been reduced from about 7,000 to 3,000. The current FPC contains 88,650 BAC clones, representing 10X coverage of the tomato genome and covering about 788 Mb of physical regions. After integrating overgo hybridization, BAC end sequence, genomic sequence, and partial PCR-based anchoring data, 457 contigs (covering about 200 Mb) were anchored to the euchromatic regions in the F2.2000 genetic map. The integrated FPC data can be accessed through WebFPC (http://tomato.genetics.ac.cn/TomatoFPC/).

Second, in silico digestion of completely sequenced BACs has been performed and integrated with the FPC data in order to ensure the integrity of the physical map and provide additional anchoring information. The sequenced BACs downloaded from GenBank were named with the GenBank accession number plus suffix -sd1, such as AF411806sd1. The sequenced BACs downloaded from SGN were named with the BAC name plus suffix -sd1, such as a0016A12sd1. The in silico digested BACs were color-coded for easy recognition in WebFPC. Third, a total of 950 genetic markers with no overgo hybridization data were selected for PCR screening of BACs containing the markers. The remaining unanchored BAC contigs will be mapped to F2.2000 using CAPS markers developed from the BAC end sequences.

## Chromosome 4 (UK)

Contact: Christine Nicholson (ckb@sanger.ac.uk)

We have now sequenced and finished two BACs from the LE\_HBa library to Phase 3 (31H5 & 198L24). There are a small number of clones also in our sequencing pipeline, however we are currently focusing on developing the physical map further prior to the selection of the majority of our BACs to sequence in the form of minimal tiling paths across the contigs located on our chromosome.

At present, we are in the process of fingerprinting the SL\_MboI library. This will augment the genomic coverage in fingerprints and will make these additional clones "visible" in our FPC map so that we can make sequence BAC selections based both fingerprint and BES data where possible. We expect to finish the laboratory-based fingerprinting in February and the fingerprint data will be available to be incorporated into FPC in April 2006.

LE\_HBa BAC 198L24 underwent FISH at the Stack Lab. The chromosome location information provided by the FISH data is most useful for confirmation of BACs. We are therefore currently verifying fifteen further BACs to send for FISH analysis at the end of January. This will further assist the identification of BACs to sequence on chromosome 4.

## Chromosome 5 (India)

Contact: Akhilesh Tyagi (akhilesh@genomeindia.org)

The sequencing group at the Indian Initiative on Tomato Genome Sequencing has confirmed 20 BAC clones from chromosome 5 with the help of markers (CT101, T1252, C2-A+1g60200, cLET-8-B23, T0876, cLED-8-G3, BS4, T1592, T1360, T1777, T1541, T1584, TG69, CT130, TG185, TG597, cLEX-13-G5 and T1746) by sequencing with marker-specific custom primers, end sequencing, and fingerprinting. Shotgun libraries have been made and high-throughput sequencing has started. Whereas, sequencing of the BACs designated C05HBa0051A13, C05HBa0042B19, C05HBa0179E24, CO5HBa0027B05, CO5HBa0239D11 has progressed to Phase I, sequencing of BACs designated C05HBa0191B01, C05HBa0261K11, C05HBa0179K09, C05HBa0006N20 has progressed to Phase II.

#### Chromosome 6 (The Netherlands)

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#### *Contact: Sander Peters (sander.peters@wur.nl)*

The Dutch CBSG tomato sequencing initiative has confirmed BACs LE\_Hba\_052-N09, LE\_Hba\_117-B06, and LE\_Hba\_060-A01 from chromosome 6 by FISH. BACs LE\_Hba\_052-N09, LE\_Hba\_117\_B06, LE\_Hba\_116-G14, LE\_Hba\_066-A20, and MboI\_009-E16 have been sequenced, Phase 2 assembled and uploaded to SGN.

MboI\_009-E16 was used to radiate out from seed BAC LE\_Hba-024-L21. Sequencing and assembly confirmed a 7.2 kb overlap. MboI\_009-E16 was found using an STC approach and high density non-selective AFLP fingerprinting. The procedure we follow will be discussed in "TOPAAS, a Tomato and Potato Assembly Assistance System for selection and finishing of BACs" by Peters *et al.*, 2006 (accepted for publication in *Plant Phys.*).

Following the STC approach, currently 24 seed BACs have been screened against ~350,000 BAC ends. We identified 387 candidate BAC clones for walking. A set of candidate BACs hitting to 11 seeds have now been AFLP fingerprinted.

#### Chromosome 7 (France)

#### Contact: Farid Regad (regad@ensat.fr)

We are happy to announce to the SOL community that we now have the official notification for the financial support of the French sequencing project. This represents the formal kick-off for the sequencing of chromosome 7. However, while waiting for this important decision, we have been making some progress on the selection of the set of BACs to be sequenced. The physical localization by FISH of the seed BACs was done in Steve Stack's lab and we recently FISH-confirmed three BACs while the FISH mapping of seven more clones is in progress.

As an update to where the French sequencing effort is currently, ten BACs were selected on the basis of anchored seed BACs and/or FISH-confirmed BACs, and sequencing is in progress (seven are in Phase 1 and three are in Phase 2). Our main concern now is to identify as many BACs as possible whose location has been securely assigned to chromosome 7. In this regard, we are interested to interact with any group interested in a locus located on chromosome 7, and we propose to process these BACs with the highest priority.

Therefore, if there are regions on chromosome 7 that are of specific interest to any research group, we would be very happy to hear about them and will direct our effort towards these regions for early BAC selection. Please send an e-mail to Farid Regad regarding this type of interaction (regad@ensat.fr).

#### Chromosome 8 (Japan)

#### Contact: Satoshi Tabata (tabata@kazusa.or.jp)

Fourteen BACs have been completed, and the sequences were uploaded onto the SGN server. We are in the process of assembling 6 BACs and there are 0 BACs in the sequencing pipeline at this time. We are now preparing for PCR check of clones chosen for 10 markers. We will need additional markers in the near future and expect to obtain new information.

#### Chromosome 9 (Spain)

#### Contact: Antonio Granell (agranell@ibmcp.upv.es)

Fifteen BACs were selected, and to date, 10 have been confirmed by several methods. Presence on chromosome 9 has been confirmed for all 10 BACs by IL mapping and 7 have been localized by FISH in the Stack lab with 3 remaining to be analyzed. One clone has been sequenced and two are currently in the sequencing pipeline. The sequenced clone has been advanced to assembly.

#### Chromosome 12 (Italy)

### Contact: Mara Ercolano (ercolano@unina.it)

To date, 18 seed BACs associated with 13 markers located on the short arm of chromosome 12 have been analyzed. Ten were found suitable as sequencing start points. The nucleotide sequence of 3 BACs has been completed to Phase 1 or Phase 2, and the remaining clones are currently in the sequencing pipeline. In order to extend the minimal tiling path around the BAC Le Hba 03K07, BAC ends of BACs belonging to the contig 1002 were sequenced. This additional work was necessary as no useful matches were found in the SGN BAC-end database. However, none of the analyzed clones allows a minimal overlap.

As for the long arm of chromosome 12, 3 clones from the Le\_Hba library (155J24 and 183M06 associated with the marker T0770, and 193C03 associated with the marker T1266) have been validated correctly by sequencing over the overgo with their respective marker specific primers. An additional 36 BACs corresponding to 19 markers ranging from offset 57.2 to 120 cM, have been selected for analyses.

The FISH technique has been set up at the University of Naples and CNR-IGV of Naples, and will be used to confirm the map positions for some of the seed BACs. An annotated EST database from dbEST (NCBI) for tomato and potato has been determined. A Gbrowse annotation interface of all currently available BACs will be released to the SOL community to provide EST mapped data for the definition of gene models.

# INSTITUTE PROFILE

# INSTITUTO DE CONSERVACIÓN Y MEJORA DE LA AGRODIVERSIDAD VALENCIANA (COMAV)

# Contributed by Fernando Nuez & Jaime Prohens

The Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) is a Research Institute recently constituted and affiliated to the Universidad Politécnica de Valencia (Spain). The objectives of COMAV are the conservation, characterisation, evaluation and utilization in of germplasm in vegetable crops breeding, and in particular crops from the Solanaceae family. COMAV is a multidisciplinary center, which includes specialists in conventional breeding, molecular biology, in vitro culture, genomics, bioinformatics, plant pathology, plant physiology, chemistry and other disciplines, with the objective



Greenhouses of COMAV

of obtaining scientific and technical information and breeding material for the development of improved varieties for a more sustainable horticulture and that produces vegetables with an improved quality.

COMAV also holds one the most important germplasm banks for Solanaceae vegetable crops with more than 6,000 accessions of cultivated and wild accessions of tomato, pepper, eggplant, and other minor Solanaceous crops, like pepino, cape gooseberry, gboma and scarlet eggplants, naranjilla or tree tomato.

At present more than 20 senior PhD scientists work at COMAV in research programs dealing with Solanaceae crops. Ongoing research programs involve:

-Conservation, morphological, agronomic and molecular characterization of germplasm.

-Molecular ecology of populations of wild species and weedy forms of tomato in its region of origin.

-Identification, isolation and characterization of allelic variants for genes of agronomic interest.

-Development of databases for the management of morphological and genomic information of germplasm accessions.

-Selection and genetic enhancement of local landraces with high guality characteristics.

-Breeding for quality (organoleptic and nutritional quality, in particular antioxidants and other compounds with healthy properties).

-Breeding for disease resistance (mostly TSWV in tomato and pepper and TYLCV and PepMV in tomato).

-Molecular and genetic mechanisms involved in parthenocarpic fruit set.

-Genetic transformation and regeneration of double-haploids.



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Diversity in germplasm of tomato

Since all the groups constituting COMAV work together under the umbrella of a single institute, new possibilities in the synergistic use of facilities and expertise of the different groups have arisen. Because of this, COMAV is now expanding its works in the field of genomics of Solanaceae vegetable crops and is seeking ways to increase its collaborations with other groups. More information on COMAV can be obtained in the web page http://www.comav.upv.es or from fnuez@btc.upv.es

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# WHAT'S NEW ON SGN?

SGN continually works to improve the website and database. Many changes are "behind the scenes", invisible to the user, but very important for future stability and scalability of the site. For example, in late 2005, the BAC end database was moved from mysql to postgresql, which has more data integrity features and transactions, and will be a great benefit in the future. In addition, the user interface is improved regularly, such as by adding better search features. The most important updates in the past two months were:

- -A new version of the F2-2000 map has been loaded into the SGN database, and contains almost 150 new PCR-based markers for a total 1,973 markers. See http://sgn.cornell.edu/cview/map.pl?map\_id=9.
- -The F2-2000 map also shows the positions of the BACs that have been fully sequenced by the tomato genome sequencing project. See http://sgn.cornell.edu/cview/map.pl?map\_id=9.
- -The FISH map has been updated with 9 new experiments, bringing the total to 18 FISHed BACs on the map. See http://sgn.cornell.edu/cview/map.pl=13. Additional experiments will be added soon.
- -The SGN GBrowse tool has been updated, most notably with BACs from chromosome 8, bringing the total of BACs that can be viewed to 48 out of 102 BACs fully sequenced by the consortium to date. See http://sgn.cornell.edu/gbrowse/.
- -A new phylogenetic tree-browsing tool is available at http://sgn.cornell.edu/tools/tree\_browser/. This tool will be tightly integrated with the *Solanaceae* gene families available in the SGN database.
- -A new mapping program, FastMapping, is available under the tools section. See http://sgn.cornell.edu/tools/fastmapping/.
- -The featured lab is The Petunia Lab at Radboud University, Nijmegen. See http://www.san.cornell.edu/community/feature/200601.pl.



## CONFERENCES

### Solanaceae - Genomics meets Biodiversity Madison, Wisconsin, July 23-27, 2006

This meeting will be a coming together of the International Solanaceae Conference (its Sixth Meeting, with meetings held every five to six years), the Third Solanaceae Genome Workshop, and the Potato Association of America (PAA; its 90th Annual Meeting. Details of this conference can be viewed at: http://www.hort.wisc.edu/PAA-Solanaceae/.

You are invited to submit abstracts for the **Tobacco Satellite Session** to be held at the conference. Chairman of the session is Paolo Donini. The session will cover aspects of tobacco genomics, and the interface of genomics with genetics, cytogenetics, metabolomics, molecular physiology, molecular pharming, etc.

## The International Plant Photobiology Meeting

Paris, France, April 24 – 28, 2006 Organizers: Chris Bowler and Margaret Ahmad Space is limited to 200 people so register ASAP at http://www.reaumur.org.

# 3rd EPSO Conference: "Plant Dynamics: from Molecules to Ecosystems"

Visegrád, Hungary, May 28 – June 1 2006 www.epsoweb.org/catalog/Conf2006.htm

# JOB OPPORTUNITIES

## POSTDOCTORAL POSITIONS AVAILABLE IN TOMATO GENOME SEQUENCING/ANNOTATION

Postdoctoral positions are available immediately in the group of Dr. Giovanni Giuliano, at the Italian National Agency for New Technologies, Energy and the Environment, Rome, Italy. The group is coordinating the Italian effort towards the sequencing of tomato chromosome 12. The ideal candidates should have a strong background in plant molecular genomics and/or bioinformatics, with particular emphasis on gene mapping/sequencing and/or genome annotation. The duties comprise BAC mapping/validation, construction of BAC-specific libraries for sequencing through our automated DNA-sequencing pipeline, assembly and annotation of the results, interfacing with the International tomato genome project. Candidates with previous experience on complex genome projects are highly welcome. The project will be running for four years. The initial appointment is for one year. Salary will be a function of the candidate's experience. Please send CV and letters of reference to: giuliano@casaccia.enea.it.



SOLANACEAE RECIPES

Thank you to Fabrizio Cillo for sharing two website links for two different recipes for Sicilian Spaghetti alla Norma. I included both versions of the recipe. Each version will serve 6 people. Enjoy!

# Sicilian Spaghetti alla Norma

First version: http://www.e-rcps.com/pasta/rcp/p\_klmn/norma.shtml

This dish from Catania is usually made with Ricotta Salata cheese. The semi-hard salted ricotta gives it a distinctive flavor and should be available in specialty shops and some supermarkets. If need be, substitute pecorino for it, it will not be the same, but still delicious. Alla Norma after the composer Bellini's (born in Catania) Norma.

2 medium eggplants, 1-inch thick slices 1/2 cup olive oil (less if baking eggplant) 1 small onion, chopped 2 cloves garlic, crushed 1 1/2 tsp fresh oregano leaves, chopped or 1/2 tsp dry oregano	<ul> <li>5 or 6 leaves fresh basil, shredded</li> <li>1/2 peperoncino or 1/2 tsp red chili flakes, more to taste</li> <li>2 lbs tomatoes, ripe, seeded and chopped or canned Italian tomatoes</li> <li>salt and freshly ground black pepper</li> <li>3/4 cup grated ricotta salata or pecorino</li> <li>1 lb spaghetti or other pasta</li> </ul>
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Sprinkle the sliced eggplant with salt. Place in colander and let stand for an hour or so. (If not bitter, omit, salting removes bitterness.). Place on paper towels to drain. Heat the olive oil in a large skillet. Brown eggplant slices on both sides. Alternatively, brush both sides of slices with olive oil, place on cookie sheet, bake in a pre-heated 450° F oven for 15 minutes or till nicely browned. Cut slices into cubes or leave whole. Heat 2 T olive oil in a pan. Add onion and sauté till golden. Add garlic and sauté for 1 or 2 minutes. Add the tomato, fresh oregano, peperoncino and basil. Raise heat so sauce cooks at a fast bubble. Cook about 10 to 15 minutes; do not allow sauce to dry out. While tomato sauce cooks, bring water to a boil. Cook pasta and drain when done. Place in a bowl, pour tomato sauce and 1/4 cup cheese and mix. Add eggplant and toss again or leave eggplant slices whole placing them on top of the pasta dressed with the tomato sauce. Serve. Pass grated cheese at table.

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Second version: http://www.italianmade.com/recipes/recipe248.cfm

1 eggplant1 lb. penne1 clove garlic10 basil leaves10 ripe tomatoes, peeled, seeded and dicedsalt3 oz. aged salted ricotta, gratedpepperolive oil0

Slice the eggplant and place on a cutting board propped on a slant, cover with salt and leave under a weight for one hour until the bitter water seeps out. Brown the garlic in oil, add the tomatoes and salt. Bring to a simmer over medium heat and continue cooking, stirring occasionally, until the sauce has reduced by 1/3. Add pinch of pepper, remove from heat and set aside. Wash the slices of eggplant and pat dry; fry in hot oil, place on paper towels to dry, then chop coarsely, and set aside. Cook the spaghetti in a large pot of boiling salted water until just al dente and drain. Quickly toss in a large skillet half of the tomato sauce, the eggplant, a few basil leaves and half of the grated cheese over a brisk flame. Then put the pasta in the serving dish, cover with the remaining half of the sauce, the rest of the grated ricotta, sprinkle with a few more basil leaves and serve.

This site suggested the following wines that would go well with this meal:

-Cerasuolo di Vittoria DOC -Rosso Barletta DOC -Sant'Anna di Isola Capo Rizzuto DOC